DISSERTATION

THE EFFECTS OF MODERATE EXERCISE ON MEASURES OF POSTPRANDIAL LIPEMIA

Submitted by
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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY KIMBERLY COX-YORK ENTITLED "THE EFFECTS OF MODERATE EXERCISE ON MEASURES OF POSTPRANDIAL LIPEMIA" BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

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ABSTRACT OF DISSERTATION

THE EFFECTS OF MODERATE EXERCISE ON MEASURES OF POSTPRANDIAL LIPEMIA

Background: Cardiovascular disease (CVD) is the leading cause of death worldwide. Impaired lipid metabolism or dyslipidemia, including elevated triglycerides (TG) and small TG particle size, is a major treatable risk factor for CVD. Lipid profiles are typically assessed after an overnight fast, however, most individuals spend the majority of the day in a fed or postprandial (PP) state, and hence this might be more reflective of CVD risk. A single bout of moderate exercise 12-16hr before a high fat meal has been shown to decrease post-prandial TGs, indicating a possible therapeutic effect. The purpose of this study was to assess the effect of a single bout of morning exercise on post-prandial lipids over an entire day with 3 mixed meals of a typical macronutrient composition (34% fat, 15% protein, 51% CHO).

Methods: Data is presented on 2 groups of men and women [26 lean subjects and 18 subjects with metabolic syndrome (MetS) as per NCEP- ATP III criteria]. Each subject completed both a rest day and an exercise day (treadmill walking, 60min at 60%VO_{2peak}), each preceded by 5 days of diet control, and 60hr abstinence from planned, vigorous exercise. Study days were randomized and 1 month apart. Study days began at 7:30 a.m. following an overnight fast.

Subjects either exercised or rested for 60 min then consumed breakfast at 8:30 a.m., lunch at 12:30 p.m. and dinner at 5:30 p.m. Meals contributed 25%, 35% and 40% of the daily energy intake respectively, calculated based on estimated energy to meet requirements of either the rest or exercise day. Blood samples were collected before exercise or rest (fasting) and continued throughout the day (26 total time points) and analyzed for TG, glycerol, FFA, total cholesterol, total apolipoprotein B and high-density lipoprotein-cholesterol (HDL-C), as well as glucose, insulin and adiponectin. A subset (10) of the plasma samples were separated into triglyceride-rich lipoprotein (TRL) subfractions (Sf>400, 20-400, <20) in which TG and cholesterol were measured. As each TRL particle from the liver contains one copy of apoB100 and each from the intestine has one copy of apoB48, these proteins were measured via SDS-PAGE in the Sf 20-400 fraction (TRL2) to assess particle origin, size and composition. Postprandial responses were expressed as area under the curve and incremental area under the curve (AUC/IAUC) and analyzed via ANOVA and t-test using SPSS 16.0.

Results: Overall, exercise had no effect on the PPTG response. However, total apoB IAUC decreased 20% with exercise ($p = 0.049$) in the group as a whole, and total cholesterol IAUC decreased 30% in the lean group with exercise ($p = 0.004$). Collectively, the men exhibited a greater PPTG response than women over the day expressed as AUC ($p = 0.046$) but not when corrected for baseline TG (IAUC $p = 0.32$), and MetS subjects started with and maintained significantly higher TG than lean subjects.

MetS women showed a decline in PPTG mid-day which remained low for the rest of the study day, with the largest decrease occurring in the large (Sf>400, TRL1) subfraction. In MetS men, however, PPTG rose consistently from breakfast to post-lunch when it leveled out and remained high. Given a decrease in PPTG without a concurrent decrease in total apoB, the average total plasma TG:apoB ratio over rest and exercise days was 30% lower in MetS women than MetS men ($p = 0.01$). TRL2 apoB-100 and B-48 were twice as high in MetS as in lean subjects, without a concomitant doubling of TG concentration, resulting in significantly smaller particle size in the MetS group ($p=0.01$).

Conclusions: These results indicate that although exercise did not have a significant effect on PPTG, there were other potentially cardio-protective effects on apoB and cholesterol levels. The lack of PPTG effect is likely due to the moderate conditions of the experiment (exercise intensity/duration; mixed, moderate fat meal), but these are more typical conditions of free-living individuals than those applied in many prior studies. This study also revealed interesting differences in PP metabolism between lean subjects and those with the clustering of symptoms characterized as MetS, as well as in men and women within this population of subjects. These are important distinctions to consider regarding the heterogeneous population at risk for CVD.

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INTRODUCTION

The expanding epidemics of obesity, metabolic syndrome (MetS) and type II diabetes mellitus (T2DM) have contributed to making cardiovascular disease CVD the leading cause of death in the Western world. CVD is characterized by atherosclerosis, the narrowing of arteries due to build-up of cholesterol-containing plaques. Fasting plasma low-density lipoprotein cholesterol (LDL-C) has been the gold standard for assessing CVD risk, but over the past decade plasma triglycerides (TG) have also been validated as an independent risk factor for CVD and fasting TG has become part of the lipid panel used in assessing CVD risk.

Fasting measures are commonly used in assessing CVD risk as the fasting state is relatively steady and measures are reproducible. The postprandial (PP) state, which is characterized by an increase in plasma TG, has since been identified as potentially atherogenic irrespective of fasting TG, as many patients with premature coronary heart disease (CHD) have normal fasting TG but most have reduced ability to clear (PP) lipids.

Plasma TG and cholesterol are transported in the circulation in triglyceride-rich lipoproteins (TRL), multi-component particles with associated proteins (apolipoproteins). Endogenous TG are transported from the liver in very-low density lipoproteins (VLDL) and dietary (exogenous) lipids are transported from the intestine in chylomicrons (CM).

Each TRL or remnant particle is associated with an apolipoprotein B moiety, apoB-100 from the liver and apoB-48 from the intestine, so named because they are from the same gene, but apoB-48 is the N-terminal 48% of the apoB-100 protein. The apoB is necessary for assembly of the particle, as well as for uptake into liver and peripheral tissues by the LDL-receptor. As the TG are hydrolyzed by lipoprotein lipase (LpL) associated with the capillary endothielium in tissues, the particles become smaller and are termed remnant particles. The smaller particles are able to penetrate and reside within the endothelial space of blood vessels, a process believed to be the initiating factor in atherosclerosis. It is also believed that apoB plays a role in the uptake of particles by the endothelium.

Indeed, apoB-containing particles have been found associated with atherosclerotic plaques and prospective studies have reported apoB to be superior to either TG or LDL-C in predicting CVD morbidity and mortality. As each TRL particle contains only one apoB, and the apoB in CM and VLDL are unique to each particle, their measurement is useful in determining the number and origin of each particle. Such analysis has yielded beneficial data regarding PP lipid metabolism and kinetics, including differences between men and women and healthy controls versus CVD patients or those at risk.

The combination of energy-dense diets and sedentary lifestyles is a key mediator in the etiology of CVD and has become the central focus of prevention and treatment strategies. Dietary advice has been mixed with regard to amounts and types of fats and carbohydrates to consume and has been the source of much consumer confusion. While recommendations for optimal amounts of physical activity have also varied, the message that increased physical activity improves health outcomes has remained for the most part, consistent.

Chronic exercise has been shown to result in lower fasting and PPTG response as demonstrated by the comparison between endurance-trained athletes and sedentary individuals. Acute exercise bouts have also been shown to reduce the PPTG response to a high fat meal. Considerable variation exists, however, based on the duration, intensity and timing of exercise as well as the macronutrient composition of the test meal. Studies using high levels of exercise and high fat meals have reported the greatest PPTG reduction while the results of studies with more moderate exercise and mixed meals are less consistent. Similarly, the majority of studies analyzed PPTG after a single meal, rather than several as would be typical in a free-living human.

The current study attempted to address these gaps and further elucidate the effect of a single bout of moderate exercise on PPTG over an entire day of mixed meal feeding. Subjects were sedentary, representing the majority of the population and men and women were compared as separate groups, controlling for menstrual cycle in women.

The exercise bout was of a duration and intensity that could realistically be incorporated in to the daily routine in an untrained individual and was administered immediately before the first test meal. The primary outcome was PPTG response with secondary outcomes of TRL TG and cholesterol, and apoB concentrations in TRL subfractions.

CHAPTER 1

Exercise and Postprandial Lipemia

Review of Literature

INTRODUCTION

Cardiovascular disease (CVD) including coronary heart disease (CHD), stroke and high blood pressure, is the leading cause of death world-wide [1, 2]. It is a multi-factorial disease and thus there are several risk factors in its development and progression, including clinical (high blood pressure, cholesterol, and diabetes), and lifestyle factors (smoking, diet, alcohol, physical inactivity), genetic susceptibility, gender, and race or ethnicity. One of the primary causes of CVD is atherosclerosis, a build-up of fatty substances, cholesterol, calcium and fibrous material collectively termed 'plaque' which occludes arteries, and disrupts blood flow, in some cases entirely. Most commonly, however, the arterial plaque becomes problematic when it attains instability and a portion breaks away from the blood vessel wall into the circulation, triggering a clotting response in another location, resulting in thrombosis and arterial blockage if severe enough. If this blockage is in a major artery supplying the brain or heart, a fatal or non-fatal stroke or heart attack can occur.

Cholesterol, lipids and CHD

Historically, cholesterol was believed to be the major contributing factor to the development of CVD. As early as 1910 [3] cholesterol was described in the lesions of diseased arteries, and has since been confirmed in both hypercholesterolemic humans and animal models. Epidemiological evidence has also revealed a strong independent relationship between serum cholesterol levels and risk for CHD [4]. As the majority of cholesterol is carried in low-density lipoproteins (LDL), LDL-cholesterol was considered the gold standard for clinical evaluation of CHD risk.

In an effort to reduce the incidence of CHD, in 1985, the National Heart, Lung, and Blood Institute (NHLBI) of the National Institutes of Health (NIH) launched the National Cholesterol Education Program (NCEP). As outlined it in its most recent set of guidelines, the Third Report of the Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults, Adult Treatment Panel III (ATPIII) [4], NCEP continues to support LDL as the primary cause for CHD and lowering plasma LDL the primary goal. Clinical trials of LDL-lowering drugs support this idea due to their effectiveness in the secondary prevention of CVD events and mortality (reviewed in [5]). Moreover, a recent meta-analysis also supports statin-mediated lowering of plasma LDL levels in primary prevention of CVD events and mortality [6].

Other lipid related blood borne factors, however, have emerged as playing a role in CHD development and progression. Indeed, the majority of patients admitted with premature vascular disease are characterized as having low concentrations of high-density lipoprotein cholesterol (HDL-C), increased small, dense, LDL, and elevated plasma concentrations of triglyceride (TG) referred to as the atherogenic lipid triad [7], indicating that a more complex risk analysis may be called for. More recently, fasting plasma TG concentration has been identified as an independent risk factor for CVD, and is now commonly used in assessing CVD risk as well. The NCEP has included fasting TG as one component of the initial screening for CHD and has defined the clinical ranges for fasting TG as:

- ✓ Normal Less than 150 mg/dL
- ✓ Borderline-high 150 to 199 mg/dL
- ✓ High 200 to 499 mg/dL
- ✓ Very high 500 mg/dL or higher

An early meta-analysis of Western, population-based, prospective studies showed a significant, independent effect of fasting total plasma TG on CVD risk [8]. In the 46,413 men and 10,864 women included in the analysis, for each 1mmol/l increase in TG the response rate (RR) was 1.37 in women versus 1.14 in men. More recently, the MELANY study, a prospective cohort study of almost 14,000 male soldiers confirmed that not only did fasting TG predict CHD incidence, but so did the change in fasting TG over time [9].

With a mean follow-up of 5 years, those men who had fasting TG in the highest quintile had a hazard ratio for CHD of 4.05 compared with those in the lowest ($p < 0.001$ for trend), after adjusting for age, BMI, HDL-C, total cholesterol, physical activity, fasting glucose, mean arterial blood pressure and smoking. Moreover, those men whose TG increased over the follow-up period had a higher risk ratio for CHD ranging from 4.9-8.2 depending on the increase, and those whose TG decreased over time had lower relative CHD risk. Even with a drop in TG over the 5 year period, those men who started the study with elevated TG still had a higher risk for CHD than those who were low at baseline and remained low at the 5-year follow up.

In other studies, the association of fasting TG with CVD risk is variable, particularly when adjusting for fasting HDL-C[10]. According to data from the Framingham study, the combination of high TG and low HDL-C is quite common and increased CHD risk by twice that of the next highest risk factor[11]. These data were supported by both the Helsinki heart study, a prospective study of 4,081 men [12] and the PROCAM, 6-year prospective study in middle-aged men [13], whereas Menotti *et. al.* found no evidence for TG as an independent risk factor when adjusting for total cholesterol in over 3,000 Roman men [14]. A meta-analysis of prospective studies of TG and CVD in Italian men and women revealed a 32% increase in disease risk with increased TG in men and a 76% increase in women[15]. Upon adjusting for HDL-C and other risk factors, these dropped to 14% and 37% respectively, but remained significant.

More recently, the results of the Women's Health Study were published. This was a prospective study of 26,509 initially healthy women enrolled between November 1992 and July 1995 and followed-up for a median of 11.4 years. At baseline, fasting TG correlated with traditional cardiac risk factors such as hypertension, smoking, BMI and physical inactivity [16]. When adjusted for these same factors at follow-up, fasting TG continued to predict CV events, however, when adjusting for total and HDL-C, and measures of insulin resistance, this relationship was significantly weakened (hazard ratio of 1.21). Moreover, Sarwar *et. al.* [17] conducted a meta-analysis of 29 prospective Western studies including more than 260,000 participants. This group found that while those in the top 1/3 for usual TG concentration had an odds ratio for CHD of approximately 2 over TG concentrations in the bottom 1/3, the odds ratio dropped by 30% when adjusted for other risk factors, especially HDL-C, which decreases with increasing concentrations of TG [18].

Postprandial lipemia and CHD

One major weakness of using fasting TG to asses CVD risk is that most individuals spend the majority of each day in a fed or postprandial (PP) state--at least 17h of a 24h period [19]. It has therefore been suggested that the PP state be recognized as the usual metabolic state for free-living humans [20]. Indeed, it has been estimated that as many as 40% of patients with premature coronary heart disease (CHD) have normal fasting TG [21], but most have decreased clearance of PP lipoproteins [22, 23].

Moreton first noted in 1947 that PP plasma lipid particles resembled those seen in hyperlipemia, leading him to posit that intimal lipid deposits in atherosclerosis are the result of the cumulative effect of many fatty meals over a lifetime [24]. This theory has since been expanded upon, most notably by Zilversmit [25-27], who's 1979 paper has been widely quoted [26].

Fasting TG can predict PPTG response, as demonstrated by Dekker *et. al.* in a recent study in middle-aged men (45-61 years). The men with the highest fasting TG had a significantly higher PPTG response to a high fat challenge than those with lower levels of fasting TG [16]. However, even manifest atherosclerosis is not necessarily characterized by elevated fasting TG as illustrated by Lupattelli *et. al.* who studied PP lipemia in subjects with peripheral artery disease (PAD). This disease is characterized by atherosclerosis of arteries in the pelvis and legs and is often associated with several lipid abnormalities. In this study, a group of men with PAD, but considered normolipemic in terms of their fasting lipid levels, were compared to a control group of age and BMI matched men for response to a high fat (65g) meal. The PAD subjects exhibited a PP increase in PPTG response, decreased HDL-C and reduced LDL particle size. They also displayed delayed PPTG clearance, with TG levels still significantly higher than controls at 8 hours post-meal.

Evidence is accumulating identifying PPTG as an independent risk factor for CVD [16, 27, 28]. The Copenhagen City Heart Study, for example, demonstrates a strong correlation between non-fasting TG levels with increased risk of myocardial infarction, ischemic heart disease and death [29]. In 7587 women and 6394 men aged 20-93, the hazard ratios (adjusted for age, total cholesterol, BMI, hypertension, diabetes, smoking, alcohol, physical inactivity, lipid lowering therapy, and, for women, postmenopausal status and hormone therapy) for myocardial infarction (MI), ischemic heart disease (IHD) and total death, increased from 1.4 to 3.3 fold per every 1mmol/L increase in non-fasting TG. Patsch *et. al.* also demonstrated a 68% prediction rate for CHD based on PPTG response in CHD patients relative to control subjects [30]. Both the maximal increase and the cumulative TG response (area under the PPTG curve) were significantly higher in patients as compared to controls.

Postprandial nutrient and lipoprotein metabolism

The PP state is characterized by a rise in glucose and TG, and while the rise in glucose is transient and usually returns to pre-meal levels within 2-3 hrs , TG levels show marked elevation within an hour and can remain elevated for 5-8 hours following ingestion of a fat-containing meal. During this time, TG-containing particles undergo extensive remodeling in the way of lipid and protein exchange, complicating evaluation of the contribution of PPTG to atherosclerosis development and progression.

As depicted in figure 1 [31], following a fat-containing meal, free fatty acids (FFA) are absorbed from the gut and converted to TG. The intestinal epithelial cells package the TG into chylomicrons (CM) which enter the intestinal lymph and then directly enter the systemic circulation, unlike dietary glucose and amino acids which enter the portal circulation and undergo first pass extraction by the liver before entering the systemic circulation. Lipoprotein lipase (LpL) is present in the capillary beds of adipose cells and muscle tissue where it hydrolyses the TG in CM to FFA. The resultant FFA are either taken up by the tissue bed and metabolized further, or can also escape into the general circulation (intravascular hydrolysis) [32, 33]. Free-fatty acids taken up by adipose tissue are predominantly re-esterified to form TG and stored within adipocytes whereas in muscle tissue FFA can either be stored or oxidized. Small quantities of FFA (depending on the type of FFA) can be used to form structural components of the cell membrane and also may be used to produce functional (signaling) moieties.

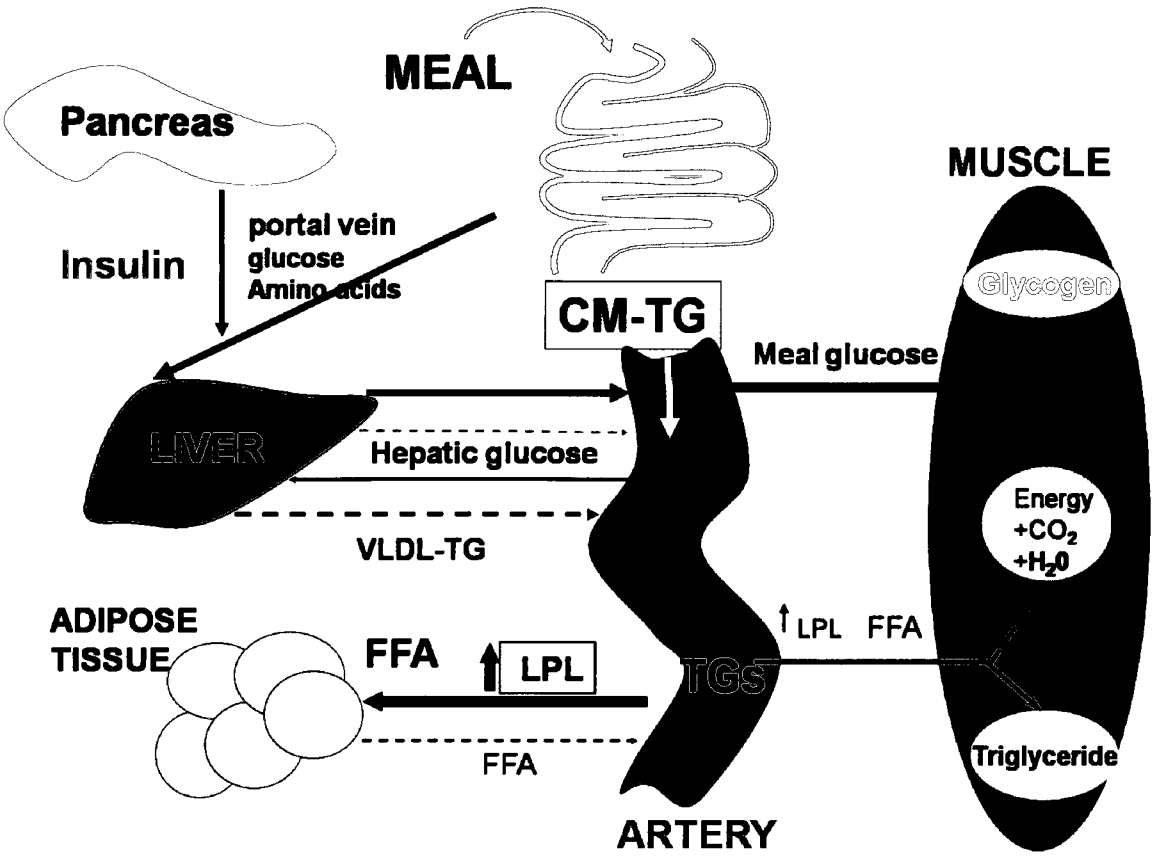

Figure 1. Postprandial lipid metabolism [31]

Abbreviations: chylomicron triglycerides (CM-TG); free fatty acids (FFA); very low-density lipoprotein triglycerides (VLDL-TG); lipoprotein lipase (LPL).

LpL, post-prandial lipemia and CHD

Dysfunction of LpL leads to hypertriglyceridemia and decreased levels of HDL-C [34, 35]. Animal studies have provided evidence that LpL activity can directly affect atherosclerosis initiation and progression. Deletion of LpL in aortic vascular walls of mice, for example, reduces or eliminates plaque formation, even under hyperlipidemic conditions [36, 37].

Conversely, decreased plasma LpL activity due to a specific gene mutation increases the incidence of atherosclerosis in humans, occurring in 1 in 20 males with proven atherosclerosis [38], and people who are heterozygous for LpL deficiency have higher TG and lower HDL-C than controls [39]. On the other hand, increased plasma LpL activity is related to reduced TG concentration and increased HDL-C concentration [40]. It is clear that LpL plays a key role in lipoprotein metabolism, and augmentation of its activity could result in lowering the risk of atherosclerosis and CVD development.

Triglyceride rich lipoproteins and the production of remnant particles, LDL and HDL.

Following the hydrolysis of circulating TGs, the remaining, TG-poor particle is taken up by the liver through binding of surface proteins to receptors. The liver synthesizes TG-rich VLDL for release into the plasma. VLDL are also hydrolyzed by LpL, converted to LDL and ultimately taken back up by the liver as well.

Collectively, CM and VLDL are termed 'triglyceride-rich lipoproteins' (TRLs), and their respective TG-poor particles are referred to as remnant-like particles (RLP). The metabolism of TRLs also involves exchange of TG and cholesterol with HDL, with the resultant HDL returning to the liver (considered to be atheroprotective). There is also inter-particle exchange of apolipoproteins which are responsible for particle formation/structure, receptor binding and enzyme activity. Under normal (healthy) conditions, these particles are rapidly taken up by the liver, relatively speaking [41], but in dyslipidemic and PP states, the number of these particles is appreciably increased and/or their hepatic clearance is delayed [42-44].

Remnant particles from TRLs have been observed in human atherosclerotic plaques [45, 46]. Importantly, retention of apolipoprotein B (apoB)-containing lipoproteins has been indicated as the key initiating process in atherosclerosis [47]. This 'Response to Retention model' of atherogenesis posits that lipoprotein retention is an absolute requirement for lesion development and is sufficient in most cases to initiate the cascade of events leading to atherosclerosis. It has thus been proposed that plasma cholesterol concentrations *per se* are not the issue, as a normal LDL cholesterol level, for instance does not mean a normal LDL *particle size and number*. Cholesterol in the arterial lumen is not of concern, but cholesterol that is contained within an atherogenic lipoprotein particle with the ability to gain access to and become trapped within the arterial wall is. It has been demonstrated that CM-RLP can also penetrate the endothelium and be retained in the intima [48-50].

The process of atherosclerosis includes infection of or damage to the endothelium followed by an inflammatory response. In this model (fig. 2), activated monocytes enter the endothelium where they differentiate into macrophages. LDL enter the vessel wall as well and are oxidized. These oxidized particles are taken up by the macrophages via scavenger receptor (CD36). Chylomicron RLP also can enter the vessel wall and be taken up by macrophages [51]. Whether oxidation of the CM-RLP makes them more, or less, proatherogenic is currently under debate [52].

As the macrophage becomes saturated with cholesterol it is transformed into a foam cell which is the major component of the plaque. This becomes a cyclic process as the activation of monocytes and macrophages results in the production of chemoattractant molecules (CAM) and cytokines that perpetuate inflammation and foam cell formation (reviewed in [53]).

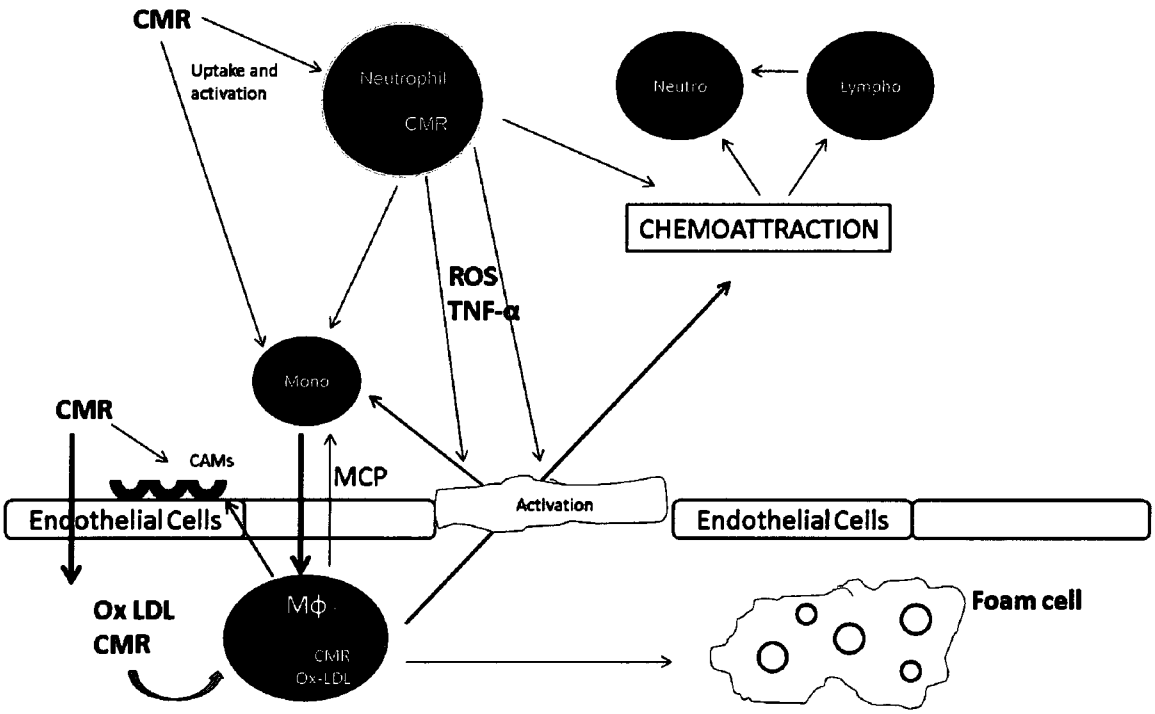

Figure 2. Inflammatory response in the process of atherosclerosis (modified from [53]).

Abbreviations: chemoattractant molecule (CAM); interleukin (IL); reactive oxygen species (ROS); monocyte (mono); neutrophil (neutro); low-density lipoprotein (LDL); oxidized LDL (Ox-LDL); macrophage (MΦ); transcription factor alpha (TNF-α).

Interestingly, Van Oostrom *et. al.* have shown that the PP rise of TG and glucose induces an acute inflammatory response characterized by an increase in neutrophil counts and concurrent production of pro-inflammatory cytokines along with oxidative stress [54, 55]. They have also shown that leukocyte activation was up-regulated PP in both healthy subjects and patients with premature atherosclerosis [56, 57].

Apolipoprotein B

ApoB is present on the surface of CM, VLDL, IDL and LDL and there is one apoB molecule per particle. Plasma apoB is therefore a direct measure of particle number, and along with plasma TG, an estimate of particle size. A direct relationship has been established between plasma apoB concentrations and the number of particles that invade and are retained in a plaque [58]. It has therefore been suggested that apoB is a better predictor of CVD risk than either TG or cholesterol (non-HDL/LDL), based on the belief that an increased number of particles within any lipoprotein fraction increases the likelihood of any member of that class invading and being retained within an arterial wall [59]. Moreover, in a post-mortem study of coronary and carotid arteries of 49 patients with early atherosclerosis, symptomatic patients had more apoB deposits in arterial plaques than patients without CV events [60].

Indeed, several prospective studies, ranging from 5-13 years have identified apoB to be superior to either TG or LDL-C in predicting CVD risk and progression. The AMORIS study followed more than 175,000 men and women for an average of 65 months and found that apoB levels were strongly and positively related to an increased risk of fatal myocardial infarction in both sexes [61]. Similarly, Pischon *et. al.* followed a portion of participants from the Health Professionals follow-up Study for 6 years [62]. They compared the value of apoB, HDL-C, and LDL cholesterol (LDL-C) as predictors for CHD and found that although both HDL-C and apoB were superior predictors than LDL-C, mutual adjustment for HDL-C and apoB left only apoB as a predictor with a relative risk of 4.18.

Methodologically, it is beneficial to use total plasma apoB as an indicator of CVD risk as total apoB is unaffected in a non-fasting or hypertriglyceridemic state [63], and can therefore be used consistently in the PP state. The measurement of apoB has also been standardized by the World Health Organization (WHO) and International Federation of Clinical Chemistry (IFCC) [64, 65] and is fully automated. Additionally, apoB is not derived from other measures, as for LDL-C which is most often calculated using the Friedwald equation. This method subtracts HDL-C and VLDL-C (estimated from TG/5) from total cholesterol to estimate LDL-C. While reasonably accurate when used with fasting samples, error increases in the Friedwald equation in the PP state and when TG are above 200 mg/dl [66]. The fact that the formula is derived from 3 different measures also imparts error intrinsic to each one. The direct measure of apoB with specific antibodies reduces this error.

Humans produce two distinct apoB isoforms, B100 and B48, so named based on their amino acid (a.a.) composition. Both are synthesized from the same gene, but apoB100 is the full-length, 4536-residue polypeptide and apoB48 is composed of 48% of the N-terminal a.a.s of apoB100. The liver incorporates one apoB100 per VLDL particle and the enterocyte incorporates one apoB48 per CM. Analytically, it is therefore possible to separate lipoprotein particles via their respective apoB particle (by size or specific antibody) and determine the origin of the particle. As the accumulation of potentially atherogenic particles is a function of their synthesis and clearance, this is potentially useful in determining the relative PP contribution of each particle type.

It is notable, however, that the laboratory methods for measuring these particles are not technically straight forward, can be time consuming and are limited by the lack of commercial availability of apoB48 antibodies and apoB48 and 100 standards. This limits the use of apoB48 and apoB100 measures in studies, not to mention clinically.

The delayed clearance of CM remnants has been found in subjects with coronary artery disease (CAD) [67, 68], and while CM particle number has been shown to rise in response to a high fat meal, Karpe *et. al.* demonstrated that the major change in lipoprotein particle number was an increase in large VLDL particles (Svedberg flotation rate (Sf) 60-400) [69]. A later study by the same group followed incorporation of meal fat into TRL and showed that dietary fatty acids are rapidly (within minutes) incorporated into the VLDL-TG pool [70].

Additionally, a recent study using incorporation of a stable isotope in a constantly fed state showed that in response to dietary fat, hepatic lipoprotein production was enhanced due to increased delivery from CM remnants (CMR). The clearance of hepatic TRLs was also delayed as a result of competition for clearance [71] with the larger, as yet, unmetabolized CM-TG particles.

The inference from this model then is that the main contribution of intestinally-derived TRLs to the atherogenic profile is to affect production and clearance of hepatic lipoproteins, rather than a direct effect on the arterial wall. In fact, although roughly 80% of the increase in TG after a fat load comes from apoB48-containing particles [72], about 80% of the increase in particle number is from apoB100 [73, 74].

Thus, while the mechanisms are debated, the outcome is becoming clear—the lipid milieu associated with the PP state is potentially atherogenic and, given the current lifestyle patterns of the Western world, worth pursuing in terms of its impact on CV health.

Variability in the postprandial evaluation of lipemia

Given that there have been significant correlations made between many lipid measures and CVD, there is now much debate about which one(s), or which combination is best used for clinical assessment. Similarly, since the PP state has been recognized as the usual state, and likely is a substantial contributor to CVD risk, the development of a fat tolerance test, similar to the standard glucose tolerance test, has been proposed.

Such a test is complicated by the fact that many external variables (eg. diet, alcohol, exercise) can greatly impact the TG response making test standardization a problem. Furthermore, it requires the generation of data establishing the relationship between the TG response to a standard fat load and CHD risk, and thus the establishment of risk cut offs. Hence, there is no established fat-tolerance test to date *per se*.

With respect to the fat content of a meal, early postprandial studies of lipid metabolism, while generating much valuable knowledge, are by no means consistent in their methods or interpretation. The variation stems from many sources including test meal composition, number of test meals administered, and subject population, as well as the method(s) used to isolate TRL fractions and analyze and interpret the data. These factors are reviewed nicely by Lairon *et. al.* [20] as factors to consider when designing a postprandial study.

Early studies used very large amounts of fat (70-100g) and those containing upwards of 50g of fat [75-77] elicit very large PPTG responses These have more recently been questioned as to their relevancy to free-living people, and more moderate doses have been used [78, 79]. Dose-response studies have revealed that fat doses from 5-15g elicit very little PPTG response, 30-50g increase PPTG in a dose-dependent manner, and doses of 80g and above elicit exaggerated responses that tend to plateau without dose effect [80-82]. Similarly, the addition of carbohydrate to a meal introduces the effect of insulin on lipid metabolism which is absent or minimal with strictly fat-containing meals [83, 84].

The number of meals is also relevant to the interpretation of PP measures. Most studies have administered a single meal in the morning after an overnight fast. While this method does give information about lipid kinetics, early single-meal studies identified subjects with 2 or 3 TG peaks over a 12-h period following a fat meal, not just one ([85, 86]. Administering the fat load 4-5 hours following a previous meal revealed an early peak about 1h post-meal, corresponding to CM and a later peak at 5-6h representing VLDL [87] suggesting that CM were being differentially released over time. More detailed analysis of the same design determined that the early, CM peak contained CM from not only the test meal, but also from the previous meal [88].

Fielding *et. al.* and Heath *et. al.* used different fats in sequential breakfast and lunch meals to further elucidate the source of TG in the postprandial peaks [70, 89]. This study confirmed that at lunch, fats from the breakfast meal were the major contributor to the "early" TG peak and the later peak was mainly derived from lunch fats. It is hypothesized that dietary fat is being stored in enterocytes and perhaps intestinal lymphatics and released following a second meal [90]. As humans typically consume several meals over the course of a day, this "second-meal" effect is a relevant factor in studying PP lipemia.

Consideration of the subject population is important when comparing PP studies, as not all populations respond equivalently. Healthy subjects tend to have lower PP responses than subjects at risk for disease or other patient populations, for instance and middle aged subjects display a significantly greater PP rise in TG relative to their younger counterparts [91].

Subjects of differing racial backgrounds also display differences in lipid profiles and response to high fat meals [92-95]. Subjects with elevated waist circumference or waist-to-hip ratio will typically exhibit elevated PP lipids, as will subjects characterized as having the metabolic syndrome (MetS)—reviewed below. Additionally, there are significant differences in the PP response to a meal in men and women as will be discussed below.

Cardiovascular Disease and Postprandial lipemia in Women

There is much research addressing CVD risk analysis and, thus far, the majority of the research has been focused on men. Historically, this was due to what was perceived by investigators to be "difficulties" in recruiting women and because of complexities in controlling for hormonal fluctuations associated with the menstrual cycle or menopause in women. Nevertheless, although CVD is typically delayed by about 10 years in women relative to men [96], after age 65, the death rate due to CVD is greater in women than in men[97], and CVD is the leading cause of death among women [98]. Moreover, CVD death rates are 2 to 3 times higher in women post-menopause than in comparably-aged women before menopause [96, 99], suggesting that female hormones not only complicate research design, they also likely play a large role in the development, or delay of CVD in women.

Sex differences in CHD risk factors

The characteristic lipid profiles of men and women are notable. Men tend to have higher plasma LDL-C and lower HDL-C concentrations when compared to age-matched women and it has also been reported that men have higher VLDL-C, TG, and apoB concentrations [92, 100-106]. It has also been widely reported that women exhibit a markedly reduced PPTG relative to age-matched men [85, 107-109]. In one study, this was demonstrated by self-collection of capillary TG, at six time points, in free living subjects over three typical days [107]. While fasting TG were not different between men and women, the women exhibited consistently lower TG over the compiled 3 days. Interestingly, a parallel study between pre- and post-menopausal women revealed that, compared to age-matched premenopausal women, the PPTG response in post-menopausal women was similar to that of the men [107].

Koutsare *et. al.* demonstrated the effect acutely with administration of high fat meals (60g) in age-matched men and women [41]. The men had a significantly greater PPTG response than the women, with regard to AUC and IAUC. Horton, *et. al.* also observed a lower PPTG response in women relative to men and further elucidated that the decrease was partly due to greater uptake of the meal-derived lipid across the leg for up to 6 hours after the meal [110], and that the women retained more lipid in the leg muscle. The results held when the test meal was administered in both the fasted and fed (4 hours post-breakfast) state, bringing relevancy to the typical PP state.

These results were confirmed by Votruba and Jensen, who also demonstrated that, while men and women take up equal amounts of fat in response to a high fat meal, women take up more fat at the leg [111]. They further found that femoral adipose LpL activity was significantly greater in women than in men in both the fed and fasted state. Moreover, these authors have also reported that fat cell size is positivity related to LpL activity in fed and fasted men and women, but that LpL activity is greater in women in all depots when expressed as μg FFA released $\cdot hr^{-1} \cdot g^{-1}$ [112].

Furthermore, Desmeules *et. al.* demonstrated elevated post-heparin LpL activity in women compared to men in the HERITAGE Family Study [113], suggesting that the lower PPTG in women compared to men could be due to increased TG clearance. In addition to increased LpL activity, women have been shown to have lower hepatic lipase (HL) activity than men [114] which would confer decreased lipolysis of TG-enriched LDL and increased TRL clearance respectively, in women (see figure 1).

In terms of sex-differences in PPTG metabolism, it is likely that estrogen plays a role. Short-term estrogen (E2) treatment has been shown to cause a decrease in HL [115] and increase adipose tissue LpL in premenopausal women [116]. A decrease in E2 with menopause could therefore affect lipoprotein metabolism in postmenopausal women. Indeed, postmenopausal women have an elevated PPTG response to a moderate fat meal (40% kcal from fat) relative to age-matched premenopausal women [117] whether this is directly related to LpL activity is unknown, but the role of LpL in lipid metabolism and CVD is an active topic of research [118-120].

Lipid metabolism in subjects with the metabolic syndrome

Along with obesity and type II diabetes mellitus (T2DM), the Metabolic Syndrome (MetS) is on the rise with 47 million people in the US having been diagnosed as of 2002. The prevalence of MetS increases with age, ranging from 6.7% of 20-29 year-olds to greater than 42% in adults 60 and older[121].

According to the NCEP ATP III guidelines, MetS is diagnosed with the coexistence of 3 of 5 of the following criteria:

- ✓ Triglyceride level of 150 mg/dL or higher.
- ✓ High-density lipoprotein (HDL) cholesterol level less than 40 mg/dL in men and 50 mg/dL in women.
- ✓ Blood pressure of 130/85 mm Hg or higher or drug treatment for hypertension.
- ✓ Fasting plasma glucose level of 100 mg/dL or higher
- ✓ Waist circumference >102cm in men and >88cm in women

As might be expected from the list of symptoms above, MetS is associated with increased risk for CVD morbidity and mortality [122-126]. In the ARIC/NHLBI study of more than 12,000 middle-aged individuals, MetS patients had an increased risk of CHD of 1.5 in men and 2.0 in women [127]. Dekker *et. al.* also found a 2-fold increased risk in fatal and non-fatal CVD in men and women respectively in the 10-year prospective Hoorn Study [124].

The predictive value of MetS *per se* is debatable, however. When compared to the Framingham Risk Score, a regression calculation based on a sample population from the Framingham heart study, MetS is consistently a weaker predictor of CVD [127-129].

In this regard, debate exists as to whether or not the number of component risk factors of MetS is important in conferring CV mortality in these subjects. Ho *et. al.* enrolled 30,365 men who presented for a preventive medical examination between 1979 and 2004 and followed them for a median of 13.6 years [130]. Contrary to the established MetS definition of 3 of 5 criteria, this group, as well as others [125, 131, 132], have seen an increase in all cause and cardiovascular mortality with the presence of just 1 or 2 components present. Existence of central obesity and hypertriglyceridemia also predicted all cause and CV mortality.

Subjects with MetS often exhibit dyslipidemia in the form of elevated TG, low HDL-C concentration and increased levels of small, dense LDL particles outlined above, which has been suggested as the major initiating factor of atherogenicity in this condition [126, 133], and are exacerbated in the PP state. Kolovou *et. al.* assessed PPTG following a high fat meal ($175g/m^2$) in men (39-62 years old) that were healthy, hypertensive or had the criteria for MetS [134]. They found that MetS men had 129% higher fasting TG than controls and a significantly higher PPTG response. Linear regression indicated that PPTG AUC went up by 8.5 mg/dl/h for every 1mg/dl increase in fasting TG.

Stable isotope tracer kinetic studies have further elucidated that the abnormalities in lipoprotein metabolism include overproduction of VLDL-apoB and decreased catabolism of CM-remnants, VLDL, intermediate-density lipoproteins (IDL) and LDL-apoB as well as increased catabolism of HDL particles. There is considerably less data in women with MetS.

Origins of the Metabolic Syndrome and the relationship to post-prandial lipemia

There is much debate on the definition of MetS, as well as the underlying causes. One theory holds that insulin resistance is the determining factor. Normally, insulin acts at the peripheral tissues to suppress lipolysis and increase LpL activity (adipose tissue), and at the liver to decrease ApoB synthesis and suppress VLDL-TG secretion. The idea first put forth by Reaven [135] is that impaired insulin signaling leads to a decreased suppression of lipolysis, defective fat storage in adipocytes, and increased flux of free fatty acids to the liver, along with decreased suppression of apoB synthesis. Together, these factors would lead to enhanced assembly and secretion of VLDL particles. Furthermore, as insulin increases LpL activity, resistance would lead to decreased clearance of triglyceride-rich particles. This idea has been challenged, however, based on the idea that in the above scenario of increased lipolysis and decreased storage, the adipocyte would shrink, which is not typically the case in an insulin resistant state [136].

More recently, it has also been proposed that insulin resistance leads to increased production of CM, not just decreased clearance of circulating TRLs [137]. Recent tracer studies have found that the apoB-48 production rate (PR) is significantly elevated in insulin resistant subjects and that the presence of diabetes was an independent predictor of apoB-48 PR [138, 139]. Experimental evidence has also been generated in the fructose-fed Syrian golden hamster model of insulin resistance. These studies demonstrate that the inflammatory response now associated with obesity and diabetes initiates intestinal lipoprotein oversecretion at many levels, including *de novo* lipogenesis, enhanced fatty acid and cholesterol absorption, and increased activity of chylomicron assembly machinery (reviewed in [140].

As MetS is most often characterized by an increased waist circumference and/or waist-to-hip ratio, abdominal obesity has become an alternative hypothesis of MetS involvement in CVD and has even been described as "the cholesterol of the 21st century" [141]. In 2003, Blackburn *et. al.* described the "hypertriglyceridemic waist" as men with a waist circumference $\geq$ 90 cm had the highest concentration of fasting TG [142]. A very recent, highly publicized study reports that abdominal obesity increases the risk of death [143]. This European, prospective study of almost 15,000 people found a doubling of relative risk for death in men and women in the highest quintile of waist circumference.

The basis for the association of body fat distribution with MetS and disease risk relates to differences in the metabolic activity of adipose tissue stored in different anatomical locations. There are three adipose tissue depots, superficial subcutaneous (found throughout the body, just beneath the skin), deep subcutaneous and visceral, the latter two compose the upper body. The deep subcutaneous depot is located below the superficial subcutaneous and the two are separated by a fascial plane. The two are morphologically different, with the superficial cells being compact and the deep being larger, more irregular and less organized [144]. It is debated whether deep subcutaneous and visceral are the same metabolically.

Visceral fat is generally associated with higher rates of transmemebrane fatty acid flux and a more unfavorable pattern of cytokine secretion [145]. In fact, the chronic, low inflammatory state now associated with obesity is believed to be due to the hypersecretion of pro-atherogenic, pro-inflammatory, and pro-diabetic adipokines from the visceral depot [146]. Conversely, adioponectin is predominantly released from subcutaneous adipose tissue and is inversely correlated with BMI and insulin action [147]. This may partly explain why individuals with high levels of visceral adipose tissue, as is characteristic of most MetS patients [148], have especially low levels of adiponectin. Various studies have reported that low plasma adiponectin is an independent predictor of future vascular disease, however there are also reports to the contrary [146]. The increasing rates of obesity, MetS and T2DM and the resulting CVD-associated morbidity and mortality make the elucidation of these mechanisms critical.

Exercise and CVD

Many of the risk factors for CVD including obesity, MetS and T2DM are modifiable. Dietary modification has waxed and waned with passing trends of low-cholesterol, low-fat and low-carbohydrate diets, along with various iterations in between. Significant data exists relating the amounts and types of macronutrients consumed to the development of these conditions, but reports are mixed and information dissemination provides considerable confusion among professionals and the general public alike. As the epidemic(s) escalate, pharmacological interventions have become more prominent in the treatment of CVD and related diseases, but these can carry heavy medical burdens with regard to side-effects and drug interactions, and monetary burdens both for individuals and a population facing astronomical health care costs.

A clear modifiable risk factor in both the prevention and treatment of CHD is physical activity. The benefits of regular physical activity are likely the result of increased insulin sensitivity [149], and decreased hypertension, obesity, and hyperlipidemia [150]. There is substantial clinical, epidemiological and basic science supporting that habitual activity delays atherosclerosis and decreases CHD incidence [151-154]. A very recent meta-analysis has shown that physically active people have lower incidence of CHD than those who are less active [155].

The benefits of chronic physical activity on CHD risk partly relates to its positive effects on known CHD risk factors, including lipid and cholesterol levels. Two studies by Wood and Stefanick *et. al.* both showed additional benefit of exercise over diet alone.

The first study saw benefit of exercise plus diet over diet alone in lowering LDL-C in men and women 30-64 years old [156]. The second trial studied the effects of a 1-year intervention of diet alone or diet plus exercise on fasting HDL-C and found that although HDL-C was not different in women with or without exercise, it increased in men who exercised over those who did not [157]. Both of these results were despite similar amounts of weight loss between the groups. However, a 2002 diet or diet plus exercise intervention study in obese women reported that while both treatments lead to weight loss, exercise conferred no additional benefit over diet alone in lowering total cholesterol and TG [158].

In contrast, the beneficial effects of exercise independent of weight loss were further demonstrated by Kraus *et. al.* who initiated an 8-month intervention in 111 sedentary, overweight men and women [159]. Subjects were randomized to 1 of 4 treatment groups including a 6-month control group, a high-amount-high-intensity, a low-amount-high-intensity, and a low-amount-moderate-intensity group, and asked to maintain their baseline body weights. Those subjects who exercised the most (17-18miles/week at a moderate jog) significantly decreased LDL particles and small LDL concentration with a concomitant increase in LDL particle number, without changing LDL cholesterol concentration. This amount of exercise also increased HDL particle number and size and decreased total and VLDL TG. The lower amounts of exercise, while not changing lipid profiles, had the secondary benefit of preventing the weight gain seen in the control group.

These results were confirmed by a similar study of twice as many subjects by Slentz *et. al.* in 2007 [160]. A recent meta-analysis of 84 exercise intervention studies (12 weeks minimum) confirmed that high intensity aerobic exercise (VO_{2max} > 60%) increased HDL-C levels, and resistance exercise trended toward lowered LDL-C, without significant effects on lipid measures with moderate exercise (VO_{2max} < 60%) [161].

While chronic physical activity or exercise training is associated with improved lipid profiles, these effects are seen in short-term trials as well. This was demonstrated by Yamada *et. al.* who investigated the differences between 5 days of exercise versus 5 days of energy restriction on serum TG and RLP-C [162]. In this case, dietary restriction produced a larger drop in body weight than exercise, yet fasting levels of TG, LDL-C and RLP-C were lower after exercise than after dietary restriction. Exercise also elicited a larger increase in HDL-C than dietary restriction. A reverse model of the short-term effect of exercise has been observed in the detraining of endurance athletes [163].

Not surprisingly, endurance-trained athletes have significantly more favorable lipid profiles than their sedentary counterparts. Lippi *et. al.* did an extensive analysis of lipid parameters in 60 sedentary men and 142 highly trained cross-country skiers and cyclists [164]. The athletes had significantly lower LDL-C, total cholesterol, and TG, and higher HDL-C than sedentary controls. Although other life-style and body composition factors could contribute to these group differences, the data are consistent with the chronic exercise data.

In addition to differences in fasting lipid levels, highly trained athletes have also been shown to have lower levels of PP lipemia in the context of a high fat meal [165, 166]. Typical PP studies in athletes were done 12-14 hours after the last bout of exercise, however, leading Hardman *et. al.* [163] to hypothesize that the most recent bout has the greatest effect on PPTG response. To this end, Hardman *et. al.* measured PP response to a high fat meal in endurance trained athletes after refraining from planned exercise for 15h, 60h and 6.5 days [163]. At the 15h measure, PPTG started at 0.85 $\pm$ .15 mM and rose to 1.5mM at 2h at which time the response leveled out for the remaining 4h study period. After 60h post-training, fasting TG had increased by 47% relative to the 15h time point and the PP response was significantly higher. Interestingly, the 6.5d test was not different than the 60h test for either fasting TG or PPTG response, indicating that the effects of exercise on PPTG are mainly acute, therefore, to maintain the PP benefit individuals must be physically active on a regular basis.

More recently, several studies have looked more closely at the effects of acute bouts of exercise on PPTG. Zhang *et. al.* compared equivalent, moderate exercise bouts (1 h at 61% of maximal oxygen uptake (VO_{2max})) administered 12 h before, 1 h before, or 1 h after a high fat meal to assess the influence of timing on the PPTG response in recreationally trained males [167]. While all three exercise bouts resulted in lower PPTG area under the curve (AUC), the 1 h post-meal bout was only 5% lower, whereas the pre-meal bouts decreased the AUC by 38% and 51% in the 1 h and 12 h trials respectively.

Importantly, when exercise was performed 12 h prior to the test meal, the fasting (pre-meal) TG were also significantly lower than the 1 h pre-meal trial. As described previously, fasting TG are highly predictive of PPTG, so assessment of PPTG should also consider baseline values. Similar results were achieved in endurance trained women with an 80 min exercise bout at 55% VO_{2max} 14 h prior to a moderate fat (35% kcal) meal [168]. Again, fasting TG were significantly lower in the exercise trial relative to no exercise yielding a lower PPTG AUC. When correcting for baseline TG with IAUC, the PPTG responses were no longer significant, however.

As previous intervention studies have suggested that higher intensity exercise has beneficial effects on PPTG over lower intensities, several groups have investigated the effects of intensity and duration of acute exercise on PPL. A study in physically active young men assessed PPTG response to a high fat meal (84% fat) consumed 1h after a low (25% VO2max) or moderate (65%VO2max) exercise bout matched for the total energy expended during exercise [169]. Only the moderate bout decreased PPTG (39%) compared to controls, even though the low bout lasted an average of 238 minutes. A low baseline PP response in this physically fit population likely decreased the ability to see an effect. At the other extreme, Zhang *et. al.* studied men with MetS under similar circumstances and saw a significant attenuation of PPTG AUC of 30%, 31%, and 38% with exercise of increasing intensity; 40%, 60% and 70% respectively [170]. The general population likely falls somewhere in between these margins, and would be the population to target with regard to CVD prevention.

With respect to studies aimed at this population, the limitations of previous PP studies outlined above are of critical importance. For application to the typical Western population, an exercise bout should be chosen that is achievable on a regular basis and test meals should be of typical macronutrient composition. Similarly, as most free-living humans consume several meals throughout the day, multiple test meals should be evaluated. The bulk of studies reviewed thus far have used single, high fat meals to study exercise effects on PPL. As mentioned, if subjects start out with a higher baseline response, i.e. to a high fat load, it is more likely that exercise will elicit an attenuated response.

Likewise, the opposing effect of exercise on insulin action will be more complex in the context of a mixed meal than one composed mainly of lipid. Nevertheless, the insulin response is relevant as this is the nature in which meals are generally consumed and the insulin response is important with respect to most aspects of post-prandial lipid metabolism.

It was the goal of the present study to control these factors and study the effect of an acute bout of moderate exercise on PPL in lean, but untrained men and women in response to 3 mixed meals over the course of a day. A group of MetS subjects was also evaluated under the same conditions to assess their response to exercise and meals across the day relative to their lean counterparts. Importantly, there is no data to date in MetS women addressing the effects of exercise on the PPTG response.

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CHAPTER 2

The Effects of a Moderate Exercise Bout on Postprandial Lipemia Over a Day of Mixed Meal Feeding

INTRODUCTION

Cardiovascular disease (CVD), including heart disease and stroke, is the leading cause of death in the United States and most developed countries, and impaired lipid metabolism (dyslipidemia) is a major treatable risk factor for CVD. It is standard practice to evaluate lipid profiles in the fasted state; however, the majority of individuals spend the better part of each day in a fed or post-prandial (PP) state. This understanding was the basis of work by Moreton, and thereafter Zilversmit that led to the "post-prandial theory of atherosclerosis", the idea that the metabolic response to individual meals over a lifetime might be the more important factor in the development of atherosclerosis [1, 2].

Evidence is accumulating that fasting plasma triglycerides (TG) are an independent predictor of CVD risk [3, 4]. Nevertheless, while fasting plasma TGs are correlated with post-prandial triglycerides (PPTG) [5-7], some patients with atherosclerosis and normal fasting TG's have PP hyperlipidemia. Indeed, it has been suggested that 40% of all patients with premature coronary artery disease have normal fasting plasma lipids, but impaired clearance of PP lipoproteins [7, 8] a response that appears to be exaggerated in obese subjects [9]. Hence, it has been suggested that better insight into aberrant lipid status might be obtained from measures made in the fed state. This is not a trivial matter, however, given the inherent inconsistency, and lack of standardization, in the evaluation of PP lipids [10].

Given the association of PPTG with CVD, there has been much interest in interventions that might minimize the postprandial lipid response to meals. Among these are dietary control (such as low saturated fat, or high monounsaturated fat diets), pharmaceutical intervention (statins, metformin) and exercise. With respect to exercise, this is an important life-style factor that can help decrease CVD risk. For example, a recent meta-analysis has shown that physically active people have lower incidence of coronary heart disease than those who are less active [11]. Although part of this beneficial effect may be due to the ability of regular exercise to increase anti-atherogenic high-density lipoprotein cholesterol (HDL-C) and decrease fasting TG levels [12, 13], exercise may also have additional positive effects with respect to PP metabolism. As exercise is less invasive than pharmaceutical intervention, which often carries undesirable or harmful side effects and monetary cost, understanding the full benefits of exercise on PP as well as fasting lipoprotein metabolism is important.

While regular, chronic, exercise habits have many beneficial effects including increases in general fitness and beneficial effects on weight loss and weight maintenance, there is evidence that a single, acute bout of exercise in and of itself can also have positive health effects, including a reduction in PPTG [14, 15]. Most studies have evaluated the effect of an acute bout of moderate exercise, performed the night before a high fat test meal (12-14 hours), on PPTG the next morning.

Such studies have generally reported a significant decrease in PPTG with prior exercise [16-21]. Other investigations, however, have reported temporal effects of exercise on PPTG, with varying results on PPTG response based on exercise intensity, duration, and time relative to meal(s) [22, 23].

Although there is a large body of literature, there are several limitations to previous studies in the field of exercise and PP lipemia. Firstly, the vast majority of studies have used a high to very high fat test meal (60-90% kcal from fat) with the aim of producing an exaggerated lipid response and hence increasing the potential for a more dramatic attenuation of PPTG with exercise. This is not a typical meal for most people, however, and the physiologic relevance is questionable.

Furthermore, subjects have typically been given a single high fat meal after an overnight fast with the PP response assessed over a 6-8 hour period. Since most people eat every 4-5 hours over the course of a day, and there is evidence of a "second meal" effect on cumulative PPL for a day [24-26], it is prudent to study more than one meal. It is also important to consider habitual diet and activity patterns prior to testing, as both the amount and type of fat and CHO in the diet, as well as exercise within 60 hrs of a test meal, can affect both fasting and PPTGs [10]. Hence, these factors need to be standardized between subjects as much as possible to ensure the validity of the results.

Finally, sex may also be a factor to consider when evaluating PP lipemia as healthy, premenopausal women are generally believed to have a lower PPTG response relative to men [27, 28]. As pre-menopausal women have a lower incidence of CVD relative to men [29] the question arises as to whether or not part of this sex-specific protection may be mediated via differences in PP lipemia. It is notable that, post-menopausal women, who quickly reach the CVD incidence levels of their male counterparts [30], have a greater PPTG response compared to pre-menopausal women [31]. In addition, whether or not exercise lowers PPTGs to the same, or a greater, extent in women as compared to men has not been directly addressed within the same study. Most studies on the effects of exercise on PPTG have used either one sex or the other, or have combined men and women as a single study group with few studies controlling for menopausal status, menstrual cycle phase and/or steroid hormone use. Evaluating the independent effects of sex, while controlling for hormonal status, is important to our understanding of potential sex differences in the benefits of exercise with respect to CVD risk.

The intention of the current study was to address the effect of acute morning exercise on PPL, from a more real-life scenario in that subjects performed a moderate exercise bout (60 minutes at 60% VO2 peak) followed by consumption of breakfast, lunch and dinner meals, moderate in fat content (35%), consumed over an entire day. Both men and pre-menopausal women were evaluated to determine sex differences in PPTG and the response to exercise.

This study also included a cohort of men and a cohort of women with at least 3 of the 5 characteristic symptoms of the metabolic syndrome (MetS). It has been well established that patients with the MetS are at a much greater risk for CVD and CVD mortality than those without, and have a larger PPTG response than normal weight (NW) subjects (reviewed in [32]). Notably, women with MetS have never been tested with regard to the effects of exercise on PPL. Therefore, evaluating the effects of exercise on PPTG in both sexes, in such an at-risk group is highly relevant.

It was hypothesized that, in NW, healthy, men and women, acute exercise would significantly decrease daytime PPTG relative to a day with no exercise, and the greatest effect would be observed later in the day. A secondary hypothesis was that the magnitude of the exercise-induced decrease in PPTG would be greater in women relative to men, and this would be associated with a lower fat oxidation in women, suggesting increased lipid storage.

A third hypothesis was that individuals with MetS would have a significantly greater PPTG response compared to NW subjects, and that exercise would decrease the PPTG response to a degree similar to that in the NW subjects.

METHODS

All subjects were studied at the General Clinical Research Center (GCRC) at the University of Colorado at Denver Health Sciences Center. Written informed consent was obtained from all subjects before participation in the study, which was approved by the Colorado Multiple Institution Review Board at the University of Colorado at Denver and Health Sciences Center.

Subjects

Two subject groups were recruited (Table 1) including NW healthy subjects (13 men and 13 women) and moderately obese subjects with MetS (9 men and 9 women). Subjects were recruited from the University of Colorado and surrounding area through email, print and posted ads.

Inclusion criteria for both groups were 20-45 years old, non-smoking, stable body weight over the previous 6 months ($\pm$ 3kg), and less than 2h/wk moderate physical activity. Women were eumenorrheic and not using steroidal contraceptives, nor were they pregnant or lactating.

Subjects were excluded for a thyroid stimulating hormone of <0.5 or >5.0 μU/ml, the presence of anemia (hemoglobin < 13.5 g/dl women or < 14.5 g/dl men), diabetes, past or present history of cardiovascular disease, and any other significant hormonal or metabolic abnormality. An apoE genotype of E2/E2 was also grounds for exclusion due to the association of this genotype with aberrant fasting and post-prandial lipoprotein levels.

NW subjects were also excluded if they had a blood glucose <65 or > 110 mg/dl, insulin > 20 μU/ml, total cholesterol >200 mg/dl and LDL cholesterol >160 mg/dl. Subjects with MetS were recruited according to NCEP ATP III criteria. Each subject was required to have at least 3 of the following 5 hallmark characteristics: a fasting glucose concentration 100-140 mg/dl, fasting TG level of > 150 mg/dl, waist circumference > 102 cm men and 88 cm women, blood pressure of 130/>85 mmHg and high-density lipoprotein cholesterol of < 40 mg/dl men and < 50 mg/dl women. Subjects were required to have a BMI less than or equal to 32 kg/m^2 in order to minimize the likelihood of other co-morbidities and to minimize body weight and body composition differences between groups, and within subjects in the MetS group.

Screening assessments

Subjects were screened for study inclusion with a health and physical examination, including blood chemistry and lipid profile. Subjects were asked to refrain from alcohol and exercise for 48 hrs before this blood draw, and were advised to consume a low carbohydrate diet before the blood draw (2 days) to minimize variability in the screening TG levels. In the NW subjects, the goal was to recruit men and women with similar fasting TGs to minimze this as a confounding factor on the PPTG response. Women had samples drawn in the follicular phase of their menstrual cycle.

A 4-day dietary record (3 weekdays, 1 weekend day) was also completed. Subjects were excluded if the percentage of total energy intake derived from nutrients was +/- 33% of the test meal nutrient composition, (e.g. fat; <20 or >40%) to avoid including individuals with extreme dietary habits and for whom the period of pre-study dietary control, and test meal, would be a drastic change from their habitual diet.

Preliminary assessments

If subjects qualified for the study, they completed the following assessments prior to the main study days.

Measurement of resting metabolic rate

This was measured in subjects using indirect calorimetry in the morning, following a 10-12 hr fast as previously described [33]. Results were used to calculate energy intake for the controlled diet phase of each study.

Peak oxygen uptake test

Subjects were studied in the morning after an overnight fast having abstained from planned exercise the day prior. Peak oxygen uptake was determined using a graded treadmill (Quinton) test using the Bruce Protocol [34] for NW subjects and a *modified* Bruce protocol for MetS subjects. Traditionally, the test starts at 2.74 km/hr (1.7 mph) and at a gradient (or incline) of 10%. At three minute intervals the incline of the treadmill increases by 2%, and the speed increases.

In the modified test, the first two stages were performed at a 1.7 mph and 0% grade and 1.7 mph and 5% grade respectively, and the third stage corresponds to the first stage of the Standard Bruce Test protocol. Heart rate was continually monitored via a 12 lead ECG with blood pressure measured in the final minute of each work level. Respiratory gas exchange was monitored continuously during the test via indirect calorimetry (Sensormedics 2900 metabolic cart system, Sensormedics, Yorba Linda, CA). The test was terminated when the subject reached volitional exhaustion or if there was any clinical abnormality noted in the ECG or blood pressure. Peak oxygen uptake was calculated from the final minute oxygen consumption prior to the test termination. Any clinically significant abnormalities noted during the test, were also cause for exclusion depending on the physicians evaluation.

Determination of body composition and anthropometry

Body composition was measured via DEXA [35] and waist and hip circumferences were measured as previously described [36].

Determination of visceral adiposity

Visceral adipose tissue was determined from computed tomography (CT). Since total and visceral fat areas from a single CT cut centered at the L4-L5 disk level are highly correlated to a patient's total and visceral fat volume ($r > 0.95$), only a single CT cut is required to accurately predict the amount of total vs. visceral fat in a given patient. These results are not presented here, however, due to delayed analysis by the contracted radiologist.

Determination of menstrual cycle phase in eumenorrheic women

Only women with a regular menstrual cycle over the preceding 6 months (cycle length 22 - 36 days) were recruited. Information on cycle length was collected at screening and thereafter. Follicular phase measurements were made between days 5-11 of a typical 28 day cycle. For longer/shorter cycles, days were adjusted accordingly.

Preparatory Dietary and Exercise Control

Subjects were fed a controlled diet for 5 days prior to each study day. The diet was prepared by the adult GCRC and no other food was permitted. The diet composition was identical to that of the meals consumed on the study day. Energy intake on the controlled diet days was calculated depending on the subjects activity level: 1.50 x RMR for a no exercise or 1.6-1.7 for an exercise day, respectively [33, 37]. Body weight was measured daily and energy intake was adjusted if a consistent weight loss or gain was observed.

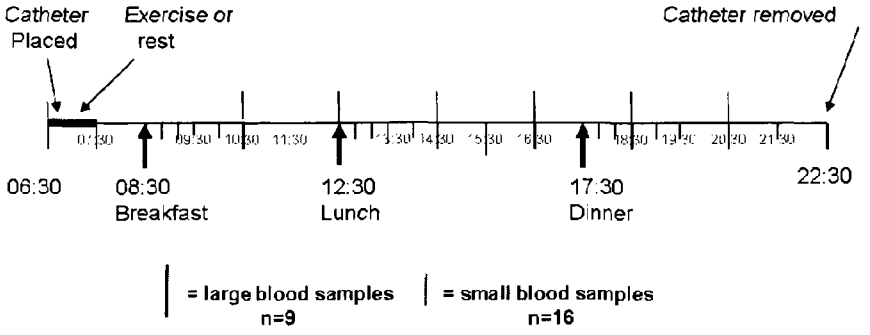
Subjects were requested not to perform planned exercise, other than activities of daily living, for 60 hrs prior to the study. Subjects spent the evening before the study on the adult GCRC and were tested the next day following an overnight fast (12 hrs).

Experimental Protocol

Study Timeline

The evening prior to each study day, subjects were admitted to the GCRC and ate their evening meal on the unit. Subjects spent the night on the GCRC and were fasted from 22:00, with only water or non-caloric/non-caffeinated beverages permitted. Testing began the next morning after an overnight fast and continued throughout the entire day as outlined below. An intravenous (IV) catheter was placed in an antecubital vein as soon as subjects awoke and was kept patent throughout the study with a slow 0.09% saline infusion. On the exercise day, subjects walked for 60 mins, at 60% of their peak oxygen uptake. Respiratory gas exchange was measured for 2 x 15-20 minute periods during the exercise bout. On the non-exercise day, this same time was spent resting during which 2 x 15-20 minute measurements of respiratory gas exchange were made.

Study Day Timeline



Subjects remained, for the most part, resting in bed during the day except to use the bathroom or stretch their legs briefly. Subjects were resting for 30 minutes prior to any sampling/testing. At the end of the first study day (~10:30pm), subjects had the option of staying a second night on the GCRC, or were allowed to leave the GCRC and asked to return the next morning for a final blood draw at 7:30 a.m. Subjects were required to fast and not consume caffeine during this time.

Study day meals

Total energy intake was calculated to meet energy requirements on the corresponding day and energy intake was divided throughout the day as follows; 25%, 35% and 40% of total daily energy intake at breakfast, lunch and dinner, respectively. The nutrient breakdown by meal, group and study day is given in table 3.

The composition of each meal was 34% fat, 15% protein and 51% carbohydrate, and was designed to maintain energy balance with energy intake calculated as follows: rest day, 1.3 x RMR; exercise day, 1.3 x RMR + net energy expenditure (EE) during the exercise + estimated excess post-exercise energy expenditure (10% of net EE in kcal/min x 30 mins). A factor of 1.3 RMR was used as subjects spent the majority of the day resting in bed, therefore, not very active. The cholesterol intake was 128 mg/1000 kcal.

Blood sampling and analysis

Blood samples were drawn via the IV catheter and placed into vacu-tubes containing EDTA (for plasma) or vacutainers without any additive (for serum).
Tubes for plasma collection were placed on ice immediately until spinning. All samples underwent centrifugation for separation of plasma or serum. Plasma/serum samples were aliquoted then frozen for analysis by the GCRC core lab.

Total TG, cholesterol, FFA, glucose and insulin were measured on all blood samples. High-density lipoprotein cholesterol (HDL-C) was measured on fasting blood samples and fasting low-density lipoprotein (LDL-C) was calculated by difference (Freidwald equation [38]). A portion of the large blood sample was also used to measure total apolipoprotein (apo) B. Estradiol and progesterone were measured on baseline blood samples only.

Plasma glucose was measured using the glucose oxidase method, insulin was determined by radioimmunoassay (Clinical Assays Gamma Coat RIA, Cambridge, MA), and FFA were measured enzymatically (Wako Chemical, Wako, TX).

Triglyceride concentrations in whole plasma were measured using an enzymatic assay (Sigma-Aldrich, St. Louis, MO) and cholesterol was assayed using the Cholesterol C kit (Wako Chemical, Wako, TX). Total TG concentrations in plasma were corrected for free glycerol concentrations measured enzymatically (Roche Diagnositics, Morristown, NJ). It was assumed that all acylglycerols detected by the assay were triacylglycerols. Adiponectin was assayed via RIA (Linco, Research, Inc., St. Charles, MO) and total plasma apoB was measured via nephelometry (Behring 100 analyzer, Siemens, Deerfield, IL). The sex steroids, estradiol, and progesterone were measured with commercial assay kits (Diagnostic Products Corporation, Los Angeles, CA).

The homeostasis model assessment of insulin-insulin resistance (HOMA-IR) was calculated using fasting values from the study day as well as the following day using the following formula [39]:

Glucose (mg/dl)/insulin (μU/ml)/405

Statistical analysis

Data were analyzed with SPSS version 16 and MS Office Excel. Area under the post-prandial curve (AUC) was calculated for plasma measures using the trapezoidal rule and incremental area under the curve was calculated by subtracting the corresponding days -75 min value x duration (840 mins) from the AUC values. AUC and IAUC results were analyzed with univariate ANOVA covarying for age.

Significant differences from ANOVA were analyzed post-hoc by t-test. Repeated measures ANOVA was used to test for significant differences in the pattern of postprandial responses. Significance was set at $p = 0.05$.

RESULTS

Subject demographics

Table 1 displays subject demographics. As a group, the women were significantly older than the men (33 $\pm$ 7 vs. 30 $\pm$ 6 yr; p=0.02), and the MetS subjects were significantly older than the NW subjects. In the NW group, the women were significantly older than the men, but there was no difference in age per sex in the Mets group.

MetS subjects had a higher BMI than NW subjects (29 $\pm$ 2.5 vs. 23 $\pm$ 2 kg/m^2; $p < 0.001$), and NW men had a higher average BMI than NW women, but there was no sex difference in the MetS group. As would be predicted from the study design, MetS subjects had significantly greater percent body fat (33 $\pm$ 5 vs. 24 $\pm$ 6 % body weight) than NW subjects, and women had a significantly greater percent body fat than men (31 $\pm$ 7 vs. 24 $\pm$ 5%). Likewise, this pattern of sex-difference in body composition was observed within each group. The expected sex difference in absolute VO_2 peak (ml/min) was observed, being greater in men vs. women ($p < 0.001$) but this was not different between groups. With VO_2 peak expressed per FFM, ie. the predominant metabolically active tissue during exercise, NW subjects had a significantly higher VO_2 peak than MetS subjects $P < 0.001$), but there were no sex differences within or between groups.

Fasting plasma values

The fasting blood values of both NW and MetS subjects are listed in Table 2, expressed as the average of the T-75 time point from each rest and exercise day. Overall, the MetS group had significantly higher fasting insulin ($p < 0.001$), glucose ($p < 0.001$), TG ($p = 0.01$), apoB ($p= 0.032$) and HOMA-IR ($p < 0.001$) than the NW group, and significantly lower levels of fasting HDL-C ($p = 0.004$) and adiponectin ($p = 0.007$). As would be expected, HDL-C was significantly higher in both NW and MetS women as compared to their respective male counterparts. Interestingly, NW women had higher levels of fasting adiponectin than NW men, but no sex differences were observed within the MetS group.

Exercise

There was no difference in the relative intensity at which subjects exercised; on average 60% of VO_2 peak, or 29 ml O_2/FFM/min. In terms of absolute VO_2 this corresponded to 2010 ml/min in NW men, 1351 ml/min in NW women, 1835 ml/min in MetS men and 1375 ml/min in MetS women. Consequently, the energy cost of the exercise was 579, 391, 550 and 412 kcals, respectively, with men having a higher energy expenditure than women.

Postprandial TG response

Figure 1 depicts the TG response curves for the NW and MetS groups (A) and the averages of the rest and exercise days for men and women (NW and MetS combined (B)). There was no significant exercise effect on the postprandial TG response over the day in either the NW or MetS groups, in either sex, nor in the group as a whole, when expressed as either AUC or IAUC (fig. 2A). Collectively, the men exhibited a 34% higher PPTG response than women over the day expressed as IAUC (5.086×10^4 vs 3.358×10^4 mg/dl/840 mins, $p = 0.037$). There was a significant overall effect of group as the MetS group had a 44% higher daily PPTG response when expressed as AUC and a 55% higher IAUC than the NW group ($p<0.001$ for both) (fig. 2B). Repeated measures ANOVA revealed a time x group x sex effect for PPTG ($p < 0.001$). A subsequent repeated measures ANOVA of just the MetS group verified a significant time x sex effect ($p = 0.032$) which was not seen in the NW group ($p = 0.28$).

Other PP response measures

The NW subjects responded equivalently between men and women, rest and exercise for glucose and insulin measures over the entire day (fig 3A & B). Free fatty acids increased significantly with exercise in both men and women, but decreased rapidly upon consumption of the breakfast meal (fig. 3C). In MetS subjects, there were no sex or day differences for glucose AUC or IAUC. However, the MetS women had a significantly higher insulin IAUC for the breakfast (T-75 to T240; $p =0.005$) and lunch (T240 to T540; $p = 0.06$) periods relative to the MetS men (fig. 3B).

The MetS women also had a significant increase in FFA from T180-T36, relative to MetS men over this period (IAUC = 25231 vs 4876 μE/L/180 mins, respectively; $p < 0.001$) (fig 3C). Exercise resulted in a 30% decrease in total cholesterol IAUC ($p = 0.003$) in the NW group (fig. 4), but had no effect on cholesterol in MetS subjects.

Total apoB AUC was 8% higher in the MetS group ($p = 0.004$), but IAUC was not different between the groups. Borderline significant overall effects were observed for day and for sex with respect to apoB IAUC ($p = 0.064$ and 0.056 respectively). Given that there is one apoB per triglyceride-rich particle, a rough measure of particle size is the ratio of TG to total apoB. This ratio revealed a significant decrease in particle size (AUC, $p = 0.025$ & IAUC, $p = 0.021$) in MetS women over the day relative to MetS men (fig 5A & B) but no sex differences in the NW group. Moreover, relative to NW subjects, MetS subjects as a group had significantly larger TG particles (IAUC $p < 0.001$).

Discussion

In contrast to what was hypothesized, in this population of NW and MetS men and women, a single bout of moderate, morning exercise did not significantly decrease the cumulative PPTG response to mixed meal feeding, measured over an entire day. This was also true for the PPTG response measured after each individual breakfast, lunch or dinner meal. Furthermore, the acute exercise bout had no effect on glucose or insulin excursions in any group.

Results presented in this paper appear contrary to what has generally been reported in the literature, that is, a lowering of PPTGs by a single bout of exercise. There are several possible explanations for the lack of effect observed in the current study, including meal composition; timing, length and/or intensity of the exercise bout and/ or the replacement of the energy expended during exercise so that subjects did not experience an energy deficit on the exercise day relative to the rest day.

As previously discussed, many prior PP lipemia studies, whether involving exercise or not, have used high to very high fat meals to elicit the PPTG response [40-42]. Several studies however have demonstrated significant differences in PPTG response with varying amounts of fat, ranging from no effect with 5-15g to a dose-dependent increase from 30-50g and non-dose-dependent increase with doses above 80g [43-45]. The content of the current meals is based more closely on the standard intake of most Americans over a typical day (NHANES III; [46]), with an average of 80 grams of fat over the day in NW and MetS subjects.

The reduced PPTG response elicited by the mixed meal consequently narrows the window for observing an exercise effect but is more relevant to normal dietary practices. A limited number of studies have employed a moderate fat meal (30-40% fat) to evaluate the ability of exercise to decrease PPTGs. Data is equivocal, however, with both a significant reduction in PPTG [47] and also no effect [48-52] being observed. Fat content of the test meal could be one, but not necessarily the only factor contributing to the lack of exercise effect on the PPTG response in the current study.

Alternatively, the exercise itself may not have been long enough and/or at a high enough intensity to elicit a change in PPTG. In response to high fat meals, exercise intensity has been reported to be a determining factor in the degree of the reduction in PPTG [17, 18, 53-56]. Moderate intensity exercise performed for as little as 30 minutes was reported to attenuate PPTG concentrations [17, 47], whereas low-intensity exercise for 90 [20]or 240 [22] minutes showed no effect. Similarly, exercise for 2 hours decreased PPTG [57] whereas 1 hour of equivalent intensity exercise had no effect [50]. Pfeiffer *et. al.* administered a mixed meal (33% fat) immediately after 30, 60, or 90 minutes of moderate (50% VO_2 max) exercise and saw no significant decrease in PPTG with any duration of exercise [51].

Higher intensity exercise has been shown to induce a greater decrease in PPTG than lower intensities as reported by both Tsetsonis [56] and Katsanos [22]. In both studies, subjects walked on a treadmill at 25-35% V_{O2max} and at 61-65% V_{O2max}, and both found that moderate intensity exercise reduced PPTG response relative to no exercise control days, whereas low intensity exercise did not.

These results are despite equivalent exercise duration (90 min, Tsetsonis) or energy expenditure (1100 kcal, Katsanos). The latter study is in line with most previous studies reporting an exercise-induced decrease in measures of PP lipemia with reported energy expenditures (EE) of at least 1000 kcal [18, 20, 22, 54, 58]. A recently released study in MetS men has reported that an exercise bout, resulting in 500 kcal EE, significantly reduced the PPTG response to a high fat meal [59]. The energy expenditure in the current study did not reach this level, with the NW subjects expending an average of 484 kcal and the MetS group only 481 kcal with the 60 min exercise session. There is evidence that subjects with lower VO_{2max} (~48 ml/kg/min) may not need to expend as much energy as those with higher VO_{2max} but it still may require an exercise EE as much as 500 kcal [21, 53, 60, 61]. The average V_{O2max} of the current subjects was 49.8 ml/kg/min.

Finally, the replacement of energy expended with exercise in the current study may have negated any effect that might have been seen had the energy not been replaced. The vast majority of previous studies of exercise and PP lipemia have not replaced the energy expended during the prior exercise bout meaning that subjects experienced an energy deficit relative to the rest/control condition. Burton *et. al.* recently reported that in overweight or obese men a moderate exercise bout (expending 27kJ/kg body mass per subject, i.e. ~400 kcal for a 70 kg individual) performed 16 hours prior to a test meal, resulted in a lower PPTG response, reduced insulin concentrations, and increased fat oxidation as compared to a non-exercise, control condition [62].

When the expended energy was replaced, however, the TG response was no longer significantly different from the control day. Of relevance, pre-meal fasting TG's were lower in the non-energy replaced condition compared to the energy replaced and control conditions, thus when baseline TG were taken into account, as IAUC, the PPTG effect was no longer significant.

There are several possible mechanisms for exercise-induced lowering of PP lipids. Firstly, the clearance of TGs from the circulation could be increased. In support of this, well-trained individuals display enhanced clearance of TG relative to untrained controls [63, 64], and prolonged exercise bouts (>3h) can induce increased TG clearance [65, 66]. The increased clearance is believed to be due to increased lipoprotein lipase activity (LpL), which has been observed to be increased in plasma 6-18 hours post-exercise [67-69]. Moreover, Oscai et. al. noted that exercise had a heparin-like effect in releasing LpL from the endothelium of active muscle tissue into the circulation, theoretically increasing TRL hydrolysis capacity [70].

Finally, Kiens et. al. have demonstrated that muscle LPL activity is increased at 4hrs but not 8 hrs after exercise [71]. However, they also observed that insulin normally decreases muscle LPL activity, which is opposite to what is observed with adipose tissue LPL [72]. Nevertheless, LPL activity in muscle after exercise plus insulin was still higher than the non-exercise condition. This would provide a mechanism for TG hydrolysis and FFA uptake at muscle for storage and/or oxidation.

In addition to plasma TG clearance, exercise could affect the appearance of TG in the circulation. From data discussed above, it appears that a single exercise bout may predominantly exert it's affects on PPTG via effects on fasting TGs, potentially suggesting effects on hepatic VLDL production and secretion rather than changes in metabolism of meal-derived lipids (CM-TG). Although a prolonged bout (2h) of moderate exercise (60%VO2max) has been shown to increase basal FFA rate of appearance (R_a), whole-body fat oxidation measured the next day and increase VLDL-TG plasma clearance rates and decrease VLDL-apoB-100 secretion rate [73], a 1h bout of similar intensity had no effect on these parameters[74]. In terms of CM-TG appearance, prior exercise has not been shown to delay the time to peak CM-TG [55, 75] and gastric emptying has not been confirmed to be slowed with exercise [75, 76]. It therefore appears likely that exercise reduces PPTG concentrations mainly by affects on TG clearance although other mechanisms cannot be ruled out.

Although not well studied, it has been reported that MetS men have significantly greater PPTG response than lean, age-matched men [77, 78]. Additionally, acute bouts of low and moderate intensity exercise have been shown to decrease the PPTG response to a single, high fat meal in these men [59, 79]. We saw no such effect, however, of exercise in the present cohort of MetS men, likely due to the factors previously discussed. Our current study is the first to report the effect of exercise on PPTG in MetS women, who also demonstrated no PPTG lowering after exercise.

Despite the lack of change in the primary outcome (PPTG), some interesting secondary results were seen in this study. Exercise significantly reduced total cholesterol in the NW subjects. The effect was greater in men ($p = 0.02$) than women ($p = 0.06$), and was not seen at all in the MetS subjects. The concentration of LDL-C, calculated using the Freidwald equation, was also significantly lower ($p = 0.02$) in the NW men with exercise, indicating that this is the main driver of the reduction in total cholesterol. It cannot be determined from the current analysis if the reduction is due to decreased particle number (apoB-100 concentration), or strictly from cholesterol, but the lowering could be considered anti-atherogenic.

As was hypothesized women, overall, had a significantly lower PPTG response compared to men. This confirms what has been observed in previous studies [27, 28, 80]. What was not expected, however, was the PPTG pattern seen in MetS women, which was driving this sex-based difference. Indeed, when sex differences were evaluated within groups, the PPTG response was not different in NW, only in the MetS. The MetS women trended toward a lower TG IAUC over the day due to a precipitous fall in TG post-lunch, and maintained a lower TG concentration for the remainder of the study day. This reduction was accompanied by a significant increase in insulin IAUC with the breakfast and lunch meals.

Information in MetS women is even more scarce than in MetS men, however one study in post-menopausal women found that those with the MetS had a significantly greater PPTG response than their normal weight, age and menopausal status-matched counterparts [81]. Adiels et. al. recently compared the PPTG response in men and women with the MetS [82]. No significant differences in PPTG were seen at 2, 4, or 6 hours after a high fat meal. At 8 h, however, the men had a significantly higher TG concentration than the women, which the authors suggest corresponds to TG clearance. This corresponds to the pattern seen in the current subject group, but the women in the current study began to separate from the men at 6 h post-meal rather than 8h, possibly due to the moderate fat content.

The MetS women in the current study had a higher HOMA-IR score than their male counterparts, indicating that the women were less insulin sensitive, possibly explaining their higher insulin excursions. Sadur and Eckel demonstrated a significant increase in adipose tissue lipoprotein lipase (AT-LPL) approximately 6 hours after insulin infusion relative to saline [72]. It can, therefore, be hypothesized that in the current study LPL activity may have peaked at about 6 hours after the breakfast meal. The elevated insulin excursion in the MetS women could have induced a greater LPL response, and augmented plasma TG removal after mid-day. A significant increase in FFA levels at mid-day in MetS women, relative to MetS men, may have been a result of reduced insulin-dependent suppression of lipolysis, due to insulin insensitivity in these women, or possibly greater intravascular lipolysis from circulating TG.

Correspondingly, the MetS women displayed a decrease in triglyceride-rich lipoprotein (TRL) particle size, estimated from the ratio of total TG to total apoB, as there is one apoB per TRL particle [83]. Total apoB did not change over the day in men or women, but the accompanying sharp decrease in TG in the women led to a smaller estimated particle size in MetS women in the second half of the day relative to MetS men. This may be clinically relevant as the influence of TRL particle size on atherogenesis has come to the fore in recent years with the discovery of remnant TRL remnant particle association with atherosclerotic plaques [84].

Smaller, denser particles are better able to enter the endothelium and reside in the endothelial space. Though the current study is not designed to determine the types (CM, VLDL, LDL) or composition of the TRL particles, the clear difference between MetS men and women speaks to the difference in TRL metabolism between the sexes. This is worthy of further investigation to determine whether the lower PPTGs but smaller TRL particle size in MetS women vs MetS men is deleterious in terms of CHD risk.

In conclusion, in the context of this well controlled study, a moderate bout of morning exercise did not have any effect on PPTG in NW or MetS men or women, measured over a full day including 3 meals. Though this is likely due to the conditions of the study i.e. moderate exercise done under fasting conditions before the consumption of moderate fat, mixed meals, these are believed to be more typical life-style patterns of free-living, Western individuals. Though PPTG did not change, exercise did decrease total cholesterol in the NW subjects, supporting the antiatherogenic potential of exercise.

In addition, the sex difference in PPTG response pattern seen in the MetS subjects was an unexpected finding, but is plausible given the increased insulin excursions in the women relative to the men. Whether this pattern is protective given the decrease in TG, or detrimental due to decreased particle size is debatable. The study underscores the heterogeneity in PP lipid response between subjects of different sexes and metabolic states, and enforces the mandate for tailored prevention and treatment strategies for CVD and related conditions.

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FIGURE LEGENDS

FIGURE 1. Average PPTG response curves for (A) rest and exercise days for normal weight (NW) men and women (n = 13 each) and metabolic syndrome (MetS) men and women (n = 9 each), arrows indicate meals. Error bars were omitted for clarity, but average SEM's were: NW women 14.2, NW men 18.5, MetS women 18.5, and MetS men 19). (B) the average rest and exercise days in NW (n = 26) and MetS (n = 18) men and women.

FIGURE 2. Average (means $\pm$ SEM) AUC and IAUC for PPTG (A) rest and exercise conditions in NW & MetS men and women. (B) NW men and women (n = 26) and MetS men and women (n = 18) average of rest and exercise conditions.

FIGURE 3. Average response curves for NW and MetS groups for insulin (A.1), glucose (B.1) and FFA (C) over 840 minutes. Average IAUC for NW and MetS subjects on rest and exercise days for insulin (A.2) and glucose (B.2). Error bars were omitted for clarity but average SEM's were: Insulin NW (1.2), Mets (3.4); glucose NW (3.1), MetS (2.3); FFA NW (30.2), MetS (30.3).

FIGURE 4. Average total cholesterol (mg/dl) IAUC for NW and MetS subjects on rest and exercise days.

FIGURE 5. Average particle size (total plasma TG:apoB mg/dl) (A) IAUC and (B) response curves.

Table 1. Anthropometric Characteristics of Subjects

	NW		MetS	
	Men	Women	Men	Women
N	13	13	9	9
Age (yr)$	26.4 ± 3.6	31.1 ± 7.1	34.9 ± 2.4	36.3 ± 2.0
Height (m)	1.83 ± .09	1.66 ± .081	1.75 ± .03	1.66 ± 0.02
Body weight (kg)$	79.7 ± 9.2	60.3 ± 7.1	92.8 ± 3.9	79.4 ± 3.0
BMI (kg/m2)$	23.7 ± 1.6	21.9 ± 1.9	30.2 ± 0.9	28.7 ± 0.91
FM (% body weight)$	20.9 ± 3.8	27.3 ± 6.5**	29.3 ± 1.2	36.0 ± 1.2**
FFM (% body weight)$	80.0 ± 3.8	72.8 ± 6.5**	70.6 ± 1.3	63.6 ± 1.3**
VO_2 Peak (mL/min)	3308 + 394	2231 + 319	3163 ± 264	2353 ± 331
VO_2 Peak (mL/kgFFM/mn)$	52.7 ± 1.04	51.1 ± 1.4	48.6 ± 1.4	46.9 ± 1.6

Values = Mean +/- SD. BMI = body mass index, FM = fat mass, FFM = fat-free mass, VO2 Peak = peak maximal oxygen uptake

$p < 0.05$ = *
$p < 0.01$ = ** } within group
$p < 0.001$ = #
$p < 0.05$ $ = between groups

Table 2. Fasting Plasma Measures

	Normal Weight		MetS	
	Men	Women	Men	Women
Total TG (mg/dl)$	103.8 ± 7.7	94.3 ± 7.4	173.9 ± 11.0	151.1 ± 12.4
Total apoB$ (mg/dl)	78.0 ± 2.7	77.6 ± 4.9	82.8 ± 4.1	91.9 ± 4.4
Total Cholesterol (mg/dl)	161.1 ± 4.7	169.8 ± 8.4	153.3 ± 7.0	168.4 ± 7.2
FFA (μE/L)$	434.9 ± 25.1	410.4 ± 28.5	489.1 ± 47.7	502 ± 38.5
HDL-C (mg/dl)$	41.9 ± 1.6	46.8 ± 1.8*	31.2 ± 1.1	37.4 ± 0.87 #
LDL-C (mg/dl)	98.5 ± 4.4	104.1 ± 7.1	87.3 ± 6.5	100.7 ± 5.7
Insulin (uU/ml)$	5.2 ± 0.51	5.3 ± 0.51	11.7 ± 1.3	15.4 ± 1.7
Glucose (mg/dl)$	86.7 ± 1.2	84.5 ± 1.3	91.6 ± 1.9	92 ± 1.6
HOMA-IR$	1.12± .11	1.10 ± .11	2.60 ± 0.29	3.57 ± 0.44**
Adiponectin$	6.7 ± 0.66	9.1 ± 0.83*	3.99 ± 0.43	3.2 ± 0.42
Estradiol	30.1 ± 1.8	84.1 ± 10.4	35.0 ± 2.0	60.3 ± 8.8**
Progesterone	1.00 ± 0.08	0.68 ± 0.08	0.59 ± 0.033	0.72 ± 0.01

$p < 0.05 = *$
$p < 0.01 = **$
$p < 0.001 = \#$
} within group

$p < 0.001 = \$$ between groups

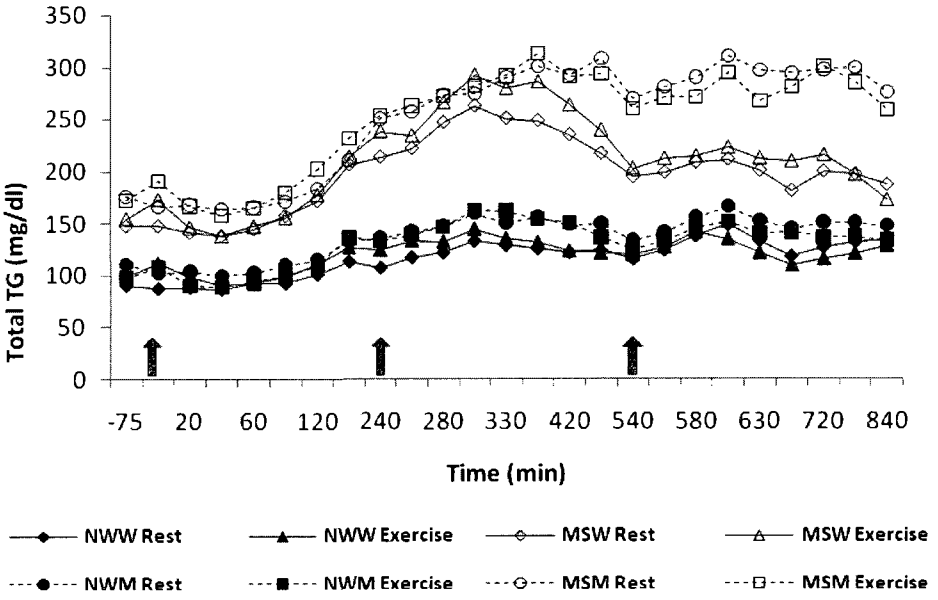
Table 3. Study Day Meals

NW	Breakfast		Lunch		Dinner	
	Men	Women	Men	Women	Men	Women
REST						
Energy (kcal)	555 ± 12	433 ± 10	777 ± 16	606 ± 14	888 ± 18	692 ± 16
Fat (g)	22 ± 0.4	17 ± 0.4	30 ± 0.6	24 ± 0.6	34 ± 0.7	27 ± 0.6
Carbohydrate (g)	73 ± 1	57 ± 1	102 ± 2	80 ± 2	116 ± 2	91 ± 2
Protein (g)	21 ± 0.5	17 ± 0.4	30 ± 0.6	23 ± 0.5	34 ± 0.7	27 ± 0.6
Fiber (g)	7.1 ± 0.1	5.6 ± 0.2	9.9 ± 0.2	7.8 ± 0.2	11 ± 0.2	9 ± 0.3
EXERCISE						
Energy (kcal)	694 ± 18	519 ± 10	971 ± 25	726 ± 15	1110 ± 29	830 ± 17
Fat (g)	27 ± 0.7	20 ± 0.4	38 ± 1	28 ± 0.6	43 ± 1	32 ± 0.7
Carbohydrate (g)	91 ± 2	68 ± 1	127 ± 3	95 ± 2	145 ± 4	109 ± 2
Protein (g)	27 ± 0.7	20 ± 0.4	37 ± 1	28 ± 0.6	43 ± 1	32 ± 0.7
Fiber (g)	8.8 ± 0.2	6.6 ± 0.2	12 ± 0.3	9.3 ± 0.2	14 ± 0.3	11 ± 0.2

MetS	Breakfast		Lunch		Dinner	
	Men	Women	Men	Women	Men	Women
REST						
Energy (kcal)	598 ± 21	501 ± 17	837 ± 30	701 ± 24	957 ± 34	801 ± 28
Fat (g)	23 ± 0.8	19 ± 0.7	33 ± 1	27 ± 0.9	37 ± 1	31 ± 1
Carbohydrate (g)	79 ± 3	66 ± 2	110 ± 4	92 ± 3	126 ± 4	105 ± 3
Protein (g)	20 ± 2	19 ± 0.7	28 ± 3	27 ± 0.9	33 ± 4	31 ± 1
Fiber (g)	7.8 ± 0.3	6 ± 0.2	11 ± 0.4	9 ± 0.3	12 ± 0.5	10 ± 0.4
EXERCISE						
Energy (kcal)	728 ± 25	603 ± 16	1019 ± 35	844 ± 23	1164 ± 39	964 ± 26
Fat (g)	28 ± 0.9	23 ± 0.6	39 ± 1	33 ± 0.9	45 ± 1	37 ± 1
Carbohydrate (g)	95 ± 3	79 ± 2	133 ± 4	110 ± 3	152 ± 5	126 ± 3
Protein (g)	28 ± 0.9	23 ± 0.6	39 ± 1	33 ± 0.8	45 ± 1	37 ± 1
Fiber (g)	9.3 ± 0.3	7.6 ± 0.2	13 ± 0.4	11 ± 0.3	15 ± 0.5	12 ± 0.3

Figure 1. Postprandial Triglyceride Response

A.



B.

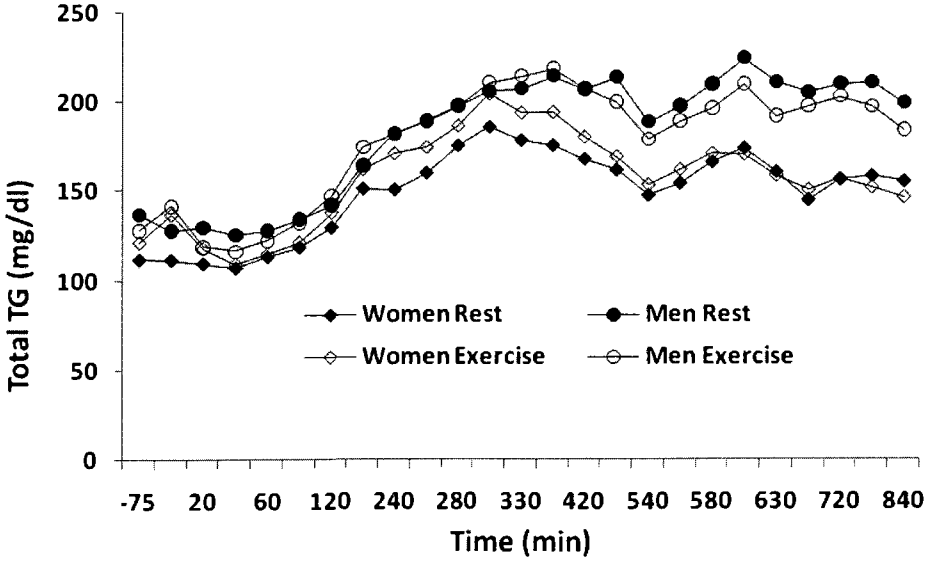
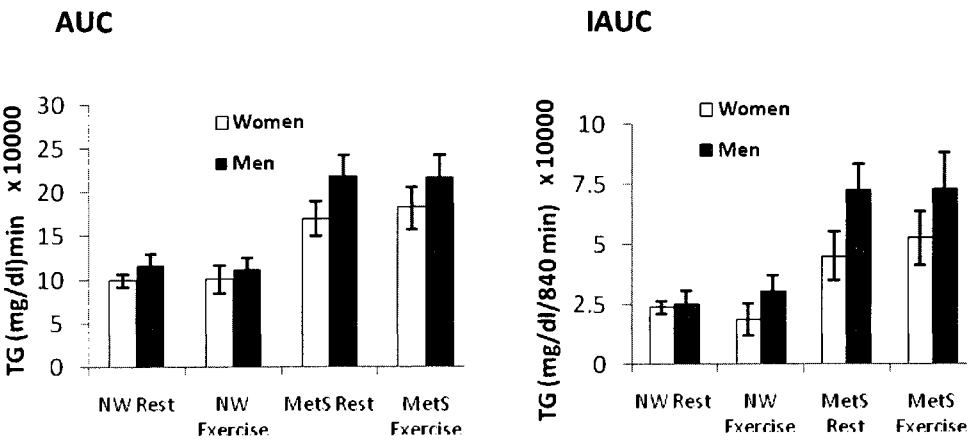


Figure 2.

A. Total TG



B. Overall PPTG AUC and IAUC

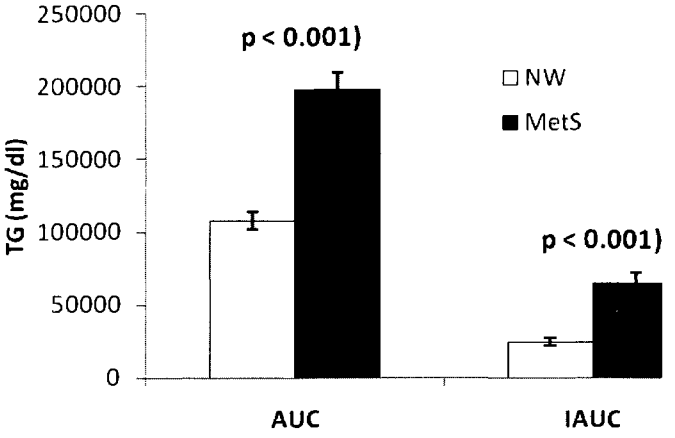
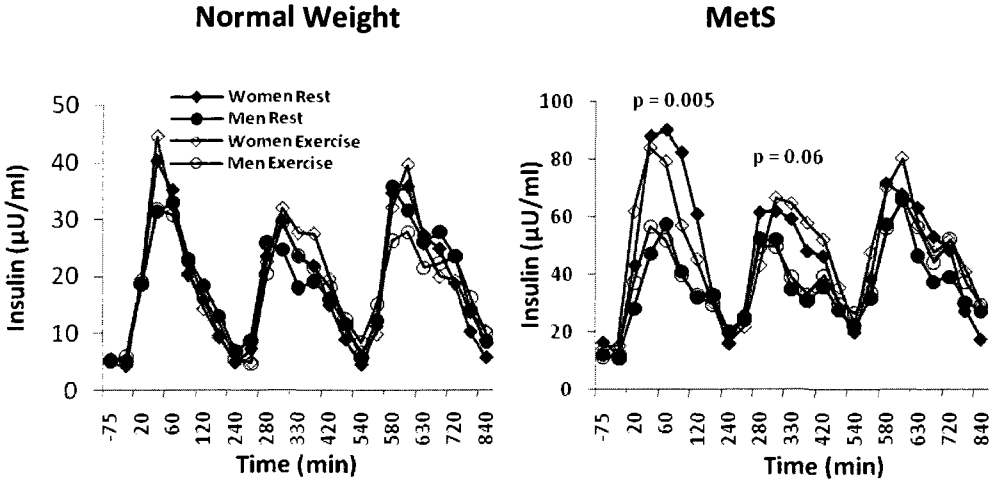


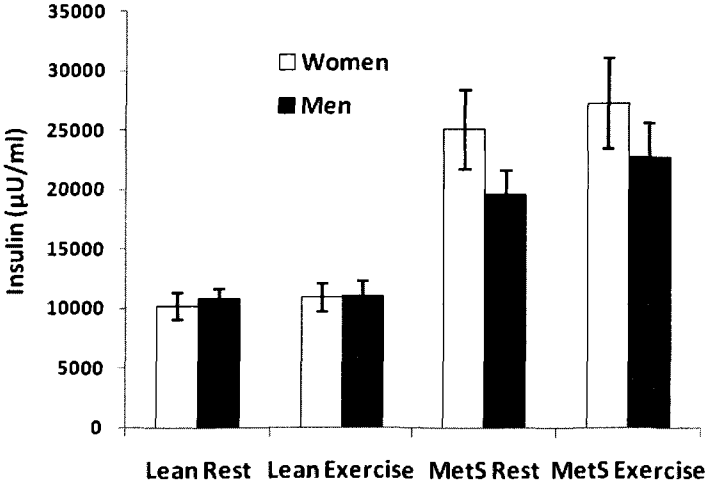
Figure 3. Insulin, glucose, and FFA

A. Postprandial Insulin

1. Response Curves

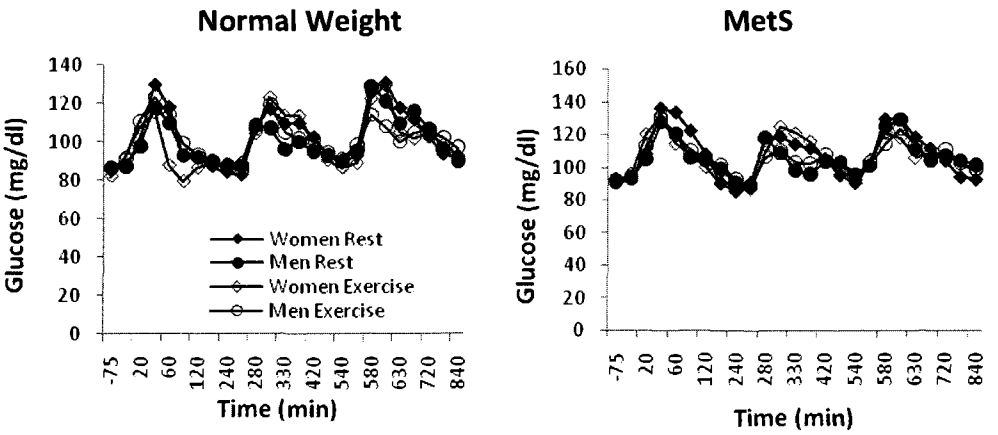


2. Insulin IAUC

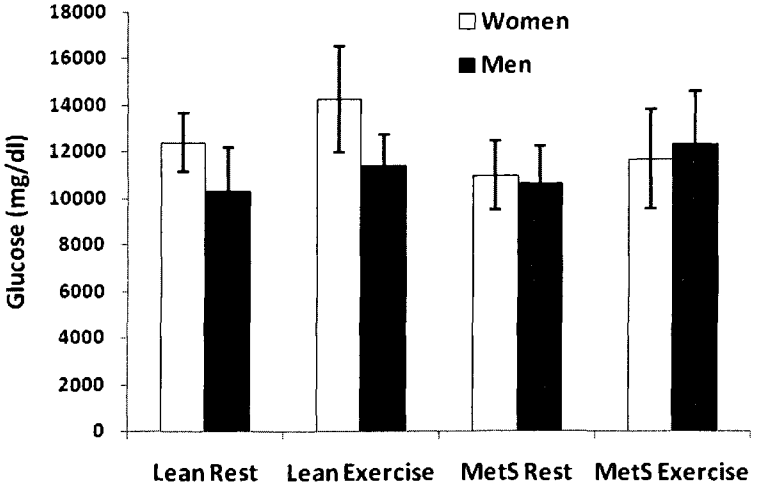


B. Postprandial Glucose

1. Response curves



2. Glucose IAUC



C. Postprandial Free Fatty Acids

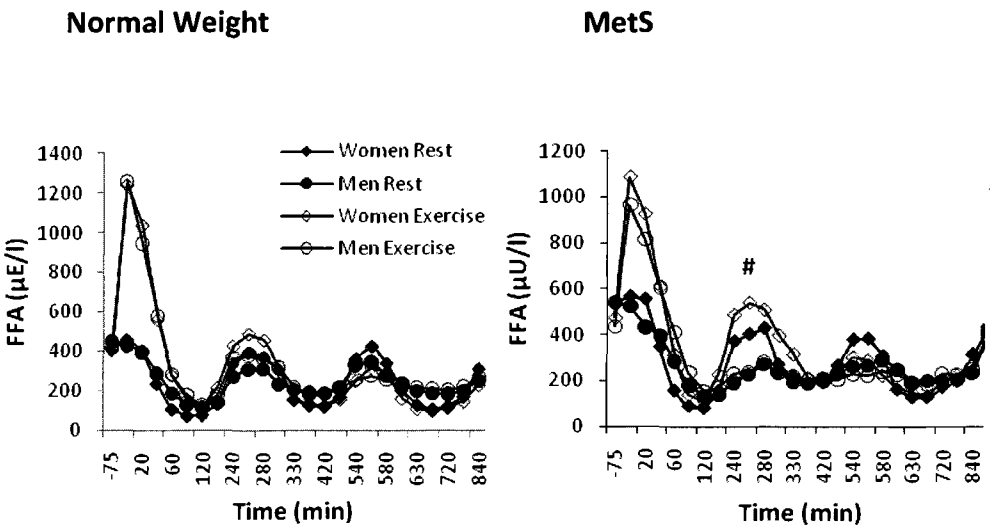


Figure 4. Total Cholesterol (IAUC)

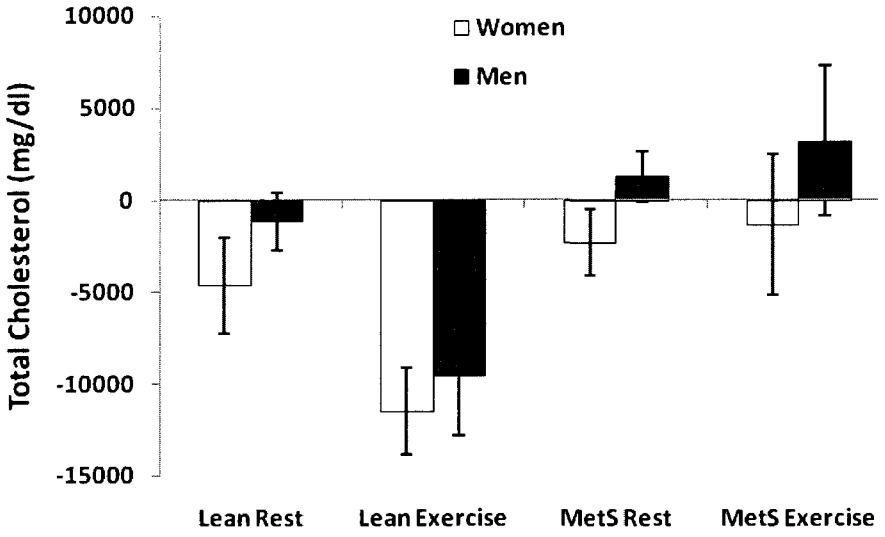
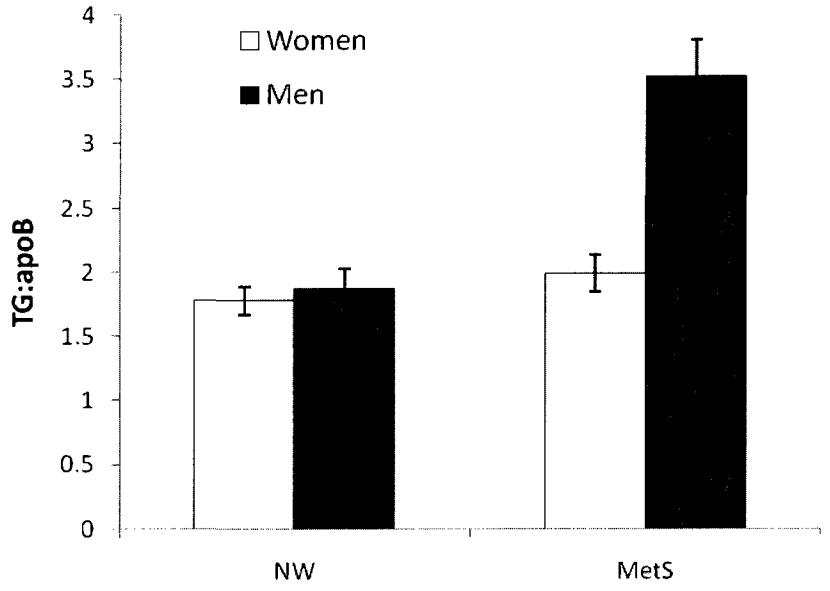
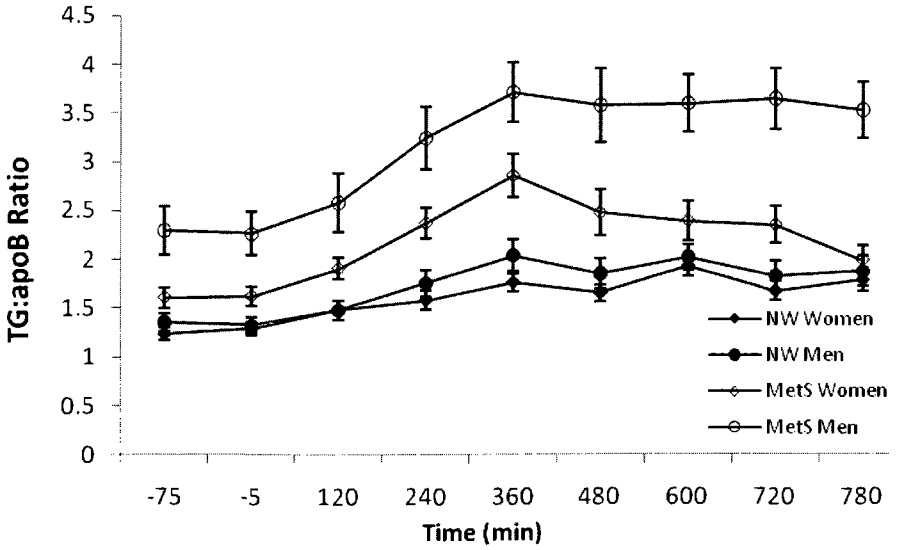


Figure 5. TRL Particle Size

A. TG:apoB (IAUC)



B. TG:apoB response curves



CHAPTER 3

Triglyceride-rich Lipoprotein (TRL) Response to Exercise and Mixed Meal Feeding

INTRODUCTION

There is now clear evidence that elevated fasting triglycerides (TG) are an independent risk factor for cardiovascular disease (CVD) [1, 2]. The majority of individuals, however, spend up to 17 hours of a 24 hour period in the fed or postprandial (PP) state [3], leading Moreton, and later Zilversmit to pursue the theory of "postprandial atherosclerosis" [4, 5]. Indeed, since this time, several studies have established PPTG as an independent risk factor for CVD [1, 6, 7]. Nonetheless, the exact contribution of elevated TG is complicated by the different triglyceride-rich lipoproteins (TRLs).

The major TRL contributing to fasting plasma TGs is very low-density lipoprotein (VLDL) TG secreted from the liver. Under fed conditions (mixed meal), although VLDL-TGs are still present, by far the major TRL contributing to PPTGs are chylomicron (CM) TGs, derived from the absorption of meal fat. Both these TRLs contribute the majority of the PPTG response, and by their dynamic metabolism, provide the macromolecular mode of TG and cholesterol transport. Additionally, high levels of TRLs are often associated with other lipid abnormalities such as decreased high-density lipoprotein cholesterol (HDL-C) and increased abnormally small low-density lipoprotein (LDL) cholesterol concentrations, together known as the atherogenic lipid triad [8].

Chylomicrons and VLDL are metabolized in the capillary beds of adipose tissue, and to a lesser extent in muscle, by lipoprotein lipase (LPL) resulting in relatively TG-poor, cholesterol-rich remnant-like lipoproteins (RLP). These particles are normally quickly taken up by the liver [9], but in dyslipidemic and PP states, the number of these particles is appreciably increased and their hepatic clearance is delayed [10-12]. Remnant particles from TRLs have been observed in human atherosclerotic plaques [13, 14], thus elevated circulating TRLs increase the likelihood that more RLPs are generated and that these RLPs will invade the intima of the arterial wall.

Specifically, retention of apolipoprotein B (apoB)-containing lipoproteins (which are present in CM, VLDL and their respective remnants, as well as LDL) has been indicated as the key initiating process in atherogenesis [15]. ApoB binds the particle it is associated with to proteoglycans on both endothelial cells and in the matrix of the plaque, and a direct relationship has been established between plasma apoB concentrations and how many particles get into and are retained in a plaque [16].

There are two forms of the apoB lipoprotein; hepatically derived apoB 100 which is associated with VLDL-TG and it's metabolic breakdown products including LDL, and intestinally derived apoB48 which associates with CM-TG and its remnants. ApoB is present on the surface of CM, VLDL and LDL and there is one apoB molecule per particle. Plasma apoB is therefore a direct measure of particle number, and along with plasma TG, can provide a general estimate of average particle size. It has been suggested that apoB is a superior predictor of CVD risk than either TG or cholesterol (non-HDL/LDL) [17-20].

Methodologically, measuring total apoB concentration can be a relatively more robust and reliable indicator of CVD risk. It is unaffected in a non-fasting state as CM-associated apoB-48 contributes little to total plasma apoB concentration, even after a fat-rich meal [17, 21]. Additionally, total ApoB levels are determined directly, rather than derived by difference from other lipid measures, as for LDL-C. Furthermore, the apoB assay has been standardized by the WHO/IFFC [22, 23], and is automated.

Several studies have shown differences in the PPTG response between men and women [24-27], and men tend to have higher plasma LDL-C and apoB levels and lower HDL-C concentrations than premenopausal women of similar age [28]. Recently, Matthan et. al. reported sex differences in the kinetics of nonfasting apoB-containing lipoproteins, and Knuth & Horowitz have observed increased clearance of CMTG in women compared to men of the same age [29, 30]. The sex differences in postprandial lipid metabolism may be one factor explaining some of the sex differences in CVD incidence. Specifically, men have a higher prevalence of CVD than women aged 20 to 44 years, a similar prevalence between 45 and 54 years and higher prevalence in women after 55 years of age [31].

This age related increase in women is believed to be due to the onset of menopause as CVD death rates are 2 to 3 times higher in post-menopausal women than in age-matched women before menopause [32, 33]. Postmenopausal women have been shown to have higher plasma total cholesterol, LDL-C, VLDL-C and TG concentrations than premenopausal women [28, 30, 34].

They have also been reported to have an elevated post-prandial lipemic response compared to pre-menopausal women [35], similar to what is observed in men relative to pre-menopausal women [36, 37], supporting a role of estrogen in the atheroprotection exhibited in premenopausal women.

Cardiovascular disease occurrence is generally assumed to be much greater in patients with the clustering of symptoms known as the metabolic syndrome (MetS), though contradictory definitions of the syndrome give rise to variation in the degree of risk associated specifically with MetS (reviewed in [38]). Often, MetS is characterized by some degree of insulin resistance, which has been suggested to be the underlying factor in the concomitant occurrence of the atherogenic lipid triad. Insulin resistance is believed to result in reduced lipoprotein lipase (LPL) activity, increased hepatic lipase (HL) activity and decreased apoB degradation leading to elevated TRL and prolonged residence time of pro-atherogenic particles [39-41]. It has been reported that MetS subjects exhibit higher PPTG than their respective controls [42], which could contribute to their increased health risk.

From studies elucidating CVD risk factors and their causative mechanisms, it is possible to target strategies that may slow the development and minimize the effects of CVD. The primary interventions to date include dietary control (such as low saturated fat, or high monounsaturated fat diets), pharmaceutical intervention (statins, metformin) and exercise. Exercise is an important life-style factor that can help decrease CVD risk.

For example, a recent meta-analysis has shown that physically active people have a lower incidence of coronary heart disease than those who are less active [43]. Although part of this beneficial effect may be due to the ability of regular exercise to increase anti-atherogenic high-density lipoprotein cholesterol (HDL-C) and decrease fasting TG levels [44, 45], exercise may have additional positive effects with respect to PP metabolism. Holme et. al., for example, reported a significant decrease in apoB and small, dense LDL particle number in the Oslo Diet and Exercise Study, a 1-year randomized diet and exercise trial [46]. As exercise is less invasive than pharmaceutical intervention, which often carries undesirable or harmful side effects and monetary cost, understanding the full benefits of exercise on PP as well as fasting lipoprotein metabolism is important.

While chronic exercise is generally associated with lower levels of postprandial lipids [47, 48] and increased TG clearance [49, 50], the association is complicated by studies indicating that the effect is due to the most recent bout of exercise [51, 52]. Gill et. al. recently reported that a moderate exercise bout (90min at 50% VO2max), on the day prior to an oral fat tolerance test, decreased concentrations of large $VLDL_1$ particles, RLP-cholesterol and RLP-TG [53]. It is important to point out however, that baseline values of these measures were significantly lower, complicating the interpretation of exercise effect on postprandial lipids *per se*. The lowering of (pre-meal) fasting TGs is a common result among studies examining exercise effects on PP lipemia [53-55] and this effect is not always taken into account when considering the effects on meal-derived TG changes.

Also common, is the use of a high fat (60-100 g) test meal [54, 56-58], the applicability of which is questionable with regard to interpreting PPL in a more standard setting, as mixed meals elicit a relatively decreased PPTG response [59-62].

The objective of the current study was to address some of the weaknesses in previous studies in the area of exercise and PP lipemia. To this end, studies were performed in both men and women, over an entire day, with the meals consumed being representative of the typical US diet (moderate fat content). Testing was performed in both low risk (normal weight (NW) subjects) and higher risk (MetS subjects) individuals. Further, the primary focus was to determine the contribution of different TRL subfractions to the total TG response. In this way, better insight may be obtained into the particular components of the PP response that may be contributing to, what may be considered, a pro- or anti- atherogenic environment.

METHODS

All subjects were studied at the General Clinical Research Center (GCRC) at the University of Colorado at Denver Health Sciences Center. Written informed consent was obtained from all subjects before participation in the study, which was approved by the Colorado Multiple Institution Review Board at the University of Colorado at Denver and Health Sciences Center.

Subjects

Two subject groups were recruited (Table 1) including normal weight (NW) healthy subjects (13 men and 13 women) and moderately obese subjects with MetS (9 men and 9 women). Subjects were recruited from the University of Colorado and surrounding area through email, print and posted ads. Inclusion criteria for both groups were 20-45 years old, non-smoking, stable body weight over the previous 6 months ($\pm$ 3kg), and less than 2h/wk moderate physical activity. Women were eumenorrheic and not using steroidal contraceptives, nor were they pregnant or lactating. Detailed exclusion criteria are reported elsewhere [63].

Screening assessments

Subjects were screened for study inclusion with a health and physical examination, including blood chemistry and lipid profile. Subjects were asked to refrain from alcohol and exercise for 48 hrs before this blood draw, and were advised to consume a low carbohydrate diet before the blood draw (2 days) to minimize variability in the screening TG levels. Women had samples drawn in the follicular phase of their menstrual cycle.

A 4-day dietary record (3 weekdays, 1 weekend day) was also completed. Subjects were excluded if the percentage of total energy intake derived from nutrients was +/- 33% of the test meal nutrient composition, (e.g. fat; <20 or >40%) to avoid including individuals with extreme dietary habits and for whom the period of pre-study dietary control, and test meal, would be a drastic change from their habitual diet.

Preliminary assessments

If subjects qualified for the study, they completed several assessments prior to the main study days, including resting metabolic rate (RMR), peak oxygen uptake test, body composition assessment (DEXA, CT scan), and determination of menstrual cycle in women as previously described [63].

Preparatory Dietary and Exercise Control

Subjects were fed a controlled diet for 5 days prior to each study day. The diet was prepared by the adult GCRC and no other food was permitted. The diet composition was identical to that of the meals consumed on the study day.

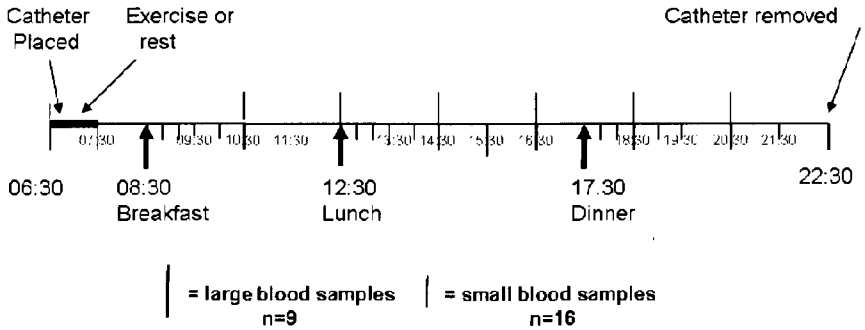
Energy intake on the controlled diet days was calculated depending on the subjects activity level: 1.50 x RMR for a no exercise or 1.7 for an exercise day [64, 65]. Body weight was measured daily and energy intake was adjusted if a consistent weight loss or gain was observed. Subjects were requested not to perform planned exercise, other than activities of daily living, for 60 hrs prior to the study. Subjects spent the evening before the study on the adult GCRC and were tested the next day following an overnight fast (12 hrs).

Experimental Protocol

Study Timeline

The evening prior to each study day, subjects were admitted to the GCRC and ate their evening meal on the unit. Subjects spent the night on the GCRC testing began the next morning after an overnight fast and continued throughout the entire day as detailed elsewhere [63]. Briefly, an intravenous (IV) catheter was placed in an antecubital vein as soon as subjects awoke. On the exercise day, subjects walked for 60 mins, at 60% of their peak oxygen uptake. Respiratory gas exchange was measured via indirect calorimetry (Sensormedics 2900, Sensormedics, Loma Linda, CA) for 2, 15-20 minute periods during the exercise bout. On the non-exercise day, this same time was spent resting during which 2, 15-20 minute measurements of respiratory gas exchange were made. Subjects remained resting in bed for the remainder of the day with the option of staying on the CRC overnight for the final fasting blood draw at 7:30 the following morning.

Study Day Timeline



Total energy intake was calculated to meet energy requirements on the corresponding day and energy intake was divided throughout the day as follows; 25%, 35% and 40% of total daily energy intake at breakfast, lunch and dinner, respectively. The composition of each meal was 34% fat, 15% protein and 51% carbohydrate, and was designed to maintain energy balance with energy intake calculated as follows: rest day, 1.3 x RMR; exercise day, 1.3 x RMR + net energy expenditure (EE) during the exercise + estimated excess post-exercise energy expenditure (10% of net EE in kcal/min x 30 mins). A factor of 1.3 RMR was used as subjects spent the majority of the day resting in bed, therefore, not very active. The cholesterol intake was 128 mg/1000 kcal.

Blood sampling and analysis

Blood samples were drawn via the IV catheter into vacu-tubes and placed on ice immediately until spinning. All samples were centrifuged for 10 minutes at 2800 rpm at 4°C, and plasma/serum samples were aliquoted then frozen for analysis by the GCRC core lab.

Total TG, cholesterol , FFA, glucose and insulin were measured on all blood samples and have been reported in detail previously [63]. On the large blood draws, HDL-C was measured [63] and LDL-C calculated by difference (Freidwald equation [66]). A portion of the large blood sample was also used to measure total apolipoprotein (apo) B. Two ml of plasma from the large blood samples was also used for the TRL separation as described below. Estradiol and progesterone were measured on baseline blood samples only.

The homeostasis model assessment of insulin-insulin resistance (HOMA-IR) was calculated using fasting values from the study day as well as the following day using the following formula [67]:

Glucose (mg/dl)/insulin (μU/ml)/405

Separation of TRL sub-fractions

Plasma was separated as described and subjected to two-stage flotation ultracentrifugation for separation of TRL. Sub-fractions of TRL were separated according to the following criteria: large (Svedberg flotation rate (Sf >400), medium (Sf 20-400), and small TRL (Sf <20). Similar designation has been used previously to identify lipoprotein sub-fractions (70). It was assumed that the large TRL sub-fraction (TRL1), was predominantly CM's with small amounts of larger VLDL particles; the medium sub-fraction (TRL2) was mostly VLDL lipoproteins (small and large) but also would include larger CM remnants, and the small TRL fraction (TRL3) would consist of intermediate density lipoproteins (IDL), remnant particles (VLDL and CM), low density-lipoproteins (LDL) and high density lipoproteins (HDL).

Specifically, 2ml of plasma was centrifuged in an Optima TLX-CE Table Top Ultracentrifuge with a TLA-100.3 rotor (Beckman Coulter, Inc., Fullerton, CA). at 100k rpm for 15 minutes at 15^{o}C. Tubes were carefully removed from the centrifuge, covered with parafilm and left to stand for approximately 30-60 minutes before removing the top, opaque layer which was visualized in a low light (xenon flashlight beam) against a dark background.

The layer was removed gradually using a calibrated pipette and transferred to a labeled micro-Eppendorf tube and the volume recorded. The remaining plasma was overlaid with 1ml (plus enough to balance tubes if necessary) NaCl density = 1.006 g/ml. Samples were then centrifuged again at 100k rpm for 2.5 hours at 15°C, removed from the centrifuge, covered with parafilm and left to stand for at least 60 minutes. The top layer was then removed under the same conditions as above, the volume recorded and stored.

Following removal of the second fraction, the infranatant (NaCl) was removed, and the volume recorded. This layer was not saved. The remaining layer was removed and stored as well. This volume was not measured, but calculated by subtraction of other layers from the original 2.0 ml of plasma. The three fractions represent the TRL1, TRL2 and TRL3 subfractions, respectively. During each stage of the separation process about 50-100ul of each subfraction was collected and kept for analysis of TG and cholesterol and the remaining volume was transferred to separate tubes for use in the apolipoprotein analysis. All TRL fractions were stored at -20°C until analysis.

apoB SDS-PAGE

Apolipoprotein B (apoB) was analyzed in the medium density (TRL2) fraction via SDS-polyacrylamide electrophoresis (SDS-PAGE) to separate and quantify levels of apoB-48 and apoB-100. This method was attempted on the low density (TRL1) and high density (TRL3) fractions as well, but the percentage of apoB relative to other protein species in these samples was too low for detection by this method. The TRL2 fraction includes particles with densities of Sf 20-400 and so contains VLDL's as well as some CM remnants, both of which are thought to be the prevailing apoB species in postprandial lipemia.

TRL2 fractions were thawed on ice and diluted in ddH20 (1:5 for NW samples and 1:10 for MetS samples) as determined by previous serial dilution experiments. 5ul of diluted sample was added to 10ul of ddH20 and 4ul 5xSDS loading buffer (250mM Tris-HCl pH 6.8, 10% SDS, 7.5% beta-mercaptoethanol, 25% glycerol, 0.02% bromophenol blue). The entire 20ul volume of each sample was loaded onto a 3-8% tris-acetate midi-gel (Invitrogen, Carlsbad, CA). Gels were run 4 at a time, with each gel containing 16-17 samples (blood samples T-75 through T720 or 780, rest and exercise per subject, and one gel per 4 had a control sample for assay calibration). Initial analysis was done with 17 samples per gel but it was found that the last band (the T780 sample) would split, compromising quantification. Future gels were therefore run excluding the T780 sample (16 samples).

As the apoB levels hardly changed over time, excluding this final sample unlikely had any impact on the results. For the NW subjects, gels were run in batches according to pair matching of male and female subjects for comparable plasma TG levels, MetS samples were run such that the rest & exercise days for 2 men and 2 women were run at the same time.

A standard curve was prepared using a TRL2 sample from a subject with very little or no apoB48. Sample concentration was determined with the BioRad DC, Lowry-based protein assay with addition of SDS to a final concentration of 2% as previously reported [68] to decrease turbidity. Standard curve concentrations ranged from 0-2ug per previous reports that this is the maximum linear concentration for apoB. At the higher concentrations (1-2ug), apoB48 as well as some smaller proteins (e.g. albumin) were visible; therefore concentrations of apoB 100 and apoB48 were calculated both with and without correction for these bands.

All gels, including the standard curve were run at 50V for 20 minutes and 100V for 2 hours. Gels were subsequently stained in separate containers but with the same batch of silver stain (Silver Stain Plus kit, BioRad) for 14 minutes per the BioRad protocol. Staining was stopped with the addition of 5% acetic acid for 15 minutes. After rinsing the gels with ddH20, they were immediately photographed (UVP Bioimaging System, UVP, Inc., Upland, CA) and density was analyzed (LabWorks 45, UVP, Inc. Upland, CA). Concentrations were calculated as mg/dl and μmol/l based on the concentration calculated from the band, multiplied by the volume of the subfraction removed, and adjusted for the initial volume of plasma removed (2ml).

Statistical analysis

Data were analyzed with SPSS version 16 and MS Office Excel. Area under the post-prandial curve (AUC) was calculated for plasma measures using the trapezoidal rule and incremental area under the curve was calculated by subtracting the corresponding days - 75 min value x duration, from AUC values. AUC and IAUC results were analyzed with univariate ANOVA covarying for age. Significant differences from ANOVA were analyzed post-hoc by t-test, where appropriate. Significance was set at $p = 0.05$.

RESULTS

Subject demographics

Table 1 displays subject demographics and these results have previously been reported [63]. As a group, the women were significantly older than the men (33 $\pm$ 7 vs. 30 $\pm$ 6 yr; p=0.02), and the MetS subjects were significantly older than the NW subjects. In the NW group, the women were significantly older than the men, but there was no difference in age per sex in the Mets group. Differences in body weight, body composition and VO2 max between groups and the sexes were generally as would be expected from the study design as previously reported [63].

Fasting values

The fasting blood values of both NW and MetS subjects are listed in Table 2, expressed as the average of the rest and exercise days at the T-75 time point. Overall, the MetS group had significantly higher fasting insulin, glucose, TG, and HOMA-IR ($p < 0.01$ for each) than the NW group, and significantly lower levels of fasting HDL-C and adiponectin ($p < 0.01$ for each). As would be expected, HDL-C was significantly higher in both NW and MetS women as compared to their respective male counterparts. Interestingly, NW women had higher levels of fasting adiponectin than NW men, but no sex differences were observed within the MetS group.

Exercise

There was no difference in the relative intensity at which subjects exercised; on average 60% of VO_2 peak, or 29 ml O_2/FFM/min. In terms of absolute VO_2 this corresponded to 2010 ml/min in NW men, 1351 ml/min in NW women, 1835 ml/min in MetS men and 1375 ml/min in MetS women. Consequently, the energy cost of the exercise was 579, 391, 550 and 412 kcals, respectively, with men having a higher energy expenditure than women.

Postprandial Triglycerides

Data on the total PPTG response have previously been reported [63] and the salient results are summarized herein (Table 3). There was no significant exercise effect on the postprandial TG response over the entire day in either the NW or MetS groups, nor in the group as a whole (NW plus MetS) when expressed as either AUC or IAUC. Collectively, the men exhibited a greater PPTG response than women over the day, expressed as IAUC (5.086×10^4 vs 3.358×10^4 mg/dl/840 mins, $p = 0.037$). The MetS group had a 44% higher PPTG response over the day when expressed as AUC and a 55% higher IAUC than the NW group ($p<0.001$ for both). Repeated measures ANOVA revealed a time x group x sex effect for PPTG ($p < 0.001$). A subsequent repeated measures ANOVA of just the MetS group verified a significant time x sex effect ($p = 0.032$) which was not seen in the NW group ($p = 0.28$).

Triglyceride response in each of the three TRL subfractions

For the TRL1 and TRL2 subfractions, ANOVA revealed no significant effect of exercise on the TG response, either overall or for any interaction by group and/or sex. Data were, therefore, combined for the 2 study days to better illustrate the group and sex differences in the diurnal pattern of TRL TG response (Figures 1 and 2, Tables 4 and 5). The MetS group had a 90% greater TRL1 TG AUC than the NW group ($p <0.001$) and men had a 15% larger AUC than women ($p = 0.01$), but there were no group or sex differences when expressed as IAUC.

TRL2 TG AUC was also significant for group (85% higher in MetS, p<0.001). With respect to IAUC, this was negative in all groups due to a decrease in TRL2 TG relative to baseline. MetS subjects had a lower TRL2 IAUC vs NW (p = 0.052) due to a greater decrease in TRL2 TG over the day.

As exercise significantly affected certain comparisons for TRL3 data, table 6 includes exercise and rest day results for each group and sex and figure 3 A and B illustrates the TRL3 response for the NW and MetS subjects on the 2 study days. The AUC for TRL3 TG was significantly different between groups (70% higher in MetS, p < 0.001) as well as between the sexes (19% higher in women, p=0.002). When adjusted for baseline values (IAUC), there was a significant day x group effect (p = 0.026) such that NW TRL3 TG decreased across the day with exercise (t-test p = 0.027), whereas in MetS subjects, TG increased. A significant sex x group interaction for IAUC (p = 0.001) was also observed as MetS women overall had a 69% higher IAUC than the MetS men (t-test p = 0.003). The largest effect was still by group with a significantly higher IAUC in MetS relative to NW (p = 0.003).

Cholesterol changes in each of the three TRL subfractions

No difference in AUC for total cholesterol (TC) was observed for any factor or combination of factors. Total TC IAUC was significantly (30%) lower in NW subjects with exercise (p = 0.003) (figure 4), while there was no exercise effect on TC in the MetS group.

A significant group effect was seen for TRL1 cholesterol AUC (MetS 35% higher than NWs; $p < 0.001$), but this was driven by differences in baseline cholesterol levels as there were no differences in IAUC (Figure 5A). For TRL2, there was no significant difference in either AUC or IAUC (figure 5B) for cholesterol. ANOVA for TRL3 AUC cholesterol revealed a significant sex-by-group effect ($p=0.013$), with NW men having higher cholesterol than NW women, and a significant day-by-group effect for IAUC ($p=0.016$, figure 5C). A post-hoc t-test established a significantly greater decrease in TRL3 cholesterol (34%) in NW subjects with exercise than in MetS subjects with exercise ($p = 0.002$).

Apolipoprotein B-100 and B-48 concentration in the TRL2 subfraction

Analysis of apoB-100 and B-48 in the TRL2 subfraction (VLDL and some CM remnants) revealed a group effect for apoB-100 AUC (MetS 56% greater than NW; $p <0.001$) and apoB-48 AUC (57% higher in the MetS group than the NW group; $p < 0.001$) (Tables 7 & 8). Adjusting for baseline gave a significant apoB-100 IAUC day x group effect ($p = 0.05$) and a t-test of MetS revealed a significant decrease in IAUC with exercise ($p = 0.04$). Overall, apoB-48 IAUC was significantly higher in the Mets vs. NW group ($p = 0.01$). ApoB100 levels were approximately 10 fold higher than ApoB48 levels for each group/sex/day. Although there were some slight changes from rest to post-meal, there was relatively no change in ApoB100 and 48 concentrations over time after consumption of the meal in any group or on any day.

The apoB-100-to-apoB-48 ratio is an estimate of VLDL-to-CM remnant particles in the TRL2 subfraction. The B-100:B-48 ratio was significantly higher in the NW subjects at all time points relative to the MetS subjects ($p < 0.05$). The difference was particularly large at the last time points of the day (T600 & 720), as the ratio in the NW subjects increased significantly over earlier NW time points (fig. 6).

The ratio of TRL2 TG to total apoB (B-100 plus B-48) is a rough estimate of particle size in the fraction. Unfortunately, the methodology employed does not enable determination of the TG quantity that was directly associated with the apoB-100 or apoB-48 protein, which would have given particle size for hepatic VLDL and CM remnants, respectively. This ratio was also significantly higher in NW subjects relative to their MetS counterparts ($p < 0.01$), indicating larger overall particle size in the NW subjects. The opposite pattern was seen in the TG:apoB ratio, with the particle size in the NW subjects declining after the breakfast meal (T-5) whereas the TG:apoB ratio remained relatively unchanged over the course of the study days (fig. 7). The plasma total TG:apoB ratio is shown in figure 8 for reference.

DISCUSSION

In this population of NW and MetS men and women, a single bout of moderate morning exercise did not change the postprandial TG response over an entire day of mixed meal feeding. The lack of exercise effect could be due to the amount or intensity of exercise, or the use of moderate fat, mixed meals, or factors specific to the study population, as reported previously [63]. There were, however, other changes in the lipid profiles directly attributable to the exercise bout that could be considered beneficial. Specifically, in the NW subjects, there was a significant exercise-induced decrease in total cholesterol over the full day, mainly due to a significant lowering of cholesterol in the TRL3 subfraction, the subfraction that contains remnant particles and LDL (albeit also HDL).

In the MetS subjects, a significant lowering of apoB-100 in the TRL2 subfraction was observed with exercise and a modest decrease in total apoB in the group as a whole was observed. Finally, an increase in the TG content of the TRL3 fraction in MetS subjects with exercise, but no change in cholesterol, suggests more lipid and less cholesterol enriched particles in this dense sub-fraction. Such responses further support a beneficial contribution of exercise in CVD outcomes.

The exercise-induced lowering of total cholesterol in the NW subjects was more significant in men ($p = 0.02$) than women ($p = 0.06$), and was not seen at all in the MetS subjects. Analysis of TRL cholesterol revealed a significant day x group effect for cholesterol in the TRL3 fraction only ($p = 0.016$), indicating that this is where the drop is occurring. Cholesterol in this subfraction is contained in the dense TRL particles, namely IDL, LDL, HDL and to a lesser extent remnants from VLDL and CMs. One potential mechanism for the fall in cholesterol level in the TRL3 subfraction could be increased reverse cholesterol transport activity (RCT) with exercise, as supported by studies of the effects of exercise on lecithin:cholesterol acetyltransferase (LCAT).

Olchawa et. al., have reported that trained men have higher fasting levels of LCAT activity without increased protein expression than untrained controls [69]. The athletic men also had higher fasting levels of HDL-C, as expected, and plasma from the trained men induced a 16% greater cholesterol efflux from a macrophage cell line, suggesting that they had greater reverse cholesterol transport (RCT) capability. Furthermore, Frey et. al. measured LCAT activity in trained and untrained men at baseline and immediately following a maximal aerobic stress test [70]. They found that while LCAT did not differ between the groups at baseline, its activity increased significantly in both the trained and untrained subjects with the exercise bout.

The NW subjects in the present study did have higher fasting total HDL-C and maintained a higher level than the MetS subjects over the day (data not shown). There were no significant differences in HDL-C between the rest and exercise days, but it does appear to decrease appreciably in the NW men on the exercise day.

This could also be explained by an exercise-induced increase in cholesterol ester transfer protein (CETP) which has been reported to increase with exercise relative to a non-exercise control day [71], and to both decrease HDL cholesterol [72] and increase reverse cholesterol transport [73]. Analyzing plasma LCAT and CETP activity and HDL-C in the TRL3 subfraction could help elucidate the mechanism for the lowering of TC in this sub-fraction and therefore plasma TC.

While chronic exercise is often promoted as lowering cholesterol, a recent review of 84 exercise training trials (ie. chronic activity) found that only 7-10% of these studies reported significant decreases in fasting TC [74]. The present data might suggest, however, that the dynamics of the metabolism of cholesterol-containing particles and how exercise might influence these dynamics is as important as the total cholesterol level.

Total plasma apoB was modestly reduced with exercise in the group as a whole ($p = 0.064$). It appears that the main exercise effect was in the men, whose apoB tended to decline over the day with little change in the levels in women. The NW women maintained a lower total apoB concentration over an average of the rest and exercise days than NW men. The apoB concentration of the men on the exercise day declined to the level of the women by 120 minutes post-meal. The MetS group, interestingly, exhibited the opposite pattern, with the women maintaining higher apoB levels over both days. While MetS men had slightly lower apoB concentrations on the exercise day, the decline was not as great as in the NW men.

The significant decline in apoB100 and thus total apoB in the TRL2 subfraction of the MetS group as a whole was also likely to contribute to the decrease in total plasma apoB. Given that CVD is elevated in MetS in this age group, this data suggests that these populations may derive particular benefit from moderate exercise with regard to circulating atherogenic, apoB-containing particles.

Perhaps the most intriguing finding from the current study was the marked decline in PPTG in the MetS women in the second half of the study day(s). The same pattern was seen in the TRL1 TG fraction. This has been described previously by Knuth and Horowitz [29], who studied the response to a high fat meal in NW men and women. Using stable tracers, they demonstrated the well-reported lower PPTG AUC response in women relative to men [26, 75, 76], and further identified the difference as a significantly lower CM-TG enrichment in the women, beginning at about 6h post-meal. This is precisely the time of the decline in the MetS in the current study. These data suggest that MetS women were either more effectively clearing large TRL particles, (predominantly CM TG), or had decreased appearance of large TRL particles (lower absorption of CM-TG or reduced secretion of larger VLDL-TG) relative to the MetS men.

Sadur and Eckel have demonstrated a significant increase in adipose tissue lipoprotein lipase (AT-LPL) approximately 6 hours after insulin infusion relative to saline [77]. Though LpL was not measured in the current study, it is possible that the significantly greater insulin response to both the breakfast and lunch meals in the MetS women relative to MetS men, initiated increased LpL activity which peaked at the 6 h time point and resulted in the subsequent decrease in TG concentrations.

Votruba and Jensen have characterized LpL activity in men and women and have determined that LpL activity in women is greater than in men per gram of adipose tissue, and that the effect is particularly significant at the thigh [78]. Horton et. al. have also characterized this sex difference as increased uptake of CM-TG across the leg in women as compared to men [26].

The MetS women also had a significant increase (to about baseline) in FFA at mid-day relative to MetS men. It is possible that more FFA were released into the circulation as a result of increased TRL lipolysis, as has been reported in limb-balance and enzyme binding studies [79, 80], if that is indeed the cause of the decline in plasma TG at mid-day.

With the intention of identifying the source (liver or intestine) of PP lipids and estimating particle size (TG: apoB) in the TRL2 (Sf 20-400) subfraction, apoB-100 and apoB-48 were measured in addition to TG and cholesterol. While the MetS group had only an 8% greater average total plasma apoB concentration than the NW, both apoB-100 and apoB-48 concentrations were twice that of the NW subjects in the TRL2 subfraction. This doubling of apoB moieties without a concomitant doubling of TG concentration in the MetS subjects yields TRL particles that are significantly smaller than those in the NW subjects ($p < 0.001$). The significance of smaller particles is that they are better able to penetrate the endothelium and contribute to plaque formation [13, 14, 17]. Encouragingly, however, exercise marginally, but significantly decreased apoB-100 in the TRL2 subfraction (comprised mainly of VLDL) in the MetS group (p 0.044), supporting a therapeutic role in lipid lowering in this population.

The main limitation of the current study was the inability, due to technical restraints, to measure apoB-100 and 48 in the TRL1 and TRL3 subfractions. While the TRL2 fraction contains the majority of apoB-containing TRL particles, it would have been useful to have estimates of particle sizes in the other fractions to complete the story. Plasma and/or tissue LpL measures would also have been helpful in elucidating the mechanism of the mid-day decline in TG in the MetS women. The elevated insulin early in the day inducing LpL activity 6h later fits nicely with the current data, but would need to be validated with enzyme measures. Similarly, the mechanism of exercise-induced cholesterol lowering in the NW subjects could be further elucidated with HDL-C and LCAT/CETP measures in the high-density subfraction. Analysis of visceral fat in the current study population might also be useful given the contribution of visceral fat to insulin action and lipid metabolism (reviewed in [81]).

The present study underscores the heterogeneity in PP lipid response between subjects of different sexes and metabolic states, and enforces the mandate for tailored prevention and treatment strategies for CVD and related conditions. The current data support exercise as a major modifiable behavior in the reduction of CVD risk.

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FIGURE LEGENDS

FIGURE 1. TRL1 PPTG response curves (mg/dl) in (A) NW men (n = 13) and women (n = 13) and (B) MetS men (n = 9) and women (n = 9) on rest and exercise study days. Values are averages plus/minus SEM.

FIGURE 2. TRL2 PPTG response curves (mg/dl) in (A) NW men (n = 13) and women (n = 13) and (B) MetS men (n = 9) and women (n = 9) on rest and exercise study days. Values are averages plus/minus SEM.

FIGURE 3. TRL3 PPTG response curves (mg/dl) in (A) NW men (n = 13) and women (n = 13) and (B) MetS men (n = 9) and women (n = 9) on rest and exercise study days. Values are averages plus/minus SEM.

FIGURE 4. Total cholesterol IAUC (mg/dl/840 min) in NW and MetS men and women on rest and exercise days. Values are averages plus/minus SEM.

FIGURE 5. TRL cholesterol response curves (mg/dl) (A) TRL1, (B) TRL2, and (C) TRL3 for NW (a) and MetS (b) subjects. Values are averages plus/minus SEM.

FIGURE 6. TRL2 apoB-100:apoB-48 response curves (mg/dl/840min) for NW and MetS subjects (average of rest and exercise days). Values are averages plus/minus SEM.

FIGURE 7. TRL2 TG:apoB response curves (umol/l) for NW and MetS subjects (average of rest and exercise days). Values are averages plus/minus SEM.

FIGURE 8. Total plasma TG:apoB response curves (mg/dl) for NW and MetS men and women on rest and exercise days. Values are averages plus/minus SEM.

Table 1. Anthropometric Characteristics of Subjects

	NW		MetS	
	Men	Women	Men	Women
N	13	13	9	9
Age (yr)	26.4 ± 3.6	31.1 ± 7.1	34.9 ± 2.4	36.3 ± 2.0
Height (m)	1.83 ± .09	1.66 ± .081	1.75 ± .03	1.66 ± 0.02
Body weight (kg)	79.7 ± 9.2	60.3 ± 7.1	92.8 ± 3.9	79.4 ± 3.0
BMI (kg/m2)	23.7 ± 1.6	21.9 ± 1.9	30.2 ± 0.9	28.7 ± 0.91
FM (% body weight)	20.9 ± 3.8	27.3 ± 6.5**	29.3 ± 1.2	36.0 ± 1.2**
FFM (% body weight)	80.0 ± 3.8	72.8 ± 6.5**	70.6 ± 1.3	63.6 ± 1.3**
VO2 Peak (mL/min)	3308.4 + 394	2231.4 + 319	3162.6 ± 264	2352.9 ± 331
VO2 Peak (mL/kgFFM/mn)	52.7 ± 1.04	51.1 ± 1.4	48.6 ± 1.4	46.9 ± 1.6

Values = Mean +/- SD

$p < 0.05$ = *
$p < 0.01$ = **
$p < 0.001$ = # } within group

$p < 0.05$ = $ between groups

Table 2. Fasting Plasma Measures

	Normal Weight		MetS	
	Men	Women	Men	Women
Total TG (mg/dl)$	104 ± 7.7	94.3 ± 7.4	174 ± 11	151 ± 12
Total apoB (mg/dl)	78 ± 3	78 ± 5	83 ± 4	92 ± 4
Total Cholesterol	161 ± 5	170 ± 8	153 ± 7	168 ± 7
FFA (μE/L)$	435 ± 25	410 ± 28	489 ± 48	502 ± 38
Glycerol (uM/L)	55 ± 4	68 ± 5	76 ± 7	83± 5
HDL-C (mg/dl)$	41.9 ± 1.6	46.8 ± 1.8*	31.2 ± 1.1	37.4 ± 0.87 #
LDL-C (mg/dl)	45.7 ± 0.8	45.5 + 1.3	52.1 + 3.4	54.1 + 3.6
TRL1 TG (mg/dl)$	42.9 ± 4.0	40.7 ± 7.4	112.5 ± 7.8	96.1 ± 9.0
TRL2 TG (mg/dl)	22.0 ± 2.3	19.4 ± 1.7	26.9 ± 3.2	19.3 ± 2.2
TRL3 TG (mg/dl)	17.6 ± 1.1	22.7 ± 1.1	19.3 ± 1.2	20.1 ± 1.2
TRL1 Cholesterol$	18.0 ± 2.4	17.5 ± 3.6	39.4 ± 3	38.1 ± 3.3
TRL2 Cholesterol	10.8 ± 0.9	9.4 ± 1.3	10.1 ± 1	8.6 ± 1
TRL3 Chlesterol	124 ±4.7	130.3 ± 8.3	105.4 ± 6.3	128.7 ± 5.4*
Insulin (uU/ml)$	5.2 ± 0.51	5.3 ± 0.51	11.7 ± 1.3	15.4 ± 1.7
Glucose (mg/dl)$	86.7 ± 1.2	84.5 ± 1.3	91.6 ± 1.9	92 ± 1.6
HOMA-IR$	1.12± .11	1.10 ± .11	2.60 ± 0.29	3.6 ± 0.44**
Adiponectin$	6.7 ± 0.66	9.1 ± 0.83*	3.99 ± 0.43	3.2 ± 0.42
Estradiol	30.1 ± 1.8	84.1 ± 10.4	35.0 ± 2.0	60.3 ± 8.8**
Progesterone	1.00 ± 0.08	0.68 ± 0.08	0.59 ± 0.033	0.72 ± 0.01

$p < 0.05 = *$
$p < 0.01 = **$ } within group
$p < 0.001 = \#$
$p < 0.001 = \$$ between groups

Table 3. Integrated Postprandial Triglyceride Response (mg/dl/840min)

	Study Day	NW men N= 13	NW women N=13	MetS men N=9	MetS women N=9
AUC*	Rest	117374 ± 12064	99793 ± 7292	219928 ±23107	170287 ± 20230
	Exercise	112018 ± 12916	101315 ±16014	217828 ±24251	182384 ± 23902
IAUC*$	Rest	24780 ± 5889	23967 ± 2654	72554 ± 10693	44894 ± 10104
	Exercise	30215 ± 6969	18801 ± 6463	73021 ± 14944	52651 ± 11254

* = $p < 0.001$ between groups (NW vs. MetS)
$ = $p < 0.05$ Men vs. Women

Figure 1. TRL1 PPTG

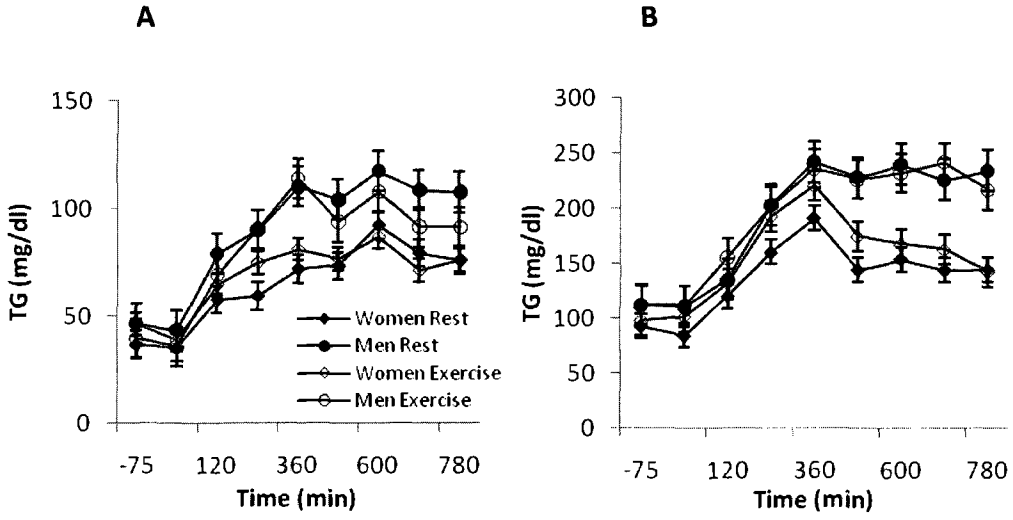


Table 4. Integrated TRL1 TG response (mg/dl/780 min)

	NW men N= 13	NW women N=13	MetS men N=9	MetS women N=9
AUC*$	71722 ± 6705	61273 ± 6956	703713 ± 33847	596090 ± 56278
IAUC	38259 ± 4945	29698 ± 2889	72086 ± 26484	46472 ± 31524

* = $p < 0.001$ between groups (NW vs. MetS)

$ = $p < 0.05$ Men vs. Women

Figure 2. TRL2 PPTG

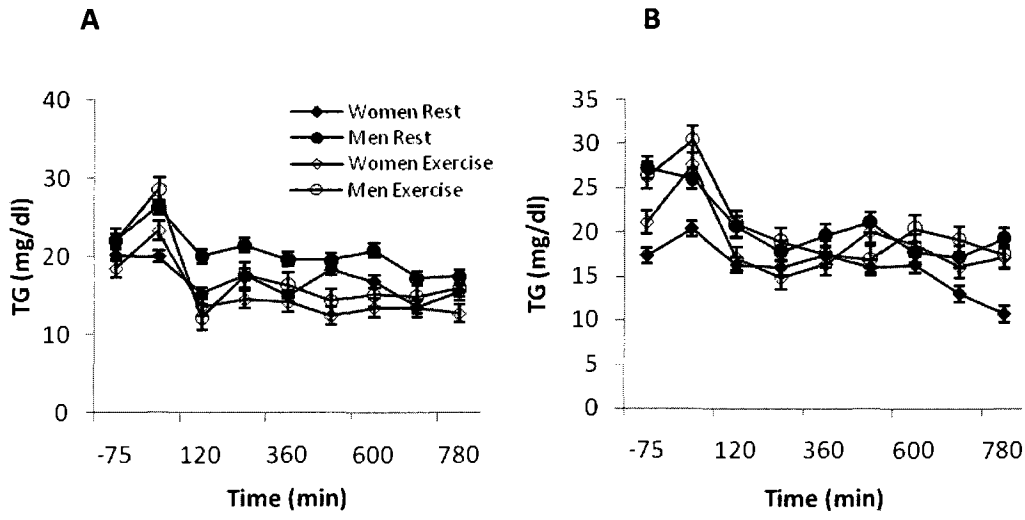


Table 5. Integrated TRL2 TG response (mg/dl/780 min)

	NW men N= 13	NW women N=13	MetS men N=9	MetS women N=9
AUC*	13810 ± 1384	11565 ± 1002	86432 ± 13000	85125 ± 10882
IAUC*	(-)3295 ± 1064	(-)3364 ± 1114	(-)33255 ± 13814	(-)30510 ± 11429

* = $p < 0.001$ between groups (NW vs. MetS)

Figure 3. TRL3 PPTG

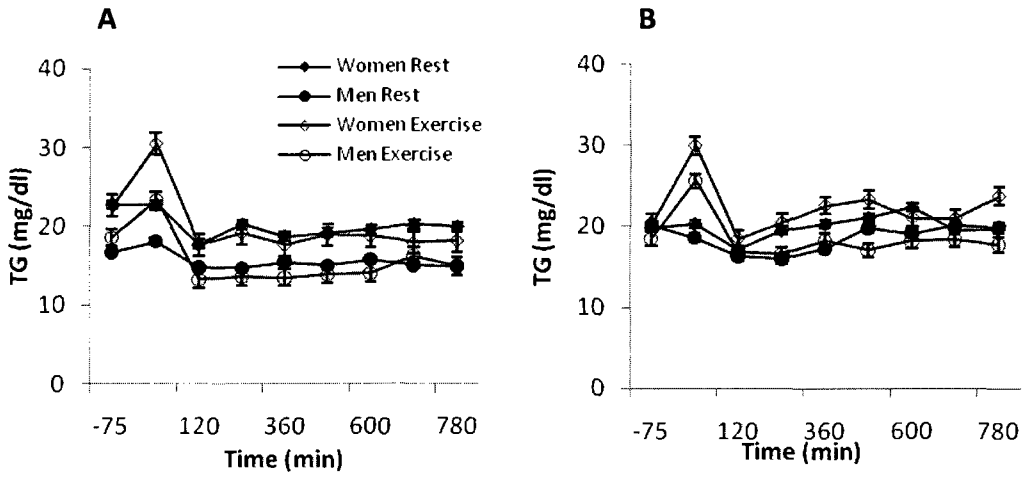


Table 6. Integrated TRL3 TG response (mg/dl/780 min)

	Study Day	NW men N= 13	NW women N=13	MetS men N=9	MetS women§ N=9
AUC*$	Rest	11846 ± 736	15218 ± 1087	39488 ± 3553	45422 ±3190
	Exercise	11030 ± 510	14484 + 1086	39267 ± 3451	49115 ± 4468
IAUC#Ŧ	Rest ¥	(-)1171 ± 988	(-)2522 ± 581	1658 ± 1216	6118 ± 1156
	Exercise	(-)3891 ± 1198	(-)2177 ±1780	2737 ± 1631	7992 ± 1968

* = p < 0.001 between groups (NW vs. MetS)
$ = p < 0.01 Men vs Women
# = p <0.05 Day x Group effect
Ŧ = p < 0.001 Gender x Group effect
¥ = p = 0.03 NW Rest vs Exercise
§ = p = 0.003 MetS Women vs. Men

Figure 4. Total Cholesterol IAUC

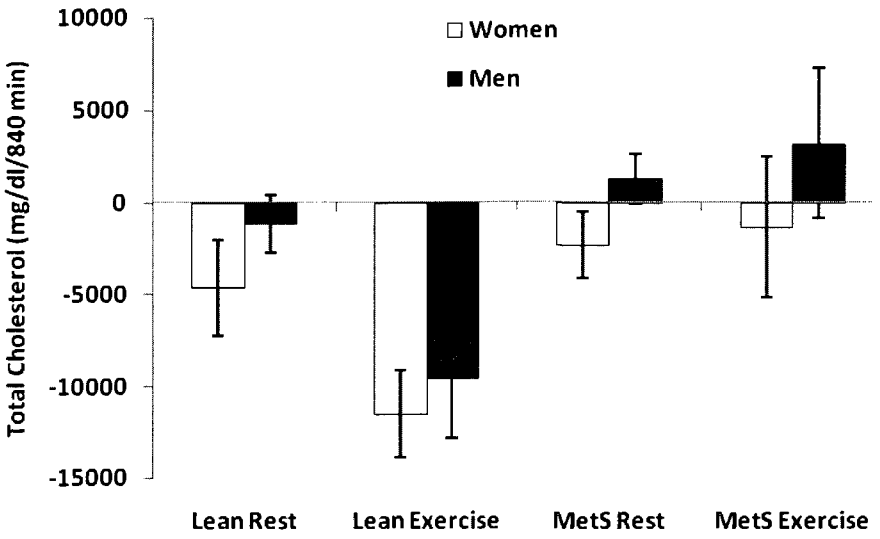


Figure 5. TRL Cholesterol

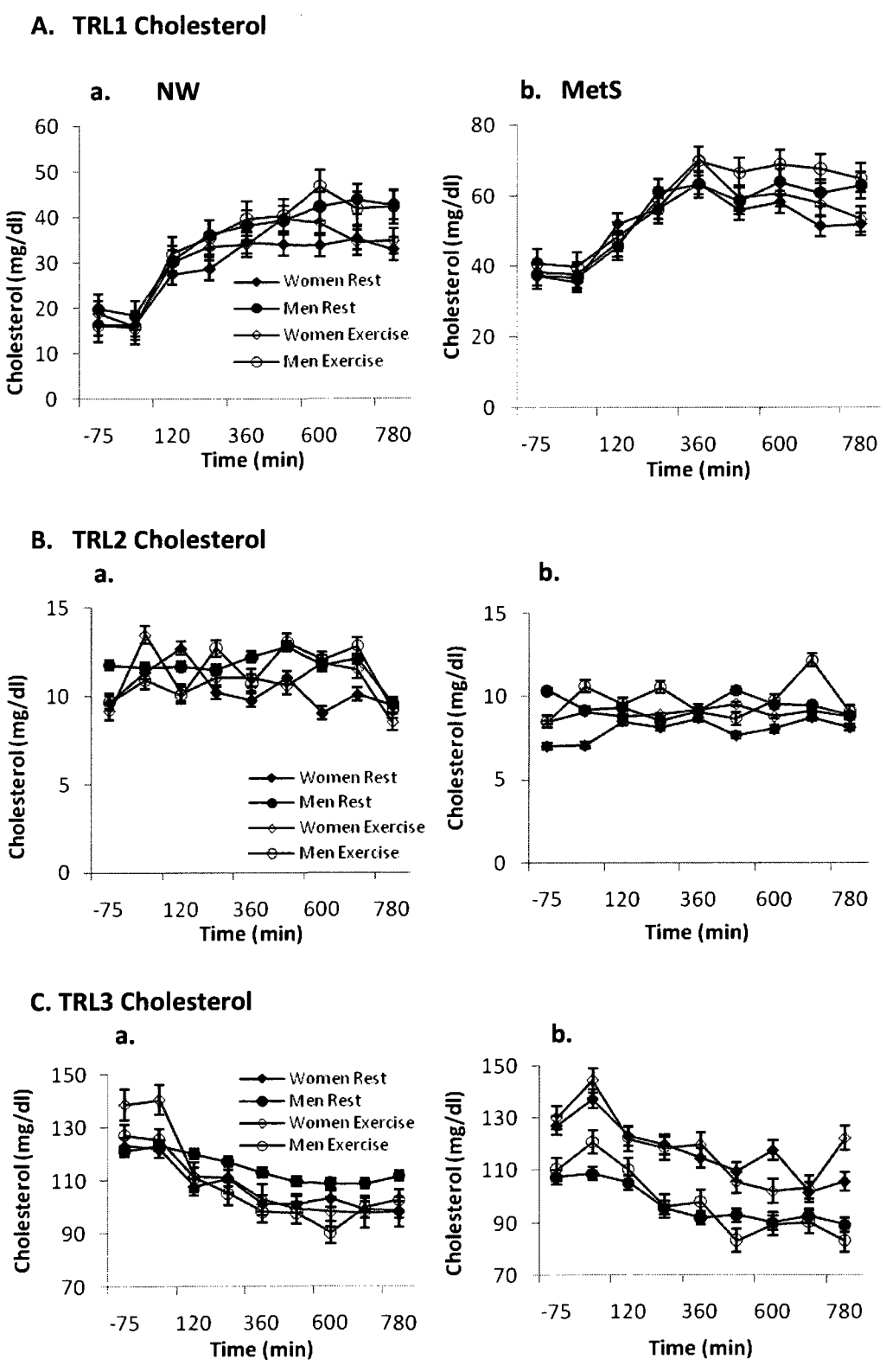


Table 7.
Integrated apoB-100 Response in TRL2 Subfraction (µmol/l/720 mins)

	Study Day	NW men N= 13	NW women N=13	MetS men N=9	MetS women N=9
AUC *	Rest	133.8 ± 21	144.5 ±23	238 ± 24	289.3 ± 48
	Exercise	112.4 ± 20	124 ± 19	246 ± 20	324.5 ± 35
IAUC#	Rest ¥	(-)9.3 ± 18	23 ± 25	13.9 ± 31	65.3 ± 25
	Exercise	14.9 ± 15	1.3± 15	(-)6.9 ± 44	30.6 ± 41

* = p < 0.001 between groups (NW vs. MetS)
# = p = 0.054 Day x Group effect
¥ = p = 0.044 Rest vs. exercise (MetS)

Table 8.
Integrated apoB-48 Response in TRL2 Subfraction (µmol/l/720 mins)

	Study Day	NW men N= 13	NW women N=13	MetS men N=9	MetS women N=9
AUC*	Rest	19.6 ± 6	15 ± 3	38. ± 5	33.9 ± 6
	Exercise	13.5 ± 5	14.2 ± 3	32 ± 4	29.5 ± 4
IAUC**	Rest	7.7 ± 2.8	3.3± 3	9.1 ± 4	15.3 ± 4
	Exercise	3.7 ± 4	1.8 ± 3	1.0 ± 7	8.3 ± 3

* = p < 0.001 between groups (NW vs. MetS)
** = p =0.01 between groups (NW vs. MetS)

Figure 6. TRL2 apoB-100:apoB-48

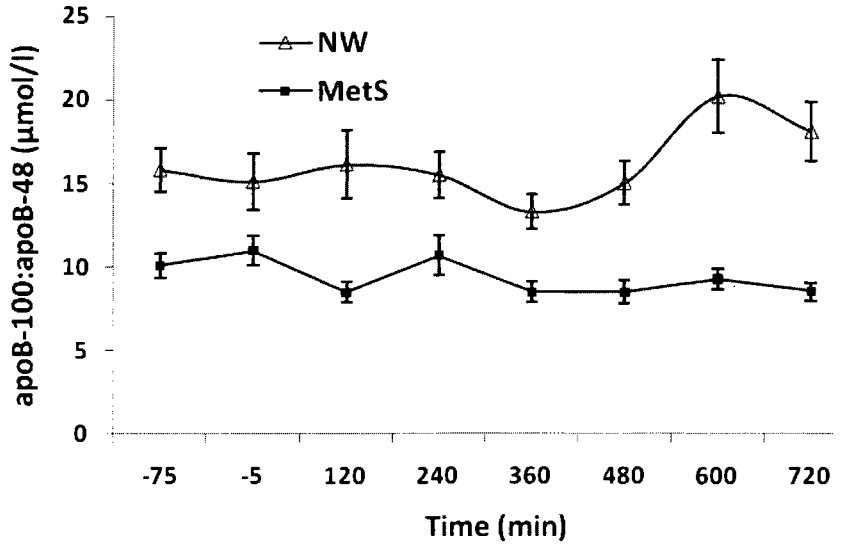


Figure 7. TRL2 TG:apoB

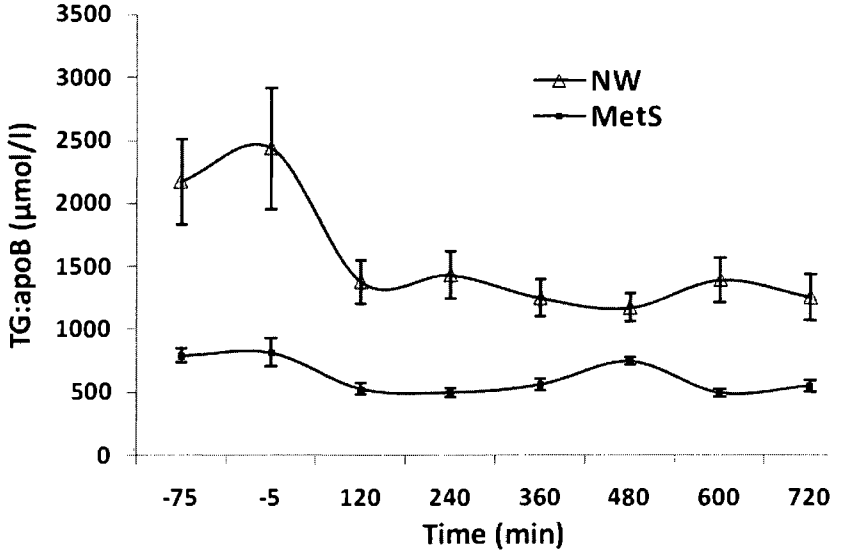
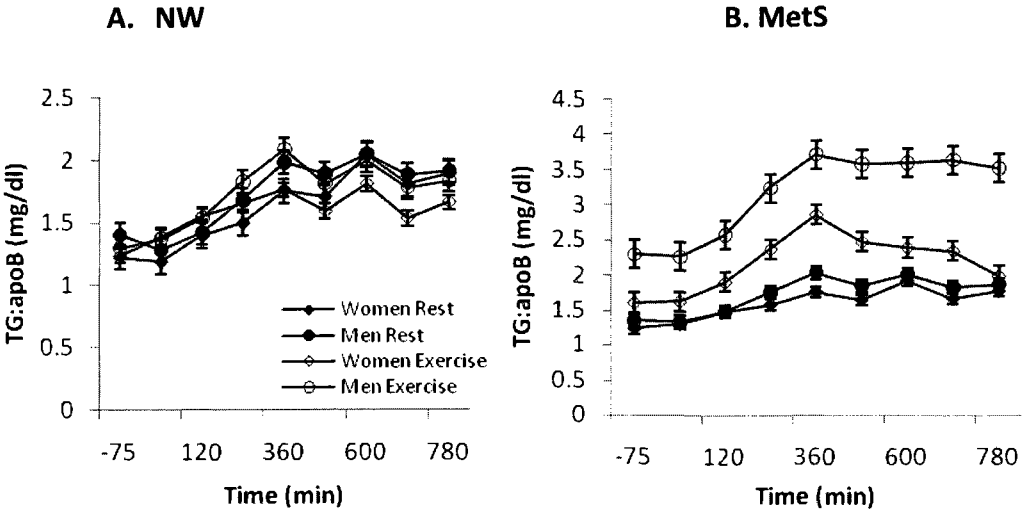


Figure 8. Total Plasma TG:apoB



CHAPTER 4

Discussion

DISCUSSION

A major aim of the present study was to address several gaps in the literature in the area of exercise and postprandial lipemia (PPL) namely by a) using a moderate exercise bout, in terms of intensity and duration, applicable to the general population; b) controlling diet and exercise leading into the study to minimize confounding effects due to subject variation in usual habits; c) feeding mixed meals with moderate fat content (35% kcal), typical of the US diet, spaced over the day as might be expected in a usual day; d) studying pre-menopausal women in the follicular phase of the menstrual cycle to minimize effects of estrogen; and e) including men, women, NW and MetS subjects for direct comparison under identical conditions. The primary outcome was the PPTG-lowering potential of the exercise bout, with secondary outcomes being changes in triglyceride rich lipoprotein (TRL) and apolipoprotein (apoB) concentrations.

It was hypothesized that a moderate exercise bout would decrease the PPTG response in men and women and that women would have a greater PPTG lowering than men in response to the exercise bout. It was also hypothesized that exercise would "normalize" PPTG in MetS subjects to a level closer to that of the NW subjects without exercise. In this population of NW and MetS, men and women, however, a single bout of moderate morning exercise did not change the PPTG response over an entire day of mixed meal feeding.

There were, however, other changes in the lipid profiles directly attributable to the exercise bout including a significant decrease in total cholesterol (mainly in the TRL3 subfraction) and TRL2 TG in the NW subjects; a significant lowering of apoB-100 in the TRL2 subfraction of the MetS subjects, and a modest decrease in total apoB in the group as a whole, further supporting a potential beneficial contribution of exercise to CVD risk factors. As described in the literature review, and outlined below, several factors contribute to the overall PPTG response and the current study illustrates the complexity of these factors in determining whether or not exercise significantly affects the final outcome.

The lack of an effect of exercise on the total PPTG response contrasts with many previous studies but not all [1-3]. Reasons for the lack of change observed in the current investigation likely relate to the study design, including rigorous control of diet and exercise pre-testing, as well as what may be considered a more relevant study design, that is, utilizing a moderate intensity/duration exercise bout, evaluating multiple mixed meals to address the full days response, exercise performed on the same day as the test meals, and replacement of activity energy expenditure to minimize energy balance differences between control and exercise days.

As previously mentioned, many prior PP studies, involving exercise or not, have used high to very high fat meals (60-100g) to elicit the PP response [4-9]. Several studies, however, have demonstrated significant differences in the PPTG response with varying amounts of fat, ranging from no effect with 5-15g to a dose-dependent increase from 30-50g and a non-dose-dependent increase with levels above 80g [10-12].

The content of the meals used in the current study was based more closely on recommendations from the USDA Food Guide Pyramid [13] and Dietary Guidelines for Americans [14] with regard to macronutrient intake, and the standard intake of most Americans over a typical day [15]. The average fat intake in the current study was about 80g per subject over each study day.

The macronutrient content of a meal can significantly alter the PPTG response irrespective of exercise and the response may also vary depending on the population studied. This is demonstrated by Dallongeville et. al., who directly compared a high fat (HF-70g) meal to a high carbohydrate (CHO-166g) meal in NW and obese women [6]. While the HF meal elicited very little glucose response in either group, the high CHO meal elicited a large glucose effect in the obese women that was significantly greater than in the NW women. The triglyceride response was considerably more dramatic, with the HF meal inducing a rapid, large, almost identical peak in both NW and obese women, whereas the CHO meal induced a relatively blunted, delayed TG peak in the obese women and almost no change in the NW women.

Similar glucose and TG responses were seen by Alssema *et. al.* in postmenopausal women who were either normoglycemic or had T2DM [16]. This group administered two meals (50 g fat + 56 g CHO or 162 g CHO + 4 g fat), however, spaced 4h apart, and included insulin measures. As would be expected, the CHO meal elicited a significantly greater insulin response than the HF meal, and the T2DM women had significantly larger insulin responses than the normoglycemic subjects.

The insulin response has the effect of decreasing hormone sensitive lipase, which decreases adipose tissue lipolysis and increases lipoprotein lipase which hydrolyzes circulating TRL lipids. These factors are important to consider when evaluating the current study due to the mixed metabolic status of the subjects i.e. the different degree of PPTG response between NW and MetS groups. Additionally, the magnitude of the exercise effect is relative to the baseline (rest day) PPTG effect, which is lower with the mixed meal than with a high fat meal.

By using a mixed meal in the current study, the PPTG response was blunted relative to the high fat meals used in other studies [8, 17, 18], creating a smaller window for observing any significant effect of exercise. Moreover, the addition of carbohydrate to the meal elicits an insulin response that could oppose some of the potential TG-lowering effect of exercise. For example, exercise increases leg muscle LpL activity [19-21] which would help to clear circulating TG, however, the insulin response to carbohydrate in the meal would lower muscle LPL activity [22] and likely decrease muscle TG clearance. Whereas Zhang *et. al.* demonstrated a significant decrease in PPTG with a moderate exercise trial (60 min at 60% VO_{2max}) just prior to a very high fat meal (90% kcal from fat) [18], Petridou *et. al.* reported no effect on the PPTG AUC with a similar amount of exercise (45 minutes of cycling at 62% VO_{2max}) just prior to a moderate fat, mixed meal (35% kcal from fat) [3]. Nevertheless, a lowering of the PPTG response has been reported after mixed meal feeding [23, 24] so the potential for observing a positive effect with such meals is apparent.

Additionally, the exercise itself may not have been long enough or at a high enough intensity to elicit a change in PPTG. Murphy *et. al.* reported that brisk walking (60% VO_{2max}) for 30 minutes prior to feeding mixed meals at breakfast, lunch and dinner decreased PPTG. This study was quite different, however, in other aspects compared to our current study design as it tested a small group of men and women (n=7) who were all overweight or obese, and older (34-66 yrs) than those in the current study (22-38 yrs). Age can affect the PPTG response with older subjects (41-50 yrs), demonstrating an increase in TGs 6 hours post-meal relative to younger subjects (31-40 yrs) whose response leveled off at this time point [24]. Even younger subjects (20-30 yrs) PPTGs tend to decrease at 6 h. Moreover, as women generally have a lower PPTG response than men [25-27], it is difficult to interpret the results with mixed sexes. Pfeiffer *et. al.* on the other hand, administered a mixed meal (33% fat) to young (25y), NW men immediately after 30, 60, or 90 minutes of moderate (50% VO_{2Max}) exercise and saw no significant decrease in PPTG with any duration of exercise, despite a 67% increase in energy expenditure between the longest and shortest bouts [28].

Moderate intensity exercise has been shown to induce a greater decrease in PPTG than lower intensities as reported by both Tsetsonis [9] and Katsanos [8]. In both studies, subjects walked on a treadmill at 25-35% V_{O2max} and at 61-65% V_{O2max}, and both found that moderate intensity exercise reduced PPTG response relative to no exercise control days, whereas low intensity exercise did not. These results were observed despite equivalent exercise duration (90 min, Tsetsonis et. al.) or energy expenditure [(EE) 1100 kcal, Katsanos et. al.].

The latter study is in line with most previous investigations reporting an exercise-induced decrease in measures of PP lipemia when exercise EE was at least 1000 kcal [8, 29-32]. A recently published study in MetS men reported that an exercise bout, resulting in 500 kcal EE, significantly reduced the PPTG response to a high fat meal [33]. Energy expenditure in the current study did not reach this level, with the NW subjects expending an average of 484 kcal and the MetS group 481 kcal with the 60 min exercise session at 60% VO_2 max. There is evidence that subjects with a lower VO_2 max (< 48 ml/kg/min) may not need to expend as much energy as those with higher VO_2 max but it still may require as much as 500 kcal [18, 34-36]. The average VO_2 max in the current subjects was 36 ml/kg/min, indicating a non-athletically trained population, but not necessarily "unfit". It is important to consider, therefore, the feasibility of expending at least 500 kcal/day in subjects who are physically active but not necessarily trained *per se*.

Independent of exercise intensity or duration, the replacement of the energy expended with exercise in the current study may have negated any affect that might have been seen had the energy not been replaced. Burton *et. al.* recently reported that a moderate exercise bout (expending 27kJ/kg body mass per subject) in overweight or obese men resulted in a lower PPTG response, reduced insulin concentrations, and increased fat oxidation the following day as compared to non-exercised controls [37]. When the energy expended with exercise was replaced, the insulin and fat oxidation effects remained, but the TG response was abolished. [38-41].

In addition, in the non-energy replaced group compared to those with energy replacement, or the control day, fasting TGs the day following exercise were significantly reduced, therefore, although the total TG response (AUC) was lower under the non-energy replaced condition, when baseline TG were taken into account as IAUC, the PPTG effect was no longer significant. While it is clinically important that fasting TG were reduced, the effect on PPTG *per se* is not immediately clear

Potential mechanisms for the PPTG lowering effect of exercise:

Relevance to lack of effect in the current study

1. Muscle Triglyceride depletion and repletion

It has been frequently suggested that the mechanism of PPTG lowering by exercise relates to muscle depletion of TG stores [18, 19, 42]. Moreover, it has been reported that muscle TG is utilized to help fuel the repletion of muscle glucose stores post-exercise [43]. Hence, when both carbohydrate and lipid are consumed in a meal this would help replete both muscle TG as well as glycogen stores depleted during exercise. In an energy replaced state, therefore, the metabolic "sink" for PPTG, that is, depleted muscle TG, may be negated to a large extent.

Thus, the energy deficit may be an important determinant of the PPTG lowering effect of exercise observed by many previous studies [30, 32, 34, 44, 45] where energy replacement did not occur. Nevertheless, replacement of the energy expended during an exercise bout effectively minimizes the energy deficit experienced by an individual relative to a non-exercise control day and so more directly addresses the effect of the exercise itself on PPL.

2) Tissue specific changes in LPL activity

The increased TG metabolism post-exercise is believed to be precipitated by a rise in exercise-induced LpL activity as outlined in a review of literature [19]. Post-exercise LpL increase has been reported to be delayed between 4-20 hours post-exercise, so in studies where the exercise bout is 12-14 h prior to the test meal, LpL activity is peaking just as the test meal is given [46-48]. In the current study, although change in LpL activity may not have occurred following the breakfast meal, enzyme activity should have increased by the time lunch and dinner meals were consumed. More vigorous exercise has been shown to induce LpL to a greater extent than lower intensities[49], and it has been estimated that at least 1000 kcal needs to be expended to significantly increase LpL activity [29]. If this is true then the current exercise bout may therefore not have been significant enough to raise LpL activity. Regularly expending 1000 kcals in activity in one exercise bout, however, is not very feasible for the majority of the population.

3) Changes in insulin sensitivity as a determinant of the PPTG response

Mechanistically, insulin plays several roles in the regulation of lipid metabolism, including regulating key hepatic and intestinal enzymes required for the assembly of TRL particles. It could be postulated, therefore, that acute changes in insulin action following exercise might impact TG metabolism. Indeed, a single bout of exercise has been shown to markedly increase glucose disposal [30, 50, 51] and increase glucose uptake [52, 53], effects which continue for up to 16-48 h after the exercise bout. It is believed that the majority of increased glucose uptake is due to increases at the exercised skeletal muscle [53, 54], however a recent study reports that hepatic glucose uptake is also increased with prior exercise [55].

It is possible that changes in insulin action, via exercise, extend to affects on hepatic TG. Acutely, insulin has been shown to decrease the assembly and secretion of VLDL particles by both increasing the degradation of apoB and decreasing the expression of microsomal transfer (MTP) in hepatocytes. [56, 57]. More specifically, insulin inhibits the hepatic production of the large, buoyant $VLDL_1$ particles, that are normally secreted only in the fasted state [58]. Much of the TG lowering effect of exercise has been attributed to reduced VLDL-TG ([44, 45, 59]. Decreased secretion [60, 61] and increased clearance [62] have both been reported as the primary means of the decrease. Exercise-induced decreases in secretion seem to be concurrent to the production of TG-richer particles [60], which are believed to be better substrates for LpL and hence are removed more rapidly from the circulation [63].

With respect to CM metabolism, there is evidence for an increased rate of basal apoB-48-containing lipoprotein secretion in insulin resistant intestines [64]. The mechanisms for increased secretion are many and include aberrant signaling at the enterocyte increased de novo lipogenesis at the intestine [65, 66] and enhanced expression of the proteins involved in CM assembly [65, 67]. Whether acute changes in insulin action, via exercise, could affect intestinal CM production is currently unknown.

In spite of the above discussion, however, data from the current study showed no effects of exercise on insulin or glucose levels, suggesting no change in insulin action. If insulin action is a factor involved in lowering PPTG with exercise, then the fact that we did not observe any insulin or glucose effects agrees with the lack of change in PPTG.

Secondary outcomes:

Despite the lack of change in the primary outcome (PPTG), some interesting secondary results were seen in this study.

Cholesterol

Exercise significantly reduced total cholesterol (TC) in the NW subjects. The effect was more significant in men ($p = 0.02$) than women ($p = 0.06$), and was not seen at all in the MetS subjects. Analysis of TRL cholesterol revealed a significant day x group effect for cholesterol in the TRL3 fraction only ($p = 0.016$), indicating that this is where the drop occurred. Cholesterol in this subfraction is contained in the dense TRL particles, namely IDL, LDL, HDL and to a lesser extent remnants from VLDL and CMs.

One mechanism for the fall in cholesterol level in the TRL3 subfraction could be increased reverse cholesterol transport activity (RCT) with exercise. In support of this hypothesis, Olchawa *et. al.* profiled a group of trained and a group of sedentary men for differences in HDL and lecithin:cholesterol acetyltransferase (LCAT) as measures of reverse RCT capability [68]. The athletic men had higher fasting levels of HDL, as expected, but they also had higher LCAT activity, without increased protein expression. Additionally, plasma from the trained men induced a 16% greater cholesterol efflux from a macrophage cell line. Furthermore, Frey *et. al.* measured LCAT activity in trained and untrained men at baseline and immediately following a maximal aerobic stress test, and found that LCAT did not differ between the groups at baseline, but increased significantly after exercise in both the trained and untrained subjects [69].

The NW subjects in the present study did have higher fasting total HDL-C and maintained a higher level than the MetS subjects over the day. Though there were no significant differences HDL-C between rest and exercise days, the NW men did tend toward decreased HDL-C with exercise. It has been shown previously that TG enrichment of HDL particles (as in the postprandial state) can significantly lower HDL-C via CETP-mediated TG exchange between TRL and HDL particles [70].

While this seems deleterious on the surface, reductions in HDL-C can be reflective of increased HDL particle turnover, resulting in increased prebeta HDL formation and increased RCT capability [71]. Analyzing plasma LCAT and CETP activity and HDL-C in the TRL3 subfraction could help elucidate the mechanism for the lowering of TC in this sub-fraction and therefore plasma TC. Other factors should also be considered, such as the associated apolipoproteins (AI, AII) and HDL particle size.

While chronic exercise is often promoted as lowering cholesterol, a recent review of 84 exercise training trials (i.e. chronic activity) found that only 7-10% of them reported significant decreases in fasting TC. The present data, evaluated the effects of a single acute exercise bout and might suggest, however, that the dynamics of the metabolism of cholesterol-containing particles is as important as the total cholesterol level.

Apolipoprotein B

In the group as a whole, total plasma apoB was moderately decreased ($p = 0.064$) with exercise, and albeit a small effect, over time this could represent a significant improvement. Similarly, although not statistically significant, some of the details in the changes in apoB are worth more discussion. Total apoB declined with exercise in the NW men in a similar fashion as total cholesterol, but there is a great deal more variability in the apoB measures, preventing a statistically clear difference.

Specifically, apoB in NW men at rest was higher over the day than in NW women at rest. Exercise, however, decreased apoB to levels similar to the women who were not visibly different on the exercise relative to rest days. Interestingly, the MetS women maintain a higher level of total apoB than MetS men over both the rest and exercise day, the opposite trend seen in the NW subjects. Similar to the NW subjects, however, the MetS men appear to have responded to exercise with a moderate lowering of apoB with little change in the women. Given the associations of apoB concentrations and CVD, this decrease might be relevant to reduced CVD risk, particularly in both NW and MetS men.

As was hypothesized women, overall, had a significantly lower PPTG response compared to men. This confirms what has been observed in previous studies [25, 27, 72]. What was not expected, however, was the PPTG pattern seen in MetS women, which was driving this sex-based difference. The TRL1 TG mirror the overall TG response, suggesting that CM were being cleared more quickly in the women than the men, again most dramatically in the MetS women. Likewise, the MetS women displayed a decrease in triglyceride-rich lipoprotein (TRL) particle size, estimated from the ratio of total TG to total apoB as there is one apoB per TRL particle. Total apoB did not change over the day in men or women, therefore, the accompanying sharp decrease in TG in the women led to a smaller particle size in MetS women in the second half of the day relative to MetS men. It is generally accepted that particles that are particularly TG-rich e.g. CM and VLDL1 are the preferred substrate for LpL [73, 74] As women have been reported to have higher LpL activity than men [75], this could facilitate the more rapid removal of CM in the MetS women relative to their male counterparts.

As Votruba and Jensen reported [76], women have greater LpL activity per gram of AT than men, particularly at the thigh. It is therefore possible that the MetS women have more AT LpL than the men as they have significantly more fat mass (36 $\pm$ 1.2 vs 27.3 $\pm$ 6.5%).

These results correspond to those reported by Knuth and Horowitz [77], who studied the response to a high fat meal in NW men and women. Using stable tracers, they demonstrated the well reported decrease in PPTG AUC response in women relative to men, but further identified the difference as a significant decrease in CMTG enrichment in the women, beginning at about 6h post-meal.

A recently published tracer study further details these events. Matthan *et. al.* maintained 8 women and 12 men (<50 years old) on a standardized Western diet (49% CHO, 15% PRO, 35% fat, mixed saturated, mono and polyunsaturated) for 4-6 weeks prior to a PP tracer study. After an overnight fast, subjects were fed 1/20 of their daily caloric intake every hour for 20 hours, and lipid kinetics was measured. This group concluded that the greatest difference between the men and women was in TRL apoB-100 fractional clearance rate (FCR), which was 1.8 times higher in premenopausal women than in men. The apoB-48 labeling was below detection in this study, so the effects on the CM fraction could not be determined. Additionally, the study showed a 30% decrease in LDL apoB-100 pool size and a 17% reduction of LDL apoB-100 concentrations. Moreover, postmenopausal women have about 50% lower TRL and LDL apoB-100 FCR relative to premenopausal women [78], suggesting the involvement of sex hormones.

These results would suggest a difference in hepatic VLDL metabolism between men and women. Indeed, Magkos et. al have reported that women produce fewer, but TG-richer VLDL particles than men [79]. While we cannot comment on this specifically from the current study, larger VLDL particles in concordance with an increase in LpL activity in the MetS women relative to MetS men would help explain the difference in TG concentrations.

Sex and group comparisons of the PPTG response

The lack of statistical difference in PPTG response in the NW women, which is contrary to what has been reported in prior studies [25, 27, 79, 80], could be due to the fact that the NW subjects were selected with borderline high fasting TG, and women usually have lower fasting TG than men. As fasting TGs are significantly associated with the PPTG response [81, 82], this may be the reason for a lack of a sex difference in PPTG in NW men and women. The fact that the NW women had elevated fasting TGs, relatively speaking, might indicate some mild "impairment" in hepatic TG metabolism. The sex difference in PPTG response pattern seen in the MetS subjects was an unexpected finding, but is reasonable given the increased insulin excursions in the women relative to the men. Whether the mechanism involves LpL cannot be determined from this study, but is plausible.

The MetS women appear to be less insulin sensitive than the men as the women had a higher HOMA-IR, as well as a significantly greater increase in insulin IAUC with the breakfast ($p = 0.005$) and lunch ($p= 0.06$) meals. Sadur and Eckel demonstrated a significant increase in adipose tissue LpL approximately 6 hours after insulin infusion relative to saline [83]. It can therefore be hypothesized that LPL activity peaked at about T360 or 6 hours after the breakfast-induced insulin excursion and this possibly augmented plasma TG removal, potentially more so for CMTG as suggested by the TRL1 TG subfraction data.

The MetS women also had a significant increase in FFA at mid-day, relative to MetS men, suggesting reduced insulin-dependent suppression of adipose tissue lipolysis, and providing further evidence of insulin insensitivity in these women. Alternatively, it is possible that more FFA were released into the circulation as a result of increased TRL lipolysis, as has been reported in limb-balance and enzyme binding studies [73, 74].

In addition to cholesterol and TG in the isolated TRL subfractions, apoB-100 and apoB-48 were measured in the TRL2 Subfraction (Sf 20-400) with the intention of identifying the source (liver or intestine) of PP lipids and estimating particle size (TG: apoB) in this fraction. There was an overall group effect for both B-100 and B-48 AUC with the MetS subjects having twice the B-100 and B-48 as the NW subjects ($p < 0.001$ for both).

This is an interesting finding given that the MetS group had only 8% more total apoB than the NW groups. The doubling of apoB in the TRL2 fraction without a concomitant doubling of TG yields particles that are significantly smaller in the MetS group in this fraction ($p<0.001$).

This was significant for the ApoB-48 in particular as these represent CM remnants and smaller particles have repeatedly been shown to be more atherogenic than larger particles, due to their ability to penetrate the endothelium [84-90]. A limitation of the current data is, however, the inability to determine the quantity of TG associated with the ApoB100 and ApoB48 particles which would provide more specific information of value. Looking in more detail at the different particle fractions is relevant because overall, the MetS group maintained a larger particle size over both the rest and exercise days, mainly due to increased TG in the TRL1 subfraction, than the NWs which might otherwise be interpreted as a positive result. Interestingly, however, exercise marginally, but significantly decreased apoB-100 in the TRL2 subfraction (comprised mainly of VLDL) in the MetS group (p 0.044), supporting a therapeutic role in lipid lowering in this population.

In conclusion, in the context of the present study, a moderate bout of morning exercise did not significantly affect PPTG in NW or MetS men or women. This is likely due to the conditions of the study i.e. moderate exercise done under fasting conditions before the consumption of moderate fat, mixed meals, and replacement of the energy expended during exercise to minimize any energy deficit.

However, these conditions are believed to be more typical of free-living, Western individuals, who are not trying to lose weight. Though PPTG did not change, exercise did decrease total cholesterol in the NW subjects, total apoB in the group as a whole, and apoB-100 in the TRL2, predominantly VLDL, subfraction in MetS subjects, supporting the antiatherogenic potential of exercise.

The main limitation of the current study was the inability, due to financial and technical constraints, to measure apoB-100 and 48 in the CM and LDL/HDL subfractions. While the VLDL fraction contains the majority of apoB-containing TRL particles, it would have been useful to have estimates of particle size and origin (hepatic vs intestinal) in the other fractions to complete the story. Plasma and/or tissue LpL measures would also have been helpful in elucidating the mechanism of the mid-day decline in TG in the MetS women. The elevated insulin early in the day inducing LpL activity 6h later fits nicely with the current data, but could be validated with enzyme measures. Similarly, the mechanism of exercise-induced cholesterol-lowering in the NW subjects could be further elucidated with HDL-C and LCAT measures in the high-density subfraction. Perhaps these can be included at a later date. Analysis of visceral fat in the current study population might also be useful given the contribution of visceral fat to insulin action and lipid metabolism (reviewed in [91]).

The present study underscores the heterogeneity in PP lipid response between subjects of different sexes and metabolic states, and enforces the mandate for tailored prevention and treatment strategies for CVD and related conditions. The subjects in this study were required to remain resting for the majority of the day, other than restroom use. Though much of the population at large is quite sedentary, the exercise bout in the morning, in combination with the activities of daily living, might change the outcomes somewhat and might be worth investigating in the future. At any rate, more physical activity cannot be a bad idea.

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