
**Effect of Acute Exercise on Postprandial Lipemia and Biomarkers of Endothelial
Dysfunction and Inflammation in Normal Weight and Overweight Adolescents**

By

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Declaration

I, the undersigned, declare that the project material, which I now submit, is my own work, that any data presented is accurate and was collected and analyzed by myself. Any assistance received by way of borrowing from the work of others has been cited and acknowledged within the work. I make this declaration in the knowledge that a breach of the rules pertaining to project submission may carry serious consequences. I am aware that the project will not be accepted unless this form has been handed in along with the project.

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ABSTRACT

Elevated postprandial lipemia (PPL) is associated with impaired endothelial function, an increase in adhesion molecule expression and inflammation. Acute exercise reduces PPL in adults. No studies have examined the effect of acute exercise on PPL in normal weight (NW) and overweight (OW) adolescents.

PURPOSE: To investigate the effect of an acute bout of exercise (600 kcal) on postprandial changes in triglycerides (TG), glucose, insulin, adhesion molecules (sICAM-1, sVCAM-1) and inflammatory markers (CRP, IL-6, TNF- α , WBC) following a high-fat meal in NW and OW adolescents.

METHODS: 10 NW (BMI: $20.9 \pm 1.7 \text{ kg m}^{-2}$, $15.6 \pm 0.7 \text{ y}$) and 8 OW (BMI: $28.3 \pm 3.6 \text{ kg m}^{-2}$, $15.9 \pm 0.4 \text{ y}$) adolescent boys underwent two 6h oral fat tolerance tests (OFTT) separated by 7 d. On the evening prior to each OFTT, subjects rested (CTL) or completed a treadmill walk/run at 70% $\text{VO}_{2\text{max}}$ until 600 kcal had been expended (EX). Blood samples were obtained at baseline and at 30 min, 1, 2, 4 and 6 h after eating.

RESULTS: Exercise reduced ($p < 0.01$) the postprandial TG area under the curve by ~25% in both the NW and OW groups. The postprandial glucose and insulin response did not differ between the control and exercise trials or between the NW and OW groups. Circulating leukocytes and plasma IL-6 levels increased ($p < 0.01$) in the NW and OW groups 6 h following the OFTT in both experimental conditions. Plasma concentrations of TNF- α were higher ($p < 0.05$) in the OW than the NW group at rest and 6 h following the OFTT in both the control and exercise experimental condition. There were no changes in CRP, sVCAM-1 or sICAM-1 following the OFTT and there were no differences between experimental condition or BMI group.

CONCLUSION: Acute exercise attenuates the postprandial TG response to a high-fat meal similarly in NW and OW adolescents but does not reduce inflammation or alter adhesion molecule expression in the postprandial period.

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CHAPTER I

INTRODUCTION

The vascular endothelium is a monolayer of cells that forms a biologic interface between circulating blood elements and the various systems in the body. It has been recognized as an active, dynamic tissue that controls many important functions, including regulation of vascular tone via nitric oxide (NO) production, regulation of platelet aggregation, leukocyte adhesion, thrombosis, fibrinolysis, and angiogenesis, as well as having an important role in the mediation of inflammation.^{67, 88, 152} Given its strategic location, the endothelium is a primary target for injury from mechanical forces and processes related to cardiovascular risk factors. Impairment of the normal function of the endothelium, a process known as endothelial dysfunction (ED), represents one of the earliest events in the pathogenesis of atherosclerosis.

High triglyceride (TG) levels and, in particular, postprandial lipemia can result in ED.^{71, 73,}
^{75, 112} Postprandial lipemia (PPL) is characterized by a rise in plasma levels of triglyceride-rich lipoproteins for up to 8 h following the consumption of a fat-rich meal. The breakdown of postprandial triglyceride-rich particles near the arterial wall plays an important role in the initiation and progression of ED.^{163, 164} Fasting TG levels do not provide information on triglyceride metabolism. The extent of postprandial TG, and the rate of its clearance after a high fat meal, can discriminate cardiovascular risk, even in individuals with normal fasting levels.^{111, 135} It is not uncommon for children and adults in industrialized nations to spend considerable time in the postprandial state in a typical day.

It is well established that inflammation plays an important role in the pathogenesis of atherosclerosis.^{87, 90, 129} Inflammatory mechanisms appear to couple dyslipidemia to atherosclerotic plaque formation by inducing the endothelium to express adhesion molecules, including intercellular adhesion molecule-1 (ICAM-1) and vascular adhesion molecule-1 (VCAM-1), on their surface. Adhesion molecules mediate the infiltration and transmigration of the vessel wall by monocytes and lymphocytes that facilitate atherosclerotic plaque development. The lipolysis products of postprandial TG-rich lipoproteins can activate endothelial cells and leukocytes, possibly by an oxidative stress mechanism.^{5, 18, 19, 143} Similar mechanisms may also lead to inflammation and leukocyte activation and there is some evidence indicating transient postprandial increases in soluble adhesion molecules, pro-inflammatory cytokines and other inflammatory markers.^{18, 62, 104, 148-151} It is not clear if overweight but otherwise healthy adolescents experience greater postprandial perturbations in these biomarkers compared to their normal weight counterparts.

Overweight and obesity in adults are associated with atherogenic dyslipidemia, chronic low-grade systemic inflammation, and endothelial dysfunction.^{1, 28, 56, 66, 85, 85, 136} Although the development of atherosclerosis occurs during childhood, clinical symptoms of the disease may not manifest until later in life. Obese adolescents typically display higher levels of fasting TG, adhesion molecules, inflammatory cytokines/biomarkers, low density lipoprotein cholesterol (LDL-C), and impaired endothelial function compared to age-matched controls.^{49-52, 101, 107, 137}

Acute exercise can significantly reduce the postprandial TG response in adults, which may contribute to the beneficial effects of exercise on endothelial dysfunction^{55, 60, 118}. A single

bout of exercise performed 4-24 h prior to feeding has been shown to reduce postprandial TG.¹⁶¹
¹⁶² This acute effect typically results in a 20-30% reduction in the area under the TG-time curve, and is consistent in a range of populations including healthy young men and women,^{2, 47, 63, 80, 81, 91, 92, 139, 142, 144, 162} healthy middle-aged men and women,^{145, 165 44 45 42} and hypertriglyceridemic and obese men.^{40, 161} It is not known if data showing attenuation of postprandial TG and markers of endothelial dysfunction and inflammation following acute exercise in adults can be extended to adolescents.

Specific Aims of the Research:

The aims of the present study are:

1. To compare the effects of a standardised high-fat mixed meal on postprandial TG in normal weight and overweight adolescent males.
2. To compare the effects of a standardised fatty meal on biomarkers of endothelial dysfunction (sICAM-1, sVCAM-1) and inflammation (interleukin-6, IL-6; tumor necrosis factor-alpha, TNF- α ; C-reactive protein, CRP; white blood cell count, WBC) in normal weight and overweight adolescent males.
3. To examine the effect of a single exercise bout on postprandial lipemia, as well as markers of endothelial dysfunction and inflammation, in normal weight and overweight adolescent males.

Study Hypotheses:

1. Serum TG concentrations will be higher after a high fat meal in overweight than normal weight adolescents.
2. Plasma concentrations of sICAM-1, sVCAM-1, IL-6, TNF- α , CRP and WBC will be higher after a high fat meal in overweight than normal weight adolescents.
3. The postprandial lipemic response will be attenuated in both normal weight and overweight adolescents, while circulating levels of biomarkers of ED and inflammation and the will be lowered only in the overweight adolescents, when exercise is performed prior to a high fat meal.

CHAPTER II:

LITERATURE REVIEW

Pathogenesis of Atherosclerosis

Atherosclerosis is the leading cause of death in industrialised countries. It is an inflammatory vascular disease characterised by the progressive accumulation of lipids and fibrous elements in the sub-endothelial space of large arteries. It frequently affects the coronary and cerebral vessels, leading to myocardial infarction and stroke. The initiating event in atherosclerosis appears to be injury to the arterial endothelium. Risk factors such as elevated LDL-C, low circulating levels of high density lipoprotein cholesterol (HDL-C), hypertension, smoking, genetic factors, advanced age, male gender, type II diabetes mellitus, obesity and a sedentary lifestyle are linked by a common mechanism through their deleterious effects on endothelial function. Structural damage to the endothelium can impair permeability, interfere with anti-thrombotic properties, and alter the release of vasoactive substances.^{35, 103} It also permits accumulation and retention of LDL-C in the sub-endothelial space. Trapped LDL-C undergoes chemical modification, including oxidation, lipolysis, proteolysis and aggregation,⁹⁰ important events in the development of atherosclerosis.

Modified LDL-C acts as a chemoattractant that recruits circulating monocytes to the vessel wall. Other factors that contribute to the recruitment of monocytes include the secretion of specific cytokines, such as TNF- α by injured endothelial cells, upregulation of monocyte

chemoattractant protein (MCP), macrophage colony stimulating factor (M-CSF), and the expression of adhesion molecules on the luminal surface of the endothelium.^{12, 77, 90} Examples of adhesion molecules involved in the atherosclerotic process include members of the immunoglobulin gene superfamily (VCAM-1, ICAM-1 and PECAM-1), and selectins (P-selectin and E-selectin).⁹⁰

Following their adherence to the intima, monocytes may penetrate into the sub-endothelial space by migrating through the junctions between endothelial cells, where they differentiate into macrophages. Macrophages engulf oxidatively modified LDL-C leading to the formation of large foam cells, a prominent characteristic of the 'fatty streak' - the initial manifestation of atherosclerosis. Autopsy findings have revealed the presence of atherosclerotic lesions and fatty streaks in the coronary arteries and aorta of children as young as 3 years, and the presence of occlusive lesions in adolescents and young adults.¹³⁸ Fatty streaks can increase in size, and progress to form fibrous plaques in advanced stages of atherosclerosis. Fibrous plaques are elevated lesions on the lining of the arteries. They are characterised by a growing mass of extracellular lipid, mainly cholesterol and cholesterol esters, necrotic cells and by the migration of smooth muscle cells (SMCs) from the arterial media to the intima and the proliferation of the SMC within the intima. The intimal SMCs secrete extracellular matrix and give rise to a fibrous cap. Plaques can ultimately narrow the lumen of the artery causing stenosis, or rupture, leading to thrombosis.

Endothelial Function

The innermost layer of blood vessels, the intima, is composed of a single layer of endothelial cells. In addition to acting as a selectively semi-permeable membrane between circulating elements in the blood and tissue, the endothelium mediates vascular tone, and actively suppresses thrombosis, vascular inflammation, and SMC migration and proliferation.^{82, 87 99} Endothelial cells (EC) are capable of sensing hemodynamic forces (i.e., shear stress and cyclic strain) and locally produced or circulating mediators, and responding to these factors by releasing a range of biologically active substances such as nitric oxide (NO), prostacyclin, endothelin-1, free radicals and platelet-activating factors.²⁰ Originally described as endothelium-derived relaxing factor, NO is a free radical generated by oxidation of L-arginine to L-citrulline. The reaction is catalyzed by endothelium-derived NO synthase (NOS).

Coronary artery endothelial function can be assessed using quantitative angiography in response to intracoronary infusions of pharmacological agents.¹⁰² Acetylcholine is the most commonly used agent to assess endothelium-mediated vasodilation in the coronary arteries.⁹ Acetylcholine acts via muscarinic membrane receptors with signal transduction through G proteins to mediate the release of NO.¹⁵ A vasodilator response to acetylcholine indicates preserved endothelial vasodilator function. Other endothelial-dependent agonists include bradykinin, substance-P, norepinephrine and serotonin.

High-resolution ultrasound is a commonly used non-invasive, technique to assess endothelial function in peripheral arteries. This method assesses the ability of the endothelium to

dilate in response to shear-induced increase in NO. Brachial artery diameter is measured at baseline and after reactive hyperemia produced by 5 min of upper or lower arm occlusion. Increased blood flow during reactive hyperemia elicits a strictly endothelium-dependent vasodilator response by activating endothelial-derived NOS. The relative increases in diameter from baseline is used as a measure of flow-mediated, endothelium-dependent dilation. Diameter changes are also measured after administration of nitroglycerin in order to assess the response of the vessel to endothelium-independent vasodilation. Studies showing a correlation between coronary and peripheral arterial function suggest that non-invasive assessment of flow-mediated dilation in peripheral arteries can be used as a surrogate for coronary artery endothelial function.

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Endothelial dysfunction refers to the physiological dysfunction of normal biochemical/biophysical processes undertaken by the endothelium that results in a reduced capacity to maintain homeostasis leading to the development of pathological inflammatory processes and vascular disease. Alterations in coronary vascular endothelial function were first described in 1986 when paradoxical vasoconstriction of atherosclerotic segments were observed after infusing acetylcholine into the left coronary artery of patients with atypical chest pain.⁸⁹ In contrast, angiographically normal, smooth segments dilated indicating that atherosclerosis leads to loss of endothelium-dependent vasodilator function.

There is accumulating evidence that endothelial dysfunction represents one of the earliest events in the pathogenesis of cardiovascular disease. It is evident as a selective

impaired response to pharmacological stimuli in patients with angiographically normal arteries¹⁰² and progresses to a loss of endothelium-mediated vasodilation in angiographically defined atherosclerotic coronary disease.⁵³ Moreover an impaired vascular response has been demonstrated in children as young as 7 years old with familial hypercholesterolemia¹³⁴ and in obese adolescents.¹⁰¹

Endothelial dysfunction is characterized in part by low levels of chronic inflammation. Evidence of inflammation is found locally in the vascular wall and systemically in the circulation. Systemic markers of vascular inflammation include an elevated WBC count, and high circulating levels of CRP, IL-6 and TNF- α .^{70, 113} In addition, endothelial dysfunction and atherosclerotic lesion progression, result in an increase in the expression of adhesion molecules on the endothelial surface.^{29, 69, 96} Upregulation of membrane-bound adhesion molecules facilitates the attachment of circulating leukocytes and monocytes to the endothelium, and leads to subsequent ED.⁹⁶ Soluble isoforms of ICAM-1 (sICAM-1) and VCAM-1 (sVCAM-1) are elevated in a number of pathologic conditions including diabetes,¹²⁸ dyslipidemia,⁵⁷ hypertension³¹ and atherosclerosis.^{13, 14, 29, 69} The appearance of soluble cell adhesion molecules in the circulation is thought to result from the release of cell surface adhesins by activated endothelial cells. Considering that the expression of cell surface adhesion molecules is tightly regulated in order to avoid uncontrolled cell migration,¹³² the level of soluble CAM in the circulation likely reflects endothelial inflammation/activation and an increase in their expression on the cell surface.^{86, 96}

Inflammatory biomarkers

CRP, an acute phase reactant synthesised primarily by hepatic and arterial tissue, is commonly used a marker of systemic inflammation. There is accumulating evidence that CRP may play a direct role in vascular inflammation.^{22, 110} CRP can bind and activate the complement system, induce expression of cell adhesion molecules and IL-6, mediate LDL-C uptake by endothelial macrophages, and induce monocyte recruitment into the arterial wall.^{87, 110, 123, 159} It is an independent predictor of cardiovascular disease risk in individuals with normal LDL cholesterol, and adds prognostic value to the Framingham risk assessment.^{79, 123-125, 146 127, 157} It is associated with obesity,¹⁰⁹ and is inversely associated with physical activity.⁶ In children, CRP is associated with ED, intima-media thickening and obesity.^{37, 74}

TNF- α is a pleiotropic pro-inflammatory cytokine derived from adipose tissue, macrophages, endothelial and smooth muscle cells.¹²² Elevated plasma TNF- α levels are positively related to obesity, insulin resistance, hypertriglyceridemia, impaired glucose tolerance and negatively related with HDL-C.^{28, 117} It has been suggested that TNF- α mediates insulin resistance in obesity, as it is over-expressed in obese rodents and humans and blocks the action of insulin.⁹⁸

There is a close relation between circulating concentrations of TNF- α , IL-6, and CRP. TNF- α stimulates SMC expression of IL-6, which is the major inductor of hepatic CRP synthesis. In addition, IL-6 has intrinsic pro-inflammatory and pro-coagulant activity and increases expression of adhesion molecules on the endothelium and levels of TNF- α . Both IL-6 and TNF- α

are over-expressed in obese individuals.^{84, 160} Adipose tissue is an important source of TNF- α , IL-6 and other cytokines, and contributes to the pro-inflammatory state associated with obesity.

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Several mechanisms may explain the pathophysiological link between TNF- α and atherosclerosis, most notably inflammatory responses. TNF- α upregulates expression of ICAM-1 and VCAM-1 in the vascular wall.¹⁰⁸ High levels of TNF- α correlate with an increased risk of recurrent events in stable post-myocardial infarction patients.¹²² Serum IL-6 levels are associated with future coronary events, even after correcting for CRP levels.¹²⁶ Circulating levels of IL-6 are increased following strenuous exercise.¹¹⁴ After exercise, IL-6 is produced in larger amounts by the contracting muscle suggesting that depleted glycogen content in the contracting muscle stimulates IL-6 release. The muscle-derived IL-6 has been shown to decrease levels of TNF- α and has been classified as both a pro-inflammatory and anti-inflammatory cytokine.^{114, 117}

Endothelial Function and Exercise

Exercise-induced increases in shear stress, blood flow and blood pressure have a positive effect on endothelial function.¹⁰⁵ Shear stress is a potent stimulus for NO release. Chronic increases in blood flow increase mRNA expression for eNOS, augment NO activity, and enhance endothelium-dependent vasodilation in coronary arteries.¹⁰⁵ Modulatory effects of exercise on CHD risk factor profile may also have a positive effect on endothelial NO production. The effect of exercise on the number and function of endothelial progenitor cells, which represent an important endogenous repair mechanism, also likely contributes to the improvement in endothelial function observed in response to exercise.^{68, 121}

The repetitive increases in coronary and peripheral blood flow in response to regular exercise may facilitate coronary artery adaptations that enhance the endothelial response to shear stress. The endothelial response to exercise training in humans appears to depend on pre-training endothelial function.¹⁰² Improved endothelial function occurs in individuals with baseline dysfunction such as the elderly, asymptomatic individuals with one or more CVD disease risk factors, and patients with CAD or heart failure,^{58, 65, 78, 130, 158} but only rarely has exercise training improved endothelial function in healthy, young subjects.^{21, 32}

Triglyceride Metabolism and Endothelial Dysfunction

Lipids are a major energy source. In addition they provide the hydrophobic barrier for cells membranes, facilitate the absorption of fat-soluble vitamins and have regulatory or co-

enzyme functions. The majority of fat in the diet is in the form of TG. The remainder consists of cholesterol, cholesterol esters, phospholipids and free fatty acids (FAA).

Dietary TG is degraded in the stomach by lipases. In the duodenum, TG are emulsified by bile salts and further degraded by pancreatic enzymes, resulting in monoacylglycerols and free fatty acids (FFA) that form mixed micelles. Unlike TG, mixed micelles can be absorbed by intestinal mucosal cells, where TG are resynthesised from their component parts. They are assembled with cholesterol esters and phospholipids and incorporated into chylomicrons, a TG-rich form of lipoprotein used to transport exogenous lipid.

Chylomicrons are released into lymph vessels and deposited into the venous circulation, where they can gain access to peripheral tissues. Chylomicrons are hydrolyzed by lipoprotein lipase (LPL), which releases FFAs for uptake into peripheral tissues. The chylomicron remnants which remain in the circulation are now largely composed of cholesterol. In the liver, they are re-exported as free cholesterol or used to form bile salts.

In the fasted state, TG are synthesized and released by the liver in the form of very low density lipoprotein (VLDL). VLDL is hydrolyzed by LPL, releasing FFAs for uptake into peripheral tissues. The remaining intermediate density lipoprotein is further processed in the circulation to form LDL particles.

The period of time when circulating levels of TG remain elevated following a meal is called the postprandial period. In the postprandial state, transient increases in plasma TG occurs, in the form of chylomicrons. Postprandial lipemia is considered a proatherogenic state.

^{163, 164} There are several proposed mechanisms through which postprandial lipemia exerts its atherogenic effects. Postprandial lipoproteins and their remnants may deposit directly into the arterial wall, where they can be oxidized and incorporated into atheromas. ^{23, 34, 39, 164} High postprandial triglyceride concentrations exert an atherogenic influence on other lipoproteins especially LDL and HDL, contributing to an 'atherogenic lipoprotein phenotype' when the postprandial lipemia state is induced consistently by the regular ingestion of high-fat meals. ¹⁶⁴ Pro-thrombotic and pro-inflammatory changes are also evident postprandially. ^{41, 83, 148, 151} Endothelial function, as assessed by flow-mediated dilation and adhesion molecule expression is impaired from repeated bouts of lipemia. ^{7, 39, 62, 73, 119, 153}

Postprandial lipemia is an independent predictor of the presence of atherosclerotic cardiovascular disease, even after controlling for fasting TG levels. ^{111, 112} There are several lines of evidence suggesting that postprandial lipemia increases risk of atherogenesis. ⁷¹ Cross-sectional studies have found that obese and diabetic individuals have greater postprandial lipemia than lean healthy subjects. ^{40, 147} Visceral obesity has been linked with disturbed free fatty acid metabolism and impaired lipolysis in the postprandial state, but it is unclear if obese individuals demonstrate specific abnormalities in postprandial metabolism, or if the findings represent a more generalized consequence of an increase in metabolic stress associated with obesity. ^{11, 26, 27}

Postprandial Lipemia in Adolescents

Postprandial lipemia is equally relevant to younger populations given that the process of atherosclerosis typically begins in the first decade of life.¹¹⁵ The Columbia Biomarkers Study showed that the magnitude of the postprandial TG response in adolescents is similar to adults.²⁵ However, age-related differences in lipid profile and stage of atherosclerosis are concerns that limit the generalizability of results seen in adults to adolescent populations.

Studies examining postprandial TG responses in children and adolescents have been limited to examinations of the influence of body composition, fat distribution and growth hormone deficiency. Fasting and postprandial TG of untreated growth hormone-deficient adolescents are positively associated with fasting and postprandial CRP, and with postprandial TNF- α and IL-6 concentrations.⁸³ Moreno and colleagues observed a significant increase in serum triglycerides at 2 h and 4 h after an oral fat tolerance test, in both obese and non-obese adolescents. The magnitude of the response was related to fat distribution. Individuals with centrally distributed fat had a greater postprandial lipemic response.¹⁰⁰ Umpaicha *et al.* observed higher TG levels in obese diabetic adolescents than non-diabetic obese and non-diabetic and healthy normal weight adolescents.¹⁴⁷ These results underline the importance of examining strategies aimed at reducing PPL in adolescents and examining possible interactions with body mass index.

Exercise and Postprandial Lipemia

Exercise attenuates postprandial lipemia. The mechanisms responsible for the exercise-related attenuation of postprandial TG are not fully understood. Exercise reduces the fasting TG pool size but the incremental area (above baseline) under the TG-time curve is also reduced compared with control conditions.^{3, 80} The lower postprandial TG concentrations after exercise also reflect an enhanced rate of removal by peripheral tissues of TG-rich lipoproteins. LPL activity is upregulated after exercise in a time-course consistent with the postprandial reduction of TG.^{131, 161, 162} This is likely to result in increased clearance of TG from the blood, but still cannot fully account for the magnitude of the attenuation observed following exercise. A decreased rate of VLDL synthesis and secretion from the liver is also a contributing factor and accounts, at least in part, for the remaining reduction in circulating postprandial TG.⁴³

The influence of exercise on postprandial lipemia was first reported in comparisons between trained individuals and sedentary individuals.⁹⁵ Detraining studies examining previously trained subjects found that the effect of exercise on postprandial lipemia resulted from acute exercise as opposed to a longer term training effect.^{41, 61} The influence of exercise intensity and substrate utilization during exercise on the subsequent attenuation of postprandial TG appears to be of relatively less importance than total energy expenditure.^{92, 118, 144} For this reason, intermittent bouts of moderate intensity exercise have been shown to be equally as effective as continuous moderate intensity exercise, provided the total energy expenditure is similar.^{3, 48} The postprandial TG reduction observed following exercise cannot be replicated by energy restriction

alone. When a caloric deficit protocol was employed to mimic the energy expenditure resulting from acute exercise, postprandial lipemia was significantly lower than a control condition but remained greater than that seen in the exercise condition.⁴²

Conclusion

In summary, since the identification of PPL as a possible contributor to atherosclerosis less than 30 years ago, an extensive evidence base has been developed linking the rise in TG after eating a high fat meal to impaired endothelial function, consistent with the hypothesis that injury to the endothelium is the initiating step in atherosclerosis. Minimizing the deleterious impact of PPL is pivotal to ensuring that the endothelium functions in an atheroprotective manner. To date, there are no conclusive data regarding the effect of acute exercise on attenuating PPL in adolescents that also examines the influence of biomarkers of inflammation and endothelial function. This represents an important void in our understanding of the vascular complications associated with PPL and is a salient question considering that of the process of endothelial dysfunction and the subsequent development of atherosclerosis is initiated at young age in humans. Furthermore, no investigations have been designed to determine whether the PPL is influenced by overweight at a young age, which would provide insight to understanding the increased cardiovascular risk associated with increased adiposity.

CHAPTER III

METHODOLOGY

Subjects

Ten normal weight (NW) and eight overweight (OW) adolescent boys were recruited. Classification of normal weight and overweight were based on based on age- and sex-specific BMI cutoffs.²⁴ Subjects were non-smokers, were not taking any medications, and had no history of diabetes, heart disease, liver dysfunction, shortness of breath or asthma with usual exercise, or other medical conditions that would contraindicate exercise participation. All subjects were moderately physically active.

Research Design

The study involved one screening visit and two experimental trials. An oral fat tolerance test (OFTT) was undertaken during each experimental trial. The experimental trials were separated by approximately 7 d. On the evening prior to each OFTT, subjects rested (control) or completed a 600 kcal treadmill jog (exercise) at approximately 65% of maximal aerobic capacity ($\dot{V}O_{2max}$). The order of the experimental sessions was randomised.

Preparation

Subjects avoided strenuous physical activity for 2 d before each visit. They recorded their normal diet for the 3 d prior to the first OFTT and repeated this diet on the 3 d prior to the second OFTT.

Screening

The nature and risks of the study were explained, a health and physical activity history questionnaire was completed, and written informed consent form was obtained in accordance with Dublin City University's research ethics committee [Appendix I]. Subjects then underwent a brief physical examination, body composition assessment and measurement of $\dot{V}O_{2max}$.

Maximal Aerobic Capacity

$\dot{V}O_{2max}$ was determined using a ramp treadmill protocol. Following a 2 min warm up at 5 or 6 mph, depending on the subject's physical activity history, the gradient was increased 0.2% every 8 sec until volitional exhaustion. Subjects were verbally encouraged to give their best effort. Respiratory metabolic measures and heart rate were determined continuously throughout the test. Maximal oxygen uptake was determined by averaging the two highest consecutive 20 s values. The test was deemed to be maximal if it satisfied at least 2 of the following criteria: leveling of oxygen consumption values as indicated by a difference in values between the final two stages of $<1.5 \text{ ml}\cdot\text{kg}\cdot\text{min}^{-1}$, RER > 1.1 and heart rate within ± 10 beats of the age predicted maximum.¹⁴¹

Experimental Trials

Subjects reported to the Cardiovascular Research Unit in DCU at approximately 8 am following an overnight fast. An intravenous catheter (21G) was inserted into a prominent forearm vein. Following a 10 min rest period, a baseline blood sample was obtained. Subjects consumed (under supervision) a mixed meal that was high in fat. 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 [Appendix III]. Water was consumed ad libitum following the first OFTT. The volume of water consumed and the time(s) of consumption relative to the meal were recorded and replicated following the subsequent OFTT.

Blood samples were obtained before the test meal was ingested and in the postprandial period at 30, 60, 120, 240 and 360 min. Subjects rested quietly during this period but were permitted to read or watch television.

Acute Exercise Bout

Data from the maximal aerobic capacity test was used to determine the speed and gradient required to elicit approximately 65% $\dot{V}O_{2max}$ using published guidelines.⁴ Subjects completed the exercise bout in the evening 16–18 h prior to ingestion of the meal. Energy expenditure and exercise intensity were recorded continuously throughout the exercise bout and the treadmill speed was adjusted accordingly if subjects could not comfortably maintain the exercise intensity. The exercise bout was completed once 600 kcal were estimated to be expended. The exact energy expenditures were later verified.

Cardiorespiratory and Metabolic Measures

Respiratory metabolic responses were determined using standard open-circuit spirometry techniques (SensorMedics Vmax 229, SensorMedics Corp., CA). Prior to testing, the gas analysers were calibrated with standard gases of known concentration. Heart rate was measured continuously during each trial via telemetry (Polar Vantage NV, Polar, NY). [Appendix II].

Body Composition

Double thickness subcutaneous adipose tissue was measured on the right side of the body using skinfold calipers (Harpenden, Cambridge Scientific Industries, MD). The following anatomical sites were used; chest, subscapular, axilla, suprailium, abdomen, triceps and thigh.⁷² A minimum of 2 measurements were taken at each site. If the measurements varied by more than 1 mm, a third measurement was taken.

Blood Sampling and Processing

Samples were collected into vacutainers containing either potassium EDTA, sodium citrate or without any additive (Becton Dickinson and Co., UK). Plasma and serum were separated by centrifugation (1620 g for 15 min at 4 °C) (PK121R centrifuge, ALC, VA) within 30 min of collection. Plasma and serum samples were stored at -20 °C and -80 °C, respectively. Repeated measures from all subjects were defrosted together and analysed in the same run.

Biochemical Assays

Immediately following collection, a complete blood count was performed on whole blood samples drawn at 0 h and 6 h in an EDTA-coated tube using an automatic hematology analyzer (Ac-T Diff 2, Beckman Coulter, CA). Serum glucose concentration was determined using an automatic blood glucose analyzer (YSI 2300 STAT, Yellow Springs Instruments, OH). Serum insulin was determined using standard fluoroimmunoassays (AutoDELFIA, Finland). Serum total cholesterol, TG, and HDL-C were determined using enzymatic assays (Liquicolor, Human, Germany/Randox direct, Randox, Northern Ireland) and measured spectrophotometrically (MODULAR, Hitachi, Japan). Fasting LDL-C was calculated using the Friedewald equation³⁸. Serum levels of high-sensitivity CRP were measured with an ultra-sensitive competitive immunoassay (Roche Diagnostics, Germany). Commercially available enzyme-linked immunoassays (R&D Systems, UK) were used to determine plasma levels of sVCAM-1, sICAM-1, IL-6, and TNF- α . Intra-assay coefficients of variation were 1.8%, 6.8%, 2.8% and 8.1%, respectively.

Calculations

Measures of hemoglobin and hematocrit at baseline and at 6 h post meal were used to monitor changes in plasma volume using the method of Dill and Costill.³³ Changes in plasma volume in the post-meal observation period did not differ between trials or BMI group and no corrections were made.

The trapezoidal rule was used to calculate the area under the TG concentration versus time curve and the incremental area (i.e. normalized to the baseline concentration) under the TG versus time curve [Appendix IV].

Energy expenditure during the acute bout of exercise was calculated using the equation of Weir: ¹⁵⁵ $EE \text{ (kJ}\cdot\text{min}^{-1}) = 16.5 \dot{V}O_2 \text{ (L}\cdot\text{min}^{-1}) + 4.63 \dot{V}CO_2 \text{ (L}\cdot\text{min}^{-1})$. The rate of carbohydrate and fat oxidation was calculated from gas exchange measurements according to the table of non-protein respiratory quotient: ¹¹⁶ $\text{CHO oxidation} = 4.85 \dot{V}CO_2 - 3.226 \dot{V}O_2$, and lipid oxidation = $1.695 \dot{V}O_2 - 1.701 \dot{V}CO_2$, with mass expressed in $\text{g}\cdot\text{min}^{-1}$ and gas volume in $\text{L}\cdot\text{min}^{-1}$. The percentage of carbohydrate and lipid oxidation during exercise was calculated using the McGilvery & Goldstein equations: ⁹⁴ $[\% \text{lipid oxidation} = ((1-\text{RER})/0.29) \times 100$, and $\% \text{CHO oxidation} = ((\text{RER}-0.71)/0.29) \times 100$. Values for $\dot{V}O_2$, $\dot{V}CO_2$ and RER were averaged every minute throughout the exercise period.

Statistical Analysis

Data were analysed using SPSS (v11.0, SPSS Inc., IL). Independent sample t-tests were used to compare baseline characteristics between the normal weight and overweight groups. A mixed model (group x experimental condition) ANOVA was used to compare blood plasma volumes changes and the postprandial lipemic, glycemc and insulinemic area under the curve values between trials. A mixed model (time x group x experimental condition) ANOVA, with repeated measures on time and experimental condition was used to compare changes in serum and plasma markers throughout trials. Where indicated by a significant F value, post hoc tests,

with a Bonferroni correction for multiple comparisons, were performed to compare specific group means. Data from the NW and OW groups were combined when there was no main effect of BMI, BMI x group or BMI x postprandial response interactions. Pearson's product-moment correlation coefficients were used to determine the relation between selected parameters. Data are presented as mean \pm standard deviation. A probability of $p \leq 0.05$ was accepted for statistical significance.

CHAPTER IV

RESULTS

A summary of subjects' physical characteristics is presented in Table 1. Body mass, BMI, sum of skinfolds and VO_{2max} ($ml \cdot kg^{-1} \cdot min^{-1}$) were lower ($p < 0.05$) in NW than OW adolescents. Fasting concentrations of total cholesterol, HDL-C, LDL-C, TG, glucose and insulin were within the normal range in both groups (Table 2). Fasting HDL-C values were higher ($p < 0.05$) and TNF- α and WBC levels were lower ($p < 0.05$) in the NW than the OW adolescents (Table 2). There were no differences in the fasting levels of IL-6, CRP, sVCAM-1 and sICAM-1 between NW and OW adolescents.

The physiological data from the exercise trial is summarized in Table 3. The exercise bout was well tolerated by all subjects. The NW group exercised at a lower % $\dot{V}O_{2max}$ ($p < 0.05$) and a higher treadmill velocity ($p < 0.05$) than the OW group. Caloric expenditure and substrate utilization during exercise were similar in both NW and OW adolescents.

Triglycerides

Exercise reduced ($p < 0.01$) the postprandial TG area under the curve by ~25% in both the NW and OW groups (Figure 1). As there was no group main effect or group x experimental condition interaction for TG AUC or TG AUC_i, the NW and OW group data were combined for further statistical analysis. The postprandial TG concentration (TG AUC) and the postprandial rise in TG concentration from baseline (TG AUC_i) were significantly lower in the exercise

condition (Figure 2). There was a significant relation between the TG AUC and sum of skinfolds ($r=0.49$, $P<0.05$ control trial; $r=0.47$, $P<0.05$ exercise trial), TG AUC and $VO_2\text{max}$ ($r=0.59$, $P<0.05$ control trial; $r=0.60$, $P<0.05$ exercise trial) and TG incremental AUC and $VO_2\text{max}$ ($r=0.55$, $P<0.05$ control trial; $r=0.53$, $P<0.05$ exercise trial).

Insulin, Glucose and Lipoproteins

The postprandial glucose (Figure 3) and insulin (Figure 4) response did not differ between the control and exercise trials or between the NW and OW group. Total cholesterol increased ($p<0.01$) between 0 h and 6 h in the control trial, but not the exercise trial, in both the NW and OW groups (Table 2). Baseline levels of HDL-C levels were higher ($p<0.01$) in the NW than OW group. Postprandial HDL-C increased ($p<0.05$) in the NW group during both the control and exercise trials but did not change in the OW group (Table 3). HDL-C levels were higher ($p<0.01$) in the NW than the OW group at all time points.

Inflammatory Markers and Adhesion Molecules

Plasma levels of CRP, sICAM-1 and sVCAM-1 were not significantly different in the NW or OW group at baseline, and did not change in either group in response to the OFTT during the control or exercise condition. WBC's increased ($p<0.01$) in the NW and OW groups 6 h following the OFTT ; the magnitude of the increases were not significantly different between trials (Table 3). Circulating levels of WBC counts were lower in NW than OW adolescents prior to the OFTT in the control trial, and at 6 h following the OFTT in the exercise trial (Table 3).

Plasma levels of IL-6 increased two-fold in the NW and OW groups in response to the OFTT at 6 h ($p < 0.01$) and this increase did not significantly differ between experimental conditions (Table 3). There was a trend for plasma IL-6 to be higher at baseline and 6 h in the exercise trial ($P = 0.06$). Plasma concentrations of TNF- α were higher ($p < 0.05$) in the OW than the NW group at rest and 6 h following the OFTT in both the control and exercise experimental condition. TNF- α levels increased ($P < 0.05$) in the OW group 6h after the OFTT in the exercise experimental condition. TNF- α did not change following the OFTT in the NW group.

Table 1: Selected subject characteristics

	Normal weight (n=10)	Overweight (n=8)
Age (y)	15.6 ± 0.7	15.9 ± 0.4
Height (cm)	176.9 ± 9.3	172.1 ± 7.5*
Body mass (kg)	65.9 ± 11.3	84.1 ± 14.1*
Body mass index (kg·m ⁻²)	20.9 ± 1.7	28.3 ± 3.6*
Body surface area (m ²)	1.8 ± 0.2	2.0 ± 0.2
Sum of skinfolds (mm)	72.6 ± 16.1	157.6 ± 48.1*
Systolic BP (mmHg)	121 ± 8	125 ± 5
Diastolic BP (mmHg)	79 ± 4	80 ± 5
$\dot{V}O_{2max}$ (ml·kg ⁻¹ ·min ⁻¹)	52.1 ± 5.1	41.8 ± 10.1*
$\dot{V}O_{2max}$ (L·min ⁻¹)	3.4 ± 0.5	3.5 ± 0.8
HR _{max} (beats·min ⁻¹)	199 ± 6	192 ± 10

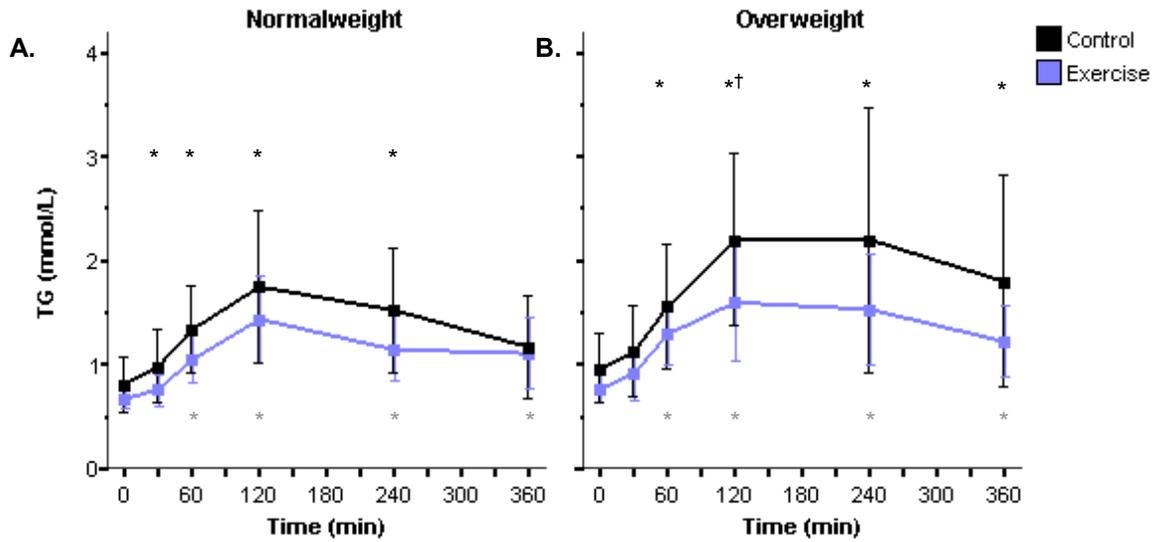
Values are mean ± SD.
*p<0.05

Table 2: Exercise trial parameters

	Normal weight (n=10)	Overweight (n=8)
$\dot{V}O_2$ (L·min ⁻¹)	2.1 ± 0.5	2.3 ± 0.5
Percentage $\dot{V}O_{2max}$ (%)	62.1 ± 6.6	68.2 ± 4.8*
Exercise time (min)	59.1 ± 14.7	52.4 ± 13.8
Average speed (k·h ⁻¹)	8.2 ± 0.6	7.3 ± 0.8*
Energy expenditure (kcal)	595.5 ± 19.7	587.9 ± 37.5
CHO oxidation (g)	119.3 ± 22.4	129.9 ± 32.7
Fat oxidation (g)	15.6 ± 7.9	11.6 ± 9.0

Values are mean ± SD.
*p<0.05

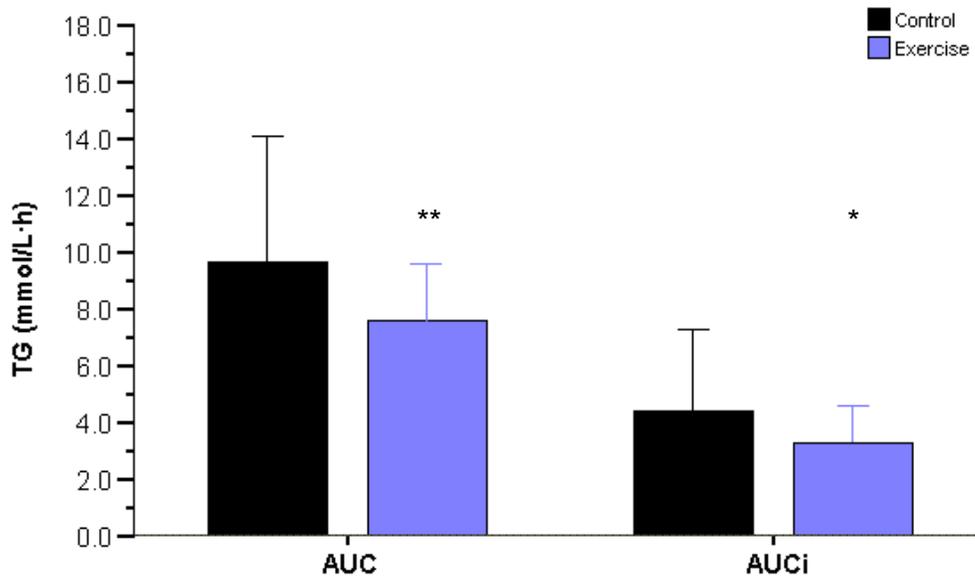
Figure 1: TG in the postprandial period during the control and exercise conditions



*Different from fasting value in the same trial, $p < 0.05$

†Difference between control and exercise trials at the same time point, $p < 0.05$.

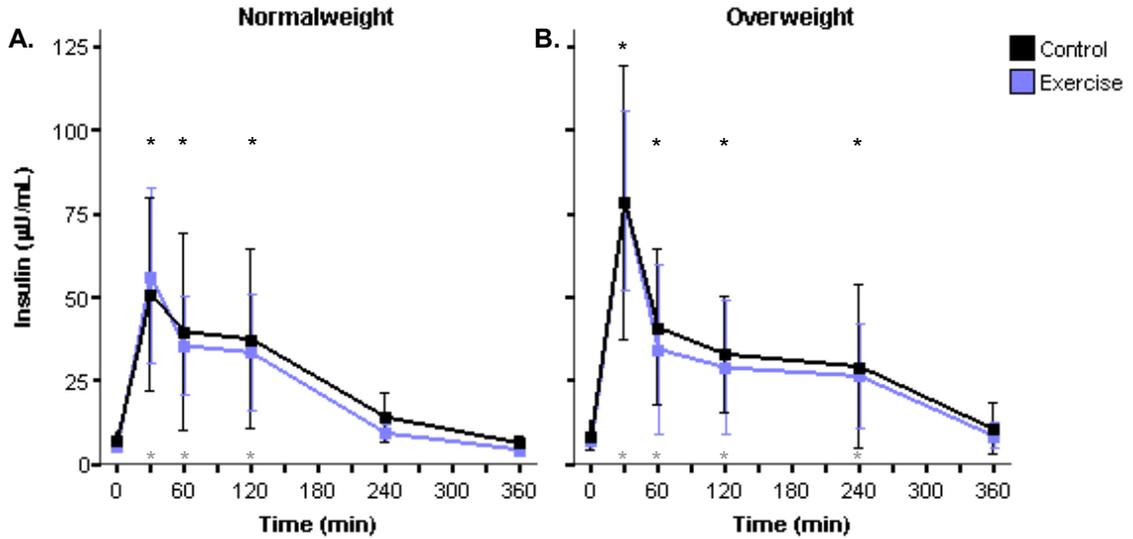
Figure 2: TG AUC and TG AUCi in the postprandial period during the control and exercise conditions



*Different from control value, $p < 0.05$

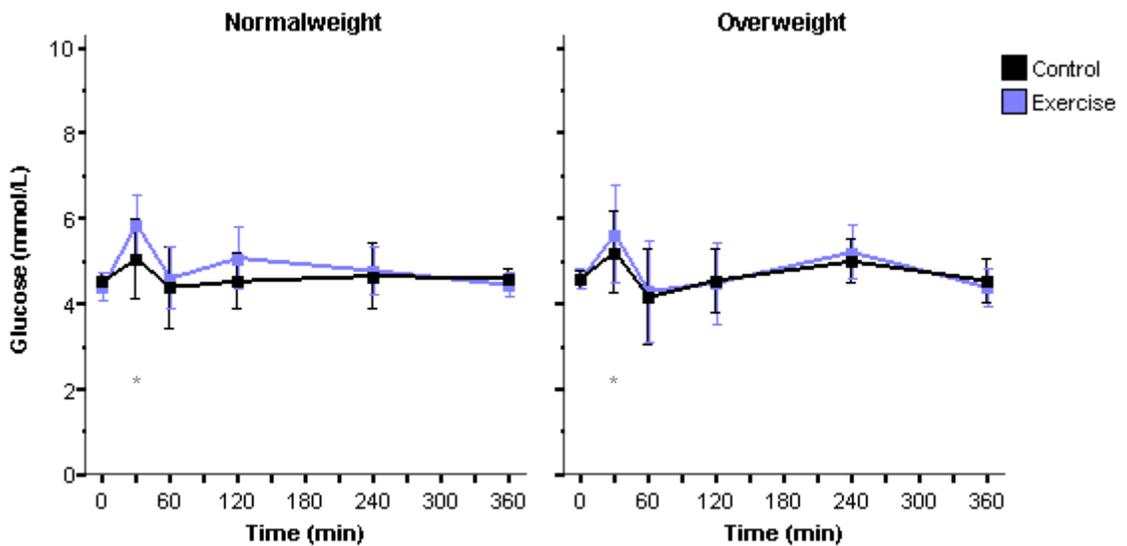
**Different from control value, $p < 0.01$

Figure 3: *Insulin in the postprandial period during the control and exercise conditions*



*Different from fasting value in the same trial, $p < 0.05$

Figure 4: *Glucose in the postprandial period during the control and exercise conditions*



*Different from fasting value in the same trial, $p < 0.05$

Table 3: Influence of acute exercise on lipid, metabolic and inflammatory markers and adhesion molecules

Variable	Group	Control		Exercise	
		0 h	6 h	0 h	6 h
Total cholesterol (mmol·L ⁻¹)	Normal weight	3.8 ± 0.9	4.0 ± 0.9*	3.7 ± 0.8	3.8 ± 0.8
	Overweight	3.7 ± 0.7	3.8 ± 0.7*	3.8 ± 0.8	3.8 ± 0.8
LDL-cholesterol (mmol·L ⁻¹)	Normal weight	2.4 ± 0.8		2.3 ± 0.7	
	Overweight	2.5 ± 0.7		2.6 ± 0.8	
HDL-cholesterol (mmol·L ⁻¹)	Normal weight	1.2 ± 0.2	1.3 ± 0.2*	1.2 ± 0.2	1.3 ± 0.2*
	Overweight	1.0 ± 0.1†	1.0 ± 0.1†	1.0 ± 0.1†	1.1 ± 0.1†
TG (mmol·L ⁻¹)	Normal weight	0.8 ± 0.3	1.2 ± 0.5	0.7 ± 0.1	1.1 ± 0.4*
	Overweight	1.0 ± 0.3	1.8 ± 1.0*	0.8 ± 0.2	1.2 ± 0.4*
Glucose (mmol·L ⁻¹)	Normal weight	4.5 ± 0.2	4.6 ± 0.2	4.4 ± 0.4	4.4 ± 0.3
	Overweight	4.6 ± 0.2	4.5 ± 0.5	4.6 ± 0.3	4.4 ± 0.5
Insulin (pmol·L ⁻¹)	Normal weight	7.3 ± 2.5	6.5 ± 2.3	5.5 ± 2.3	4.6 ± 1.3
	Overweight	8.8 ± 4.5	10.7 ± 8	7 ± 3.2	8.7 ± 4.3
HOMA-IR	Normal weight	1.5 ± 0.5			
	Overweight	1.8 ± 1.0			
WBC (cells x 10 ³ ·μL ⁻¹)	Normal weight	4.6 ± 0.8	5.9 ± 0.8*	5.3 ± 1.1‡	5.7 ± 0.7*
	Overweight	5.5 ± 0.8†	6.6 ± 1.0*	5.9 ± 1.0	6.6 ± 0.9*†
IL-6 (pg·mL ⁻¹)	Normal weight	1.4 ± 0.4	2.9 ± 1.9*	1.9 ± 1.3	3.7 ± 2.2*
	Overweight	1.6 ± 0.6	3.1 ± 1.9*	1.7 ± 0.6	3.7 ± 2.4*
TNF-α (pg·mL ⁻¹)	Normal weight	30 ± 1.9	28.2 ± 1.7	29 ± 3.4	29 ± 2.2
	Overweight	33 ± 2.3†	31.6 ± 2.7†	32 ± 1.0	36 ± 5.4*††
CRP (mg·L ⁻¹)	Normal weight	0.7 ± 0.9	0.6 ± 0.7	0.6 ± 0.8	0.7 ± 0.9
	Overweight	1.0 ± 0.4	1.0 ± 0.4	1.4 ± 1.0	1.5 ± 1.0
sVCAM-1 (ng·mL ⁻¹)	Normal weight	966 ± 263	1025 ± 321	879 ± 182	939 ± 225
	Overweight	810 ± 161	823 ± 150	789 ± 127	800 ± 125
sICAM-1 (ng·mL ⁻¹)	Normal weight	218 ± 35	222 ± 40	214 ± 41	209 ± 38

Values are mean ± SD

*P<0.05 vs. baseline in same trial (within group comparison)

‡P<0.05 vs. same time point during control trial (within group comparison)

†P<0.05 vs. same time point in NW (between group comparison)

CHAPTER V

DISCUSSION

Postprandial Triglycerides

Postprandial lipemia has generated interest as a novel prognostic indicator of cardiovascular morbidity and mortality. The rise in plasma levels of triglyceride-rich lipoproteins for up to 8 h following the consumption of a fatty meal is a better predictor of future cardiovascular disease than fasting triglycerides alone.^{8, 106, 111, 112} Acute exercise has consistently been shown to reduce PPL in adults.^{60, 118} Exercise-induced reductions in PPL likely contribute to the cardiovascular health benefits of regular exercise.⁵⁴ There is now compelling evidence that atherosclerotic-CVD processes begin early in childhood and are influenced over the life course by genetic and potentially modifiable risk factors.^{51, 101, 134} To our knowledge, only one previous study has examined the effect of acute exercise on PPL in healthy normal weight adolescents.¹⁰ They found that both continuous and intermittent activity attenuates postprandial lipemia in this cohort. The present study extends these findings to show that an acute bout of exercise also attenuates postprandial lipemia to a similar degree in overweight, otherwise healthy adolescent boys. The percentage TG reductions observed are comparable with those found in other reports on adults.⁴³

Previous studies have found higher postprandial TG in obese compared to normal weight adolescents.^{100, 147} Obese adults also exhibit markedly higher TG concentrations after eating a

high fat meal.⁴⁰ We found that the 2 h post-meal TG concentration was higher in the OW than the NW adolescents in the control trial. TG levels were similar in both groups at all other time points but, interestingly, TG levels returned to baseline values at 6 h in the NW group but were still elevated at 6 h in the OW group, suggesting a delayed clearance of TG, at least in the control trial. Coupled with the significant relation between sum of skinfolds with TG AUC, and previous evidence showing greater postprandial TG response in obese adolescents,¹⁰⁰ there remains the possibility that OW adolescents are at greater risk of postprandial exposure to atherogenic particles.

The mechanisms responsible for the reduction in PPL in the present study are unclear. One possibility is that TG are cleared faster from the circulation following acute exercise, a process mediated by an acute increase in the activity of skeletal muscle lipoprotein lipase (LPL) in a time course consistent with the postprandial reduction of TG.^{131, 161, 162} Results from short-term training and detraining studies provide evidence of pre-translational¹³¹ and post-translational¹³³ regulation of skeletal muscle LPL activity. Exercise-induced reductions in PPL have also been documented in the absence of changes in LPL activity.^{47, 64, 76} Gill and colleagues found that individuals who had the greatest increase in LPL activity following exercise tended to have the largest reductions in PPL.⁴⁷ This suggests that increases in LPL activity are not essential to attenuating postprandial lipemia after prior exercise but, where increases occur, they can have a strong influence on the magnitude of the reduction.

The exercise attenuation of fasting and postprandial TG may also be mediated by a reduction in hepatic TG synthesis and VLDL-TG secretion.⁴³ Changes in insulin sensitivity can alter these processes. A recent investigation in healthy young adults found that the addition of protein corrected the endothelial dysfunction associated with a high-fat meal. The authors concluded that the effect was mediated by insulin.¹⁵⁶ No exercise condition-related changes in the postprandial insulin response were observed in the present study and so insulin cannot explain the reduction in PPL seen in the present investigation.

Inflammatory Biomarkers

The results from the measurement of a diverse group of inflammatory biomarkers employed in this study were largely consistent with previous findings, demonstrating increased circulating WBC^{40, 149, 150} and IL-6⁴⁰ but not TNF- α ⁴¹ at 6 h following a high-fat meal. Taken together, these results indicate that elevated levels of select inflammatory markers in the postprandial state may contribute to a pro-inflammatory environment.

Diurnal variation in plasma IL-6 levels and lymphocytes may account for part of the postprandial increase in IL-6 and WBC.¹⁵⁰ The inflammatory response in the postprandial state was not attenuated by acute exercise even with substantial reductions in TG. Concentrations of IL-6 exhibited a trend towards being higher in the exercise condition, but the magnitude of the postprandial increase was similar in both trials, suggesting that the acute exercise bout resulted in a higher background IL-6 concentration, independent of the postprandial response. Gill *et al.* also found an increase in IL-6 in the exercise condition in obese subjects following a 90 min walk

in the afternoon preceding an OFTT but there were no differences at baseline prior to the meal.⁴⁰ Circulating levels of IL-6 are increased following strenuous exercise,^{114, 117} and this exercise-induced increase in circulating IL-6 may have both pro-inflammatory and anti-inflammatory activity. In contrast to previous data in adults,⁴⁰ we found that WBC were elevated at baseline in the exercise trial, further suggesting an inflammatory response to the prior exercise bout.

The higher levels of plasma TNF- α in the OW group than the NW group, may indicate chronic low-grade inflammation in the overweight subjects. Previous investigations examining the effect of a high fat meal on plasma TNF- α concentrations have been equivocal. Significant elevations in plasma TNF- α concentrations in diabetics, and up-regulated gene expression of TNF- α in leukocytes of older sedentary non-diabetics have been reported after eating.¹⁰⁴ Gill *et al.* found no change in TNF- α in normal weight endurance-trained athletes in response to a high fat mixed meal similar to that used in the present investigation.⁴¹ The significant increase in TNF- α in the present study appears to be largely driven by three of the eight OW subjects and likely reflects a degree of inter-individual variability in the TG response to the meal.

The relation of CRP to PPL has not been extensively investigated, but CRP is linked to endothelial cell activation and disturbed fat metabolism.^{59, 110} Although we found no change in CRP levels at 6 h after the OFTT, there is evidence that CRP levels increase earlier in the postprandial period (~1 h) and then quickly return to baseline.¹⁶

Adhesion Molecules

The vascular endothelium is an active, dynamic tissue that controls many important functions, including regulation of vascular tone and platelet aggregation, leukocyte adhesion, inflammation, thrombosis, fibrinolysis, and angiogenesis.^{67, 88, 152} Endothelial dysfunction represents a switch from a quiescent phenotype toward one that involves the host defense response and occurs early in the pathogenesis of atherosclerosis. The expression of adhesion molecules on endothelial cells is a critical step in endothelial dysfunction, facilitating the adhesion and trans-endothelial migration of leukocytes. Soluble levels of adhesion molecules in the circulation likely reflect an increase in their expression on the endothelium^{86, 96} and have been employed as biomarkers of endothelial dysfunction.

Fasting levels of sICAM-1 and sVCAM-1 were similar in OW and NW boys. In contrast, others have found higher levels of sICAM-1 and sVCAM-1 in obese compared with normal weight boys.^{30, 52, 107} Previous investigations have consistently shown increases in sICAM-1 and sVCAM-1 in diabetic and hypertriglyceridemic populations following an OFTT^{17, 18, 93, 104} suggesting increased endothelial cell activation in a pro-atherosclerotic environment. No postprandial changes in sICAM-1 or sVCAM-1 were found in NW or OW adolescents despite considerable increases in serum TG and plasma IL-6, both of which have been shown to induce endothelial adhesion molecule expression in vitro.^{97, 154} Others have shown small increases^{18, 104} or no changes in postprandial sICAM-1 and sVCAM-1^{41, 143} in healthy volunteers.

Limitations

There are a number of limitations to the present investigation. There is a possibility that the study was underpowered to detect group differences between NW and OW subjects. The sample sizes employed in this study were based pilot data collected from our first 3 subjects and suggested that 5 subjects were needed to detect a significant effect of exercise on postprandial TG with a power of 80%, and 6 subjects per group to detect the influence of BMI. This estimate is broadly consistent with previous cross-sectional studies that found significant differences in PPL when assigning 8-15 subjects per group.^{36, 46, 64, 120, 147} Nevertheless, we acknowledge that it is possible that a larger absolute difference in BMI, i.e. a strictly obese cohort rather than overweight, may be required to elucidate the effect of body weight on PPL.

There was a small but significant between-group difference in the relative exercise intensity between the NW and OW groups during the exercise bout. The OW group exercised at an average of 68% $\dot{V}O_{2max}$ compared with 62% $\dot{V}O_{2max}$ maintained by the NW group. This is a surprising finding that only became apparent after the data collection was completed. It is possible that the equations used to estimate exercise intensity underestimated the treadmill speed/grade in NW subjects and, while exercise intensity was monitored throughout each trial, it was not adjusted for unless the subject found it difficult to maintain (RPE > 14). It should be noted that the desired goal of the exercise bout was to expend an absolute magnitude of energy (600 kcal) rather than to exercise at a specific intensity. The influence of exercise intensity and substrate utilization during exercise on the subsequent attenuation of postprandial TG appears to

be of much less importance than total energy expenditure.^{48, 92, 118, 144} For this reason, and the fact that the absolute difference in mean exercise intensity between groups is small, it is unlikely that this had any substantive impact on the results of this investigation.

Inflammatory markers and adhesion molecules were only measured at baseline and at 6 h in the postprandial state. The possibility that changes occurred within the 6 h period and returned to baseline cannot be discounted. We and others have previously reported positive findings of increases in many of these markers at 4-6 h in adults.^{17, 18, 104, 143} Endothelium-dependent vasodilation is impaired and endothelial cell activation occur during the postprandial state and may be due to increased levels of oxidative stress.^{7, 19} Endogenous antioxidant defenses may limit endothelial cell activation in relatively healthy populations such as normal weight and overweight adolescents. As a result, increased adhesion molecule expression after an OFTT may not be evident in this population. A more sensitive method of endothelial function assessment, such as flow-mediated dilation may be necessary to examine the effect of exercise and PPL on endothelial function in adolescents.

The exclusion of female subjects from this investigation limits the generalizability of our results. Differences in the level of sexual maturation within the present cohort may have also influenced the results. There is limited research documenting the impact of maturation on the postprandial response to a high fat meal. To date, no relation has been found between postprandial TG and sexual maturity.^{10, 83}

Conclusion

In summary, an isocaloric exercise bout prior to a high fat meal effectively reduces postprandial TG concentrations to a similar degree in both NW and OW adolescents. The majority of studies to date have been undertaken in adult subjects. To our knowledge, this is the first investigation to examine PPL in both NW and OW adolescents and our findings extend the evidence base, demonstrating the efficacy of moderate exercise in reducing postprandial lipemia in both groups. Importantly, exercise does not reduce the concomitant increase in inflammatory markers and has no effect on the number of circulating adhesion molecules.

REFERENCE LIST

- (1) Al Suwaidi J, Higano S.T., Holmes Jr.D.R., Lennon R., Lerman A. Obesity is independently associated with coronary endothelial dysfunction in patients with normal or mildly diseased coronary arteries. *J Am Coll Cardiol* 2001;37(6):1523-8.
- (2) Aldred HE, Perry I.C., Hardman A.E. The effect of a single bout of brisk walking on postprandial lipemia in normolipidemic young adults. *Metabolism* 1994;43(7):836-41.
- (3) Altena TS, Michaelson JL, Ball SD, Thomas TR. Single sessions of intermittent and continuous exercise and postprandial lipemia. *Med Sci Sports Exerc* 2004;36(8):1364-71.
- (4) American College of Sports Medicine. *Guidelines for exercise testing and prescription*. 6th ed. Baltimore, MD: Lippincott Williams & Wilkins; 2000.
- (5) Anderson RA, Evans M.L., Ellis G.R. et al. The relationships between post-prandial lipaemia, endothelial function and oxidative stress in healthy individuals and patients with type 2 diabetes. *Atherosclerosis* 2001;154:475-83.
- (6) Aronson D, Sheikh-Ahmed M, Avizohar O et al. C-reactive protein is inversely related to physical fitness in middle-aged subjects. *Atherosclerosis* 2004;176:173-9.
- (7) Bae JH, Bassenge E., Kim KB. et al. Postprandial hypertriglyceridemia impairs endothelial function by enhanced oxidant stress. *Atherosclerosis* 2001;155:517-23.
- (8) Bansal S, Buring JE, Rifai N, Mora S, Sacks FM, Ridker PM. Fasting compared with nonfasting triglycerides and risk of cardiovascular events in women. *JAMA* 2007 July 18;298(3):309-16.
- (9) Baron AD. Vascular reactivity. *Am J Cardiol* 1999;84:25J-7J.
- (10) Barrett LA, Morris JG, Stensel DJ, Nevill ME. Exercise and postprandial plasma triacylglycerol concentrations in healthy adolescent boys. *Med Sci Sports Exerc* 2007 January;39(1):116-22.
- (11) Berglund L. Postprandial lipemia and obesity: any unique features? *Am J Clin Nutr* 2002;76:299-300.
- (12) Bhattacharyya G, Libby P. Atherosclerosis. In: Lilly LS, editor. *Pathophysiology of Heart Disease*. 2nd ed. Lippincott Williams & Wilkins; 1998. p. 101-18.
- (13) Blankenberg S, Barbaux S., Tiret L. Adhesion molecules and atherosclerosis. *Atherosclerosis* 2003;170:191-203.
- (14) Blann AD, McCollum CN. Circulating endothelial cell/leukocyte adhesion molecules in atherosclerosis. *Thromb Haemost* 1994 July;72(1):151-4.
- (15) Cahill PA, Redmond E.M., Sitzmann J.V. Endothelial dysfunction in cirrhosis and portal hypertension. *Pharmacology & Therapeutics* 2001;89:273-93.
- (16) Carroll MF, Schade DS. Timing of antioxidant vitamin ingestion alters postprandial proatherogenic serum markers. *Circulation* 2003;108:24-31.
- (17) Ceriello A, Assaloni R, Da Ros R et al. Effect of atorvastatin and irbesartan, alone and in combination, on postprandial endothelial dysfunction, oxidative stress, and inflammation in type 2 diabetic patients. *Circulation* 2005;111:2518-24.

- (18) Ceriello A, Quagliaro L, Piconi L et al. Effect of postprandial hypertriglyceridemia and hyperglycemia on circulating adhesion molecules and oxidative stress generation and the possible role of simvastatin treatment. *Diabetes* 2004;53:701-10.
- (19) Ceriello A, Taboga C., Tonutti L. et al. Evidence for an independent and cumulative effect of postprandial hypertriglyceridemia on endothelial dysfunction and oxidative stress generation. *Circulation* 2002;106:1211-8.
- (20) Cines DB, Pollak ES, Buck CA et al. Endothelial cells in physiology and in the pathophysiology of vascular disorders. *Blood* 1998;91(10):3527-61.
- (21) Clarkson P, Montgomery H.E., Mullen M.J. et al. Exercise training enhances endothelial function in young men. *Journal of the American College of Cardiology* 1999;33(5):1379-85.
- (22) Cleland SJ, Sattar N, Petrie JR, Forouhi NG, Elliot HL, Connell JMC. Endothelial dysfunction as a possible link between C-reactive protein levels and cardiovascular disease. *Clin Sci* 2000;98:531-5.
- (23) Cohn JS. Postprandial lipemia: emerging evidence for atherogenicity of remnant lipoproteins. *Can.J.Cardiol.* 14 (Suppl. B), 18B-27B. 1998.
Ref Type: Abstract
- (24) Cole TJ, Bellizzi M.C., Flegal K.M., Dietz W.H. Establishing a standard definition for child overweight and obesity worldwide: international survey. *Br Med J* 2000;320:1-6.
- (25) Couch SC, Isasi C.R., Karmally W. et al. Predictors of postprandial triacylglycerol response in children: the Columbia University Biomarkers Study. *Am J Clin Nutr* 2000;72:1119-27.
- (26) Couillard C., Bergeron N., Pascot A et al. Evidence for impaired lipolysis in abdominally obese men: postprandial study of apolipoprotein B-48- and B-100-containing lipoproteins. *Am J Clin Nutr* 2002;76:311-8.
- (27) Couillard C, Bergeron N., Prud'homme D. et al. Postprandial triglyceride response in visceral obesity in men. *Diabetes* 1998;47:953-60.
- (28) Das UN. Is obesity an inflammatory condition? *Nutrition* 2001;17:953-66.
- (29) Davies MJ, Gordon JL, Gearing AJ et al. The expression of the adhesion molecules ICAM-1, VCAM-1, PECAM, and E-selectin in human atherosclerosis. *J Pathol* 1993;171(3):223-9.
- (30) Desideri G, De Simone M, Iughetti L et al. Early activation of vascular endothelial cells and platelets in obese children. *J Clin Endocrinol Metab* 2005;90(6):3145-52.
- (31) DeSouza CA, Dengel DR, Macko RF, Cox K, Seals DR. Elevated levels of circulating cell adhesion molecules in uncomplicated essential hypertension. *Am J Hypertens* 1997;10:1335-41.
- (32) DeSouza CA, Shapiro L.F., Clevenger C.M. et al. Regular aerobic exercise prevents and restores age-related declines in endothelium-dependent vasodilation in healthy men. *Circulation* 2000;102:1351-7.
- (33) Dill DB, Costill DL. Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. *J Appl Physiol* 1974;37(2):247-8.

- (34) Doi H, Kugiyama K, Ohgushi M et al. Remnants of chylomicron and very low density lipoprotein impair endothelium-dependent vasorelaxation. *Atherosclerosis* 1998;137:341-9.
- (35) Drexler H, Hornig B. Endothelial dysfunction in human disease. *J Mol Cell Cardiol* 1999;31:51-60.
- (36) Eriksson JW, Burén J., Svensson M., Olivecrona T., Olivecrona G. Postprandial regulation of blood lipids and adipose tissue lipoprotein lipase in type 2 diabetes patients and healthy control subjects. *Atherosclerosis* 2003;166:359-67.
- (37) Ford ES. C-reactive protein concentration and cardiovascular disease risk factors in children. *Circulation* 2003;108:1053-8.
- (38) Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972;18(6):499-502.
- (39) Funada J, Sekiya M., Hamada M., Hiwada K. Postprandial elevation of remnant lipoprotein leads to endothelial dysfunction. *Circ J* 2002;66:127-32.
- (40) Gill JMR, Al-Mamari A, Ferrell WR et al. Effects of prior moderate exercise on postprandial metabolism and vascular function in lean and centrally obese men. *J Am Coll Cardiol* 2004;44(12):2375-82.
- (41) Gill JMR, Caslake M.J., McCallister C. et al. Effects of short-term detraining on postprandial metabolism, endothelial function, and inflammation in endurance-trained men: dissociation between changes in triglyceride metabolism and endothelial function. *J Clin Endocrinol Metab* 2003;88(9):4328-35.
- (42) Gill JMR, Hardman A.E. Postprandial lipemia: effects of exercise and restriction of energy intake compared. *Am J Clin Nutr* 2000;71:465-71.
- (43) Gill JMR, Hardman A.E. Exercise and postprandial lipid metabolism: an update on potential mechanisms and interactions with high-carbohydrate diets. *Journal of Nutritional Biochemistry* 2003;14:122-32.
- (44) Gill JMR, Herd S.L., Hardman A.E. Moderate exercise and post-prandial metabolism: issues of dose response. *J Sports Sci* 2002;20:961-7.
- (45) Gill JMR, Mees G.P., Frayn K.N., Hardman A.E. Moderate exercise, postprandial lipaemia and triacylglycerol clearance. *Eur J Clin Invest* 2001;31(3):201-7.
- (46) Gill J, Al-Mamari A, Ferrell W et al. Effects of prior moderate exercise on postprandial metabolism and vascular function in lean and centrally obese men. *J Am Coll Cardiol* 2004;44(12):2375-82.
- (47) Gill J, Herd SL, Vora V, Hardman AE. Effects of a brisk walk on lipoprotein lipase activity and plasma triglyceride concentrations in the fasted and postprandial states. *Eur J Appl Physiol* 2003;89:184-90.
- (48) Gill J, Murphy MH, Hardman AE. Postprandial lipemia: effects of intermittent versus continuous exercise. *Med Sci Sport Exerc* 1998;30(10):1515-20.
- (49) Glowinska B, Urban M. [Selected cytokines (IL-6, IL-8, IL-10, MCP-1, TNF-alpha) in children and adolescents with atherosclerosis risk factors: obesity, hypertension, diabetes]. *Wiad.Lek.*

56[3-4], 109-116. 2003.

Ref Type: Abstract

- (50) Glowinska B, Urban M., Bossowski A., Szczepanska J. Endothelial dysfunction in children and adolescents with obesity and hypertension. *Polish Heart Journal* 2001;LIV(Nr 1).
- (51) Glowinska B, Urban M., Koput A., Galar M. New atherosclerosis risk factors in obese, hypertensive and diabetic children and adolescents. *Atherosclerosis* 2003;167:275-86.
- (52) Glowinska B, Urban M., Peczynska J., Szczepanska-Kostro J. [Selected adhesion molecules: sICAM-1 and sVCAM-1 as markers of endothelial dysfunction in diabetic children and adolescence]. *Pol.Merkuriusz.Lek.* 14[81], 205-209. 2003.
Ref Type: Abstract
- (53) Gordon JB, Ganz P., Nabel E.G. et al. Atherosclerosis influences the vasomotor response of epicardial coronary arteries to exercise. *J Clin Invest* 1989;83:1946-52.
- (54) Graham TE. Exercise, postprandial triacylglyceridemia, and cardiovascular disease risk. *Can J Appl Physiol* 2004;29(6):781-99.
- (55) Graham TE. Exercise, postprandial triacylglyceridemia, and cardiovascular disease risk. *Can J Appl Physiol* 2004;29(6):781-99.
- (56) Grundy SM. Obesity, metabolic syndrome, and cardiovascular disease. *J Clin Endocrinol Metab* 2004;89(6):2595-600.
- (57) Hackman A, Abe Y, Insull W, Jr. et al. Levels of soluble cell adhesion molecules in patients with dyslipidemia. *Circulation* 1996 April 1;93(7):1334-8.
- (58) Hambrecht R, Fiehn E., Weigl C. et al. Regular physical exercise corrects endothelial dysfunction and improves exercise capacity in patients with chronic heart failure. *Circulation* 1998;98:2709-15.
- (59) Hanna FWF, Issa BG. Hyperlipidaemia and cardiovascular disease: C-reactive protein and atherosclerosis - new dimensions. *Curr Opin Lipidology* 2002;13:101-3.
- (60) Hardman AE. The influence of exercise on postprandial triacylglycerol metabolism. *Atherosclerosis* 1998;141(Suppl. 1):S93-S100.
- (61) Hardman AE, Lawrence J.E.M., Herd S.L. Postprandial lipemia in endurance-trained people during a short interruption to training. *J Appl Physiol* 1998;84(6):1895-901.
- (62) Harrison M, Nertney L, O'Gorman D, Moyna NM. Influence of acute exercise on postprandial lipemia and endothelial dysfunction in normal- and overweight men. *Med.Sci.Sport Exerc.* 36[5 (Suppl)], S218. 2004.
Ref Type: Abstract
- (63) Herd S.L., Kiens B., Boobis L.H., Hardman A.E. Moderate exercise, postprandial lipemia, and skeletal muscle lipoprotein lipase activity. *Metabolism* 2001;50(7):758-62.
- (64) Herd S, Lawrence JEM, Malkova D, Murphy MH, Mastana S, Hardman AE. Postprandial lipemia in young men and women of contrasting training status. *J Appl Physiol* 2000;89:2049-56.

- (65) Higashi Y, Sasaki S., Kurisu S. et al. Regular aerobic exercise augments endothelium-dependent vascular relaxation in normotensive as well as hypertensive subjects. *Circulation* 1999;100:1194-202.
- (66) Higashi Y, Sasaki S., Nakagawa K., Matsuura H., Chayama K., Oshima T. Effect of obesity on endothelium-dependent nitric oxide-mediated vasodilation in normotensive individuals and patients with essential hypertension. *AJH* 2001;14(10):1038-45.
- (67) Hinderliter AL, Caughey M. Assessing endothelial function as a risk factor for cardiovascular disease. *Current Atherosclerosis Reports* 2003;5:506-13.
- (68) Hoetzer GL, Van Guilder GP, Irmiger HM, Keith RS, Stauffer BL, DeSouza CA. Aging, exercise, and endothelial progenitor cell clonogenic and migratory capacity in men. *J Appl Physiol* 2007 March;102(3):847-52.
- (69) Hope SA, Meredith IT. Cellular adhesion molecules and cardiovascular disease. Part I. Their expression and role in atherogenesis. *Intern Med J* 2003;33:380-6.
- (70) Horne BD, Anderson JL, John JM et al. Which white blood cell subtypes predict increased cardiovascular risk? *J Am Coll Cardiol* 2005;45(10):1638-43.
- (71) Hyson D, Rutledge J.C., Berglund L. Postprandial lipemia and cardiovascular disease. *Current Atherosclerosis Reports* 2003;5:437-44.
- (72) Jackson AS, Pollock ML. Practical assessment of body composition. *Phys Sportsmed* 1985;13(5):76-90.
- (73) Jagla A, Schrezenmeir J. Postprandial triglycerides and endothelial function. *Exp Clin Endocrinol Diabetes* 2001;109:S533-S547.
- (74) Järvisalo MJ, Harmoinen A, Hakanen M et al. Elevated serum C-reactive protein levels and early arterial changes in healthy children. *Arterioscler Thromb Vasc Biol* 2002;22:1323-8.
- (75) Karpe F. Postprandial lipoprotein metabolism and atherosclerosis. *J Intern Med* 1999;246:341-55.
- (76) Katsanos CS, Grandjean P.W., Moffatt R.J. Effects of low and moderate exercise intensity on postprandial lipemia and postheparin plasma lipoprotein lipase activity in physically active men. *J Appl Physiol* 2004;96:181-8.
- (77) Kher N, Marsh JD. Pathobiology of atherosclerosis - a brief review. *Seminars in Thrombosis and Hemostasis* 2004;30(6):665-72.
- (78) Kobayashi N, Tsuruya Y., Iwasawa T. et al. Exercise training in patients with chronic heart failure improves endothelial function predominantly in the trained extremities. *Circ J* 2003;67:505-10.
- (79) Koenig W, Sund M., Fröhlich M. et al. C-reactive protein, a sensitive marker of inflammation, predicts future risk of coronary heart disease in initially healthy middle-aged men. *Circulation* 1999;99:237-42.
- (80) Kolifa M, Petridou A, Mougios V. Effect of prior exercise on lipemia after a meal of moderate fat content. *Eur J Clin Nutr* 2004;(58):1327-35.

- (81) Koutsari C, Hardman A.E. Exercise prevents the augmentation of postprandial lipaemia attributable to a low-fat high-carbohydrate diet. *Br J Nutr* 2001;86:197-205.
- (82) Landmesser U, Hornig B., Drexler H. Endothelial function. A critical determinant in atherosclerosis? *Circulation* 2004;109(suppl):II-27-II-33.
- (83) Lanes R, Paoli M, Carrillo E, Villaroel O, Palacios A. Peripheral inflammatory and fibrinolytic markers in adolescents with growth hormone deficiency: relation to postprandial lipemia. *J Pediatr* 2004;145:657-61.
- (84) Lau DCW, Dhillon B, Yan H, Szmitko PE, Verma S. Adipokines: molecular links between obesity and atherosclerosis. *Am J Physiol Heart Circ Physiol* 2005;288:2031-41.
- (85) Lee Y-H, Pratley RE. The evolving role of inflammation in obesity and the metabolic syndrome. *Current Diabetes Reports* 2005;5:70-5.
- (86) Leeuwenberg J, Smeets E, Neefjes J et al. E-selectin and intercellular adhesion molecule-1 are released by activated human endothelial cells in vitro. *Immunology* 1992;77(4):543-9.
- (87) Libby P. Inflammation in atherosclerosis. *Nature* 2002;440:868-74.
- (88) Libby P, Ridker P.M., Maseri A. Inflammation and atherosclerosis. *Circulation* 2002;105:1135-43.
- (89) Ludmer PL, Selwyn AP, Shook TL et al. Paradoxical vasoconstriction induced by acetylcholine in atherosclerotic coronary arteries. *N Engl J Med* 1986 October 23;315(17):1046-51.
- (90) Lusis AJ. Atherosclerosis. *Nature* 2000;407:233-41.
- (91) Malkova D, Evans R.D., Frayn K.N., Humphreys S.M., Jones P.R.M., Hardman A.E. Prior exercise and postprandial substrate extraction across the human leg. *Am J Physiol Endocrinol Metab* 2000;279:E1020-E1028.
- (92) Malkova D, Hardman A.E., Bowness R.J., MacDonald I.A. The reduction in postprandial lipemia after exercise is independent of the relative contributions of fat and carbohydrate to energy metabolism during exercise. *Metabolism* 1999;48(2):245-51.
- (93) Marchesi S., Lupattelli G., Lombardini R et al. Effects of fenofibrate on endothelial function and cell adhesion molecules during post-prandial lipemia in hypertriglyceridemia. *J Clin Pharm Ther* 2003;28:419-24.
- (94) McGilvery RW, Goldstein GW. *Biochemistry: A Functional Approach*. Philadelphia: Saunders; 1983.
- (95) Merrill JR, Holly R.G., Anderson R.L., Rifai N., King M.E., DeMeersman R. Hyperlipidemic response of young trained and untrained men after a high fat meal. *Arteriosclerosis* 1989;9(2):217-23.
- (96) Meydani M. Soluble adhesion molecules: surrogate markers of cardiovascular disease? *Nutr Rev* 2003;61:63-8.
- (97) Moers A, Fenselau S, Schrezenmeir J. Chylomicrons induce E-selectin and VCAM-1 expression in endothelial cells. *Exp Clin Endocrinol Diabetes* 1997;105 Suppl 2:35-7.

- (98) Moller DE. Potential role of TNF- α in the pathogenesis of insulin resistance and type 2 diabetes. *TEM* 2000;11(6):212-7.
- (99) Moncada S, Higgs A. Mechanisms of disease: the L-arginine-nitric oxide pathway. *N Engl J Med* 1993;329(27):2002-12.
- (100) Moreno L, Quintela I, Fleta J et al. Postprandial triglyceridemia in obese and non-obese adolescents. Importance of body composition and fat distribution. *J Pediatr Endocrinol Metab* 2001;14(2):193-202.
- (101) Moyna N, Estrada E, LaRocca GM et al. Endothelial function in adolescents with type 2 diabetes. *Med Sci Sport Exerc* 2001;33(5 Suppl 1):S395.
- (102) Moyna N, Thompson PD. The effect of physical activity on endothelial function in man. *Acta Physiol Scand* 2004;(180):113-23.
- (103) Müller MM, Griesmacher A. Markers of endothelial dysfunction. *Clin Chem Lab Med* 2000;38(2):77-85.
- (104) Nappo F, Esposito K, Cioffi M et al. Postprandial endothelial activation in healthy subjects and in type 2 diabetic patients: role of fat and carbohydrate meals. *J Am Coll Cardiol* 2002;39(7):1145-50.
- (105) Niebauer J, Cooke J.P. Cardiovascular effects of exercise: role of endothelial shear stress. *JACC* 1996;28(7):1652-60.
- (106) Nordestgaard BG, Benn M, Schnohr P, Tybjaerg-Hansen A. Nonfasting triglycerides and risk of myocardial infarction, ischemic heart disease, and death in men and women. *JAMA* 2007 July 18;298(3):299-308.
- (107) O'Brien KD, O'Gorman DJ, Hughes SM et al. Inflammatory markers and adhesion molecules correlate with BMI and aerobic fitness levels in Irish adolescents. *Med Sci Sport Exerc* 2006;38(5):S2302.
- (108) Ohta H, Wada H, Niwa T et al. Disruption of tumor necrosis factor- α gene diminishes the development of atherosclerosis in ApoE-deficient mice. *Arteriosclerosis* 2005;180:11-7.
- (109) Park H-S, Park JY, Yu R. Relationship of obesity and visceral adiposity with serum concentrations of CRP, TNF- α and IL-6. *Diabetes Res Clin Pract* 2005;69(1):29-35.
- (110) Pasceri V, Willerson J.T., Yeh E.T.H. Direct proinflammatory effect of C-reactive protein on human endothelial cells. *Circulation* 2000;102:2165-8.
- (111) Patsch JR, Miesenbock G, Hopferweiser T et al. Relation of triglyceride metabolism and coronary artery disease: Studies in the postprandial state. *Arteriosclerosis and Thrombosis* 1992;12:1336-45.
- (112) Patsch W, Esterbauer H, Föger B, Patsch JR. Postprandial lipemia and coronary risk. *Current Atherosclerosis Reports* 2000;2:232-42.
- (113) Pearson TA, Mensah GA, Alexander RW et al. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: a statement for healthcare from the Centers for Disease Control and Prevention and the American Heart Association. *Circulation* 2003;107:499-511.

- (114) Pedersen BK, Steenburg A, Fischer C et al. The metabolic role of IL-6 produced during exercise: is IL-6 an exercise factor? *Proc Nutr Soc* 2004;63:263-7.
- (115) Pepine C.J. The effects of angiotensin-converting enzyme inhibition on endothelial dysfunction: potential role in myocardial ischemia. *Am J Cardiol* 1998;82:23S-7S.
- (116) Peronnet F, Massicote D. Table of nonprotein respiratory quotient: an update. *Can J Sport Sci* 1991;16:23-9.
- (117) Petersen AMW, Pedersen BK. The anti-inflammatory effect of exercise. *J Appl Physiol* 2005;98:1154-62.
- (118) Petitt DS, Cureton K.J. Effects of prior exercise on postprandial lipemia: A quantitative review. *Metabolism* 2003;52(4):418-24.
- (119) Plotnick GD, Corretti M.C., Vogel R.A. Effect of antioxidant vitamins on the transient impairment of endothelium-dependent brachial artery vasoactivity following a single high-fat meal. *JAMA* 1997;278:1682-6.
- (120) Potts JL, Coppack SW, Fisher RM, Humphreys SM, Gibbons GF, Frayn KN. Impaired postprandial clearance of triacylglycerol-rich lipoproteins in adipose tissue in obese subjects. *Am J Physiol Endocrinol Metab* 1995;268(31):E588-E594.
- (121) Rehman J, Li J, Parvathaneni L et al. Exercise acutely increases circulating endothelial progenitor cells and monocyte-/macrophage-derived angiogenic cells. *J Am Coll Cardiol* 2004;43(12):2314-8.
- (122) Ridker P.M., Rifai N., Pfeffer M et al. Elevation of tumor necrosis factor- α and increased risk of recurrent coronary events after myocardial infarction. *Circulation* 2000;101:2149-53.
- (123) Ridker PM, Buring J.E., Cook N.R., Rifai N. C-reactive protein, the metabolic syndrome, and risk of incident cardiovascular events. *Circulation* 2003;107:391-7.
- (124) Ridker PM, Buring J.E., Shih J., Matias M., Hennekens C.H. Prospective study of C-reactive protein and the risk of future cardiovascular events among apparently healthy women. *Circulation* 1998;98(8):731-3.
- (125) Ridker PM, Cushman M., Stampfer M.J., Tracy R.P., Hennekens C.H. Plasma concentration of C-reactive protein and risk of developing peripheral vascular disease. *Circulation* 1998;97:425-8.
- (126) Ridker PM, Rifai N., Stampfer M.J., Hennekens C.H. Plasma concentration of interleukin-6 and the risk of future myocardial infarction among apparently healthy men. *Circulation* 2000;101(15):1767-72.
- (127) Ridker PM, Stampfer M.J., Rifai N. Novel risk factors for systemic atherosclerosis. *JAMA* 2001;285(19):2481-5.
- (128) Roep BO, Heidenthal E, de Vries RR, Kolb H, Martin S. Soluble forms of intercellular adhesion molecule-1 in insulin-dependent diabetes mellitus. *Lancet* 1994 June 25;343(8913):1590-3.
- (129) Ross R. Atherosclerosis - an inflammatory disease. *NEJM* 1999;340(2):115-26.
- (130) Schmidt A, Pleiner J., Bayerle-Eder M. et al. Regular physical exercise improves endothelial function in heart transplant recipients. *Clin Transplant* 2002;16:137-43.

- (131) Seip R., Mair K, Cole T.G., Semenkovich C.F. Induction of human skeletal muscle lipoprotein lipase gene expression by short-term exercise is transient. *Am J Physiol Endocrinol Metab* 1997;272:E255-E261.
- (132) Sen CK, Roy S. Antioxidant regulation of cell adhesion. *Med Sci Sports Exerc* 2001;33(3):377-81.
- (133) Simsolo RB, Ong JM, Kern PA. The regulation of adipose tissue and muscle lipoprotein lipase in runners by detraining. *J Clin Invest* 1993 November;92(5):2124-30.
- (134) Sorenson KE, Celermajer D.S., Georgakopoulos D., Hatcher G., Betteridge D.J., Deanfield J. Impairment of endothelium-dependent dilation is an early event in children with familial hypercholesterolemia and is related to the lipoprotein(a) level. *J Clin Invest* 1994;93(1):50-5.
- (135) Stampfer MJ, Krauss RM, Ma J et al. A prospective study of triglyceride level, low-density lipoprotein particle diameter, and risk of myocardial infarction. *JAMA* 1996;276(11):882-8.
- (136) Steinberg HO, Chaker H., Leaming R., Johnson A., Brechtel G., Baron A.D. Obesity/insulin resistance is associated with endothelial dysfunction. *J Clin Invest* 1996;97(11):2601-10.
- (137) Steinberger J, Daniels S.R. Obesity, insulin resistance, diabetes, and cardiovascular risk in children. *Circulation* 2003;107:1448-53.
- (138) Sternby NH, Fernandez-Britto JE, Nordet P. Pathological determinants of atherosclerosis in youth (PBDAY Study), 1986-96. *Bulletin of the World Health Organisation* 1999;77(3):250-7.
- (139) Suter PM, Gerritsen-Zehnder M., Häsler E., Gürtler M., Vetter W., Hänseler E. Effect of alcohol on postprandial lipemia with and without preprandial exercise. *J Am Coll Nutr* 2001;20(1):58-64.
- (140) Takase B, Uehata A., Akima T. et al. Endothelium-dependent flow-mediated vasodilation in coronary and brachial arteries in suspected coronary artery disease. *Am J Cardiol* 1998;82:1535-9.
- (141) Tanaka H, Monaghan KD, Seals DR. Age-predicted maximal heart rate revisited. *J Am Coll Cardiol* 2001;37(1):153-6.
- (142) Thomas TR, Horner K.E., Langdon M.M. et al. Effect of exercise and medium-chain fatty acids on postprandial lipemia. *J Appl Physiol* 2001;90:1239-46.
- (143) Tsai W-C, Li Y-H, Lin C-C, Chao T-H, Chen J-H. Effects of oxidative stress on endothelial function after a high-fat meal. *Clin Sci* 2004;106:315-9.
- (144) Tsetsonis NV, Hardman A.E. Reduction in postprandial lipemia after walking: influence of exercise intensity. *Med Sci Sport Exerc* 1996;28(10):1235-42.
- (145) Tsetsonis NV, Hardman A.E., Mastana S.S. Acute effects of exercise on postprandial lipemia: a comparative study in trained and untrained middle-aged women. *Am J Clin Nutr* 1997;65(2):525-33.
- (146) Tzoulaki I, Murray GD, Lee AJ, Rumley A, Lowe GDO, Fowkes GR. C-reactive protein, interleukin-6, and soluble adhesion molecules as predictors of progressive peripheral atherosclerosis in the general population. *Circulation* 2005;112:976-83.

- (147) Umpaichitra V, Banerji M, Castells S. Postprandial hyperlipidemia after a fat loading test in minority adolescents with type 2 diabetes mellitus and obesity. *J Pediatr Endocrinol Metab* 2004;17(6):853-64.
- (148) van Oostrom AJHMM, Rabelink TJ, Verseyden C et al. Activation of leukocytes by postprandial lipemia in healthy volunteers. *Atherosclerosis* 2004;177:175-82.
- (149) van Oostrom AJHMM, Sijmonsma TP, Rabelink TJ, van Asbck BS, Cabezas MC. Postprandial leukocyte increase in healthy subjects. *Metabolism* 2003;52(2):199-202.
- (150) van Oostrom AJHMM, Sijmonsma TP, Verseyden C et al. Postprandial recruitment of neutrophils may contribute to endothelial dysfunction. *J Lipid Res* 2003;44:576-83.
- (151) van Oostrom AJHMM, van Wijk JPH, Cabezas MC. Lipaemia, inflammation and atherosclerosis: novel opportunities in the understanding and treatment of atherosclerosis. *Drugs* 2004;64(Suppl. 2):19-41.
- (152) Vogel RA. Coronary risk factors, endothelial function, and atherosclerosis: A review. *Clin Cardiol* 1997;20:426-32.
- (153) Vogel RA, Corretti M.C., Plotnick G.D. Effect of a single high-fat meal on endothelial function in healthy subjects. *Am J Cardiol* 1997;79:350-4.
- (154) Watson C, Whittaker S, Smith N, Vora AJ, Dumonde DC, Brown KA. IL-6 acts on endothelial cells to preferentially increase their adherence for lymphocytes. *Clin Exp Immunol* 1996 July;105(1):112-9.
- (155) Weir JB. New methods for calculating metabolic rate with special reference to protein metabolism. *J Physiol* 1949;109:1-9.
- (156) Westphal S, Taneva E, Kastner S et al. Endothelial dysfunction induced by postprandial lipemia is neutralized by addition of proteins to the fatty meal. *Arteriosclerosis* 2006;185:313-9.
- (157) Willerson JT, Ridker P.M. Inflammation as a cardiovascular risk factor. *Circulation* 2004;109(suppl II):II-2-II-10.
- (158) Xiang G-D, Wang Y-L. Regular aerobic exercise training improves endothelium-dependent arterial dilation in patients with impaired fasting glucose. *Diabetes Care* 2004;27(3):801--802.
- (159) Yeh ETH. CRP as a mediator of disease. *Circulation* 2004;109(suppl):II-11-II-14.
- (160) Yudkin JS, Kumari M, Humphries SE, Mohamed-Ali V. Inflammation, obesity, stress and coronary heart disease: is interleukin-6 the link? *Atherosclerosis* 1999 February 1;148(2):209-14.
- (161) Zhang JQ, Nunez G, Feathers S, Hart CL, Yao WX. Effect of exercise timing on postprandial lipemia in hypertriglyceridemic men. *Can J Appl Physiol* 2004;29(5):590-603.
- (162) Zhang JQ, Thomas T.R., Ball S.D. Effect of exercise timing on postprandial lipemia and HDL cholesterol subfractions. *J Appl Physiol* 1998;85(4):1516-22.
- (163) Zilversmit DB. Atherogenesis: a postprandial phenomenon. *Circulation* 1979;60(3):473-85.
- (164) Zilversmit DB. Atherogenic nature of triglycerides, postprandial lipidemia, and triglyceride-rich remnant lipoproteins. *Clin Chem* 1995;41(1):153-8.

- (165) Ziogas GG, Thomas T.R., Harris W.S. Exercise training, postprandial hypertriglyceridemia, and LDL subfraction distribution. *Med Sci Sport Exerc* 1997;29(8):986-91.

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Appendix I
Ethics Submission

DUBLIN CITY UNIVERSITY
RESEARCH INVOLVING HUMAN SUBJECTS
ETHICS SUBMISSION

INVESTIGATOR: Dr. Niall M. Moyna
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STUDY TITLE: Influence Of Acute Exercise On Postprandial Lipaemia And Endothelial Dysfunction In Normal Weight And Overweight Adolescents.
STUDY ACRONYM: (OPEEC Study - Overweight, Postprandial lipemia, Endothelial function and Exercise in Children study)

1. DESCRIBE THE RATIONALE AND AIMS OF THE PROPOSED STUDY

The vascular endothelium is a single layer of cells that forms a biologic interface between circulating blood elements and the various systems in the body. It plays a vital physiological role in vascular homeostasis by synthesizing and releasing a number of biologically active factors involved in the regulation of vascular tone, platelet aggregation, monocyte and leukocyte adhesion and thrombosis. There is accumulating evidence that endothelial dysfunction (ED) represents one of the earliest events in the pathogenesis of cardiovascular disease (*Moyna et al., Acta Physiol Scand, 2004, 180:113*). A number of blood markers of endothelial dysfunction exist enabling this crucial process to be monitored.

Given its strategic location between the circulating blood elements and the various systems in the body, the endothelium is a primary target for injury from mechanical forces and processes related to cardiovascular risk factors. Hypercholesterolemia is an important risk factor for coronary atherosclerosis, and is associated with abnormal endothelial function. Recent studies have shown that triglyceride (TG) levels and in particular postprandial hypertriglyceridemia are associated with endothelial dysfunction. This is not surprising considering that majority of adults and children spend more than 17 hours per day in the postprandial state, with triglyceride-rich lipoproteins remaining in the circulation for an extended period of time, even in individuals with normal fasting TG. A recent study found impaired endothelial function in response to a high fat (50 g) meal in 20 healthy normocholesteremic subjects with total and low density lipoprotein (LDL) < 200 mg/dl and 130 mg/dl respectively (*Plotnick et al, Am J Cardiol, 1997,3:350*). Mean changes in flow mediated brachial artery vasodilation correlated significantly with the change in postprandial TG's. In contrast, there was no significant correlation between arterial response and fasting TG levels, suggesting that postprandial triglyderide-rich lipoproteins are at least partly responsible for the impairment in endothelial function.

Strategies that reverse ED have important clinical applications in a variety of cardiovascular disease states. Pharmacologic intervention, L-arginine supplementation, LDL apheresis and smoking cessation have all been shown to improve endothelial function in adults. A single bout of exercise that occurs 4-24 h prior to feeding can reduce postprandial TG. This acute effect typically results in a 20-30% reduction in the area under the TG-time curve. This finding is

consistent in a range of healthy populations including young adults, sedentary adults, trained adults, older men and postmenopausal women. To our knowledge no studies have compared the effects of acute exercise on postprandial triglycerides in adolescents with BMI < 25 kg·m² (normal weight) and a BMI > 25 kg·m² (overweight). This information is important considering that overweight/obesity is a growing health problem, and is reaching epidemic proportions among children in western societies. Overweight/obese children typically display higher fasting triglycerides and impaired endothelial function compared to age matched controls (*Moyna et al., 2000, Med. Sci. Sports Exerc, 32:5*)

Specific Aims:

1. To compare the effects of a standardised fatty meal on postprandial triglycerides in adolescent males with a BMI < 25 kg·m² and a BMI > 25 kg·m²
2. To compare the effects of a standardised fatty meal on endothelial function in adolescent males with a BMI < 25 kg·m² and a BMI > 25 kg·m²
3. To examine the efficacy of a single exercise bout in attenuating postprandial lipemia and endothelial dysfunction in adolescent males with a BMI < 25 kg·m² and a BMI > 25 kg·m²

2. OUTLINE THE DESIGN AND METHODOLOGY OF THE STUDY

Research Design Overview: Subjects will report to the Cardiovascular Research Unit (CRU) on 5 separate occasions (two screening sessions, two experimental sessions and one exercise session). During the experimental session subjects will be undergo an oral fat tolerance test (OFTT). The OFTT tests will be separated by at least 7 d and will be preceded by a single bout of submaximal exercise or rest.

Subjects: Normal weight (n=15), overweight (n=15) and obese (n=15) Adolescent males boys 16-17 years with a BMI < 25 kg·m² (N=15) and a BMI > 25 kg·m² (n=15) old will be recruited to participate in the study

Though men and women have predominately the same physiological structure there are some differences in the regulation of certain processes. Phenotypically, women have a greater body fat store and evidence is suggesting that women utilise fats with different rates of efficiency than men, especially at lower exercise intensities. However, this difference is not fully understood and work is currently underway to provide a more accurate explanation. Hormonal fluctuations resulting from the menstrual cycle have been shown to have a profound effect on substrate utilization in young women. Furthermore, circulating estrogen is known to favourably alter the lipid profile in women. On this occasion, which is a typical approach, we have decided to begin our investigation with adolescent boys only. If there is a gender specific response, there is a chance our results may be skewed by the simultaneous inclusion of boys and girls.

Exclusion Criteria: Subjects will be excluded if they have diabetes, history of heart disease, liver dysfunction, shortness of breath or asthma with usual exercise, or other medical conditions that may contraindicate exercise participation. or if they are They will not be receiving medication known to alter lipid metabolism. Smokers will also be excluded, as the free radicals generated are likely to increase lipoprotein oxidation and endothelial dysfunction.

Preparation: Prior to each OFTT, subjects will be required to abstain from alcohol, and strenuous physical activity for a minimum of 2 d. They will record their normal diet (food diary) for the 3 d prior to the first experimental visit and repeat the recorded diet for the 3 d before the second experimental visit.

3. DESCRIBE THE RESEARCH PROCEDURES AS THEY AFFECT THE RESEARCH SUBJECT AND ANY OTHER PARTIES INVOLVED.

Oral Fat Tolerance Test: Subjects will report to the CRU at approximately 8 a.m. following an overnight fast. An intravenous catheter (21G) will be inserted into a prominent forearm vein. Following a 15 min rest period, a baseline blood sample will be obtained. Subjects will then consume the test meal (mixed meal but high in fat, i.e., 1.0g fat/ kg body mass). Blood samples will be obtained in the postprandial (after feeding) period and at 30 min, 1, 2, 4, 6 and 8 h. Subjects will rest quietly during this period but will be permitted to read or watch television. Subjects will be allowed to consume water ad libitum.

Screening Session 1: During the first screening visit, the nature and risks of the study will be explained, the health history questionnaire will be completed and written informed consent will be obtained. Subjects will then undergo a brief physical examination, body composition assessment and measurement of maximal oxygen uptake ($VO_2\text{max}$).

Screening Session 2: During the second screening visit, subjects will perform a submaximal exercise test in order to determine the exercise intensity corresponding to 75% $VO_2\text{max}$.

Exercise session: Subjects will exercise on the evening prior to one OFTT. This will consist of treadmill walking/running until they expend 700 Kcal. The order of the exercise and control trials will be randomised. Expired air will be collected for determination of energy expenditure and substrate utilisation using indirect calorimetry.

Body composition: Lange skinfold callipers (Cambridge Scientific Industries, MD) will be used to measure double thickness subcutaneous adipose tissue on the right side of the body. The following anatomical sites will be used; chest, subscapular, axilla, suprailium, abdomen, triceps and thigh. A minimum of 2 measurements will be taken at each site. If the measurements vary by more than 1 mm a third measurement will be taken. Percent body fat will be calculated according to the Jackson and Pollock equation (*Jackson et al 1985, Physician Sport Med, 13, 76*). Waist and hip circumference will also be measured using a tape measure.

Heart Rate: Heart rate will be measured continuously during each training session using a wireless Polar heart rate monitor (Polar Vantage NV™ Polar, Port Washington, NY). The Polar Vantage NV™ records 134 hours of performance data.

Ratings of Perceived Exertion: RPE will be obtained using the 15-point Borg category RPE scale. Prior to each maximal exercise test subjects will be read a standard set of perceptual scaling instructions. These instructions will follow an established format used in previous investigations. Low and high “perceptual anchors” will be established during the maximal exercise test. This involves asking subjects to assign a rating of 7 (low anchor) to the lowest exercise intensity, and 19 (high anchor) to the highest exercise intensity. During each session subjects will be instructed to make their subjective assessments of perceived exertion relative to these minimum and maximum standards (perceptual anchors).

Peak Oxygen Consumption ($VO_2\text{max}$): Maximal aerobic capacity ($VO_2\text{max}$) will be determined using a Modified Astrand treadmill exercise test. Each test will be voluntarily terminated when the subject can no longer continue to exercise because of fatigue. Subjects will be verbally encouraged to exercise to volitional exhaustion. Respiratory metabolic measures, heart rate and ratings of perceived exertion will be determined continuously throughout the test. Maximal oxygen uptake will be determined by averaging the two highest consecutive 30 s values.

4. DESCRIBE THE LABORATORY PROCEDURES

Expired Air Analysis: Expired oxygen, carbon dioxide, ventilatory volume and respiratory exchange ratios (RER) will be determined using a Sensormedics Vmax 229 metabolic system (SensorMedics Corp., Yorba Linda CA). Prior to testing, the gas analysers will be calibrated with standard gases of known concentration.

Biochemical Assays: Plasma/serum will be obtained from blood samples by centrifugation and stored at -20°C or -70°C . Repeated measures from each subject will be defrosted together and analysed in the same machine run. Plasma triglycerides, total cholesterol, HDL-cholesterol, free

fatty acids, 3-hydroxybutrate and glucose will be determined by colourimetric enzymatic assay and measured on a spectrophotometer. Insulin, VCAM-1, ICAM-1, interleukin-6, TNF- α and endothelin-1 will be measured using an ELISA. Remnant lipoproteins (VLDL-C and VLDL-TG) will be analysed using an immunoseparation technique (Nakajima *et al*, 1993, *Clin Chim Acta* 223: 53). Apo B-48 and apo B-100 will be determined using analytical SDS-PAGE (Karpe and Hamsten, 1994, *J Lipid Res*, 35; 1311).

Substrate Utilisation: The percentages of carbohydrate and lipid oxidation will be calculated using the McGilvery & Goldstein equations (McGilvery & Goldstein, 1983, *G.W. Biochemistry: A Functional Approach. Philadelphia: Saunders, 1983, pp 976*): %lipid = $((1-RER)/0.29) \times 100$, and %CHO = $((RER-.71)/0.29) \times 100$. The RER value will represent the average of each 5-min period throughout the study. The rate of CHO and fat oxidation will be calculated from gas exchange measurements according to the table of non-protein respiratory quotient (Peronnet & Massicote, 1991, *Can. J. Sport Sci.* 16:23): CHO = $4.85 \times VCO_2 - 3.226 \times VO_2$, and lipid = $1.695 \times VO_2 - 1.701 \times VCO_2$, with mass expressed in g/min and gas volume in L/min. The VO_2 and VCO_2 values will represent the average of each 5-min period throughout the study. Energy expenditure will be calculated using the equation of Weir (1949): $EE \text{ (kJ/min)} = 16.5 \text{ } VO_2 \text{ (L/min)} + 4.63 \text{ } VCO_2 \text{ (L/min)}$.

Statistical Analysis

A one way ANOVA will be used to compare baseline characteristics of each group. A 3 way (time x group x experimental condition) ANOVA will be used to compare changes in serum markers. Pearson product-moment correlations will be used to determine the association between selected parameters. Probability values ≤ 0.05 will be considered to indicate statistical significance.

5. WHAT IN YOUR OPINION ARE THE ETHICAL CONSIDERATIONS INVOLVED IN THIS PROPOSAL? (YOU MAY WISH FOR EXAMPLE TO COMMENT ON ISSUES TO DO WITH CONSENT, CONFIDENTIALITY, RISK TO SUBJECTS, ETC.)

- A. Anthropometric measurements: Subjects may be sensitive to the anthropometric measurements. All measurements will be taken in a private examination room.
- B. Anonymity and confidentiality: Subjects personal identity or personal information will not be revealed, published or used in further studies. Subjects will be assigned an ID number under which all personal information will be stored in a secure file and saved in password protected file in a computer. The study results may be used as part of a series of studies and study findings may be presented at scientific meetings and published in scientific journals.
- C. Risks of Exercise: Exercise testing carries with it a very small risk of abnormal heart rhythms, heart attack or death, but these are very rare in children. Subjects may experience some muscle soreness in their arms and legs following maximal exercise. If the experimental protocol is adhered to, the likelihood of these risks occurring is minimal. We will adhere to standard absolute indicators for terminating an exercise test (J. Am. Coll. Cardiol. 1997;30:260-315). Subjects with diabetes, history of heart disease, liver dysfunction and other major signs or symptoms suggestive of cardiovascular (CVD) and pulmonary disease (angina, dizziness or syncope, orthopnea or paroxysmal dyspnea, asthma, ankle edema, palpitations or tachycardia, intermittent claudication, known heart murmur, or unusual fatigue or shortness of breath with usual exercise) will be excluded from participating in the study. The pre-test likelihood of coronary artery disease in asymptomatic adolescents is very low. Subjects will be allowed to stop the test at any time. It is standard procedure to inform subjects that they are allowed to stop at any time during a maximal exercise test. No participant will be excluded from the study if they terminate their maximal exercise test early. However, they will be asked to repeat the test to insure that they meet at least 2 of the following criteria: 1) a plateau of VO_2 as indicated by a difference in values between the final 2 stages of the test of $< 2.1 \text{ ml/Kg/min}$; 2) a respiratory exchange

ratio of ± 1.10 and 3) heart rate within ± 5 beats/min of each subjects age predicted maximal value.

- D. Early withdrawal: Subjects may withdraw from the study at any point. There will be no penalty if withdrawal occurs before testing is complete.
- E. Blood sampling: Research procedures require blood samples to be taken at regular intervals. An individual trained in phlebotomy will insert the IV catheter. There is a very small risk of infection from having blood drawn. Every effort will be made to ensure that the procedures used to take blood adhere to safety standards. There is a very small risk of infection from having blood drawn but every safety precaution will be taken to ensure that does not happen. The total amount of blood taken during the entire study will be approximately 200 ml. This is much less than the 570 ml (pint) of blood that is usually donated at blood banks. The fluid volume is replaced within 24 hours and the red blood cells will be replaced within 2 weeks.
- F. Emergency crash cart: In the event of an emergency, the research laboratory is equipped with an emergency crash cart and defibrillator.
- G. Recruitment procedure:

We will be using two recruitment methods. The first will involve local sports clubs. Permission will be sought from the sports clubs to speak to team(s) in the appropriate age group. A brief 10 min presentation will be undertaken (Dr. Niall Moyna) to explain the nature of the study. A question and answer session will follow the presentation. Anyone interested in participating in the study will be asked to discuss his potential involvement with his parent(s)/guardian(s). A parent/guardian will be asked to contact Dr. Moyna to confirm that they are considering allowing their son to participate in the study. The parent(s)/guardian(s) and son(s) will be invited to DCU to discuss the study. They will be told that by agreeing to visit DCU they are not obligated to agree to allow their son participate in the study. The benefits, risks and discomforts of the study will be explained. If parent(s)/guardian(s) and son(s) agree to participate they will be asked to sign an informed consent and assent form respectively.

The second recruitment method will involve local GPs. The physicians will describe the nature of the study to potential participants. Anyone interested in participating in the study will be asked to discuss his potential involvement with his parent(s)/guardian(s). A parent/guardian will be asked to contact Dr. Moyna to confirm that they are considering allowing their son to participate in the study. The parent(s)/guardian(s) and son(s) will be invited to DCU to discuss the study. They will be told that by agreeing to visit DCU they are not obligated to agree to allow their son participate in the study. The benefits, risks and discomforts of the study will be explained. If parent(s)/guardian(s) and son(s) agree to participate they will be asked to sign an informed consent and assent form respectively.

6. OUTLINE THE REASONS, WHICH LEAD YOU TO BE SATISFIED THAT THE POSSIBLE BENEFITS TO BE GAINED FROM THE PROJECT JUSTIFY ANY RISKS OR DISCOMFORTS INVOLVED

The risks and discomforts associated with participation in the study are minimal. Subjects will receive a copy of their personal results which will include their fitness level, body composition, triglyceride and cholesterol measurements as well as a summary of the overall results. The commercial rate for these tests is in excess of € 500.00.

7. WHO ARE THE INVESTIGATORS (INCLUDING ASSISTANTS) WHO WILL CONDUCT THE RESEARCH AND WHAT ARE THEIR QUALIFICATIONS AND EXPERIENCE

Dr. Niall M. Moyna. Dr. Moyna is an Exercise Physiologist and Senior Lecturer in the Faculty of Science and Health at Dublin City University. He is Head of the Centre for Sport Science and

Health and Associate Director of the Vascular Health Research Centre at DCU. He was Director of the Applied Physiology Laboratory in the Division of Cardiology at the University of Pittsburgh Medical Center, and a Senior Research Scientist and Director of the Clinical Research Laboratory in the Division of Cardiology at Hartford Hospital, Connecticut, USA.

Dr. Noel McCaffrey is a physician with an adjunct position in the Faculty of Science and Health. He will act as the medical advisor for the study.

Mr. Owen MacEneaney is a graduate student in the Centre for Sport Science and Health at DCU. He has an undergraduate degree in Sport Science and Health.

8. ARE ARRANGEMENTS FOR THE PROVISION OF CLINICAL FACILITIES TO HANDLE EMERGENCIES NECESSARY? IF SO, BRIEFLY DESCRIBE THE ARRANGEMENTS MADE.

The CRU is fully equipped with an emergency crash-cart and defibrillator. An individual trained in resuscitation will be present during each test.

9. IN CASES WHERE SUBJECTS ARE IDENTIFIED FROM INFORMATION HELD BY ANOTHER PARTY (FOR EXAMPLE, A DOCTOR OR HOSPITAL), DESCRIBE THE ARRANGEMENTS WHEREBY YOU GAIN ACCESS TO THIS INFORMATION.

N/A

10. SPECIFY WHETHER SUBJECTS WILL INCLUDE STUDENTS OR OTHERS IN A DEPENDENT RELATIONSHIP.

N/A

11. SPECIFY WHETHER THE RESEARCH WILL INCLUDE CHILDREN OR THOSE WITH MENTAL ILLNESS, DISABILITY OR HANDICAP. IF SO, PLEASE EXPLAIN THE NECESSITY OF USING THESE SUBJECTS.

The study will include boys in mid-to-late adolescence. The prevalence of overweight and obesity in Irish children and adolescents has reached alarming levels. Greater body weight has been found to predispose children and adolescents to many of the medical complications of obesity found in adults, such as hypertension, dyslipidemia, impaired glucose homeostasis, steatohepatitis, sleep apnea, and intracranial hypertension, and to problems unique to childhood and adolescence, including accelerated pubertal and skeletal development and orthopedic disorders, such as slipped capital femoral epiphysis. Severely obese children and adolescents who seek obesity treatment have more than a 5-fold increased risk of reporting a low health-related quality of life compared with a reference sample of healthy children and adolescents—a risk similar to that previously described for children and adolescents diagnosed as having cancer. Overweight during childhood and adolescence also appears to be an important independent predictor of health risks and mortality in later life, even when adult weight is taken into account. Thus, it seems clear that one of the most compelling medical challenges of the 21st century is to develop effective strategies to treat the complications of childhood obesity.

12. WILL PAYMENT BE MADE TO ANY RESEARCH SUBJECT?

No

13. DESCRIBE THE PROCEDURES TO BE USED IN OBTAINING A VALID CONSENT FROM THE SUBJECT. PLEASE SUPPLY A COPY OF THE INFORMATION SHEET PROVIDED TO THE INDIVIDUAL SUBJECT.

The nature and risks involved in the study will be explained prior to the starting the study and a contact number will be provided. Parental signature and a witness signature will be required on the informed consent. Participants will be required to sign an assent form.

- 14. COMMENT ON ANY CULTURAL, SOCIAL OR GENDER-BASED CHARACTERISTICS OF THE SUBJECT WHICH HAVE AFFECTED THE DESIGN OF THE PROJECT OR WHICH MAY AFFECT ITS CONDUCT.**

None

- 15. GIVE DETAILS OF THE MEASURES WHICH WILL BE ADOPTED TO MAINTAIN THE CONFIDENTIALITY OF THE RESEARCH SUBJECT**

Each participant's personal identity or personal information will not be revealed, published or used in further studies. Subjects will be assigned an ID number under which all personal information will be stored in a secure file and saved in password protected file in a computer at DCU. The principle investigator, graduate student and medical director will have access to the data. The study results may be used as part of a series of studies being conducted in the CRU.

- 16. WILL THE INFORMATION GAINED BE ANONYMIZED? IF NOT, PLEASE JUSTIFY.**

Yes. In order to insure that all personal and medical information remains confidential, each subject will be identified by a unique ID number.

- 17. WILL THE INTENDED GROUP OF RESEARCH SUBJECTS, TO YOUR KNOWLEDGE, BE INVOLVED IN OTHER RESEARCH? IF SO, PLEASE JUSTIFY.**

No

- 18. DATE ON WHICH THE PROJECT WILL BEGIN May 2004 AND END May 2005**

- 19. PLEASE STATE LOCATION(S) WHERE THE PROJECT WILL BE CARRIED OUT.**

Cardiovascular Research Unit in the Centre of Sport Science and Health, Dublin City University

Signed _____
(Principle Investigator)

Date _____

Signed _____
(Head of Department)

Date _____

DUBLIN CITY UNIVERSITY
Research - Parental consent form

- I. **Project Title:** *Influence Of Acute Exercise On Postprandial Lipaemia And Endothelial Function In Normal Weight And Overweight Adolescents.*

- 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 are left with a high concentration of fat in their bloodstream over a prolonged period. This may leave them at a higher risk of heart disease.

- III. **I am being asked to allow my child to participate in this research study. The study has the following purpose:** This is a research study investigating the effect of exercise on the body's ability to digest a high fat meal and the possible implications in the development of heart disease. We will compare the responses of normal and overweight adolescents to a high fat meal. Research studies include only subjects who choose to take part. You are being asked to let your child take part in this study. Please take your time to make your decision. Discuss it with your child and family. Be sure to ask questions about anything you don't understand. My child cannot take part if he has diabetes, history of heart disease, liver dysfunction, or if he is a smoker.

- IV. **This research study will take place at the Centre for Sport Science and Health, Dublin City University, and will last approximately 2 weeks. This is what will happen during the research study:**
 1. My child will visit the Cardiovascular Research Unit in the Centre for Sport Science and Health on 4 separate days. The first visit will be for screening purposes. My son will be asked to eat a high fat meal on the morning of the second and third visit to the Cardiovascular Research Unit. He will also be asked to visit the laboratory at DCU for one hour on the night before one of the fat meals. During this visit my son will exercise on a treadmill for between one and one and a half hours. My son will be required to fast for 4 hours before visit one and for 12 hours (overnight fast) before each fat meal. He will also be asked to abstain from alcohol for 3 days prior to each visit.
 2. During the first visit, my child will have a brief medical examination and will perform an exercise test to determine his fitness level. This will involve running/walking on a treadmill at increasing speeds until his maximal exercise capacity is reached. For this, and all subsequent exercise tests, he will be fitted with a mouthpiece connected to a machine and computer to measure the composition of gases in his breath. He will also have his height, weight, waist circumference and hip circumference measured. Skinfold calipers will be used to estimate the amount of fat tissue and muscle tissue in his body.
 3. During the second and third visits, my son will have a small amount of blood drawn after having eaten a high fat meal. On the day of each of the high-fat meals, my child will report to the Cardiovascular Research Unit in the Centre for Sport Science and Health at 8 a.m. He will have a small plastic tube, called a catheter, inserted into a vein in his arm taken at regular intervals for 8 hours. During this time he will be free to read, watch television or play computer games. The total amount of blood taken during the entire study will be approximately 200 ml. This is much less than the 570 ml (pint) of blood that is usually donated at blood banks. The fluid volume is replaced within 24 hours and the red blood cells will be replaced within 2 weeks.
 4. On the evening before one of the fatty meals, my child will go to DCU to walk/jog on a treadmill until he burns 700 calories. Depending on his fitness level, this will take between 50 and 90 minutes. On the evening before the other fatty meal test, he will rest quietly at home.
 5. For 2 days before each fatty meal test my child will not be allowed to do any exercise or strenuous physical work.

6. For 3 days before the first fatty meal test, he will record the food he eats in a diary. He will follow the same diet for the 3 days before the second fatty meal test.
7. My child will be asked to give his best effort during each exercise test. However, he should let the researchers know if he experiences discomfort during any of the exercise sessions. If he wishes, he can stop exercising at any time during any of the exercise sessions.

V. Sometimes there are side effects from performing exercise tests. These side effects are often called risks and for this project the risks are:

1. Because my child will be asked to give a maximum effort, he may experience some muscle soreness or nausea following the maximal exercise test. It should be noted that if the experimental protocol is adhered to, the likelihood of these risks occurring is minimal.
2. My child may feel a slight pain when the catheter is inserted and 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 the blood. There is a very small risk of infection from having blood drawn but every safety precaution will be taken to ensure that does not happen. The amount of blood drawn is not harmful. However, if my child has a history of anemia or a fear of needles, I should inform the researchers.
3. My child may be allergic to part of the test meal. I will report any food allergies that my child is known to have to the researcher.

VI. There may be benefits from my child's participation in this study. These are:

1. My child will receive a copy of his personal results, including fitness level, percentage body fat, triglyceride and cholesterol measurements as well as a summary of the overall results.
2. **No other benefits have been promised to me, or my child.**

VII. My child's confidentiality will be guarded: DCU will protect all the information about your child and his part in this study. Your child's 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 and may also be presented in academic publications and conference papers.

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

IX. My child's participation in this study is my decision. If I do agree to allow my child to take part in the study, I can stop his participation at any time, including during an exercise test. There will be no penalty if I withdraw my child before he has completed all stages of the study. However, once my child has completed the study, I will not be allowed to have his personal information or results removed from the database. If I decide to stop my child from participating in this study, it is recommended that I first talk to Dr. Moyna.

X. 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 allow my child to take part in this research project entitled: *"Influence of acute exercise on postprandial lipaemia and endothelial function in normal weight and overweight adolescents."*

Signed: _____
Signature

Date: _____

(Block Capitals)

Witness: _____

Date: _____

Signature

DUBLIN CITY UNIVERSITY

Assent Form

I. Project Title: *Influence Of Acute Exercise On Postprandial Lipaemia And Endothelial Function In Normal Weight and Overweight Adolescents.*

II. I am being asked to take part in a scientific study. I have told my parent(s)/guardian(s) about my interest in taking part in the study. We have discussed what will happen during the study and they have agreed to allow me to participate. The study will examine how exercise affects how the body processes fat that we eat in our diet, and will seek to find out if normal weight and overweight adolescents of my own age process the fat differently.

III. This research study will take place at the Centre for Sport Science and Health in Dublin City University and will last for 2 weeks. I will have to go to DCU on 4 separate days.

IV. I understand the reason for the study and why I am being asked to take part in it. I have been told that, if I agree to be in this study, I will do the following:

1. I will visit the Centre for Sport Science and Health on 4 separate days.
2. On the first day, a doctor will examine me, and I will perform an exercise test on a treadmill for about 8 to 10 minutes to determine how fit I am. I will breath through a special mouthpiece during this test to measure the oxygen and carbon dioxide in my breath. I will have my height, weight, waist circumference and hip circumference measured with measuring tape. The researcher will also use special calipers to estimate the amount of fat and muscle in my body.
3. During the next two visits to DCU I will be asked to eat a fatty meal. On the evening before one of these tests, I will go to DCU to run on the treadmill for about an hour and a half. Before I eat the meal, a plastic tube will be placed in a vein in my arm so that blood can be taken without me feeling anything. I may experience a little pain when the plastic tube is being placed in my arm. There is a very small risk of infection and bruising from the needle. I will remain at DCU for 8 hours. During this time I will be free to read, watch television or use a computer.
4. I cannot eat for 4 hours before visit one and for 12 hours (overnight fast) before each fat meal. I cannot drink alcohol for 3 days before each visit. I cannot do any strenuous exercise for 2 days before each fatty meal test.
5. I will be asked to keep a record of all the food I eat for 3 days before the first fatty meal test. I will follow the same diet for the 3 days before the second fatty meal test.

V. I should talk this over with my parents before I decide whether or not to participate.

My parents will also be asked to give their permission for me to take part in this study, but even if they say "yes", I can still decide not to do it. I know that I can ask questions about this study at any time. Being in this study is up to me and no one will be upset if I don't want to do it or even if I change my mind later and want to stop. If I have questions about the research project, I am free to call Dr. Niall Moyna at 01-7008802.

VI. Signature: Signing my name at the bottom means that I agree to be in this study. My parents and I will be given a copy of this form after I have signed it.

Signed: _____ **Date:** _____
Signature

(Block Capitals)

Witness Signature: _____ **Date:** _____

Response to Reviewer

1. Comment re explanation of the link between the measures and the aims of the research “it is not made clear, in the application how endothelial dysfunction will be measured (there are many measures listed but none are clearly identified - for the lay reader - as relevant for measuring endothelial dysfunction or function)”.

Endothelial dysfunction is characterized, in part, by chronic low levels of inflammation. This manifests as increased plasma levels of cell adhesion molecules (ICAM-1, VCAM-1), interleukins (IL-6) and C-reactive protein (CRP). Tumor necrosis factor-alpha (TNF- α) and endothelin-1 (ET-1) levels also increase in response to endothelial damage. Plasma levels of the above parameters can used to give an indirect assessment of endothelial dysfunction.

2. Comment re blood sampling safety: “Risks associated with insertion of a catheter and that remains in place for a period of time include: bruising, infection and local discomfort. In addition, blood sampling from the catheter could introduce pathogens into the blood stream. The researchers propose to use an individual with training in phlebotomy to insert the catheter: that should reduce these risks, however, I would hope that all blood sampling will meet appropriate safety standards to further reduce risk of infection”.

We agree that any invasive procedure performed during clinical research studies should strictly adhere to safety standards. The following sentences have been added to the blood sampling in section 5 of the ethics submission: There is a very small risk of infection from having blood drawn. Every effort will be made to ensure that the procedures used to take blood adhere to safety standards

3. Comment re exercise safety: “I assume that among the exclusion criteria will be participants with conditions like asthma (probably covered by “shortness of breath with usual exercise”)”.

Risks of exercise in section 5 of the ethics submission now reads as follows: Subjects with diabetes, history of heart disease, liver dysfunction and other major signs or symptoms suggestive of cardiovascular (CVD) and pulmonary disease (angina, dizziness or syncope, orthopnea or paroxysmal dyspnea, asthma, ankle edema, palpitations or tachycardia, intermittent claudication, known heart murmur, or unusual fatigue or shortness of breath with usual exercise) will be excluded from participating in the study.

4. Comment re consumption of a fatty meal safety: “It would be helpful if the researchers considered identifying conditions that would merit exclusion (medical expertise would be required to ascertain the relevant conditions). Further it would be worth considering whether participants might have food allergies to the experimental meal, and excluding such participants”.

The mixed meal used for the OFTT (1g fat/kg body weight) should be well tolerated by most subjects. OFTTs have been used successfully with adolescent subjects in previous investigations without reporting problems (J. Pediatr. Endocrinol. Metab. Feb;14(2):193-202, 2001; Am. J. Clin. Nutr. Nov;72(5):1119-27, 2000). The following sentence has been added to section V of the parental consent form:

My child may be allergic to part of the test meal. I will report any food allergies that my child is known to have to the researcher.

5. Comment re information sheet: “The information sheets do not mention that the research intends to compare normal, overweight and obese adolescents responses to fatty meals and exercise: this is

deception. Similarly in the parental consent form IV2, the researchers write 'Finally, his total amount of muscle will be estimated using skin calipers' when the researchers intend to measure fat, not muscle".

After deliberating with a number of clinicians we initially decided not to use the term overweight or obese on the consent or assent forms because it may stigmatize the child. We appreciate that the omission of the terms overweight and obesity could be construed as deception. However, this was never our intent. We have now decided to only use two research groups in the study, and to select the subjects based on body mass index (BMI). Subjects with a BMI < 25 will be classified as normal weight and those with a BMI > 25 will be classified as overweight. This is now clearly stated in the ethics submission, and the informed consent and assent forms.

Section II of the assent form now reads as follows: I have told my parent(s)/guardian(s) about my interest in taking part in the study. We have discussed what will happen during the study and they have agreed to allow me participate. The study will examine how exercise affects how the body processes fat that we eat in our diet, and will seek to find out if normal weight and overweight adolescents of my own age process the fat differently.

We are interested in estimating muscle because it is a metabolically active tissue, and is a likely putative mechanism to explain difference in postprandial responses between adolescent boys with a BMI < 25 and a BMI > 25. Postprandial changes will be corrected for lean body mass. We are not interested in estimating body fat because we are using BMI to classify subjects. However, since we do not want to give the impression that we are deceiving the subjects we have added the following sentence to section IV(2) of the assent form: The researcher will use special calipers to estimate the amount of fat and muscle in my body.

The following sentence has been added to section IV (2) of the parental consent form: He will also have his height, weight, waist circumference and hip circumference measured. Skinfold calipers will be used to estimate the amount of fat tissue and muscle tissue in his body.

6. Comment re information sheets: "omit a range of information that may be relevant to the child's assent including the screening tests, food diary, risks associated with the blood sampling, etc. I would think that a simplified version of the 9 steps in item IV of the parental consent form would be more appropriate"

Section IV of the assent form now reads as follows:

IV. I understand the reason for the study and why I am being asked to take part in it. I have been told that, if I agree to be in this study, I will do the following:

- 1. I will visit the Centre for Sport Science and Health on 5 separate days.***
- 2. On the first day, a doctor will examine me, and I will perform an exercise test on a treadmill for about 8 to 10 minutes to determine how fit I am. I will breath through a special mouthpiece during this test to measure the oxygen and carbon dioxide in my breath. I will have my height, weight, waist circumference and hip circumference measured with measuring tape. The researcher will also use special calipers to estimate the amount of fat and muscle in my body.***
- 3. During my second visit to DCU I will run on a treadmill at a comfortable pace for about 30 minutes. I will breath through a special mouthpiece again during this test.***
- 4. During the next two visits to DCU I will be asked to eat a fatty meal. On the evening before one of these tests, I will go to DCU to run on the treadmill for about an hour and a half. Before I eat the meal, a plastic tube will be placed in a vein in my arm so that blood can be taken without me feeling anything. I may experience a little pain when the plastic tube is being placed in my arm. There is a very small risk of infection and bruising from***

the needle. I will remain at DCU for 8 hours. During this time I will be free to read, watch television or use a computer.

5. I cannot eat for 4 hours before visit one and visit two and for 12 hours (overnight fast) before each fat meal. I cannot drink alcohol for 3 days before each visit. I cannot do any strenuous exercise for 2 days before each fatty meal test.

6. I will be asked to keep a record of all the food I eat for 3 days before the first fatty meal test. I will follow the same diet for the 3 days before the second fatty meal test.

7. Comment re contact number: "I would recommend that a contact number for Dr Moyna be provided in the youth assent form so that the adolescent could make contact if he had concerns without having to raise these first with his parent"

The following sentence has been added to section V of the youth assent form: *If I have questions about the research project, I am free to call Dr. Niall Moyna at 01-7008802.*

8. Comment re: emotional or psychological harm: "A further risk that could be associated with this research is emotional or psychological harm caused by stigmatization of adolescents as overweight or obese. The information and consent material for parents and adolescents does not refer to weight (nor to the anthropometric measures to be undertaken in the first visit), and so it might be thought that the researchers have minimized the risk of stigmatization on the basis of weight (by non-disclosure of one key intent of the research). However, because no information has been provided about how participants are to be recruited (through schools? Sporting clubs? physicians?), nor on how participants will first find out about the project, it is impossible to determine from the information given, whether this risk is adequately addressed in the recruitment process"

As noted above (see 5) we have decided to classify the children as normal weight or overweight. The subjects will be told that the weight classification will be based on BMI. The consent and assent forms refer to weight and the anthropometric measures to be taken during the first visit. The recruitment procedures (outlined below) will involve local sports clubs and physicians. These procedures are designed to insure that any involvement in the study will be initiated by the child.

9. Comment re recruitment: "Nothing is mentioned about how parents or adolescents are approached for consent. The Adolescent information sheet implies that the boys are approached after their parents. It would be useful to know who seeks consent (the researcher, teachers, physician, other?) to help assess whether the consent can be assumed to be freely given".
10. Comment re recruitment: "No information has been provided about initial recruitment: I would think that there are a range of ethical issues associated with recruiting adolescents into this project including: whether overweight and obese adolescents are identified as such prior to consenting to participate (with the attendant risks of harm to self-esteem); whether the adolescents are recruited directly or through another responsible body (e.g. school or physician) and hence whether undue pressure is placed on adolescents to consent, etc"

The following section has been added to the ethics submission section 5 (G):

Recruitment procedure:

We will be using two recruitment methods. The first will involve local sports clubs. Permission will be sought from the sports clubs to speak to team(s) in the appropriate age group. A brief 10 min presentation will be undertaken (Dr. Niall Moyna) to explain the nature of the study. A

question and answer session will follow the presentation. Anyone interested in participating in the study will be asked to discuss his potential involvement with his parent(s)/guardian(s). A parent/guardian will be asked to contact Dr. Moyna to confirm that they are considering allowing their son to participate in the study. The parent(s)/guardian(s) and son(s) will be invited to DCU to discuss the study. They will be told that by agreeing to visit DCU they are not obligated to agree to allow their son participate in the study. The benefits, risks and discomforts of the study will be explained. If parent(s)/guardian(s) and son(s) agree to participate they will be asked to sign an informed consent and assent form respectively.

The second recruitment method will involve local GPs. The physicians will describe the nature of the study to potential participants. Anyone interested in participating in the study will be asked to discuss his potential involvement with his parent(s)/guardian(s). A parent/guardian will be asked to contact Dr. Moyna to confirm that they are considering allowing their son to participate in the study. The parent(s)/guardian(s) and son(s) will be invited to DCU to discuss the study. They will be told that by agreeing to visit DCU they are not obligated to agree to allow their son participate in the study. The benefits, risks and discomforts of the study will be explained. If parent(s)/guardian(s) and son(s) agree to participate they will be asked to sign an informed consent and assent form respectively.

Appendix II

Data Collection Sheets

Subject Details

Name _____ Subject ID _____

Address _____

Phone # _____(H) Phone # _____(M)

D.O.B. _____ Height (cm) _____

Age _____ Weight (kg) _____

Blood Pressure _____ BMI/BSA _____

During the last 6 months, on average how many times per week do you exercise vigorously?
_____ Time(s) per week

Body Composition

Triceps _____ Mid-axillary _____ % Body Fat _____

Pectoralis _____ Suprailiac _____ Lean Body Mass _____

Subscapular _____ Thigh _____ Fat Mass _____

Abdominal _____

Comments

Health History Questionnaire

Do you suffer from any of the following?

- | | | |
|---|------------------------------|-----------------------------|
| Pain or discomfort in the chest, neck, jaw, | Yes <input type="checkbox"/> | No <input type="checkbox"/> |
| Shortness of breath at rest or with mild exertion | Yes <input type="checkbox"/> | No <input type="checkbox"/> |
| Unusual fatigue at rest or with usual activities | Yes <input type="checkbox"/> | No <input type="checkbox"/> |
| Dizziness | Yes <input type="checkbox"/> | No <input type="checkbox"/> |
| Swelling at the ankles | Yes <input type="checkbox"/> | No <input type="checkbox"/> |
| Palpitations or racing heart beat | Yes <input type="checkbox"/> | No <input type="checkbox"/> |
| A heart murmur | Yes <input type="checkbox"/> | No <input type="checkbox"/> |
| Difficulty with breathing lying down or waking at night short of breath | Yes <input type="checkbox"/> | No <input type="checkbox"/> |
| Cardiovascular disease | Yes <input type="checkbox"/> | No <input type="checkbox"/> |
| Pulmonary disease | Yes <input type="checkbox"/> | No <input type="checkbox"/> |
| Metabolic diseases | Yes <input type="checkbox"/> | No <input type="checkbox"/> |
| Contraindications or prior difficulties with exercise | Yes <input type="checkbox"/> | No <input type="checkbox"/> |

Have any of your first-degree male relatives (father, brother, half-brother) been diagnosed with coronary heart disease (heart attack, angina, by-pass surgery) before the age of 55?

Yes No

Have any of your first-degree female relatives (mother, sister, half-sister) been diagnosed with coronary heart disease (heart attack, angina, by-pass surgery) before the age of 65?

Yes No

Have you ever been told you have high blood pressure or been on medication for blood pressure?

No Yes

Have you ever been told you have high cholesterol or been on medication for cholesterol?

Yes No

Are you currently a cigarette smoker or have you quit in the last six months?

Yes No

Do you undertake moderate intensity physical activity (e.g. walking, gardening, heavy manual work)?

Yes No

If yes, how often _____

Are you currently taking vitamin supplements?

If so please state brand and dosage _____

Please list any medications you are currently taking or related medical condition (including insulin, allergy shots or pills, asthma inhalers, mineral supplements, anti-inflammatories, including aspirin).

Have you any known allergies to medications? If yes, please give details

Comments

**Screening Session
Exclusion Criteria**

- Physical activity within 24h? Yes No
- 4 hour fast? Yes No
- Alcohol within 3 days? Yes No
- Satisfactory Medical History? Yes No

VO₂ max Exercise Test

Treadmill Protocol _____

Time: (min)	Stage	RPE-O	RER	HR: (bpm)
0-1	Warm-up			
1-2	Warm-up			
2-3	Exercise			
3-4	Exercise	*		
4-5	Exercise			
5-6	Exercise	*		
6-7	Exercise			
7-8	Exercise	*		
8-9	Exercise			
9-10	Exercise	*		
10-11	Exercise			
11-12	Exercise	*		
12-13	Exercise			
13-14	Exercise	*		
14-15	Exercise			
15-16	Exercise	*		

Results:

Total Exercise Time: _____ Predicted HR max: _____

VO₂max (mL·kg⁻¹·min⁻¹) _____ HR max: _____

VO₂max (L·min⁻¹) _____ %HR max: _____

Workload corresponding to 70% VO₂max _____

Energy expenditure at 70% VO₂max (kcal·min⁻¹) _____

Exercise time to 600 kcal _____

Comments:

Walking Eqn (1.9 - 3.7 mph): VO₂ (mL·kg·min⁻¹) = (0.1·Speed [m/min])+(1.8·Speed·Grade [%])+3.5

Running Eqn (>3.7 mph): VO₂ (mL·kg·min⁻¹) = (0.2·Speed) + (0.9·Speed·Grade) + 3.5

**Exercise Session
Exclusion Criteria**

- Physical activity within 24h? Yes No
- 4 hr fast? Yes No

Alcohol within 3 days?

Yes

No

Time	HR (bpm)	RPE	VO ₂ (ml/kg/min)	RQ	Fat oxidation (g/min)	CHO oxidation (g/min)	EE (kcal)
10min							
20min							
30min							
40min							
50min							
60min							

Prescribed exercise bout completed?

Yes

No

Total fat oxidation (g)

Total carbohydrate oxidation (g)

Total energy expenditure (kcal)

Comments:

Oral Fat Tolerance Test 1

Date _____ Trial (Contr/Ex) _____

Supervised by _____ Canula insertion _____

Control of prior exercise, diet and alcohol

Have you consumed any alcohol during the last 72 hours? Yes No

Apart from the treadmill walk in the laboratory, have you undertaken any exercise or prolonged strenuous physical work over the last 48 hours? Yes No

Have you closely followed the diet you recorded prior to your first fatty meal test? Yes No

Have you been fasting since 7 pm last night (control trial only) or since the you finished the laboratory treadmill walk (exercise trial) ? Yes No

Test meal

Body Surface Area (m²) _____

Ice-cream (180g/2m²) _____ 48%

Croissants (100g/2m²) _____ 26%

Butter (35g/2m²) _____ 9%

Chocolate (33g/2m²) _____ 9%

Pringles (30g/2m²) _____ 8%

Total fat content (g) _____

Total carbohydrate content (g) _____

Test meal started _____

Test meal finished _____

Water consumed during test

Water consumed with meal (ml) _____

Time	Volume
_____	_____
_____	_____
_____	_____
_____	_____

Blood samples

Meal finished _____

Blood Sample	Due time	Time taken if different	Vacutainers
0 hr	_____	_____	RR P B _____
30 min	_____	_____	R P _____
1 hr	_____	_____	R P _____
2 hr	_____	_____	RR P B _____
4 hr	_____	_____	RR P B _____
6 hr	_____	_____	RR P B _____

Complete Blood Count

0 hrs		6 hrs	
WBC	LY	WBC	LY
RBC	MO	RBC	MO
Hgb	GR	Hgb	GR
Hct	LY#	Hct	LY#
MCV	MO#	MCV	MO#
MCH	GR#	MCH	GR#
MCHC	RDW	MCHC	RDW
Plt	MPV	Plt	MPV

Comments:

Oral Fat Tolerance Test 2

Date _____ Trial (Contr/Ex) _____

Supervised by _____ Canula insertion _____

Control of prior exercise, diet and alcohol

Have you consumed any alcohol during the last 72 hours? Yes No

Apart from the treadmill walk in the laboratory, have you undertaken any exercise or prolonged strenuous physical work over the last 48 hours? Yes No

Have you closely followed the diet you recorded prior to your first fatty meal test? Yes No

Have you been fasting since 7 pm last night (control trial only) or since the you finished the laboratory treadmill walk (exercise trial) ? Yes No

Test meal

Body Surface Area (m²) _____

Ice-cream (180g/2m²) _____ 48%

Croissants (100g/2m²) _____ 26%

Butter (35g/2m²) _____ 9%

Chocolate (33g/2m²) _____ 9%

Pringles (30g/2m²) _____ 8%

Total fat content (g) _____

Total carbohydrate content (g) _____

Test meal started _____

Test meal finished _____

Water consumed during test

Water consumed with meal (ml) _____

Time	Volume
_____	_____
_____	_____
_____	_____
_____	_____

Blood samples

Meal finished _____

Blood Sample	Due time	Time taken if different	Vacutainers
0 hr	_____	_____	RR P B _____
30 min	_____	_____	R P _____
1 hr	_____	_____	R P _____
2 hr	_____	_____	RR P B _____
4 hr	_____	_____	RR P B _____
6 hr	_____	_____	RR P B _____

Complete Blood Count

0 hrs		6 hrs	
WBC	LY	WBC	LY
RBC	MO	RBC	MO
Hgb	GR	Hgb	GR
Hct	LY#	Hct	LY#
MCV	MO#	MCV	MO#
MCH	GR#	MCH	GR#
MCHC	RDW	MCHC	RDW
Plt	MPV	Plt	MPV

Comments:

Appendix III
Meal Composition and Nutritional Information

Name	Energy (KJ/100g)	Energy (Kcal/100g)	Protein (g/100g)	CHO (g/100g)	Fat (g/100g)
Darina Allen Vanilla Ice-cream	965	231	4.2	19.8	15.6
Cadbury's Buttons	2195	525	7.6	56.2	29.9
Mayfair Croissants	1647	393	8.6	48.4	18.3
Kerrygold Butter	3031	737	0.5	0.0	80.0
Sour Cream & Onion Pringles	2325	559	4.7	46.0	37.0

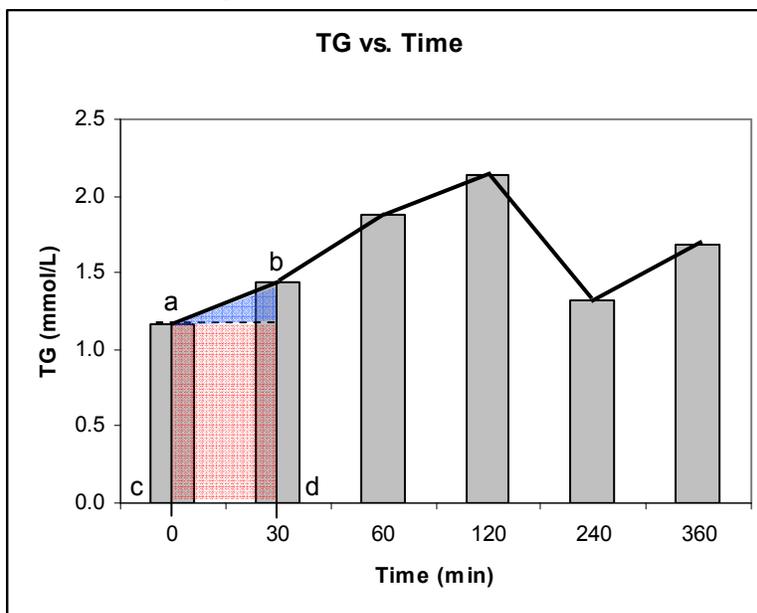
Appendix IV

Worked examples of Trapezoid Rule for Calculation of Area Under the Curve

The following values are taken a subject who completed the study procedures outlined in Chapter 3. For illustrative purposes, only the values from the control trial are shown.

Time (min)	TG (mmol·L ⁻¹)
0	1.16
30	1.44
60	1.88
120	2.14
240	1.32
360	1.69

When plotted on a graph, the values look like this:



The area of the rectangle ($ac \cdot cd$) plus the area of the triangle ($cd \cdot (bd - ac) / 2$) make the area of the trapezoid $abcd$. Adding the areas of each trapezoid will result in a close approximation of the area under the curve. In the example above, the AUC would be calculated as follows:

0	0-30	30-60	60-120	120-240	240-360	AUC
	0.65	0.83	2.01	3.46	3.01	9.96

The incremental area under the curve is calculated in a similar manner with the exception that only area above the initial baseline point is included. In the example above, the AUCi would be calculated as follows:

0	0-30	30-60	60-120	120-240	240-360	AUCi
	0.07	0.25	0.85	1.14	0.69	3.00

Appendix V **Response to External Review**

Name of Candidate: Owen MacEaney
Student ID: 99006339
Award Sought: MSc
Thesis Title: Effect of Acute Exercise on Postprandial Lipemia and Biomarkers of Endothelial Dysfunction and Inflammation in Normal-weight and Overweight Adolescents.

COMMENTS AND RECOMMENDATIONS FOR CHANGES TO THE THESIS

Introduction

Page 2, 4th line from bottom. Check period placement.

- Period placement corrected.

Page 3, lines 2 & 3, Change to “It is not known if data showing attenuation of postprandial TG *and markers of endothelial dysfunction and inflammation* following acute exercise in adults can be extended to adolescents.”

- The passage now reads: “It is not known if data showing attenuation of postprandial TG *and markers of endothelial dysfunction and inflammation* following acute exercise in adults can be extended to adolescents.”

Under **Specific Aims of the Research:**

Change #3 to read “To examine the effect of a single exercise bout on postprandial lipemia, as well as markers of endothelial dysfunction and inflammation in normal weight and overweight adolescent males.”

- The passage now reads: “To examine the effect of a single exercise bout on postprandial lipemia, as well as markers of endothelial dysfunction and inflammation in normal weight and overweight adolescent males.”

Under **Study Hypotheses:**

In my opinion, a very interesting question is being ignored. Hypothesis #3 addresses between group differences in post-exercise postprandial lipemia. However, I would suggest a 4th hypothesis addressing *within group* changes/differences.

- This is an excellent suggestion. The results section already addresses the within group changes but the hypothesis has been updated to read: “The postprandial lipemic response will be attenuated in both normal weight and overweight adolescents, while circulating levels of biomarkers of ED and inflammation and the will be lowered only in the overweight adolescents, when exercise is performed prior to a high fat meal.”

Methods

Page 17, Subheading **Subjects**

Please state if subjects were previously inactive and for how long. If this is not the case and not all subjects were inactive, this needs to be explained.

- The following text has been added to the Methods section: “All subjects were moderately physically active.”

Page 18, Subheading **Maximal Aerobic Capacity**

One of the criteria for a max test was plateau in $\dot{V}O_{2max}$ as indicated by a difference in values between the final two stages 2.0 ml/kg/min. This seems large given the protocol. I would suggest the commonly applied criterion of 1.5 ml/kg/min.

- This is another good suggestion. This criterion was applied and the test results were reanalyzed but no differences were found in the quantification of $\dot{V}O_{2max}$. The following text has been added to the Methods section: “The test was deemed to be maximal if it satisfied at least 2 of the following criteria: leveling of oxygen consumption values as indicated by a difference in values between the final two stages of $<1.5 \text{ ml}\cdot\text{kg}\cdot\text{min}^{-1}$, RER > 1.1 and heart rate within ± 10 beats of the age predicted maximum.”

Pages 19 – 21

The description of the metabolic system and its calibration is much, much too long. This should be no more than one page – at the most!

- The reviewer is correct that this section was verbose and unnecessary. It has been shortened and now reads as follows: “**Cardiorespiratory and Metabolic Measures** Respiratory metabolic responses were determined using standard open-circuit spirometry techniques (SensorMedics Vmax 229, SensorMedics Corp., CA). Prior to testing, the gas analysers were calibrated with standard gases of known concentration. Heart rate was measured continuously during each trial via telemetry (Polar Vantage NV, Polar, NY).”

On the other hand, there is no description of the exercise intervention and how it was monitored. Given that this is an extremely important variable, this needs to be addressed.

- It is agreed that this is an important aspect of the methodology that needs further description. The following text has been added to the Methods section: “**Acute Exercise Bout:** Data from the maximal aerobic capacity test was used to determine the speed and gradient required to elicit approximately 65% $\dot{V}O_{2max}$ using published guidelines.⁴ Subjects completed the exercise bout in the evening 16–18 h prior to ingestion of the meal. Energy expenditure and exercise intensity were recorded continuously throughout the exercise bout and the treadmill speed was adjusted accordingly if subjects could not comfortably maintain the exercise intensity. The exercise bout was completed once 600 kcal were estimated to be expended. The exact energy expenditures were later verified.”

Page 21, Subheading **Body Composition**

Which formulas were used to calculate percent body fat?

- No formula was used to calculate percent body fat, and percent body fat *per se* is not presented in the results section. The only published equations to estimate % body fat using skinfold measures in adolescents we found to be overly simplistic, employing only 2 or 3 skinfold sites, and lead to somewhat crude estimates. The 7-site procedure, although more comprehensive, has not been validated in this age group and we felt it would be inappropriate to present %

body fat data as a result. The sum of skinfolds itself, though not incorporated into a % body fat estimate, is still an informative index of adiposity and so the data was presented in this form.

Page 22, Subheading **Blood Sampling and Processing**

It states that “Repeated measures from each subject were defrosted and together and analyzed in the same run.” I would hope that ALL samples were defrosted and analyzed in the same run!

- We apologize for this misleading terminology – all samples were defrosted and analyzed in the same run and we have updated the text to reflect this fact.

Results

Page 25, paragraph 2

Why were there between-group differences in the relative intensity and treadmill velocity? This needs to be addressed in the discussion.

- This is an important point and we thank the reviewer for his insight. The following text has been added to the discussion that we feel addresses this point appropriately:
“There was a small but significant between-group difference in the relative exercise intensity between the NW and OW groups during the exercise bout. The OW group exercised at an average of 68% VO_{2max} compared with 62% VO_{2max} maintained by the NW group. This is a surprising finding that only became apparent after the data collection was completed. It is possible that the equations used to estimate exercise intensity underestimated the treadmill speed/grade in NW subjects and, while exercise intensity was monitored throughout each trial, it was not adjusted for unless the subject found it difficult to maintain (RPE > 14). It should be noted that the desired goal of the exercise bout was to expend an absolute magnitude of energy (600 kcal) rather than to exercise at a specific intensity. The influence of exercise intensity and substrate utilization during exercise on the subsequent attenuation of postprandial TG appears to be of much less importance than total energy expenditure. For this reason, and the fact that the absolute difference in mean exercise intensity between groups is small, it is unlikely that this had any substantive impact on the results of this investigation.”

Page 26, line 1

Please explain the term “incremental AUC”

- Incremental AUC is described in the methods section under “Calculations” and there is a worked example in Appendix IV.

Page 26, Subheading **Insulin, Glucose, and Lipoproteins**

Change “Total cholesterol increased ($p<0.01$) between 0 h and 6 h in the control trial in both the NW and OW groups (Table 2).” to “Total cholesterol increased ($p<0.01$) between 0 h and 6 h in the control trial, but not the exercise trial, in both the NW and OW groups (Table 2).

- The text now reads: “Total cholesterol increased ($p<0.01$) between 0 h and 6 h in the control trial, but not the exercise trial, in both the NW and OW groups (Table 2).”

Line beginning “Postprandial HDL-C increased ($p<0.05$) . . . “ doesn’t seem to make sense.

- This ill-worded sentence has been rewritten to clarify our point. It now reads: “Postprandial HDL-C increased ($p<0.05$) in the NW group during both the control and exercise trials but did not change in the OW group (Table 3).”

“HDL-C levels were higher ($p < 0.01$) in the NW than the OW group at 6 h following the OFTT during the control and exercise trials.” I can’t help but wonder if this was a function of higher baseline values in the NW group. Was this addressed?

- This sentence does not accurately convey the HDL-C data. It now reads “HDL-C levels were higher ($p < 0.01$) in the NW than the OW group at all time points.” Certainly, the NW group had higher HDL-C at baseline than the OW group, but the statistical analysis showed a significant interaction whereby HDL-C increased in response to the OFTT only in the NW group. The study is not powered to address this point in further statistical depth by employing ANCOVA correcting for baseline values. Moreover, as HDL-C is a secondary endpoint and the magnitude of change is quite small (<5%) we feel further analysis would add little to the interpretation of the results.

Subheading **Inflammatory Markers and Adhesion Molecules**

In several places, change “were similar” to “were not significantly different ($P > 0.05$)”

- We have removed this statistically incorrect terminology and the text now reads: “Plasma levels of CRP, sICAM-1 and sVCAM-1 were not significantly different in the NW or OW group at baseline, and did not change in either group in response to the OFTT during the control or exercise condition. WBC’s increased ($p < 0.01$) in the NW and OW groups 6 h following the OFTT ; the magnitude of the increases were not significantly different between trials (Table 3). Circulating levels of WBC counts were lower in NW than OW adolescents prior to the OFTT in the control trial, and at 6 h following the OFTT in the exercise trial (Table 3). Plasma levels of IL-6 increased two-fold in the NW and OW groups in response to the OFTT at 6 h ($p < 0.01$) and this increase did not significantly differ between experimental conditions (Table 3). There was a trend for plasma IL-6 to be higher at baseline and 6 h in the exercise trial ($P = 0.06$). Plasma concentrations of TNF- α were higher ($p < 0.05$) in the OW than the NW group at rest and 6 h following the OFTT in both the control and exercise experimental condition. TNF- α levels increased ($P < 0.05$) in the OW group 6h after the OFTT in the exercise experimental condition. TNF- α did not change following the OFTT in the NW group.”

Table 3 The alignment of the rows is off and the last row appears to be cut off.

- This has been corrected.

Discussion

The between-group differences in the relative intensity and treadmill velocity of the exercise intervention needs to be addressed and the potential implications, if any, for the results of the study.

- We have added a discussion of this issue to the ‘Limitations’ section as outlined above.

Please provide references for each of the following statements: “Acute exercise has consistently been shown to reduce PPL in adults. Exercise-induced reductions in PPL likely contribute to the cardiovascular health benefits of regular exercise. There is now compelling evidence that atherosclerotic-CVD processes begin early in childhood and are influenced over the life course by genetic and potentially modifiable risk factors.”

- We apologize for this oversight – the appropriate references have been added.

Change “Taken together, these results indicate that selective inflammatory in the postprandial state may contribute to a pro-inflammatory environment.” to “Taken together, these results indicate that elevated levels selective inflammatory markers in the postprandial state may contribute to a pro-inflammatory environment.”

- We have changed the text to read: “Taken together, these results indicate that elevated levels selective inflammatory markers in the postprandial state may contribute to a pro-inflammatory environment.”

The use of subheadings might be helpful to the reader

- We agree with the reviewer and have added subheadings to the discussion.

Appendix V

Invited Slideshow of Results Presented at the American College of Sports Medicine Annual Meeting in Denver (June 2006)



Effect of Acute Exercise on Postprandial Lipemia and Biomarkers of Endothelial Function and Inflammation in Adolescents

Owen J. MacEneaney, Michael Harrison, Donal O'Gorman, Elena Pankratieva, Paul O'Connor, and Niall M. Moyna FACSM.

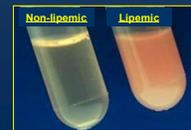
Science & Health
Dublin City University



BACKGROUND

Background

- Elevated postprandial triglycerides (TG) linked to atherosclerosis
 - Most of day spent in postprandial state
- Postprandial TG vs. fasting TG
 - 'Challenge' of a fat load more revealing



Background

- Endothelial function is impaired following a high-fat meal
- Pro-inflammatory changes are evident postprandially

Background

- Exercise acutely reduces PPL
- The effect is related to the energy expenditure during exercise
- Consistent finding with many adult healthy and at-risk groups

Specific Aims

- To compare the effects of a standardized high-fat meal on:
 - TG in normal-weight and overweight adolescent males
 - Adhesion molecules (sICAM-1, sVCAM-1) and inflammation (IL-6, TNF- α , WBC, CRP)
- To examine the efficacy of acute exercise in attenuating postprandial TG, adhesion molecules and inflammation

METHODS

Subjects

- Normotensive
- Normolipidemic
- Normoglycemic
- Non-smokers
- Not taking any medication or vitamins
- Free of overt cardiovascular disease

Experimental Protocol

- Oral fat tolerance test (OFTT)
 - Mixed meal: 61% Fat; 33% CHO; 6% Protein
 - Amount based on BSA
 - Water ad libitum
- Exercise Trial
 - 60-70% $\dot{V}O_{2max}$ until 600 kcal expended
 - 12 h prior to one OFTT (order randomized)
 - Control condition: no exercise prior to OFTT
- Control of Lifestyle

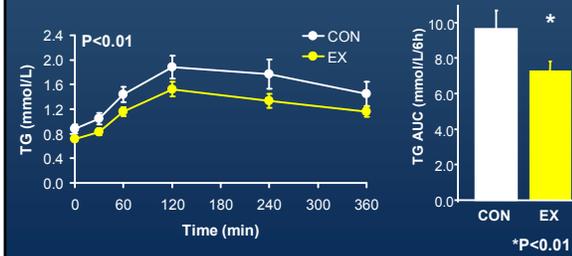
RESULTS

Selected Subject Characteristics

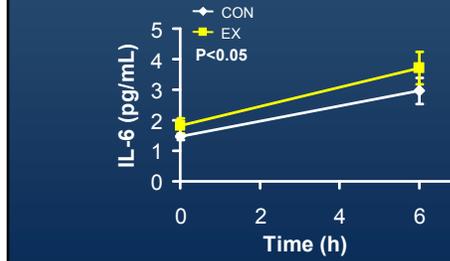
Variable	Normalweight (n=10)	Overweight (n=8)
Age (years)	16 \pm 0.2	16 \pm 0.1
BMI (kg.m ⁻²)	20.9 \pm 0.5	28.3 \pm 1.3*
BSA (m ²)	1.8 \pm 0.1	2.0 \pm 0.1
$\dot{V}O_{2max}$ (L.min ⁻¹)	3.4 \pm 0.5	3.5 \pm 0.8
Total-C (mmol.L ⁻¹)	3.8 \pm 0.3	3.7 \pm 0.2
HDL-C (mmol.L ⁻¹)	1.2 \pm 0.1	1.0 \pm 0.0*
LDL-C (mmol.L ⁻¹)	2.4 \pm 0.3	2.5 \pm 0.3
Glucose (mmol.L ⁻¹)	4.5 \pm 0.1	4.6 \pm 0.1
Insulin (pmol.L ⁻¹)	43.5 \pm 5.2	52.5 \pm 9.5
HOMA-IR	1.5 \pm 0.2	1.8 \pm 0.3

Values are mean \pm SEM.
*p<0.01 vs. NW.

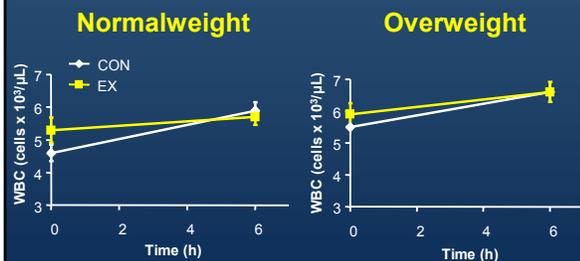
Postprandial Lipemia and Exercise



Inflammation: IL-6

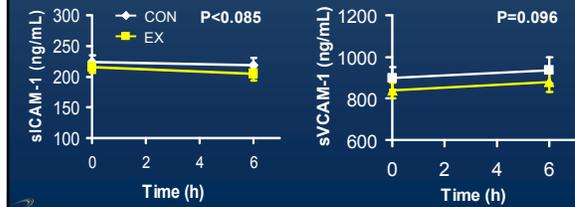


Inflammation: WBC



Adhesion Molecules

- No postprandial response
- No apparent effect of exercise condition



CONCLUSIONS

Summary

- High-fat meal causes PPL in adolescents
- Acute exercise attenuates PPL response
- No concomitant decrease in inflammation
- No effect on adhesion molecules

Conclusions

- Collectively, these results indicate:
 - prior exercise reduces PPL but does not eliminate all of the cardiovascular abnormalities associated with the PPL state in adolescents

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- Elena Pankratieva
- Louise Reilly
- Kevin O'Brien
- Dr. Donal O'Gorman
- Prof. Niall M. Moyna
- Research Participants



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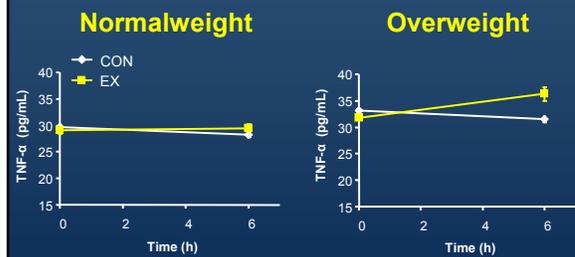


Selected Exercise Characteristics

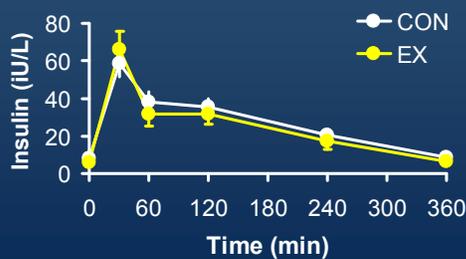
Variable	Normalweight (n=10)	Overweight (n=8)
$\dot{V}O_2$ (L.min ⁻¹)	2.1 ± 0.1	2.3 ± 0.2
% $\dot{V}O_2$ max	62 ± 2	68 ± 2*
Exercise time (min)	59 ± 5	52 ± 5
Average speed (km.h ⁻¹)	8.2 ± 0.2	7.3 ± 0.3*
Energy Expenditure (kcal)	596 ± 6	588 ± 13
Total CHO (g)	119 ± 7	130 ± 12
Total Fat (g)	16 ± 2	12 ± 3

Values are mean ± SEM.
*p<0.05 vs. NW.

Inflammation: TNF- α



Postprandial Insulinemia & Exercise



Postprandial Glycemia & Exercise

