INTERACTION OF EXERCISE AND FISH OIL ON POSTPRANDIAL LIPEMIA

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In Partial Fulfillment
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Master of Arts

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by

YING LIU

Dr. Tom R. Thomas, Thesis Supervisor

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The undersigned, appointed by the Dean of the Graduate School,
Have examined the thesis entitled

INTERACTION OF EXERCISE AND FISH OIL
ON POSTPRANDIAL LIPEMIA

Presented by Ying Liu

A candidate for the degree of Master of Arts

And hereby certify that in their opinion it is worthy of acceptance.

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Professor Grace Sun

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Professor Marilyn James-Kracke

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INTRODUCTION

The leading causes of death in industrialized nations are lifestyle related diseases, caused in part by imbalanced diet, lack of exercise, poor stress management, and smoking. The character of this life style is high energy intake and low energy consumption, which leads to a metabolic syndrome characterized by weight gain, dyslipidemia, insulin resistance, and hypertension. This metabolic syndrome is the precursor of lifestyle related diseases.

Inflammation is a key component of the metabolic syndrome and has been shown to be involved in atherosclerosis, diabetes, cancer, and other lifestyle diseases. Among all the lifestyle related diseases, cardiovascular diseases (CVD) are the number one killer of women and men. One in five males and females has some form of CVD. CVD accounted for 38.5 percent of all deaths or 1 of every 2.6 deaths in the United States in 2001. CVD mortality was about 60% of "total mortality". Almost 150,000 Americans killed by CVD each year are under 65. In 2004 the estimated direct and indirect cost of CVD is $368.4 billion (9); the cost has nearly tripled in the last two yeas!

There are 18.2 million American; in 2002 approximately 6.3% of the population had diabetes, among them, 90% to 95% had type 2 diabetes. This number is increasing by 1.3 million per year. The total annual economic cost of diabetes in 2002 was estimated to be $132 billion, or one out of every 10 health care dollars spent in the United States (8). Cancer is the second leading cause of death in the US, causing 1 of every 4 deaths. Overall costs of cancer in 2004 was $189.8 billion (6).

The role of inflammation in atherogenesis

Signs of inflammation accompany the earliest accumulation of lipid within the arterial wall. The diseased endothelium begins to express adhesion molecules that selectively bind to circulating leukocytes. These cell adhesion molecules include intercellular adhesion molecule-1 (ICAM-1).

Once adherent to the endothelium, inflammatory cells migrate into the subendothelial space, and leukocytes contribute to the local inflammatory response. Macrophages, endothelial cells, and smooth muscles cells produce the pleiotropic cytokine tumor necrosis factor-α (TNF-α), which along with interferon-γ and interleukin-1 (IL-1) stimulate smooth muscle cell production of IL-6. IL-6 is the main stimulant for the hepatic production of C-reactive protein (CRP) (40).

As the atherosclerotic lesion matures, the accumulation of foam cells leads to the formation of a lipid pool, rich in pro-thrombotic tissue factor. Smooth muscle cells produce collagen which contributes to the strength of the fibrous cap, shielding the circulating blood from the pro-thrombotic lipid pool. The synthesis and breakdown of collagen in the fibrous cap is dynamically mediated by inflammatory signals (49). If pro-inflammatory forces predominate, the fibrous cap may thin and eventually rupture, with release of the pro-thrombotic lipid pool into the lumen. This may cause the acute onset of an ischaemic event.

PPL and inflammation

Postprandial lipemia (PPL) is the sustained and elevated concentration of plasma triglycerides (TG) that occurs following a meal. Americans ingest approximately 33% of

their daily calories from fat (3) and spend most of their day digesting a meal, placing them in a postprandial state for the majority of the day. Hypertriglyceridemia leads to high plasma concentration of low-density lipoprotein cholesterol (LDL-C), and low plasma concentration of high-density lipoprotein cholesterol (HDL-C). It has been shown that plasma concentration of TG, and ratios of TC/HDL-C, LDL-C/HDL-C were positively correlated to CRP (63, 95). Plasma concentration of HDL-C was shown to be negatively correlated to CRP (27). Studies have found that hypertriglyceridemia actived leukocytes, in vivo (68). Since activation of leukocytes is obligatory for inflammation and atherogenesis by adhering to the endothelium via specific ligands, hypertiglyceridemia thus induces adhesion. On the other hand, hypertiglyceridemia was found to be correlated to cell adhesion molecules, thus contributing to inflammatory procedure and atherosclerosis (45). Elevated or exaggerated PPL is often observed in CVD patients, and is a major risk factor for CVD (71). Based on these results, exaggerated PPL TG makes inflammation worse. Omega-3 fatty acid (n-3FA) and aerobic exercise are two strategies that have been shown to reduce PPL.

Fish oil reduces PPL

N-3FAs are long chain fatty acids (FA) that are primarily found in fish oil. The two primary n-3FAs found in fish oil are eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3). Researches have found n-3FA supplementation reduced PPL (37, 79, 85, 94). The reduction in PPL following n-3FA supplementation is hypothesized to be due to either decreased hepatic TG secretion (very low-density lipoprotein, VLDL) and/or decreased intestinal TG secretion (chylomicrons and VLDL) (38). This inhibition of TG secretion reduces the amount of TG that is present in the

plasma, thus lowering PPL. Study also found n-3FA supplementation increased lipoprotein lipase activity (LPLa) in human subjects (79). Since lipoprotein lipase (LPL) is the major enzyme to hydrolysis circulating TG, by increasing LPLa, n-3FA attenuates PPL.

Exercise reduces PPL

Ziogas et al (95) demonstrated that exercise training was associated with significantly lowered PPL. Zhang et al (97) showed that a session of aerobic exercise before ingestion of a high-fat meal significantly decreased PPL up to 50%, and elevated high density-lipoprotein cholesterol (HDL-C) concentrations. Others also have observed a marked attenuating effect of aerobic exercise on PPL (88). The reduction of PPL is due to an increase in LPLa (96). LPL is an enzyme that is attached to the endothelial layer of the vascular walls of the capillary beds within skeletal muscle and adipose tissue. Exercise enhances LPLa in skeletal muscle and adipose tissues (13, 69). Exercise was also found to induce LPL gene expression in skeletal muscle (51, 77).

Exercise, fish oil, and PPL

In a study in which fish oil was coupled with exercise, Warner et al (93) found that exercising 3 days/wk for 12 wk in conjunction with fish oil supplementation can lower fasting TG levels. However, they only measured fasting TG, without measuring PPL TG. Thomas et al (85) found the combination of fish oil and an acute exercise had no effect on the PPL TG response in sedentary subjects; the combination of the two treatments produced interference. But Smith et al (79) found in trained subjects, the combination of fish oil supplementation with an acute exercise bout produced an additive effect on attenuating PPL TG. These results raised two important questions: 1) Is the

recommendation of combining fish oil supplementation with exercise training valid in sedentary people? 2) How long does it take for this interference effect to convert to a beneficial combination in sedentary people who start exercise training? The answers to these questions would be scientifically relevant to the large population of sedentary people recommended by physicians to take exercise and fish oil supplements. This recommendation is published in many popular magazines.

Halle et al (35) exercise trained subjects for 4wk, and found plasma TG was significantly decreased. Hamazaki et al (36) found plasma TG decreased after 4 weeks fish oil supplementation. Based on these previous studies, we hypothesize that fish oil or exercise for 4 weeks should decrease TG significantly. However, no studies have combined these treatments.

Exercise, fish oil, and inflammation

Exercise and inflammation

The pathology of atherosclerosis may be initiated by inflammation. Monocytes adhere to endothelialcells, and subsequently transmigrate into the vascular intima as a key event in the development of atherosclerosis. Exposure of leukocyte and vascular adhesion molecules such as ICAM-1 on the surface of monocytes and endothelial cells causes them to adhere. High blood TG is correlated to the expression of cell adhesion molecules; thus TG also contributes to atherosclerosis (45). By attenuating the levels of PPL TG, exercise indirectly inhibits development of atherosclerosis. Acute exercise induces inflammation in the body (59, 67, 83). However, long-term aerobic exercise training seems to inhibit inflammatory processes and thus reduces the risk of lifestyle

related diseases (57, 64). There were studies (14, 15) which explored the anti-inflammatory effect of different types of exercise, but the conclusion has yet to be drawn.

Fish oil and inflammation

Epidemiological studies have shown that men who ate at least some fish weekly had a lower mortality rate from CHD than men who did not (47). The Physicians' Health Study reported an inverse relationship between blood levels of long-chain omega-3 fatty acids and risk of sudden death in men without a history of cardiovascular disease (CVD) (2).

Available randomized controlled trials show n-3FA reduced overall mortality, mortality due to myocardial infarction, and sudden death in patients with CHD (55, 56). There was in vitro evidence that DHA reduced endothelial expression of ICAM-1and IL-6 in stimulated cells (23, 24).

Another potential antiatherogenic mechanism of omega-3 fatty acids is their interference with the arachidonic acid cascade that generates a wide variety of eicosanoids. EPA not only can replace arachidonic acid in phospholipid bilayer, but it is also a competitive inhibitor of cyclooxygenase, reducing the production of the prostaglandins, thromboxanes, and prostacyclin and the leukotrienes (92). Some studies have shown that omega-3 fatty acids may increase the susceptibility of LDL to oxidation (39, 81), whereas others have not (12, 42).

Adhesion molecules, including ICAM-1 participate in the adhesion of leukocytes on endothelium. Once adherent to endothelium, macrophages, endothelial cells and smooth muscles cells produce pleiotropic cytokine. Pleiotropic cytokine stimulates the production of IL-6, which further induces CRP. Fish oil intake decreases the

concentrations of inflammatory markers in the blood; thus it is believed fish oil has anti-inflammatory effect by decreasing IL-6 level (19, 22, 24). Eschen and Miles (30, 60) suggested the impact of fish oil supplementation on sICAM-1 varied with dose of the oil as well as age and gender of the subjects.

No studies were found to investigate the combined effect of fish oil and exercise on inflammatory markers. It is possible that for sedentary people, fish oil may attenuate the inflammatory effects of starting an exercise program.

Purpose and Hypothesis

Purpose:

The purpose of the study was to examine: (1) whether fish oil and exercise training together can attenuate PPL in sedentary subjects better than fish oil alone; (2) whether fish oil supplementation can attenuate the inflammatory effects of starting exercise in previously sedentary subjects.

Hypothesis:

In pre treatment PPL and exercise PPL tests:

Acute exercise 12 hours before high fat meal attenuates PPL TG.

In non exercise PPL tests:

After 4 wk treatment:

(1) Both fish oil supplementation and fish oil plus exercise training will attenuate PPL TG, but the combination treatment will attenuate PPL TG better than fish oil supplementation alone.

(2) Both fish oil supplementation and fish oil plus exercise training will attenuate CRP, IL-6 and sICAM-1 induced by exercise, but the combination treatment will attenuate CRP, IL-6 and sICAM-1 more.

In exercise PPL tests:

After 4 wk treatment:

(4) a. In sedentary subjects who take fish oil supplement alone, acute exercise will cause interference in PPL.

b. Fish oil supplementation plus exercise training will attenuate acute exercise PPL TG better than fish oil supplementation alone.

(5) Both fish oil supplementation and fish oil plus exercise training will attenuate CRP, IL-6 and sICAM-1 induced by acute exercise, but the combination treatment will attenuate CRP, IL-6 and sICAM-1 more.

METHODS

Subjects

22 healthy men and women, age 18 to 55, were recruited to participate in this study. There were altogether 37 people signed the consent forms, among them, 22 finished the study. People dropped out the study for the following reasons: 1) failed to carry out exercise, 1 subject; 2) blood draw difficulties, 4 subjects; 3) busy schedule, 3 subjects; 4) VO_2max indicated having good aerobic training , 1 subject; 5) not able to tolerate PPL blood draw, 2 subjects; and 6) no specific reason, 6 subjects. Among the 22 people who finished the study, 10 were males, 12 were females. There were one African American (male), two Egyptians (one male, one female), one Asian (female), and all others were Caucasians. The characteristics of the subjects were shown in Table 1.

Table 1. Characteristics of subjects

Variables	Groups	Baseline
Age	FO+ExTr (n=12)	32.2 ± 2.0
	FO (n=10)	33.1 ± 3.1
Gender	FO+ExTr	male=6, female=6
	FO	male=4, female=6
Cholesterol (mg/dl)	FO+ExTr	192.0 ± 8.9
	FO	182.8 ± 7.7
Glucose (mg/dl)	FO+ExTr	99.2 ± 2.5
	FO	99.9 ± 2.9
Body Weight (kg)	FO+ExTr	78.0 ± 2.6
	FO	78.3 ± 2.8
BMI (kg/m^2)	FO+ExTr	26.4 ± 0.6
	FO	26.9 ± 0.8
VO2max (L/min)	FO+ExTr	2.8 ± 0.2
	FO	2.8 ± 0.3

Note: There was no significant difference between two groups in characteristics of subjects

Subjects were physically sedentary, which was defined as physical exercise less than one hour per week within the past 3 months. Subjects had no more than one major cardiovascular disease risk factor determined by American College of Sports Medicine (ACSM) or any other disease symptom according to the guideline of ACSM (7). Their body mass index (BMI) was 25-29.9 kg/m^2. No subject was taking any medication or fish oil supplement within the past 6 months. Subjects were required to stop taking multiple vitamin and other healthy supplements for 2 wk before they could enroll into the study.

Before any participant began the testing period of the study, they had their responsibilities and obligations to the research explained to them, and gave informed consent. All subjects completed a health screen, informed consent, and exercise/activity

questionnaire. Fasting blood glucose, cholesterol and blood pressure were also checked before they could be enrolled. Women also completed menstrual cycle questionnaires. Subjects were compensated. This study was approved by Health Sciences Institutional Research Board.

Experimental Design

A base line PPL (Pre-PPL) was performed on every subject, after a 12h fast overnight. Subject took a high fat meal, which consisted of a standardized high fat shake. Blood samples were taken pre, 2h, 4h, 6h, and 8h after the high fat meal. Subjects also took an acute exercise at 60% VO_2max 60 min, before 12h fast overnight; another PPL was performed afterwards (Ex-PPL). In exercise PPL trials, blood samples were also taken right after exercise bout. The two postprandial trials were randomized. There was one week between these two trials. Subjects were then randomly divided into two groups; group one took fish oil supplementation (4g n-3 FA/day) for 4 wk; group two took fish oil supplementation together with exercise (5 times/wk, 45 min/day at 60% VO_2max (~75%maximal heart rate estimated) for 4 wk. When the treatment was finished, a non exercise PPL (Post-PPL) and an exercise PPL (Post-Ex-PPL) were performed on every subject. The two postprandial trials were randomized. There was one week between these two trials. The timeline of the study is shown in Figure 1. The timeline of PPL trail is shown in Figure 2.

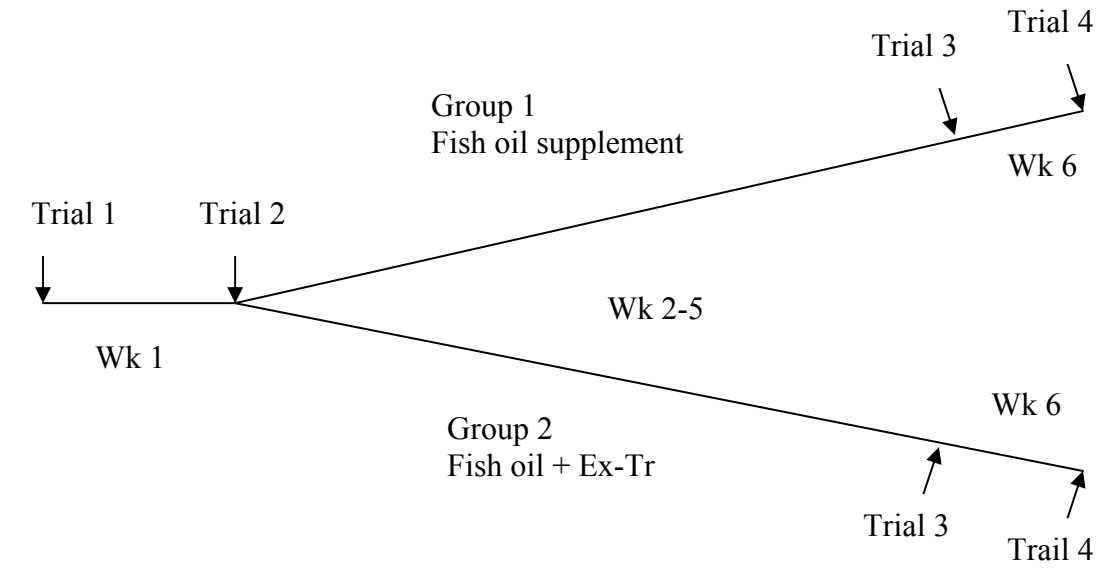

Figure1: Study timeline
Note: Trial 1 and 2 were PPL or Ex-PPL, the order was randomized. Trial 3 and 4 were PPL or Ex-PPL, order was randomized.

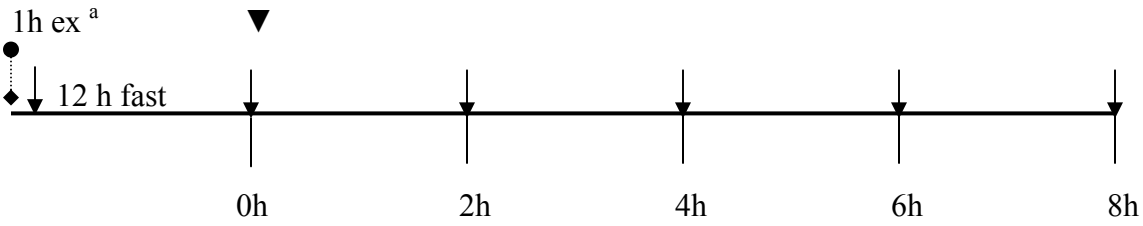

Figure 2: PPL trial timeline
▼ = high fat meal ↓ = Blood sample
[a] For exercise trials, each subject completed a 1 h treadmill exercise at 60% of VO_{2max}, 12 h before the high fat meal.

Body Mass Index

Body weight was measured to the nearest 0.05 kg by using a scale (Toledo Honest Weight Inc., Toledo, IA). Height was measured to the nearest 0.1 cm by using calibrated wall chart. Body mass index (BMI) was calculated by dividing the subjects' weight in kg by the square of their height in m.

Diet

The three day diet log in the beginning of the study from 10 subjects (4 in FO, 6 in FO+ExTr), were collected. There was no difference between groups in the amount of nutritional factors intake, and the portion of each nutritional factors contributed to the calories intake (Table 2). Subjects were instructed to maintain their diet habit during the study. The food intake on the day before the first PPL trial was documented, and kept by the subject. On the days before every PPL trials afterwards, they followed the diet of the day before the first PPL trial.

Table 2. Dietary characteristics of the subjects

Variable	Group	Values
Total Kcal	FO (n=4)	2056.0 ± 166.9
	FO+ExTr (n=6)	2736.2 ± 352.1
	two groups (n=10)	2464.1 ± 239.5
CHO (g)	FO	258.3 ± 21.1
	FO+ExTr	317.5 ± 46.1
	two groups	293.8 ± 29.4
Protein (g)	FO	86.7 ± 10.1
	FO+ExTr	93.8 ± 7.2
	two groups	90.9 ± 5.7
Fat (g)	FO	79.3 ± 10.1
	FO+ExTr	123.5 ± 20.2
	two groups	105.8 ± 14.2
Sat Fat (g)	FO	23.7 ± 6.6
	FO+ExTr	37.5 ± 6.8
	two groups	31.9 ± 5.1
CHO (% kcal)	FO	49.4 ± 2.7
	FO+ExTr	46.3 ± 3.3
	two groups	47.6 ± 2.2
Fat (% kcal)	FO	34.0 ± 3.0
	FO+ExTr	38.8 ± 3.7
	two groups	36.9 ± 2.5
Protein (% kcal)	FO	16.6 ± 1.7
	FO+ExTr	14.2 ± 1.1
	two groups	15.1 ± 1.0

Note: There was no significant difference between two groups in dietary characteristics.

High fat meal

The size of the high fat meal was based upon the body weight of each subject, and contains 1.3g fat, 0.06g proteins and 0.3g of carbohydrate per kg of body weight. For an example, the high fat meal of a 75kg subject contained 97.5g fat, 4.2g protein, and 25.4g of carbohydrate; and the total energy provided by the meal was 1087.8 kcal. This diet has been used in other studies of our group (4, 79).

PPL

Subjects were required to stop physical exercise 30 h to 36 h before fasting started in non exercise PPL trials. Subjects who participated in exercise training did 1 h exercise at 60% VO_2max during Ex-PPL to substitute the training session for that day. Subject reported to the lab in the morning, after 12h of fasting overnight. The body weight of the subject was obtained right before the high fat meal was prepared, during each every PPL trial. After the fasting blood samples were taken, subjects ingested the high fat meal. Venus blood samples were taken before, 2h, 4h, 6h and 8h after the high fat meal. Subjects were allowed to leave the lab after every blood sampling, but they were required to only drink water before the blood samples of all the time points were taken.

Exercise

A. Exercise PPL. Each subject reported to the lab 12 hours prior to the high fat meal to complete this aerobic exercise session. The session consisted of exercising on the treadmill for 60 min at 60% of their previously determined VO_2max. Heart rate monitor (Polar E600, Polar Electro Inc., Woodbury, NY) was used to monitor the heart rate during the test. Subjects started walking at a low intensity treadmill speed that increased every minute. The 60 min session started after the 9 min warm-up. Treadmill speed was

adjusted to allow the heart rate to reach and maintain the 75% HR max. At the end of the exercise session, subjects were allowed to cool down at their own paces to achieve a near resting heart rate. Subjects then started fasting for 12h overnight, and they reported to the lab in the morning. After the fasting blood samples were taken, subjects ingested the high fat meal. Venus blood samples were taken before, 2h, 4h, 6h and 8h after the high fat meal. This test was performed before and after the treatment. It was performed a week before or after each common PPL.

B. VO_2max test. Each subject completed a VO_2max test on a treadmill (Quinton Model 18-60) to determine baseline fitness status, and appropriate intensity for the submaximal exercise training prescription. The protocol that was used for the VO_2max test was slightly modified from the protocol that has been previously described by our group (88). Initially, subjects warmed up by walking 3.0 mph on the treadmill. All subjects began the first workload stage of the VO_2max test at 3.5 mph. The first workload stage was maintained for 2 min. After the first workload stage, the treadmill speed was increased by 0.5 mph/min at the end of each minute until reaching 6.0 mph (beginning of 7^{th} minute of test). The pace (6.0 mph) was held for 1 min and maintained throughout the remainder of the test. After the end of the 7^{th} minute, treadmill grade was increased by 2%/min until subjective exhaustion. The highest VO_2 value obtained was considered the subject's VO_{2max}. Physiological criteria for a VO_2max test included a maximal respiratory exchange ratio (RER) $\geq$ 1.1, maximal heart rate within 10% of age predicted maximum, and leveling of O_2 consumption ($\leq$ 2 ml/kg/min difference between two successive work loads). We required at least two of these criteria to be met in order for the highest VO_2max value to be considered a true VO_2max.

Gas collection and analysis during VO_2max tests were performed in the Exercise Physiology Lab using the Parvo Medics TrueOne 2400 metabolic cart (Parvo Medics Inc, Sandy, UT). The headgear used for gas collection during treadmill maximal tests was a Hans Rudolph head support (Hans Rudolph model # 2726, Kansas City, MO) that held a bi-directional valve attached to a rubber mouthpiece. The valve was connected to 4 ft. of tubing that was directly connected to the pneumotach of the TrueOne 2400 metabolic cart.

C. Exercise training. Subjects were randomly assigned to fish oil supplementation (FO group) or exercise training and fish oil supplementation group (FO+ExTr group). Subjects in FO+ExTr group participated walking or jogging on treadmill 5 time/wk, 45 min/day, for 4 wk at 60% VO_2max (75% HR max). The intensity of the training was monitored using heart rate (with the goal 75% HR max as measured on the VO_2max test) and Rating of Perceived Exertion (RPE). The training followed a one week progression: In wk 1 subjects were trained 30 min, 60% VO_2max (75% HR max) for the first three days; by the end of wk 1, they started training at 45min, 60% VO_2max; and maintain this intensity 5d/wk, for the rest of the 4 wk training section. Most of the exercise trainings were carried out under the supervision in Exercise Physiology Lab. For every subject, one training session per week was allowed to be done on his/her own. Subject borrowed heart rate monitor from the lab, monitored his/her heart rate when walking on a treadmill or outdoor. However, most of the subject came to the lab every time.

Fish oil supplementation

After the completion of the Pre-test which including baseline PPL and Ex-PPL, fish oil capsules (Super EPA-500, Bronson Pharmaceutical, St. Louis, MO) were provided to

all the subjects once a week for 4wk. Each capsule contained 300 mg of EPA and 200 mg of DHA (500 mg n-3FA/capsule). Every subject took 8 capsules, which equaled to 4g n-3 FA/day. Subjects were instructed to take two capsules with each meal (breakfast, lunch, and dinner) and two before going to bed.

Supplement was provided to the subjects once a week. Pill counts were used to track compliance in taking the supplement. Each subject was provided with 2 or 3 more capsules every week than they supposed to take and the number was recorded. When they returned to the lab for more capsules each week, the number of remaining capsules was counted. Failure to ingest the required number of capsules may have resulted in dismissal from the experiment. Overall subject compliance was calculated by taking the average of all individual subject compliances.

Compliance = (Amount taken/Amount should have taken)*100

Blood Collection

Blood was collected from a butterfly needle inserted into an antecubital vein. In every time point, 10 ml blood samples were collected into a 10ml tubes containing EDTA (anticoagulant and chelating agent).

All EDTA blood samples were separated by centrifugation at 4^0C for 15 min at 2000g in a Marathon 22100R centrifuge (Fisher Scientific, Pittsburgh, PA). The separated plasma was transferred to 0.5ml and 1.8 ml cryogenic vials and stored at -70^0C for later analysis.

Plasma Volume. Hematocrit (Hct) values were used to calculate plasma volume (PV) changes. Hct was measured in 4 subjects before acute exercise and at every time point of Ex-PPL trial. % change of plasma volume in comparing to pre exercise was calculated as

previously described by Beaumont et al (11). The changes in PV were assessed to determine if corrections were necessary.

Hct was measured immediately following the blood draws. Before the blood was transferred to vacutainers, two micro hematocrit capillary tubes were filled two-thirds full with whole blood and sealed with capillary tube sealant. The capillary tubes were spun in a micro hematocrit centrifuge (Model MB, International Equipment Company, Needham Heights, MA) for 3 min. Hct was measured with a hematocrit tube reader (Model CR, International Equipment Company, Needham Heights, MA), using the directions provided by the manufacturer.

Plasma TG Analysis

Plasma TG concentration was measured to determine the PPL response. The PPL response was defined as the total area under the TG curve (TG-AUC_T), the incremental TG-AUC (TG-AUC_I), and the TG peak response. The TG-AUC_T was calculated using the trapezoidal method as described by Tai (84). The TG-AUC_I also was calculated using the trapezoidal method, but the baseline TG values were subtracted from each TG value before completing the calculations. The TG-AUC was calculated by the trapezoidal method using the following formula:

$$TG\text{-}AUC_T = 0.5*[X_1(y_0+y_2) + X_2(y_2+y_4) + X_3(y_4+y_6) + X_4(y_6+y_8)]$$

$$TG\text{-}AUC_I = 2* (y_2 + y_4 + y_6) + y_8 - 7*y_0$$

where:

$X_1 = x_2-x_0$, $X_2 = x_4-x_2$, $X_3 = x_6-x_4$, and $X_4 = x_8-x_6$

x_n = n hour

y_n = the TG concentration at n hours

The TG peak response was defined as the difference between greatest plasma TG concentration minus the 0 h plasma TG value.

TG concentrations were measured from the blood drawn at pre, 2, 4, 6, and 8 h post high fat meal. This was done to determine PPL response to the meal. The area under the curve and peak response determined the magnitude of the response. Plasma TG was measured enzymatically using a diagnostic kit (Infinity^{TM}, Thermo DMA, Inc. Louisville, CO). Measurements are made using a Beckman DU 530 spectrophotometer (Beckman Instruments Inc. Fullerton, CA) using known standards. Infinity ^{TM} TG Reagent (1.0 ml) was pipette into 13*100disposable culture tubes. Standard (10 µl) (TR 22923, Thermo DMA, Inc. Louisville, CO) and plasma (10 µl) were pipetted into appropriately labeled tubes. After the addition of the reagent and the plasma, the tubes were briefly vortexed and incubated for five minutes at 37^0C. The absorbance of TG was read at 520 nm. Plasma TG concentration was calculated using the following equation: (absorbance of sample/absorbance of calibrator) * concentration of the calibrator (200 mg/dl). In order to eliminate inter assay variability; all samples from a single subject were analyzed together for each assay. For this procedure, the within assay mean coefficient of variation (CV) was 1.4%.

HDL Analysis

Total HDL-C and HDL_3-C were measured enzymatically using diagnostic kits. All samples for an individual subject were included in the same run, with one to two subjects' samples being run together in the same day. Infinity^{TM} Cholesterol Reagent (1.0 ml) was pipetted into appropriately labeled falcon tubes for each plasma sample (500 µl). A 1:1 heparin/$MnCl_2$ (50 µl) solution was added to each tube and vortexed.

Following 20 min of refrigerated incubation, samples were centrifuged at 1500g for 20 min at 0^0C.

Supernatant (200 µl) was pipetted into a new set of falcon tubes for each sample. Dextralip 15 (20 µl) solution was added to each sample, which were then vortexed and incubated at room temperature for 20 min. While those tubes were incubating, the supernatant remaining in the first set of falcon tubes was analyzed for total HDL-C.

Supernatant of each sample (50 µl) from the first set of tubes was combined with Infinity™ Cholesterol Reagent (1.0 ml) and incubated for 5 min at 37^0C. Samples were then transferred into cuvettes and placed into the spectrophotometer read at 450 nm. Total HDL-C was calculated using the following equation: (absorbance of sample/absorbance of calibrator) * concentration of the calibrator (50 mg/dl) * 1.10.

Following the incubation period, the second set of falcon tubes were again centrifuged at 1500g for 20 min at 20^0C. Samples were then analyzed for HDL_3-C concentration in the identical manner total HDL-C was analyzed. HDL_3-C was calculated using the following equation: (absorbance of sample/absorbance of calibrator) * concentration of the calibrator (50 mg/dl) * 1.21. HDL_2-C was calculated using the following equation: total HDL-C (mg/dl) - HDL_3-C (mg/dl). The CV for HDL-C concentration was 0.85%.

LDL-C Calculation

Plasma LDL-C concentration was calculated by using the Friedewald equation (30). LDL-C (mg/dl) = TC (mg/dl) – HDL-C (mg/dl) - TG/5 (mg/dl).

CRP, IL-6 and sICAM-1 Assays

Commercially available solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle was used to determine fasting serum concentrations of

the CRP (#30-9710S, ALPCO Diagnostics, Windham, NH), IL-6 (#88-7066, eBioscience San Diego, CA) and sICAM-1 (BBE-1B, R & D Systems Inc. Minneapolis, MN). In brief, antibodies specific for each marker were pre-coated onto a microtitre plate. Standards and samples were pipetted into the wells and the immobilized antibody binds any marker present. After washing away any unbound substances, an enzyme-linked antibody specific for the marker was added to the wells. Following a second wash to remove any unbound antibody-enzyme reagent, an acidic substrate solution was added to the wells and color develops in proportion to the amount of marker bound in the initial step. A stop solution was added and the intensity of color (read at wavelengths of 450 nm and 630 nm reference) is measured. The coefficient of variation for each of these variables was less than 6%.

Statistical Analysis

2-way repeated measures ANOVA was performed by using SigmaStat 2.03 under the following conditions:

(1) Within non exercise PPL data, the two factors were groups (fish oil vs. fish oil plus exercise training) and time (pre treatment vs. post treatment).

(2) Within exercise PPL data, the two factors were groups (fish oil vs. fish oil plus exercise training) and time (pre treatment vs. post treatment).

(3) HDL-C values of the two groups in the same kind of PPL trial, pre and post treatment. HDL-C values of the two groups in PPL and Ex-PPL trials before treatment as well as after treatment. Same method was applied to HDL2-C and HDL3-C values.

(4) LDL-C values of the two groups in fasting samples, pre and post treatment.

(5) CRP values of the two groups in fasting samples, pre and post treatment. CRP values of the two groups in 12h after acute exercise samples, pre and post treatment.

(6) IL-6 values of the two groups in Ex-PPL 2h samples, pre and after the treatment.

(7) sICAM-1 values of the two groups in fasting samples, pre and post treatment.

One-way ANOVA was performed by using SigmaStat 2.03 in:

(1) Comparing the difference between the two treatment groups in age, cholesterol, glucose, body weight, BMI, waist:hip ratio, % fat, VO2max, maxHR, and the dietary characteristics before treatment.

(2) Comparing the PPL TG values in the same trial of the two groups before treatment as well as after treatment.

One-way repeated measures ANOVA was performed by using SigmaStat 2.03 in:

(1) Comparing PPL TG values in PPL and Ex-PPL trials within the same treatment group in pre treatment as well as in post treatment.

(2) Comparing PPL TG in same group, same PPL trial pre and post treatment values.

(3) HDL-C values within the same treatment group, same kind of PPL trial, pre and post treatment. Same method was applied to HDL2-C and HDL3-C values.

(4) CRP values of same group, in fasting samples and 12h after acute exercise samples.

RESULTS

PPL samples were successfully taken in all time points of 21 subjects (10 in FO group, 11 in FO + ExTr group). There was one subject missed 8h time point sampling in PPL trial in pre testing, due to the difficulty of venipuncture. The value was estimated by using the following equation: 8h TG Value of the subject = (Mean 8h TG of FO+ExTr group / Mean 0h TG of FO+ExTr group) * 0h TG of the subject. Overall subject

compliance was 104.0% since most subjects left no fish oil capsules when they came to refill, although they were provided 2 or 3 more.

Body Composition

There were no differences in body weight, BMI, waist/hip ratio, % fat, VO_2max, and maxHR between the two groups before treatment. There was no change of body weight, BMI, and waist/hip ratio in both groups after the treatment. % fat was significantly decreased in FO+ExTr group (Table 3).

Aerobic Capacity

There was no significant change of VO_2max and maxHR in FO group. In FO+ExTr group VO_2max were significantly increased, while maxHR did not change after the treatment (Table 3).

Plasma TG

Pre treatment

TG-AUC_T, TG-AUC_I, TG-$Peak_T$ and TG-$Peak_I$ values are shown in Table 4. There were no significant differences in any PPL parameters (Table 4, wk 0), and TG values at any time points (Table 5, wk 0) between two groups in PPL trials before the treatment (Table 6). There was no significant difference in any PPL parameters (Table 4, wk 0), and TG values at any time points (Table 5, wk 0) between two groups in Ex-PPL before treatment (Table 6). There were no differences in any PPL parameters (Table 4, wk 0), and TG values at any time points (Table 5, wk 0) between PPL and Ex-PPL trial within the same group or two groups together before treatment (Table 6).

Post treatment

The summary of the PPL TG changes is in Table 6. There were no significant differences in any PPL parameters between two groups in PPL trials after the treatment (Table 4, wk 4). There were significant changes in TG-AUC_T in two groups together after the treatment, in PPL trial (Table 4, wk 0 vs. wk 4).

There was no significant difference in any PPL parameters between two groups after treatment, in Ex-PPL (Table 4, wk 4). In FO+ExTr group, there were significant changes in TG-AUC_T and TG-$Peak_T$ after the treatment, in Ex-PPL (Table 4, wk 0 vs. wk 4, Figure 7, Figure 8). There were significant changes in TG-AUC_T and TG-$Peak_T$ in two groups together after the treatment, in Ex-PPL trial (Table 4, wk 0 vs. wk 4). There were no differences in any PPL parameters between PPL and Ex-PPL trial within the same group or two groups together after treatment (Table 4, wk 4).

Among PPL trials, in FO group, TG was significantly lower at 0h and 4h after treatment (Table 5, Figure 3); in FO+ExTr group, TG was significantly lower at 0h and 8h after treatment (Table 5, Figure 4). There were significant decreases of TG in 0h, 4h and 8h in two groups together after the treatment, in PPL trials (Table 5, Figure 9).

Among Ex-PPL trials, in FO group, there were significant decreases of PPL TG at 0h and 6h after the treatment (Table 5, Figure 5). In FO+ExTr group, there were significant decreases of PPL TG at 0h, 2h, 4h, and 6h after the treatment (Table 5, Figure 6). TG peaked at 2h in both pre and post Ex-PPL trials. There were significantly decreases of TG in all 5 time points in two groups together after the treatment in Ex-PPL trial (Table 5, Figure 9).

HDL-C and subfractions

Pre treatment

HDL_2-C and HDL_3-C values were significantly different in two groups together between fasting samples and exercise samples (12h after acute exercise) (Table 7, wk 0).

Post treatment

There were significant increases of HDL-C and HDL_2-C and decrease of HDL_3-C in two groups together, in fasting samples after the treatment (Table 7). HDL_2-C and HDL_3-C values were significantly different in two groups together between fasting samples and exercise samples (12h after acute exercise) (Table 7). There was a significant increase of HDL_2-C, but HDL-C and HDL_3-C were unchanged in FO group (Table 7, Figure 10). There were significant increases of HDL-C and HDL_2-C and decreases of HDL_3-C in FO+ExTr group after the treatment (Table 7, Figure 11).

LDL-C

There was no significant change of LDL-C in each group, or two groups together after the treatment (Table 8).

CRP

There were no differences of CRP in fasting samples and exercise samples (12h after acute exercise) between the two groups, before treatment (Table 9, wk 0). There were no significant changes of CRP in fasting samples in both groups, after the treatment (Table 9). In the exercise samples (12h after acute exercise), there were no significant changes in CRP values in both groups, after treatment (Table 9). Within the same treatment group, there was no significant difference between fasting samples vs. exercise samples pre or post treatment in CRP values (Table 9).

IL-6

IL-6 values were undetectable in the exercise samples (12h after acute exercise). In Ex-PPL 2h samples, there was no significant difference of IL-6 values before and after the treatments (Table 10).

sICAM-1

There was no significant difference in sICAM-1 values in fasting samples before and after the treatments (Table 11).

Plasma Volume

There was a significant difference of % change of plasma volume in 6h, comparing with 0h (Table 12). The trend of plasma volume change showed a decrease after exercise and then gradually back up after 16h to 18h.

Table 3. Changes in characters of subjects

Variables	Groups	0 weeks	4 weeks
Body Weight (kg)	FO	78.3 ± 2.8	78.4 ± 2.4
	FO+ExTr	78.0 ± 2.6	78.5 ± 2.6
BMI (kg/m^2)	FO	26.9 ± 0.8	26.9 ± 0.7
	FO+ExTr	26.4 ± 0.6	26.6 ± 0.6
Waist : Hip Ratio	FO	0.80 ± 0.02	0.81 ± 0.02
	FO+ExTr	0.81 ± 0.02	0.81 ± 0.02
% fat (skin fold)	FO	28.0 ± 4.0	26.3 ± 4.0
	FO+ExTr	26.2 ± 2.3	24.1 ± 2.1*
VO2max (L/min)	FO	2.8 ± 0.3	2.9 ± 0.3
	FO+ExTr	2.8 ± 0.2	3.0 ± 0.2*
max HR	FO	190.2 ± 4.0	187.9 ± 3.7
	FO+ExTr	184.7 ± 4.5	183.5 ± 4.5

Values are reported as Means± SE. * Significant difference between pre and post treatment in each group, $P<0.05$.

Table 4. PPL TG values of each group and two groups together

Group	Trial	Measure	Week 0	Week 4
FO	PPL	TG-AUC_T	1319.2 ± 178.9	1013.1 ± 53.3
	PPL	TG-AUC_I	390.9 ± 113.2	255.2 ± 42.2
	PPL	TG-$Peak_T$	214.1 ± 29.3	163.0 ± 7.8
	PPL	TG-$Peak_I$	98.0 ± 20.9	68.3 ± 7.1
	Ex-PPL	TG-AUC_T	1321.6 ± 143.8	1077.5 ± 85.8
	Ex-PPL	TG-AUC_I	344.2 ± 64.4	273.1 ± 55.2
	Ex-PPL	TG-$Peak_T$	206.0 ± 24.5	177.5 ± 15.8
	Ex-PPL	TG-$Peak_I$	83.8 ± 14.3	76.9 ± 12.6
FO + ExTr	PPL	TG-AUC_T	1438.9 ± 183.8	1354.7 ± 226.3
	PPL	TG-AUC_I	347.8 ± 82.0	418.7 ± 103.6
	PPL	TG-$Peak_T$	207.9 ± 27.1	212.1 ± 39.5
	PPL	TG-$Peak_I$	71.6 ± 14.3	95.1 ± 24.5
	Ex-PPL	TG-AUC_T	1457.4 ± 206.9	1153.6 ± 127.6*
	Ex-PPL	TG-AUC_I	327.6 ± 92.9	273.4 ± 31.6
	Ex-PPL	TG-$Peak_T$	217.9 ± 28.9	169.9 ± 19.2*
	Ex-PPL	TG-$Peak_I$	76.7 ± 13.8	59.8 ± 7.9
two groups	PPL	TG-AUC_T	1384.5 ± 126.7	1199.4 ± 128.7*
	PPL	TG-AUC_I	367.4 ± 66.6	344.4 ± 61.1
	PPL	TG-$Peak_T$	210.7 ± 19.4	189.8 ± 22.1
	PPL	TG-$Peak_I$	83.6 ± 12.3	82.9 ± 13.8
	Ex-PPL	TG-AUC_T	1395.7 ± 128.4	1119.0 ± 78.5*
	Ex-PPL	TG-AUC_I	335.2 ± 57.2	273.2 ± 29.6
	Ex-PPL	TG-$Peak_T$	212.5 ± 18.9	173.3 ± 12.4*
	Ex-PPL	TG-$Peak_I$	79.9 ± 9.7	67.6 ± 7.2

Values are reported as Means± SE. * Significant difference between pre and post treatment within the same row, $P< 0.05$. There was no significant change between two groups in PPL trials before the treatment, as well as after treatment. There was no significant difference between two groups in Ex-PPL before treatment, as well as after treatment. There was no change between PPL and Ex-PPL trial within the same group or two groups together before treatment, as well as after treatment.

Table 5. PPL TG values at every time point

Group	Trial	Measure	Week 0	Week 4
FO	PPL	0h	116.0 ± 10.7	94.7 ± 7.8*
	Ex-PPL		122.2 ± 14.1	100.6 ± 9.1*
FO+ExTr	PPL		136.4 ± 14.7	117.0 ± 16.2*
	Ex-PPL		141.2 ± 16.3	110.0 ± 12.9*
two groups	PPL		127.1 ± 9.4	106.9 ± 9.6*
	Ex-PPL		132.6 ± 10.9	105.7 ± 8.1*
FO	PPL	2h	191.6 ± 25.2	154.7 ± 11.1
	Ex-PPL		190.3 ± 21.8	168.9 ± 15.7
FO+ExTr	PPL		187.2 ± 21.5	179.7 ± 22.6
	Ex-PPL		203.8 ± 25.7	159.5 ± 17.4*
two groups	PPL		189.2 ± 16.0	168.3 ± 13.3
	Ex-PPL		197.7 ± 16.8	163.8 ± 11.6*
FO	PPL	4h	184.1 ± 22.2	132.8 ± 8.0*
	Ex-PPL		193.6 ± 22.9	146.3 ± 16.3
FO+ExTr	PPL		203.7 ± 27.6	192.0 ± 40.5
	Ex-PPL		192.4 ± 29.4	162.3 ± 19.1*
two groups	PPL		194.8 ± 17.8	165.1 ± 22.9*
	Ex-PPL		193.0 ± 18.7	155.0 ± 12.6*
FO	PPL	6h	164.3 ± 33.9	123.4 ± 10.2
	Ex-PPL		158.9 ± 19.6	127.5 ± 10.6*
FO+ExTr	PPL		187.2 ± 27.8	184.6 ± 36.5
	Ex-PPL		186.5 ± 30.3	137.6 ± 14.7*
two groups	PPL		176.8 ± 21.2	156.8 ± 21.1
	Ex-PPL		173.9 ± 18.6	133.0 ± 9.2*
FO	PPL	8h	123.3 ± 16.9	96.6 ± 6.3
	Ex-PPL		113.7 ± 11.1	91.7 ± 7.1
FO+ExTr	PPL		146.4 ± 18.7	125.2 ± 17.3*
	Ex-PPL		150.6 ± 26.3	124.9 ± 16.6
two groups	PPL		135.9 ± 12.7	112.2 ± 10.1*
	Ex-PPL		133.8 ± 15.4	109.8 ± 10.1*

Values are reported as Means± SE. * Significant difference between pre and post treatment within the same row, $P < 0.05$. There was no change between PPL and Ex-PPL trial within the same group or two groups together before treatment, as well as after treatment.

Table 6. Summary of PPL TG changes

	Pre treatment	Post treatment
FO vs. FO+ExTr	↔	↔
FO		
PPL		TG↓ 0h, 4h
Ex-PPL		TG↓ 0h, 6h
FO+ExTr		
PPL		TG↓ 0h, 8h
Ex-PPL		TG-AUCT↓, TG-PeakT↓ TG↓ 0h, 2h, 4h, 6h
2 groups		
PPL		TG-AUCT↓ TG↓ 0h, 4h, 8h
Ex-PPL		TG-AUCT↓, TG-PeakT↓ TG↓ 0h, 2h, 4h, 6h, 8h

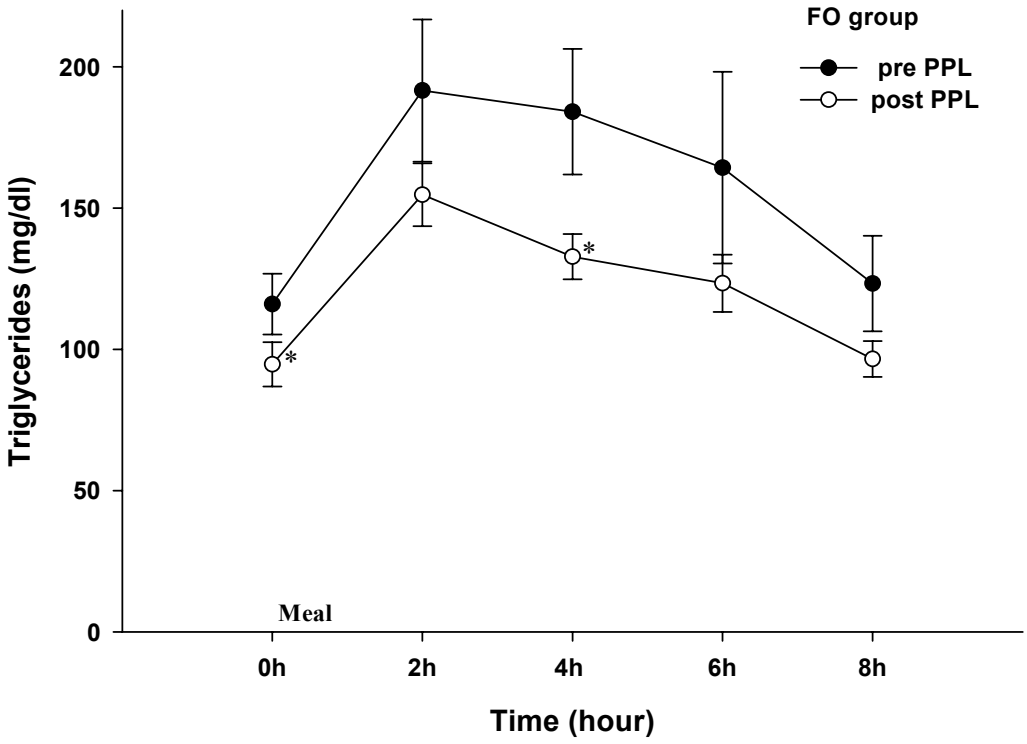

Figure 3. TG changes at time points after high fat meal in FO group, before and after treatment. Values are reported as Means ± SE. * Significant difference between pre and post samples, $p<0.05$.

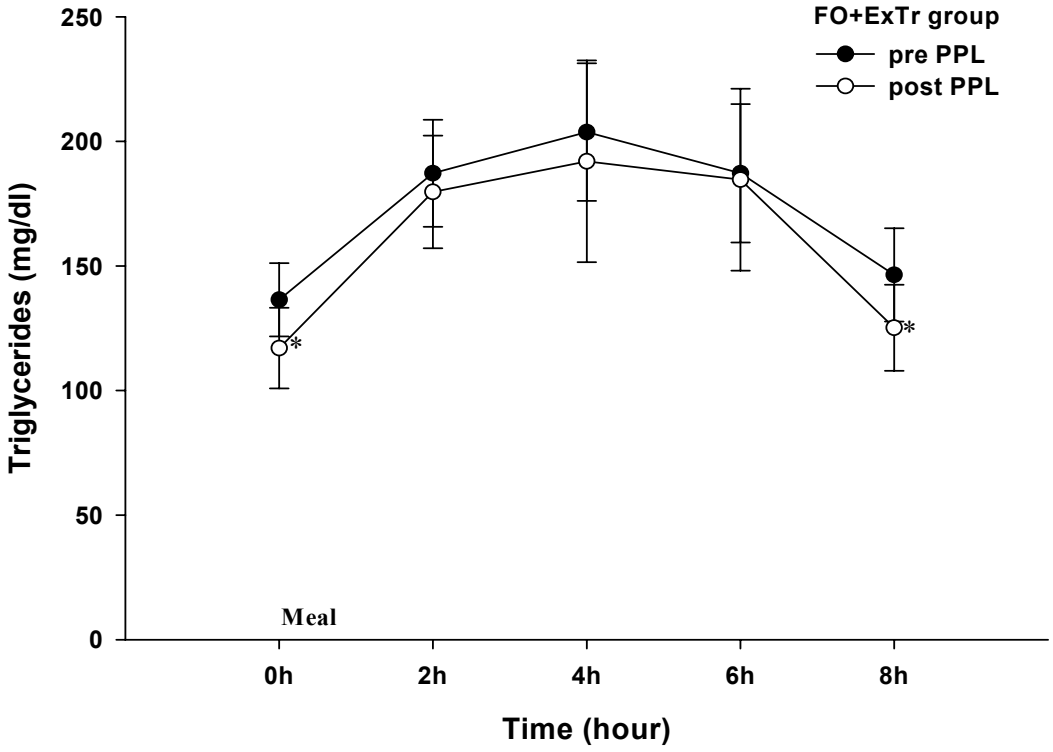

Figure 4. TG change at 5 time points after high fat meal in FO+ExTr group before and after treatment. Values are reported as Means ± SE. * Significant difference between pre and post samples, $p<0.05$.

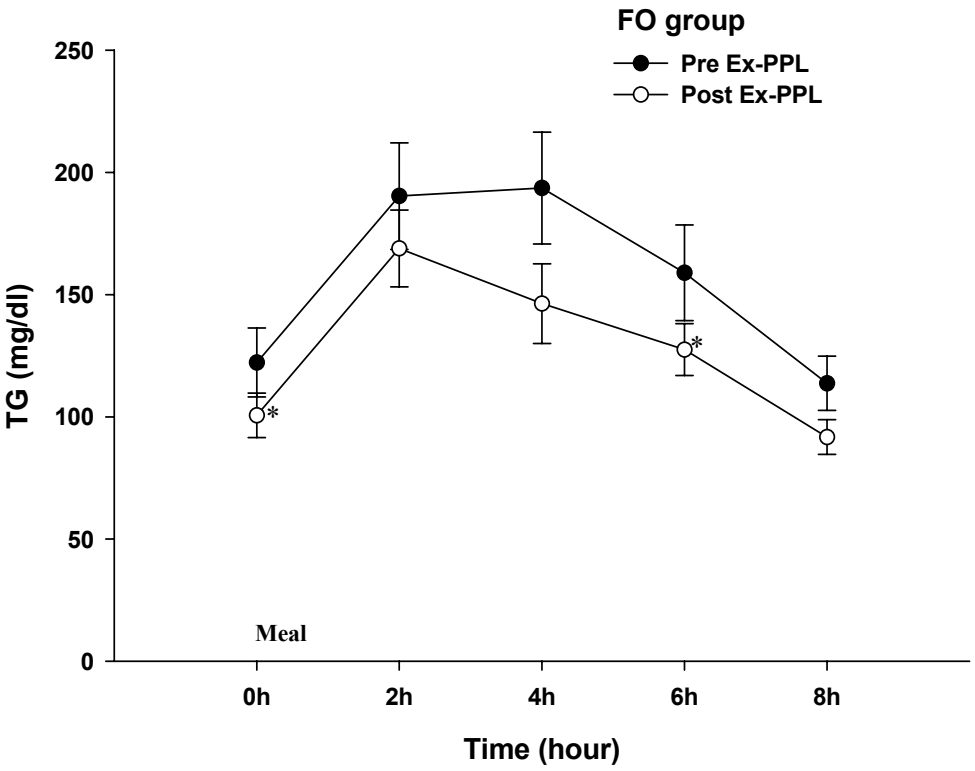

Figure 5. TG changes at time points in FO group, Ex-PPL trail pre vs. post treatment. Values are reported as Means ± SE. * Significant difference between pre and post samples, $p<0.05$.

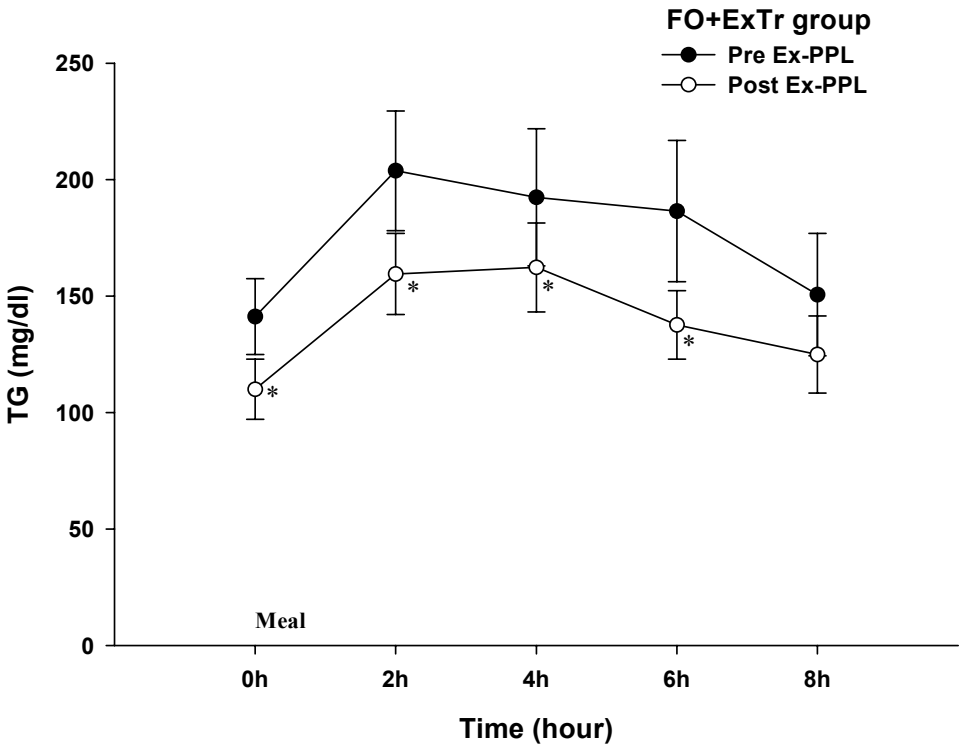

Figure 6. TG changes at time points in FO+ExTr group, Ex-PPL trail pre vs. post treatment. Values are reported as Means ± SE. * Significant difference between pre and post samples, $p<0.05$.

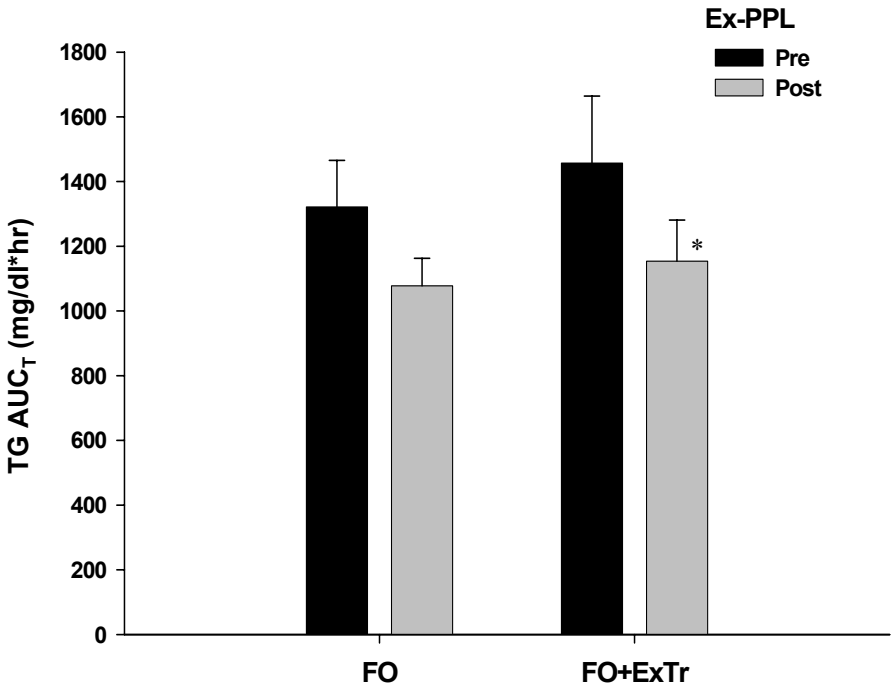

Figure 7. TG AUCT in Ex-PPL pre vs post in each group. Values are reported as Means ± SE. * Significant difference between pre and post samples, $p<0.05$.

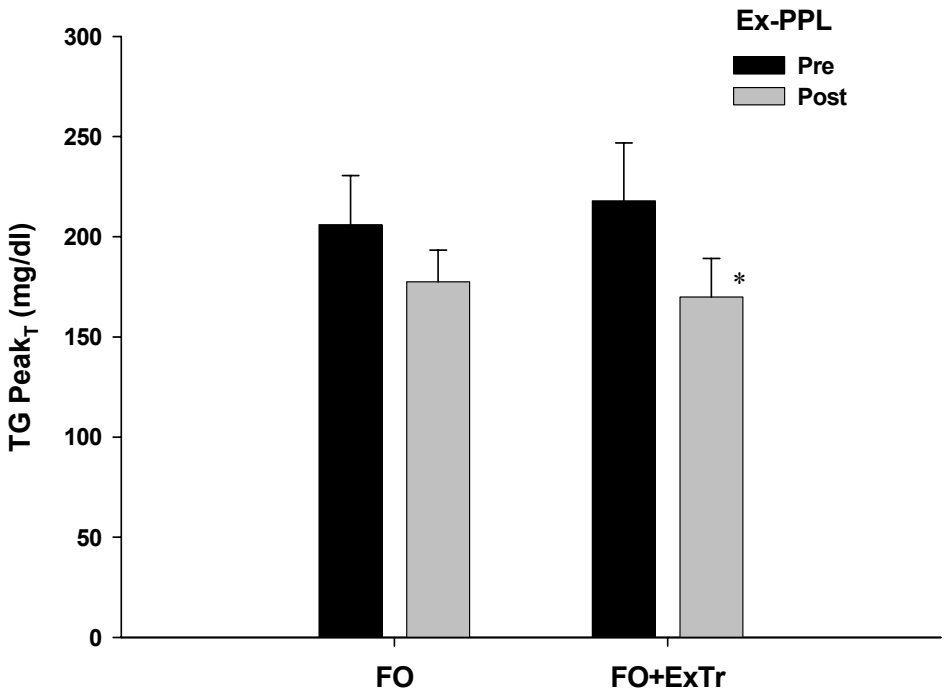

Figure 8. TG Peak$_T$ in Ex-PPL pre vs post in each group. Values are reported as Means ± SE. * Significant difference between pre and post samples, $p<0.05$.

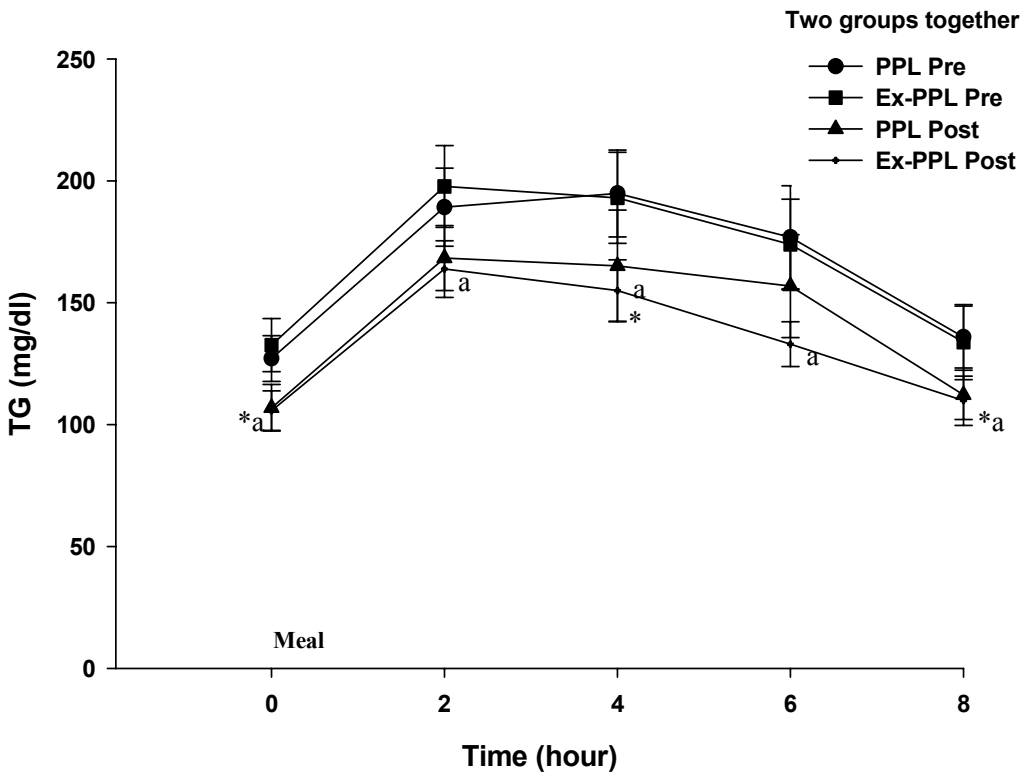

Figure 9. TG values of 5 time points of two groups together in PPL and Ex-PPL trials before and after treatment. Values are reported as Means ± SE (Table 5). * Significant difference between pre and post PPL, $p<0.05$. a Significant difference between pre and post Ex-PPL, $p<0.05$. There was no significant difference between PPL and Ex-PPL in pre testing or in post testing.

Table 7. HDL-C and subfraction values

Group	Trail	HDL-C (mg/dl)		HDL_2-C (mg/dl)		HDL_3-C (mg/dl)	
		week 0	week 4	week 0	week 4	week 0	week 4
FO	Non Exerc.	50.3 ± 3.6	51.7 ± 4.0	16.6 ± 2.6	19.8 ± 3.4*	33.7 ± 1.3	31.9 ± 1.0
FO+ExTr	Non Exerc.	53.7 ± 5.1	57.4 ± 5.4*	20.3 ± 3.6	26.8 ± 4.2*	33.4 ± 1.9	30.6 ± 1.4*
2 groups	Non Exerc.	52.2 ± 3.2	54.8 ± 3.4*	18.6 ± 2.3	23.6 ± 2.8*	33.6 ± 1.1	31.2 ± 0.9*
FO	Exercise	51.3 ± 4.8	51.9 ± 4.2	12.9 ± 2.9	15.7 ± 3.2	38.4 ± 2.1	36.2 ± 1.2
FO+ExTr	Exercise	52.7 ± 4.8	54.4 ± 5.0	14.1 ± 2.8	19.8 ± 3.8	38.6 ± 2.3	34.6 ± 1.9
2 groups	Exercise	52.1 ± 3.4	53.3 ± 3.3	13.6 ± 2.0**	17.9 ± 2.5**	38.5 ± 1.5**	35.4 ± 1.1**

Values are reported as Means ± SE. FO group, n=10; FO+ExTr group, n=12.
* Significant difference after the treatment within the same group. ** Significant difference between non exercise and exercise trial within the same group, $p < 0.05$.

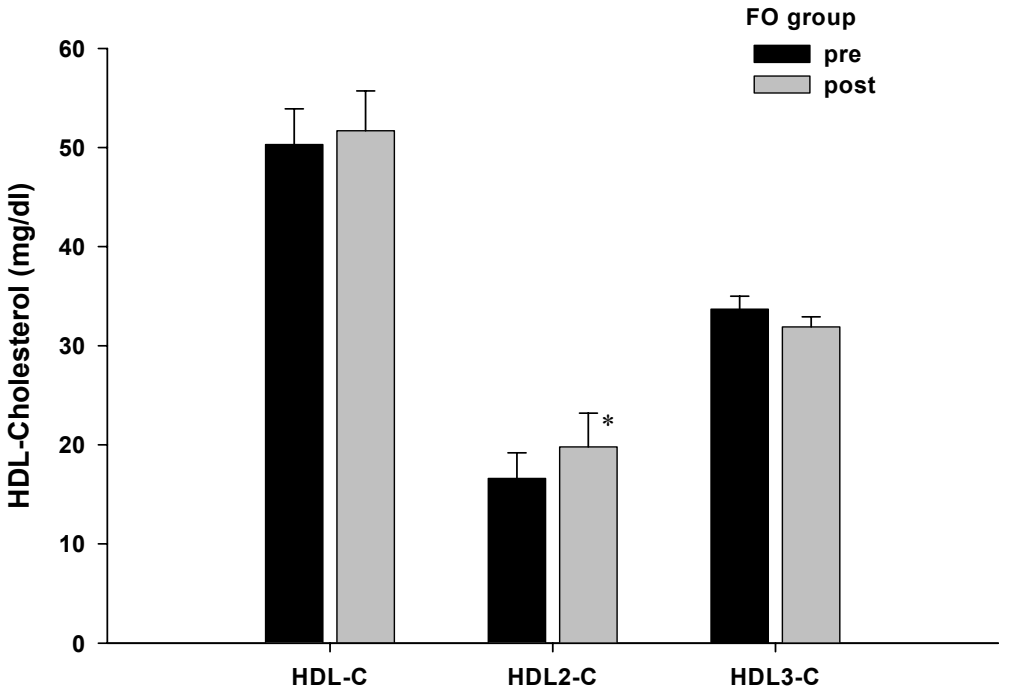

Figure 10. HDL-C and subfraction before and after the treatment in FO group. Values are reported as Means ± SE. * Significant change between pre and post samples, $p<0.05$.

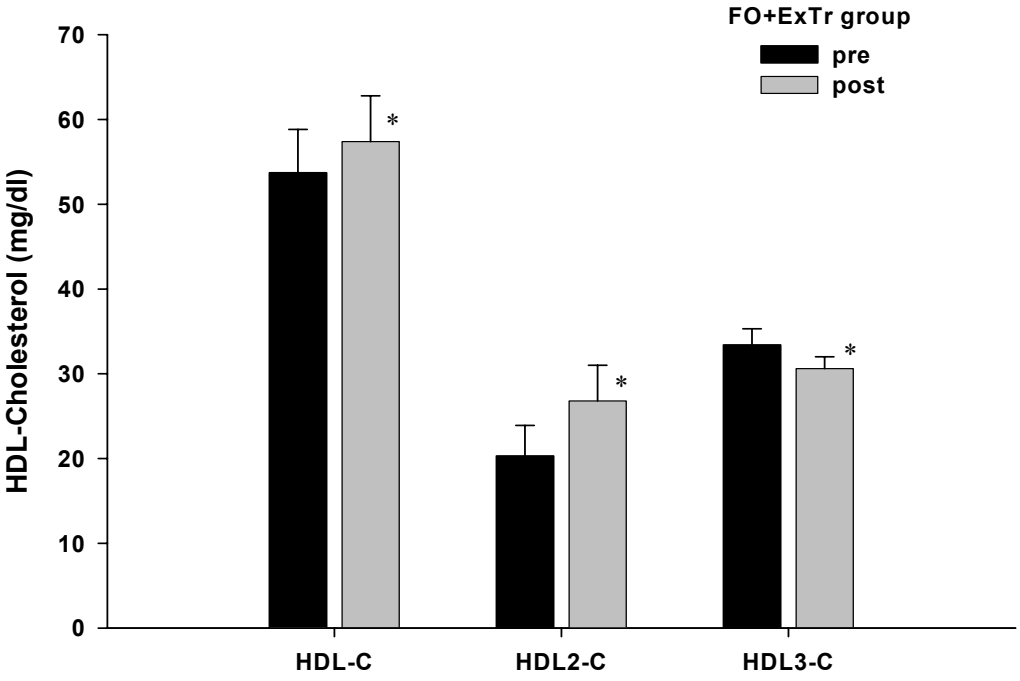

Figure 11. HDL-C and subfraction before and after the treatment in FO+ExTr group. Values are reported as Means ± SE. * Significant change between pre and post samples, $p<0.05$.

Table 8. Calculated LDL-C values

Group	LDL-C week 0 (mg/dl)	LDL-C week 4 (mg/dl)
FO (n=10)	109.4 ± 7.1	106.4 ± 6.3
FO+ExTr (n=12)	111.1 ± 7.2	117.7 ± 4.6
two groups (n=22)	110.3 ± 4.9	112.5 ± 3.9

Values are reported as means ± SE. There was no significant change between pre and post treatment.

Table 9. Plasma CRP concentrations

Group	Measurement	CRP wk 0 (mg/L)	CRP wk 4 (mg/L)
FO (n=10)	non exerc.	4.6 ± 1.9	5.7 ± 1.4
	exercise*	3.7 ± 1.3	6.7 ± 2.7
FO+ExTr (n=12)	non exerc.	4.0 ± 2.3	3.1 ± 1.4
	exercise	3.1 ± 1.6	3.2 ± 1.2
two groups (n=22)	non exerc.	4.3 ± 1.5	4.3 ± 1.0
	exercise	3.4 ± 1.0	4.8 ± 1.4

Values are reported as means ± SE. There was no significant change between pre and post treatment. There was no significant change between non exercise and exercise samples pre treatment, as well as post treatment. * 12h after acute exercise.

Table 10. Plasma IL-6 concentrations in Ex-PPL 2h samples

Group	IL-6 wk 0 (pg/ml)	IL-6 wk 4 (pg/ml)
FO (n=10)	2.7 ± 0.2	2.9 ± 0.2
FO+ExTr	2.9 ± 0.4 (n=6)	2.8 ± 0.2 (n=10)

Values are reported as means ± SE. IL-6 was only detectable in 6 subjects before treatment and in 10 subjects after treatment in FO+ExTr group. There was no significant change between pre and post treatment.

Table 11. Plasma concentration of sICAM-1

Group	sICAM-1 wk 0 (ng/ml)	sICAM-1 wk 4 (ng/ml)
FO (n=10)	60.9 ± 8.3	56.6 ± 7.8
FO+ExTr (n=12)	48.8 ± 10.5	47.2 ± 9.1
two groups (n=22)	54.3 ± 6.8	51.4 ± 6.1

Values are reported as means ± SE. There was no significant change between pre and post treatment.

Table 12. Hct and plasma volume pre and post exercise bout

Subject	Variable	Pre Ex	0h	2h	4h	6h	8h
n=4	Hct	42.1 ± 1.3	43.2 ± 1.3	42.6 ± 2.0	41.9 ± 1.9	42.3 ± 1.3	43.0 ± 1.6
n=4	% change in PV		-4.1 ± 1.3	-1.5 ± 3.4	1.2 ± 3.6	-0.6 ± 1.3*	-3.2 ± 2.6

Note: Values are reported as Means ± SE. * Significant difference between 6h and 0h, $p<0.05$.

DISCUSSION

As reported in the results, the characteristics of the subjects in FO group did not change, while in FO+ExTr group %fat decreased, VO_2max improved. Subjects were required to maintain the diet habit, but without checking diet again at the end of the study, it was unclear how much they kept the diet habit; and this was a limitation of the study. However, because there was no change in body weight, BMI, and waist:hip ratio after treatment in both groups (Table 3), it was reasonable to believe the subjects did not change their dietary habits. Exercise training improved the body composition without changing BMI and waist/hip ratio. Fish oil supplement on the other hand, caused a boarder line decrease in %fat (P=0.084). Four weeks of aerobic exercise training significantly improved VO_2max of the previously sedentary subjects, while fish oil showed no improvement (Table 3). Similar result of combination treatment improving aerobic capacity was also shown in the study by Warner et al (93). The results of improved VO_2max in combination treatment indicated fish oil supplementation plus aerobic exercise training improved aerobic capacity in sedentary subjects. Since the improvement of body composition and aerobic capacity will benefit the prevention of life style related diseases, it would be a better way to provide combination treatment on the sedentary subjects on this variable.

PPL

Our group has done fish oil and PPL studies in sedentary people and recreational trained people. In recreational trained subjects, Zhang et al (97) found acute exercise 12h before high fat meal attenuated TG AUC by 51%. Smith et al (79) found fish oil supplement (4 g/day, 5 wk) plus acute exercise attenuated PPL TG more effectively than fish oil alone. These results indicated acute exercise in combination of fish oil supplementation had an additive effect on attenuating PPL TG, in recreational trained subjects.

In sedentary subjects, Thomas et al (85) provided subjects fish oil 4 g/day for 3 wk. They found a significantly decreased baseline TG in fish oil alone and fish oil in combination with acute exercise two groups together after the treatment. However, there were no differences in PPL parameters in any group when comparing with control group. Our group has demonstrated acute exercise 12h before high fat meal attenuated PPL TG (88, 97), but fish oil in combination with acute exercise did not attenuate PPL TG (85), suggesting that fish oil supplementation had interference with acute exercise in attenuating PPL TG. According to these results, one could conclude: 1) 3 wk fish oil supplementation was not sufficient to attenuate PPL TG. 2) fish oil supplementation had interference with acute exercise in attenuating PPL TG, in sedentary subjects. Warner et al (93) trained sedentary subjects (3 d/ wk), and having them taking fish oil supplement (17.5 ml/day) for 12 wk. They found both fish oil alone and fish oil in combination with exercise training attenuated fasting TG. However there were no differences between these two groups. They did not investigate PPL TG. According to the results of these previous studies, it was unclear how fish oil supplementation in combination with

exercise training influences PPL TG in sedentary subjects, especially when they just start exercise training.

The design of the current study filled the blank of the previous studies in sedentary subjects, since Thomas et al did not train subjects, and Warner et al did not measure PPL TG; while the current study did both. The basic thought of the current study is that when sedentary people participate into exercise program, in time they will respond to fish oil supplementation and acute exercise (additive effect) in PPL TG just like trained subjects. But before they reach that point, there are questions need to be answered: 1) Is the recommendation of combining fish oil supplementation with exercise training valid in sedentary people? 2) How long does it take for this interference effect to convert to a beneficial combination in sedentary people who start exercise training?

The current study provided subjects n-3FA 4 g/day for 4 wk; about half of the subjects also took aerobic exercise training 5 d/wk. Although there were no differences in FO group in PPL parameters pre vs. post treatment, there was a significant decrease in TG-AUC_T in two groups together after the treatment. This might because the sample size of FO group was not great enough to bring a statistic change; while two groups together overcome this problem. On the other hand, there were significantly lowered TG values in several time points in each group and two groups together in post treatment PPL trails, in the current study (Table 5, Figure 3, Figure 4). These results indicated 4 wk fish oil supplementation attenuated PPL TG. Thomas et al (85) provided subjects fish oil supplement for 3 wk at the same dose, but they did not found statistical change in PPL parameters; they also did not find TG values decreased significantly in the time points other than 0h. This might because in the current study treated subjects with fish oil

supplement for 4 wk, while Thomas et al only did for 3 wk. We analyzed the 3 day diet log and found the intake of n-3 FA was insufficient in the subjects; the average amount was 0.25 g/day. According to the recommendation of American Heart Association, EPA+DHA 0.5 to 1.8 g/day, there might be n-3 FA deficiency in the subjects before the study started. If that was the case, it may take shorter time for people who do not have n-3 FA deficiency to react to fish oil supplementation in PPL TG, than the subjects of the current study.

Exercise training did provide some additive effects in attenuating PPL TG, as in FO+ExTr group, there were significant changes in TG-AUC_T and TG-$Peak_T$ after the treatment, in Ex-PPL (Table 4, wk 0 vs. wk 4, Figure 7, Figure 8). There was no change in PPL parameters in FO group, although during the comparison of TG AUCT pre vs post, p= 0.077 showed the trend of decrease after treatment. Combination treatment also decreased PPL TG values in more time points than FO alone, in Ex-PPL trial (Table 5). In FO group, TG values decreased only at 2 time points after treatment, although there was a trend of decrease in 2 other time points after treatment. Although there were no differences between the two groups after 4 wk treatment, it is very possible that by extending exercise training period, sedentary subjects would gradually experience additive effect of the combination treatment in Ex-PPL TG, as observed by Smith et al (79).

Based on the results of the current study, the answers to the previous two questions would be: 1) The recommendation of combing fish oil supplementation with exercise training should be avoided in the first a few weeks of therapy, in sedentary people. 2)

There is evidence that the interference effect starts to convert into beneficial effect four weeks after sedentary people start exercise training program.

Exercise and PPL

Many studies have indicated that exercise attenuates PPL TG (88, 97, 98). Surprisingly exercise 12h before high fat meal did not attenuate PPL TG in either treatment group in the current study (Table 3, Table 4). However, the exercise bout was carried out by every subject in the lab under supervision.

One of the possibilities could be the diet of the subjects on the day before the fat loading test provided varied fat and/or calorie intake between PPL and Ex-PPL trials. Although we had instructed subjects to follow the same diet they took the day before the first PPL, in all the PPL trials afterward, it was still hard to confirm that they followed the instruction. This dietary factor may contribute to the fact that there was no significant difference of TG values in 0h samples between PPL and Ex-PPL trials. In fact 11 subjects had their exercise fasting TG (0h sample in Ex-PPL) higher than non exercise fasting TG (0h sample in PPL) in pre testing; while this happened in 12 subjects in post testing. This suggested the diet control was not sufficient. In the future study, it would be ideal to provide a standard meal at least for the meal before fasting the day before the fat loading test. This would minimize the impact of different diet on the baseline level of TG.

The second explanation was that the mode of exercise influenced results. During pre testing, all the subjects did fast walking in the acute exercise bout; while in post testing, 5 subjects from FO+ExTr group did slow jogging. The target heart rate was 75% maxHR which was determined by VO_2max test. Although the target heart rate was the same with

the one used in the study of Zhang et al (97), their subjects did jogging instead of walking. Thomas et al (85a) investigated energy expenditure in walking and jogging in 9 young men. They found at the same exercise heart rate, jogging caused higher energy expenditure and higher fat energy expenditure than walking. This result indicated that different exercise mode had significant impact on fat utilization, which might influenced plasma TG level. On the other hand, there have been several walking studies that had subjects expend between 800 and 1,000 kcal per exercise session (90, 91). Despite the fact that they expended more kcal during their exercise session, the reduction in PPL was smaller when compared to the running trials (79, 97). This also is the case with the cycling study (41). During the 90 min bout of cycling, subjects expended 1,070 kcal of energy, and the reduction in PPL was less than the running studies (79, 97) and similar to the walking studies (90, 91). Since exercise modes influence energy expenditure and fat metabolism, it is important to use jogging during the acute exercise in Ex-PPL study.

The primary difference between the modes of exercise is that the eccentric component of muscular contractions is much greater in running than in walking and cycling. This eccentric component may alter fat metabolism. Researchers have reported that fat energy expenditure was greater during jogging over a 60 min exercise session than cycling (86). It was concluded that this increase in fat energy expenditure was probably related to the eccentric component of the jogging. Since the eccentric component of walking is less than jogging, it would be reasonable to assume that this would be the case if jogging were to be compared to walking. Based on these findings, it seems that running is more effective in reducing PPL than walking and cycling when the activities are performed at the same intensity levels. In the current study, 50% of the subjects in FO+ExTr group; or

25% of subjects in two groups together did slow jogging during post testing. This percentage was not large enough to lead to a statistically significant change between PPL and Ex-PPL in post testing. However, among the 5 subjects who jogged during post testing, 3 of them showed decreased TG-AUC_T, TG-AUC_I, TG-$Peak_T$ and TG-$Peak_I$ in acute exercise PPL, in comparing to non exercise PPL. This indicated the mode of exercise did influence the results. On the other hand, among these 5 subjects, 3 of them had higher 0h TG values in Ex-PPL trial than in PPL trial in post testing. This again indicated the impact of diet on baseline (0h) TG values.

The third explanation is that in the current study, the exercise bout did not induce enough LPL change relative to the amount of fat intake. Exercise has been shown to induce LPL gene expression in skeletal muscle (78). LPL activity becomes elevated shortly after an exercise session, but the activity does not peak until 18h after an exercise session and remains elevated for up to 30h (44). It is believed that the increased LPL activity in muscle may play the most important role in the attenuated TG response (51). Study has shown that exercise induces human LPL gene expression predominantly in skeletal muscles, and more vigorous skeletal muscle activity may result in greater increase in LPL production (78). Because jogging involves more muscle activity than walking does, jogging may induce grater LPL activity than walking does. Zhang et al (97) found TG AUC was 51% lower in Ex-PPL in comparing with PPL; and Smith et al (79) found a 50% decrease in TG peak response in Ex-PPL. Since these two studies all used jogging during acute exercise, it is very possible that the exercise in the current study might not be intensive enough to induce great LPL release. Since the current study

did not measure the LPLa pre and post treatment, there was no direct evidence to prove this explanation.

Smith et al (79) measured LPLa in recreational trained subjects during PPL and Ex-PPL trial. They found although TG-AUC_I during Ex-PPL trial was 40% less than the TG-AUC_I during the PPL trial, there were no statistically significant differences between the LPLa for these two trials. This result suggested the change of PPL TG was not parallel to that of LPLa. It begged the question, if there is anything to do with the source of LPLa. If adipose tissue is the primary source of LPLa during the PPL trial, maybe exercise increased muscle LPLa which hydrolyzed TG at the greater rate than LPLa in adipose tissue during Ex-PPL trial. If this is the case, it is easy to think that in the current study, exercise did not induce enough LPLa change in skeletal muscle as it was in other studies. Again it may due to the mode or intensity of the exercise. It would be useful if tissue samples were taken to measure the LPLa activity in adipose tissue and skeletal muscle in the future study.

The fourth reason may be due to the variability associated with the PPL responses. There were 6 subjects who had TG peak response less than 50 mg/dl; and 2 subjects who had TG peak response greater than 150 mg/dl in PPL trial during pre testing in the current study. After 4 wk treatment, there were 3 subjects who had TG peak response less than 50 mg/dl; and 4 subjects had TG peak response greater than 150 mg/dl during post testing. This range of responses resulted in large amount of variability in TG values and in any variable that was calculated from these values. The interesting phenomenon was the number of subjects, who had TG peak response lower than 50 mg/dl or higher than 150 mg/dl, decreased after 4 wk of treatment. It seems fish oil supplementation

normalized the response to high fat meal. This may due to the positive impact that fish oil has in fat metabolism.

Although single bout of exercise before fat loading did not decrease PPL TG, exercise training for 4 wk did provide a lower TG-AUC_T and TG-$Peak_T$ in FO+ExTr group in comparing with before treatment in Ex-PPL trials. On the other hand, among the four subjects who had TG peak response greater than 150 mg/dl in post testing, three of them were from FO+ExTr group. This suggested the combination treatment group had better response to acute exercise in PPL. This result agreed with other studies (79, 98). In recreational trained subjects in the study of Smith et al, fish oil supplementation in combination with acute exercise, PPL parameters were significantly lower than fish oil alone. Ziogas et al (98) investigated PPL TG in sedentary, recreational trained, and endurance trained subjects. They found recreational trained, and endurance trained subjects, TG-AUC_T was significantly lower than sedentary subjects. These results indicated that exercise training attenuates PPL TG; exercise training induced greater response in acute exercise PPL. Studies have found exercise training increases LPLa, but this increase was transient (77, 78). If exercise training induced LPLa could not be accumulated, there would not be difference between sedentary and trained subjects in acute exercise PPL, after trained subjects stopped exercise training for a day or two. But that was not the case in the study of Ziogas et al (98); their subjects did not participate into vigorous exercise 36h prior PPL test. The most recent study by Pilegaard et al (75) suggested that the cumulative effects of transient increases in transcription during recovery from consecutive bouts of exercise may represent the underlying kinetic basis for the cellular adaptations associated with exercise training. If this is true, it may

explain why subjects in the study of Smith et al responded to acute exercise PPL better than those in the current study. Since the subjects in the study of Smith et al (79) participated in daily physical activities longer than those in the current study.

Since both n-3FA and exercise training increase LPL, it is reasonable that the two methods in combination would provide an even better result in attenuating PPL TG. However, it remains unclear why acute exercise causes interference in the effect of fish oil in attenuating PPL TG, in sedentary subjects. The mechanism of how exercise training interacts with fish oil supplementation to improve the response of human subject to acute exercise in PPL has yet to be discovered too.

Fish oil and PPL

Researches have found n-3FA supplementation reduce PPL TG (37, 79, 85, 94). The reduction in PPL following n-3FA supplementation is hypothesized to be due to decreased hepatic TG secretion (very low-density lipoprotein, VLDL) and/or decreased intestinal TG secretion (chylomicrons and VLDL) (38). This inhibition of TG secretion reduces the amount of TG that is present in the plasma, thus lowering PPL. In the current study, there were significant changes in TG-AUC_T in two groups together after the treatment, in PPL trial. There were significant changes in TG-AUC_T and TG-$Peak_T$ in two groups together after the treatment, in Ex-PPL trial. This result agreed with previous studies that fish oil supplementation attenuates PPL TG. Despite of the significant changes in two groups together, there was no change in FO group. This might because the sample size in FO group was not large enough (n=10), but when two groups were combined, the sample size increased (n=20), the changes became significant.

While postprandial lipemia is one of the most accurate predictors of CVD risk, fasting TG remain to be a valuable parameter. In the current study, both groups had decreased TG in fasting samples and acute exercise sample (12h after acute exercise) after the treatment. However, there was no difference between the two groups in fasting TG after the treatment. This result agreed with Warner et al (93) who found exercising 3 days/wk for 12 wk in conjunction with fish oil supplementation lowered fasting TG level. However, the response was the same in combination treatment group and fish oil only group. In considering Warner trained the subject for 12 wk, which was 3 times longer than the current study, combination treatment might not attenuate fasting TG more than fish oil supplementation alone even in a long run.

HDL and LDL

The effects of fish oil supplementation on HDL and LDL have not been consistent in previous studies (72, 81, 82). Zhang et al has reported that acute exercise (exercise on treadmill for 1h at 60% VO2max) increases HDL-C (97), which was not the case in this study. However, acute exercise 12 h before blood sampling did increase HDL_2-C and decrease HDL_3-C in two groups together in this study. As previously discussed, the intensity of walking and jogging is very different; it is possible that the intensity of the acute exercise in the current study was too low to induce HDL-C change. It is also possible that different modes of exercise influenced HDL-C differently, since Zhang et al (97) used jogging, while most of the subjects in the current study did walking.

This study found increased HDL-C and HDL_2-C and decreased of HDL_3-C in FO+ExTr group after the treatment. There was an increase of HDL_2-C, but HDL-C and HDL_3-C were unchanged in FO group. This result suggested that both treatment groups

had improved HDL profile, while the improvement in combination treatment group seems to be more ideal. Other investigators have reported that n-3 FA supplementation does not affect baseline HDL-C (28, 85). This was confirmed in the current study. There was no difference between FO and FO+ExTr group in HDL-C and subfractions after the treatment. Acute exercise combined with fish oil supplementation (85), or exercise training combined with fish oil supplementation (current study and Warner et al) all did not showed additive effects for HDL-C or subfractions in sedentary people. This seems to be true in active people (79).

There were several possibilities why a larger increase in HDL cholesterol did not occur in the exercise group. First, the intensity of exercise may have been too low. Because the combination treatment group showed significant improvement of VO2max and the subjects were working at 75% maximal HR, it is unlikely that intensity was too low to effectively increase HDL-C. Second, the frequency or duration of exercise training may not have been sufficient. Warner et al trained subject 3 d/wk for 12 wk still did not improve HDL-C, while another study which used the same training protocol successfully induced HDL-C change (43). The exercise training was more frequent than Warner et al. It is possible that after a longer period of time (8-12 wk), HDL-C change would be induced. Finally one could not rule out the possibility that fish oil and exercise interacted and affected the mechanism of HDL-C production and removal.

It has been a concern about prescribing n-3 FA due to the potential to elevate LDL-C (20, 61). Fish oil did not significantly increase LDL-C concentration in the current study. On the other hand, Warner et al (93) has concluded that aerobic exercise significantly adds to the hypolipidemic effects of fish oil by causing a decrease in LDL-C and by

improving the composition of LDL. The current study did not find a decrease of LDL-C in any treatment group. This may because of the exercise training duration of the current study was shorter than that of Warner.

Since we did not measure the concentration of some important enzymes in lipoprotein metabolism, like LPL, lecithin cholesterol acyltransferase (LCAT), and cholesterol ester transfer protein (CETP), it would be difficult to analyze more detailed metabolic change. CETP catalyzes the exchange of TG from TG-rich lipoproteins with cholesterol from HDL, especially HDL_2-C. Elevated activity of this enzyme may increase CVD because of the potential for lowering HDL-C and HDL_2-C. CETP has been shown to be decreased by exercise training, but Zhang et al (96) found no change of this enzyme activity over 24 h following a single aerobic exercise session. LCAT binds CEs to HDL, which may enhance cholesterol uptake from tissues. Exercise has been shown to stimulate the activity of this enzyme, but the results have not been consistent (26, 96). Thomas et al (87) reported LCAT and CETP functions were not stimulated by fish oil supplementation and exercise.

Inflammatory markers

Hypertriglyceridemia-induced endothelial cell dysfunction plays a critical role in the pathology of atherosclerosis. Many researches have supported fatty acid-mediated endothelial activation and dysfunction as a consequence of hypertriglyceridemia (10, 32). Hypertriglyceridemia leads to high plasma concentration of low-density lipoprotein cholesterol (LDL-C), and low plasma concentration of high-density lipoprotein cholesterol (HDL-C). It has been shown that plasma concentration of TG, and ratios of TC/HDL-C, LDL-C/HDL-C were positively correlated to CRP (63, 95). Plasma

concentration of HDL-C was shown to be negatively correlated to CRP (27). Hypertriglyceridemia is also found to be correlated to cell adhesion molecules, thus contributing to inflammatory procedure and atherosclerosis (45).

Researches have found that fish oil decreases the concentrations of inflammatory markers in the blood; thus it is believed fish oil has anti-inflammatory effect (19, 24). There were studies reported that fish oil intake decreased IL-6 level (19, 22). Researches by Eschen and Miles (30, 60) suggest the impact of fish oil supplementation on sICAM-1 varied with dose of supplement as well as age and gender of subjects. On the other hand, it is also believed that exercise training inhibits inflammatory process and thus reduces the risk of lifestyle related diseases (57). Acute exercise however, has been proved to induce inflammation (59, 80).

Based on these understandings, the current study tried to investigate if fish oil has inhibitory effects on acute exercise induced inflammation. Similar method has been used by Phillips et al (74) on a dietary supplement study, during which they successfully induced CRP and IL-6 by eccentric exercise. In the current study, CRP and IL-6 were measured in exercise samples (12h after acute exercise), while CRP was also measured in fasting samples pre and post treatment. Since previous studies have conflict results on sICAM-1 concentration changes after acute exercise (58, 62, 64), the current study only measure the sICAM-1 concentration on fasting samples pre and post treatment. In the current study, since acute exercise was not sufficient to cause inflammation, the ability of fish oil to attenuate inflammation induced by exercise could not be investigated.

CRP

CRP is an acute phase inflammatory protein that is released in response to acute injury, infection, or other inflammatory stimuli. There was no significant change of CRP after the treatment in fasting samples in both groups in this study. This suggested neither fish oil, nor exercise training attenuated CRP level in sedentary subjects after 4 wk treatment. On the other hand, CRP values in exercise samples were not different from the non exercise samples pre treatment. This indicated acute exercise had no significant impact on CRP values in this study. Since acute exercise did not successfully stimulate CRP, it is impossible to investigate whether fish oil and exercise training attenuated CRP reaction after acute exercise in this study.

There were studies showed CRP levels after exercise correlated to the muscle damage level (5, 73). Paulsen et al (73) measured maximal force-generating capacity, creatine kinase (CK), CRP, and IL-6 in 11 healthy men before and after 300 maximal eccentric actions with m. quadriceps. They found a positive correlation between acute loss of force and changes in CK, and between the acute loss of force and changes in CRP. If that was the case in the current study, it would be easy to explain the negative response of CRP after the exercise session, since the intensity of the exercise may not be enough to induce CRP change.

However, there is also a possibility that the time of sampling was impropriate to detect the CRP change. Some studies showed CRP peaked a day after the exercise bout (59, 33), Paulsen et al (73) reported CRP peaked 2 days after exercise. Byrne et al (17) had subjects performed military exercise, prolonged moderate physical exertion in full battledress, covering approximately 10 miles. They reported no change of CRP concentration 12h after exercise. The more reasonable analysis is that the exercise bout

in the current study did not induce detectable CRP change at 12h. If the intensity was higher, it would be possible to see an increase of CRP concentration at 12h if not peaked.

Among these studies which investigated how exercise affected CRP; there was a large variety of mode and intensity of exercise, including military exercise (17), bench stepping (33), cycling (59), and maximal eccentric action of a certain muscle (73). This could be one of the possibilities which caused different results, since as previous discussed, different modes and intensity of exercise affect energy expenditure, fat metabolism and muscle damage differently. This may have indirect impact on CRP concentration after exercise, and also make it hard to compare the results among studies.

Although fish oil has been associated with anti-inflammatory effect, evidence regarding the effects of omega-3 fatty acids on hs-CRP is inconclusive (18, 29, 53, 54). In terms of the impact of DHA on acute phase proteins, published results range from no effect to a lowering effect on CRP levels (29, 54). The difference in study populations, particularly from an age and health standpoint, may account for the differences observed in these studies.

There has been argument about how much change of CRP value indicates the inflammatory change of the body. Kushner et al (48) believed researchers should be cautious when explain the CRP values between 3 and 10 mg/L. It may only imply a mild degree of tissue stress or injury, suggesting the presence of distressed cells, rather than a resulting inflammatory response. If this is true, exercise induced CRP has to reach a certain level before one could interpret it as a systemic inflammatory response. This will increase the difficulties and intensities of exercise bouts.

IL-6

IL-6 is the main stimulant for the hepatic production of CRP. Researchers have indicated that IL-6 also predict future cardiovascular events (76). Elevated IL-6 levels amongst patients presenting with acute coronary syndromes were a strong independent predictor of increased mortality (50).

Investigators have pointed out that IL-6 is the central mediator of exercise immunology (66, 67). It has been found that plasma IL-6 starts to increase within the first hour of prolonged exercise and continues to rise depending on the duration of exercise (65, 70). On the other hand, other studies have indicated that shorter intensive or mild prolonged exercise of less than 1h seems to be insufficient to significantly alter plasma IL-6 concentration (14, 25). There has been research indicated that IL-6 levels after exercise correlated to the muscle damage (15). In the current study, IL-6 was not detectable in most of the samples 12 h after exercise, but was detectable in the 2h samples in Ex-PPL. Based on the results of previous studies, it is more likely that the high fat meal stimulated IL-6 release in the current study. Few studies reported high fat meal induced IL-6. Corpeleijn et al (21) investigated IL-6 change at postprandial stage in male subjects with impaired glucose tolerance. They found high-fat meal can evoke IL-6 release from muscle. However, it is unknown if this is the case in healthy people. In the current study high fat meal successfully induced IL-6 in both pre and post treatment Ex-PPL trials. The future study may be able to investigate if fish oil supplement inhibits inflammation induced by high fat meal. Although exercise and high fat meal both can induce inflammation, one should be cautious when combining these two factors in inflammatory study; since the combination effect of these two factors on inflammation has yet to be determined.

Researches have found fish oil intake decreased IL-6 level (19, 22). There was in vitro evidence that DHA reduced endothelial expression of IL-6 in stimulated cells (23, 24). De Caterina et al (24) used a variety of stimulating stressors to invoke an inflammatory response and observed a significant reduction in IL-6 levels in the presence of DHA. Similarly, Phillips et al (74) used eccentric exercise to induce IL-6 and CRP, and found a dietary supplement mainly DHA attenuate the IL-6 and CRP in the subjects after exercise. Based on the success of these studies, it seems to be a practical idea to magnify the inflammatory marker change by exercise or other methods, before investigating if a factor could decrease these markers.

sICAM-1

The pathology of atherosclerosis has been regarded to be an inflammatory procedure. One of the key initial events in the development of atherosclerosis is the adhesion of monocytes to endothelial cells, with subsequent transmigration into the vascular intima. Leukocyte and vascular adhesion molecules such as ICAM-1 play critical roles in the adhesion of monocytes to endothelial cells. Research also has shown high blood TG is correlated to cell adhesion molecules, thus contributing to atherosclerosis (45).

The current study did not find sICAM-1 change after 4 wk treatment in either group. Studies in terms of exercise induced sICAM-1 have provided conflicting results. Nielsen et al (64) used subjects who participated in marathon or half-marathon, and found increased sICAM-1 after both treatments. Mizia-Stec et al (62) used patients with coronary artery disease to complete treadmill stress test, and found no change in sICAM-1. Meksawan et al (58) combined dietary fat intake with maximal exercise test. They found subjects who had fat intake equaled to 19% total calories, which put them in

negative energy balance, resulted in increased sICAM-1. Akimoto et al (5) had healthy male subjects to perform three different kind of exercise, bicycle ergometer at 80% VO2max for 16 min, 42 km endurance running, and 30-min downhill running at intensity of ventilation threshold. They found plasma sICAM-1 concentration increased a day after the endurance running (12%) and downhill running (14%), but not after ergometer exercise. Plasma CRP and creatine kinase concentration also increased a day after running. They concluded exercise associated with muscle damage and/or inflammation results in increased levels of plasma sICAM-1. If this is the case, the current study might not provide sufficient muscle damage to induce sICAM-1, because of the mode of exercise, as previously discussed.

The impact of fish oil on plasma sICAM-1 concentration has been investigated by previous studies. Lopez-Garcia et al (52) analyzed the dietary intake of 727 women, aged 43 to 69 y. They found CRP levels were 29% lower among those in the highest quintile of total n-3 fatty acids, compared with the lowest quintile; IL-6 levels were 23% lower, E-selectin levels 10% lower, sICAM-1 levels 7% lower, and sVCAM-1 levels 8% lower. Long-chain n-3 fatty acids (EPA and DHA) were inversely related to sICAM-1 and sVCAM-1. However these associations were not modified by intake of vitamin E, dietary fiber, trans fatty acids, or by the use of postmenopausal hormone therapy. Abe et al (1) treated hypertriglyceridemia patients with n-3 fatty acids 4g/d for at least 7 months, and found reduced sICAM-1, E-selectin and TG levels. This result support previous in vitro data showing that disorders in triglyceride and HDL metabolism influence CAM expression and treatment with fish oils may alter vascular cell activation.

However, it seems the effect of n-3 FA on sICAM-1 is complex and may depend on gender and dose. Eschen et al (30) performed a double blind study with 60 healthy subjects. Subjects were randomly assigned to receive a daily supplement of n-3 FA 6.6 g, n-3 FA 2.0 g, or olive oil for 12 weeks. Among the women, there was a significant decrease in sICAM-1 in the n-3 FA 2.0 g group and a significant increase in sVCAM-1 in the n-3 FA 6.6 g group. In the men, after supplementation with 6.6 g of n-3 FA, there was a significant decrease only in sP-selectin. The subjects in the current study received 4.0 g n-3 FA daily for 4 weeks, and there were no significant changes in sICAM-1 concentrations of all the subjects after the treatment. When sICAM-1 concentrations of female subjects were processed by 2-way repeated measures ANOVA, there were no significant changes in FO vs. FO+ExTr and pre vs. post treatment. This may because the dose and treatment length of n-3 FA supplementation in the current study were less than the study of Eschen et al.

Plasma Volume Change

Investigators have indicated that plasma volume (PV) is affected up to 24h after an acute bout of high-intensity or endurance exercise (89). During exercise, blood colloid osmotic pressure increases, and leads to the fluid goes towards interstitial spaces, thus decreases the PV. A few hours after exercise, blood pressure decreases because of vasodilation; baroreceptor input decreases; antidiuretic hormone (ADH) secretion decreased; kidney reserves liquid, and thus increases plasma volume (34). The PV change in the current study agreed with this theory. By measuring Hct in blood samples at Ex-PPL 0h which was 12h after acute exercise, we found a decreased PV, the average change of PV was -4.1 ± 1.3% in comparing to pre exercise. When Ex-PPL 0h TG

values were corrected according to this PV change, the statistic results of PPL TG 0h vs. Ex-PPL TG 0h remained to be the same. At PPL 6h which was 18h after exercise, there was a significant increase of PV in terms of % change of PV, -0.6 ± 1.3%, between PPL 6h and 0h samples. When PPL TG values at 6h were corrected according to PV changes, the statistical results remained to be the same.

Limitation to the study

The acute exercise session in Ex-PPL did not provide enough effect as it should. As previously discussed, this mainly because of the walking mode may not have been sufficient. The diet the day before PPL and Ex-PPL trials were not sufficiently controlled. These two factors covered the effect of acute exercise on attenuating PPL-TG. The low intensity exercise may also responsible for the failure in inducing inflammatory markers, and HDL-C. The small number of subjects, n=10 in each group in PPL TG study is a limitation to the study. This small sample size along with the variability of PPL response reduced the statistical power of the result. Inflammatory markers in more time points should be analyzed due to the different peaking time of these proteins after exercise. On the other hand, if high fat meal induced IL-6, it might also induce other inflammatory markers in PPL trial.

Summary of results

The current study found acute exercise did not attenuate PPL TG parameters, and TG values at any time points. Fish oil supplement only and fish oil in combination with aerobic exercise training attenuated PPL TG. Combination treatment had no interference on n-3FA in attenuating PPL TG in previously sedentary people after 4 wk treatment. Aerobic exercise training improved body composition and aerobic capacity of the

previous sedentary subjects. Combination treatment provided more ideal HDL-C and subfraction change than fish oil only, although the difference between the two treatments was very mild. Fish oil supplementation did not alter LDL-C in sedentary subjects. Exercise training at 60% VO2max for 60 min did not successfully induce inflammatory marker change, but high fat meal increased plasma IL-6 concentration. Neither fish oil supplementation, nor combination treatments inhibit inflammation and cellular adhesion.

Conclusions

N-3 FA supplementation attenuates PPL in sedentary subjects. Exercise training has no interference or additive effects on n-3FA in attenuating PPL. N-3FA does not attenuate inflammation; this may because our acute exercise did not induce inflammation.

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APPENDIX A

EXTENDED LITERATURE REVIEW

Dietary fat and inflammation

Life style related diseases have been the leading cause of death in industrialized nations. The character of this life style is high energy intake and low energy consumption, which leads to a metabolic syndrome. Dietary factors especially dietary fat intake has caught attentions in understanding of the impact of high energy intake on lifestyle diseases. The Third Report of the National Cholesterol Education Program (NCEP) Adult Treatment Panel III focuses on therapeutic lifestyle changes as the cornerstone of therapy of CHD. The components include reduced intakes of saturated fat and cholesterol, increased dietary fiber, inclusion of plant sterols/stanols, increased physical activity, and weight control (10). Since inflammation is a key component of the metabolic syndrome, and has been shown to be involved in atherosclerosis, diabetes, cancer and other lifestyle diseases; it is necessary to discuss the relationship between dietary fat and inflammation.

Saturated fatty acid and trans fatty acids induce inflammatory procedure

High fat diet always leads to positive energy intake, which causes obesity. Obesity is highly correlated with inflammation with the fact that elevated CRP levels have been associated with obesity (52). On the other hand, weight loss causes decreased CRP in obese subjects (17). Animal study also indicated that many inflammatory and macrophage-specific genes are dramatically upregulated in white adipose tissue in obesity induced by high fat diet (55). These results indicated the chronic effect of high fat diet characterized by high energy intake and obesity, leads to inflammation.

There have been several studies that reported a positive correlation between diets with a high content of saturated and trans fatty acids (TFAs) and biomarkers of inflammation.

King et al (20) examined the National Health and Nutrition Examination survey data from 1999 to 2000; and revealed a modest association between saturated fat consumption and elevated CRP. Analysis of data from the Nurses' Health Study I cohort revealed a 73% higher level of CRP in women in the highest quintile of TFAs intake compared with the lowest quintile (26). TFAs intake was positively related to plasma concentration of CRP, soluble tumor necrosis factor receptor (TNFR2), E-selectin, sICAM-1, and sVCAM-1 in linear regression models after controlling for age, BMI, physical activity, intake of saturated fatty acids et al (27). These large sample studies indicated saturated fatty acids and TFAs induce inflammatory procedure.

According to previous studies, there are two mechanism of TFAs affects inflammation. First, TFAs has negative impact on lipoprotein profile, and the impaired lipoprotein metabolism induces inflammation. Numerous studies have reported increases in triglyceride levels ranging from 1.0 to 24 mg/dl, with an average increase of 3.0 mg/dl per 2 percent of energy intake from TFAs (25, 46, 58). Human study also indicated that TFAs diet not only increase LDL-C but also decrease HDL-C level (31, 58). Impaired lipoprotein metabolism induces inflammation. It has been shown that plasma concentration of TG, and ratios of TC/HDL-C, LDL-C/HDL-C were positively correlated to CRP (34, 57). Plasma concentration of HDL-C was shown to be negatively correlated to CRP (9). Studies have found that hypertriglyceridemia actived leukocytes, in vivo (38). Since activation of leukocytes is obligatory for inflammation and atherogenesis by adhering to the endothelium via specific ligands, hypertiglyceridemia thus induces adhesion. On the other hand, hypertriglyceridemia was found to be correlated to cell adhesion molecules, thus contributing to inflammatory procedure and atherosclerosis

(19). LDL-C is a major cause of injury to the endothelium and underlying smooth muscle (12, 32, 33). When LDL particles become trapped in an artery, they can undergo progressive oxidation and be internalized by macrophages by means of the scavenger receptors on the surfaces of these cells (12, 22, 32, 33). The internalization leads to the formation of lipid peroxides and facilitates the accumulation of cholesterol esters, resulting in the formation of foam cells. Since hypertriglyceridemia and increased LDL-C play such important roles in inflammation and atherosclerosis, TFAs thus indirectly induces inflammation.

Second, there was evidence showed TFAs related to endothelial dysfunction (6, 26), but the biological mechanisms underlying the adverse effects of TFAs on endothelial function are not clear. TFAs are incorporated into endothelial cell membranes and thus could alter cellular and macromolecular components acting at the interface of the blood vessel wall. This could result in changes in the antihemostatic properties, altered vascular tone, hyperadhesiveness to blood leukocytes, and increased cytokine and growth factor production, all of which are characteristics of endothelial dysfunction (6).

Hypertriglycerdemia leads to endothelial cell dysfunction

High fat diet is directly related with postprandial triglyceridemis. Chronic hypertriglyceridemia or postprandial triglyceridemia, elevated levels of triglyceride-rich lipoproteins, including chylomicrons, very low density lipoproteins (VLDL) and their remnants, are an independent risk factor in the development and progression of atherosclerosis. Hypertriglyceridemia, particularly when associated with decreased HDL and abdominal or visceral obesity, is a highly atherogenic phenotype (24).

There is evidence that hypertriglyceridemia-induced endothelial cell dysfunction plays a critical role in the pathology of atherosclerosis, and researches have detected the following mechanisms for this procedure. First, hypertriglyceridemia can lead to endothelia cell dysfunction associated with increased vascular superoxide anion production and a subsequent decrease in nitric oxide bioavailability (23). Second, it has been reported that leukocyte, and especially monocyte, adhesion to the endothelial surface is stimulated by triglyceride-rich lipoproteins (4, 7). Third, endothelial dysfunction was observed after consuming a high-fat meal and is associated with augmented oxidative stress manifested by the depletion of serum antioxidant enzymes and increased excretion of oxidative modification products (48**)**. Research has indicated that endothelia cell activation during metabolic states of hypertriglyceridemia and PPL is redox sensitive (8). Doi et al reported that a-tocopherol or N-acetylcysteine can suppress endothelia expression of ICAM-1, VCAM-1 and tissue factor, mediated by remnant-like lipoprotein particles isolated from plasma of hyerptriblyceridemic patients.

Fourth, high LPL activity is associated with endothelial cell dysfunction and atherosclerosis. In atherosclerosis, LPL is overexpressed in vascular lesions and also produced by monocytes, marcrophages and smooth muscle cells (37, 43, 56). It was shown in both cultured human umbilical vein and human coronary artery endothelial cells that inflammatory cytokines which are implicated in the etiology of vascular diseases, such as TNF-alpha and IL-1 beta, could upregulate endothelial cell-derived LPL mRNA (15). These results indicated that high LPL activity related with inflammation may play a critical role in the pathology of atherosclerosis. Furthermore, Chung et al (3) found that lipoprotein remnants derived from human triglyceride-rich lipoproteins produced after a

meal rich in polyunsaturated fat were more injurious to cultured vascular endothelial cells than those produced after a meal rich in saturated fat. These data support the hypothesis that PPL hypertriglyceridemia and the simultaneous release of free fatty acids during LPL-mediated TG hydrolysis in the proximity of the endothelium can cause endothelial cell injury.

N-3 fatty acids and inflammation

Epidemiological studies showed an inverse correlation between dietary fish or fish oil consumption and biomarkers of inflammation (28), although the results from intervention trials have not yet confirmed. The mechanism of the anti-atherosclerosis effect of N-3 fatty acids has been investigated by many laboratories. The research group of De Caterina et al (29) investigated the intracellular generation of reactive oxygen species (ROS) in endothelial cells treated with various fatty acids. It is believed that ROS, generated in low concentrations, may function intracellularly as second messengers in mediating the activation of NF-κB and activator protein 1. De Caterina et al (6) stimulated endothelial cells with cytokines or lipopolysaccharide. Preincubation of endothelial cells with the DHA blunted the endothelial response to the proinflammatory stimulation in terms of VCAM-1 surface expression or the release of macrophage colony stimulating factor. Such effects were accompanied by a decreased production of ROS and a sparing in the depletion of the intercellular antioxidant reduced glutathione. These results indicated that n-3 fatty acids may exert a direct vascular atheroprotective effect by inhibiting endotherlial activation through a quenching of stimuli-induced increase of ROS.

The second possible mechanism is that EPA and DHA alter the metabolism of adhesion molecules such as VCAM-1, E-selectin, and ICAM-1. Abe et al (1) reported a 9% reductionin sICAM-1 and a 16% reduction in sE-selectin, in hypertriglyceridemic subjects receiving 3.4 g/d of high purified n-3 fatty acid ethyl esters for 7 to 12 months. There is also in vitro evidence that DHA reduces endothelial expression of VCAM-1, E-selectin, ICAM-1, IL-6 and IL-8 in stimulated cells (5, 6). These results were confirmed by studies in smokers and in coronary patients (18, 41).

The third potential anti-atherogenic mechanism of n-3 fatty acids is their interference with the arachidonic acid cascade. EPA not only can replace arachidonic acid in phospholipids bilayers, but it is also a competitive inhibitor of cyclooxygenase, reducing the production of prostaglandins and thromboxanes (49). EPA and DHA can inhibit platelet affregaton by reducing the affinity of platelet TxA_2/PGH_2 receptor for its ligand (2).

Although there are a lot of intervention studies about n-3 fatty acids influences on inflammation, the results have been conflicting. The reason for this is that there are a lot of differences among the studies, the dose of n-3 fatty acids, and the duration of treatment; the character of the subject population, the standard and method of evaluation, the existent of other therapy, et al. Among these factors, the quality and stability of n-3 fatty acids preparation has not been widely known until recently. Highly concentrated fish oils usually have markedly lower stability than natural fish oils. It has been shown that intake of highly concentrated n-3 fatty acids preparations can have adverse effects (14, 44). The adverse effects were ascribed to increased lipid peroxidation in the

subjects. Based on these understandings, one need to be cautious, and take all the relative factors into consideration, when comparing different results of the studies.

The prevention effect of physical exercise to inflammation

In order to prevent lifestyle related diseases, it is probably the most important strategy to maintain a healthy lifestyle. Despite of controlling dietary factors which influence the energy intake, it is equally significant to maintain the energy output by doing physical exercise. Physical activity is associated with a range of health benefits, and its absence can have harmful effects on health and well being, increasing the risk for coronary heart disease, diabetes, certain cancers, obesity, and hypertension (50). In fact exercise training plays such an important role that it is recommended by many institutes including Centers for Disease Control and Prevention (CDC), and American College of Sports Medicine (ACSM) to the general population to have a beneficial effect on their health (39).

Studies have reported exercise training decreased inflammation in both healthy and high risk subjects (13, 30, 51, 53). Postmenopausal women, senior citizen and CVD patients are three important groups of people who have high risk of atherosclerosis. Studies (13, 51, 53) in these populations indicated exercise decrease the level of blood inflammatory markers, thus benefit prevention of atherosclerosis. On the other hand, different types of exercise have been investigated in influencing inflammatory markers. King et al (21) analyzed the adults, age 17 and over who participated in the National Health and Nutrition Examination Survey (NHANES) III, total of 4072 people. They found a significant lower likelihood of elevated inflammatory markers among regular participants in jogging, swimming, cycling, and aerobic dancing. A nine-month

marathon training in 14 subjects also inhibited CRP (30). Based on these studies, aerobic training decrease levels of the inflammatory marker, so that benefits the prevention of atherosclerosis. The results of how resistance training influences inflammatory markers were inconsistent (11, 36, 42), thus the mode, intensity, and duration of resistance training in inhibiting inflammation has yet to be determined. Despite of the effect of exercise in inhibiting inflammation, exercise training was also proved to improve endothelial function, by both animal and human studies (45, 54).

Scientists have tried to explain why acute exercise stimulates inflammation, while long term exercise training suppresses inflammatory process; although the mechanism remains to be unclear. Physiologic study (40) has demonstrated that contracting skeletal muscle acutely produced IL-6, a cytokine that has dual functions in inflammation. Increased levels of inflammatory markers also have been demonstrated in the blood stream acutely after weight lifting and marathon running (35, 47). While some scientists thought this elevation is due to muscle damage during exercise (42), some evidence showed different possibilities, as cytokine levels did not correlate with parameters of muscle damage, such as release of creatine kinase (16). It may be due to intense exercise or a substantial mechanical load on the muscle. Regular exercise training appears to modify this response, possibly due to a training effect, as CRP level actually decreased with regular endurance training (30). More research needs to be conducted in this area.

Generally speaking, it is clear that in order to decrease the risk of life style related diseases; one should control diet, especially dietary fat intake, and to participate adequate amount of physical exercise. This could help to inhibit pathology procedure of inflammation, and ultimately prevent people from lifestyle related diseases.

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APPENDIX B

INFORMED CONSENT

CONSENT FORM TO PARTICIPATE IN A RESEARCH STUDY

Tom R. Thomas, Ph.D.

Project # 1049539

FOR HS IRB USE ONLY
APPROVED _____ ____ HS IRB Authorized Representative Date **EXPIRATION DATE:** _____

STUDY TITLE: EFFECTS OF EXERCISE TRAINING PLUS OMEGA 3 FATTY ACIDS ON FAT HANDLING AND INFLAMMATION

INTRODUCTION

This consent may contain words that you do not understand. Please ask the investigator or the study staff to explain any words or information that you do not clearly understand.

This is a research study. Research studies include only people who choose to participate. As a study participant you have the right to know about the procedures that will be used in this research study so that you can make the decision whether or not to participate. The information presented here is simply an effort to make you better informed so that you may give or withhold your consent to participate in this research study.

Please take your time to make your decision and discuss it with your family and friends.

You are being asked to take part in this study because you are an untrained person who may benefit from initiating an exercise program.

This study is being sponsored by the Department of Nutritional Sciences and the Exercise Physiology Laboratory.

In order to participate in this study, it will be necessary to give your written consent.

WHY IS THIS STUDY BEING DONE?

Both long-term exercise and fish oil (omega-3 fatty acids) supplementation may be prescribed to help reduce blood fats after a meal (postprandial lipemia) and chronic inflammation. It may be best to supplement with fish oil prior to starting an exercise program so that inflammation will be reduced. The purpose of this research is to examine the interaction between exercise and fish oil intake on blood parameters related to the risk of cardiovascular disease. Specifically, we will explore the effect of exercise and fish oil intake on blood fats and/or markers of inflammation.

HOW MANY PEOPLE WILL TAKE PART IN THE STUDY?

About 50 people will take part in this study at this institution.

WHAT IS INVOLVED IN THE STUDY?

If you volunteer, your participation will consist of the following:

1. You will complete an initial screening consisting of the following: medical, diet, activity, bleeding disorders, and menstrual cycle (females only) questionnaires, a vigorous treadmill test to determine maximal oxygen consumption, and have your body composition (% fat) determined by skinfold measurements.
2. You will record your food intake for three days and follow a 24-hour prescribed diet, including a 12-hour fast prior to the pre and posttesting sessions.
3. You will complete four fat load trials, two before and two after the treatment. During each trial, you will eat a high fat meal. The high fat meal will consist of a milk shake that is based on your body weight and is made of ice cream and heavy whipping cream. A 60 minute treadmill brisk walk/jog of moderate intensity will be included with two of the four trials. The walk/jog will take place in the laboratory.
4. You will have about 120 ml of blood withdrawn during each trial from a vein in your arm. This is less than the 450 ml of blood collected when you donate blood. During each trial, you will be required to return to the lab 5-6 times in an eight hour period to have 10-40 ml of blood drawn each time. These visits will last approximately 15 minutes. Each trial will be separated by 1-4 weeks. The total number of visits to the lab is 28 for the fish oil only group and 48 for the fish oil plus exercise group. The total amount of time in the lab for all trials is about 10 hours for the fish oil group and about 30 hours for the fish oil plus exercise group.
5. Treatment: You will ingest fish oil supplements (gel tabs, 4g/day) each day for four consecutive weeks. Half the subjects also will exercise on a treadmill at moderate intensity (brisk walk or slow jog) for 45 minutes, 5 days per week for 4 weeks. You will need to come to the lab each week to pick up the fish oil gel tabs. For the exercise group, the total amount of time for exercise over 4 weeks is about 20 hours.
6. You will not receive any placebos, and you will act as your own control.
7. You will not change your exercise or activity other than what the research requires, and you otherwise will attempt to maintain the level of activity you had before you began the study.

8. Blood will be kept in storage until all tests have been run in case of errors in the lab testing process. Once tests are completed, blood will be discarded.

HOW LONG WILL I BE IN THE STUDY?

If you volunteer, your participation will last 7-9 weeks, but it will be ended at any time at your request, and Dr. Thomas may end it at any time that, if in his judgment, it is in your best interest to do so. Depending on the group in which you are assigned, your time commitment should be less than 30 hours.

The investigator and/or your doctor may decide to take you off this study if the fish oil tablets cause nausea or other discomfort.

If you are a woman and become pregnant, you also will be removed from the study.

WHAT ARE THE RISKS OF THE STUDY?

While in the study, you are at risk for the side effects described below. You should discuss these with the investigator and/or your doctor. There may be other side effects that we cannot predict.

1. Potential lightheadedness and tiredness at the end of the exercise test to voluntary exhaustion. This also could occur during the submaximal exercise sessions.
2. Heart problems may occur during the exhaustive exercise test or the exercise training sessions. These potential problems include irregular heart beats and chest pain. In the event you experience any heart problems, we will stop the exercise session immediately and evaluate you to determine if you need medical attention.
3. You may experience muscle soreness from the exercise.
4. Temporary increases in low density lipoprotein (LDL) cholesterol levels may occur due to the fish oil supplements in subjects with high triglyceride levels. This risk is temporary, but elevated LDL levels are associated with increased risk of heart disease.
5. You may experience temporary discomfort and bruising where a needle is inserted to collect blood.
6. Mild nausea may be caused by the fish oil supplements.
7. As is true of all medications and medical treatment, there is always the possibility of a new or unexpected risk.

For reasons stated above we will observe you closely while giving you the treatment described. If you have any worrisome symptoms or symptoms that my associates and I have described to you, notify me immediately. My telephone number is **(573) 882-8191**.

ARE THERE BENEFITS TO TAKING PART IN THE STUDY?

If you agree to take part in this study, there may or may not be direct medical benefit to you.

You may expect to benefit from taking part in this research to the extent that you are contributing to the medical knowledge. Other benefits include: Fitness evaluation, dietary analysis, and plasma lipid profile.

WHAT OTHER OPTIONS ARE THERE?

An alternative to taking part in this research would be to not participate in this research.

WHAT ABOUT CONFIDENTIALITY?

Information produced by this study will be stored in the investigator's file and identified by a code number only. The code key connecting your name to specific information about you will be kept in a separate, secure location. Information contained in your records may not be given to anyone unaffiliated with the study in a form that could identify you without your written consent, except as required by law. If the investigator conducting this study is not your primary, or regular doctor, he must obtain your permission before contacting your regular doctor for information about your past medical history or to inform them that you are in this trial.

Results of this research may be published and reports may be made to government agencies, funding agencies, manufacturers or scientific bodies, but you will not be identified in any such publication or report. In addition, the Federal Food and Drug Administration, other government agencies, and the manufacturer of the drug(s) used in this study may inspect and copy your medical records that apply to this research. In all cases, information about you will be treated confidentially.

WHAT ARE THE COSTS?

Examinations and tests for this research will be paid for by the Exercise Physiology Laboratory.

WILL I BE PAID FOR PARTICIPATING IN THE STUDY?

You will be compensated $50.00 for your participation.

WHAT IF I AM INJURED?

It is not the policy of the University of Missouri to compensate human subjects in the event the research results in injury. The University of Missouri, in fulfilling its public responsibility, has provided medical, professional and general liability insurance coverage for any injury in the event such injury is caused by the negligence of the University of Missouri, its faculty and staff. The University of Missouri also will provide, within the limitations of the laws of the State of Missouri, facilities and medical attention to subjects who suffer injuries while participating in the research projects of the University of Missouri. In the event you have suffered injury as the result of participation in this research program, you are to contact the Risk Management Officer, telephone number (573) 882-1181, at the Health Sciences Center, who can review the matter and provide further information. This statement is not to be construed as an admission of liability.

WHAT ARE MY RIGHTS AS A PARTICIPANT?

Participation in this study is voluntary. If you do not volunteer or if your participation is ended for any reason, this will not affect any care or consideration to which you are entitled. In addition, the investigator of this study may decide to end your participation in this study at any time after Dr. Thomas has explained the reasons for doing so and has helped arrange for your continued care by your own doctor, if needed.

You will be informed of any significant new findings discovered during the course of this study that might influence your health, welfare, or willingness to continue participation in this study.

WHOM DO I CALL IF I HAVE QUESTIONS OR PROBLEMS?

Please ask any questions you have about this research or how it will affect you, and I will answer them. In addition, if you have any questions during your participation I, or one of my associates, will be glad to discuss them with you. You may call me at **(573) 882-8191 or 882-0062**.

If you have any questions regarding your rights as a participant in this research and/or concerns about the study, or if you feel under any pressure to enroll or to continue to participate in this study, you may contact the University of Missouri Health Sciences Institutional Review Board (which is a group of people who review the research studies to protect participants' rights) at (573) 882-3181

A copy of this consent form will be given to you to keep.

Signature
I confirm that the purpose of the research, the study procedures, the possible risks and discomforts as well as potential benefits that I may experience have been explained to me. Alternatives to my participation in the study also have been discussed. I have read this consent form and my questions have been answered. My signature below indicates my willingness to participate in this study.

Subject/Patient*	Date
Legal Guardian/Advocate/Witness (if required)**	Date
Additional Signature (if required) (identify relationship to subject)***	Date

*A minor's signature on this line indicates his/her assent to participate in this study. A minor's signature is not required if he/she is under 7 years old. Use the "Legal Guardian/Advocate/Witness" line for the parent's signature, and you may use the "Additional Signature" line for the second parent's signature, if required.

**The presence and signature of an impartial witness is required during the entire informed consent discussion if the patient or patient's legally authorized representative is unable to read.

***The "Additional Signature" line may be used for the second parent's signature, if required. This line may also be used for any other signature which is required as per federal, state, local, sponsor and/or any other entity requirements.

"If required" means that the signature line is signed only if it is required as per federal, state, local, sponsor and/or any other entity requirements.

SIGNATURE OF STUDY REPRESENTATIVE

I have explained the purpose of the research, the study procedures, identifying those that are investigational, the possible risks and discomforts as well as potential benefits and have answered questions regarding the study to the best of my ability.

Study Representative**	**Date**

****Study Representative is a person authorized to obtain consent. Per the policies of the University of Missouri Health Care, for any 'significant risk/treatment' study, the Study Representative must be a physician who is either the Principal or Co-Investigator. If the study is deemed either 'significant risk/non-treatment' or 'minimal risk,' the Study Representative may be a non-physician study investigator.

APPENDIX C

SUBJECT FORMS

University of Missouri-Columbia
Exercise Physiology Lab
Health History and Medical Questionnaire

Name:_____Date:_____ Age:_____

Address:_____ Sex: Male Female

_____Height:_____ Weight:_____

Telephone: _____

Email:_____

Personal Physician's Name:_____

Blood Pressure:_____/_____

Address &

Telephone:_____

Health History:

Have you ever had: High Blood Pressure **Y / N** Low Blood Pressure **Y / N**
Heart Disease **Y / N**
Irregular Heart Beat **Y / N** Diabetes **Y / N** Heart Murmurs **Y / N** Chest Pain **Y / N**
Thyroid Disease **Y / N** Lung Disease **Y / N** Arthritis **Y / N**
High Blood Cholesterol **Y / N**
Has a parent or sibling had any heart disorders prior to age 55? **Y / N**
Has a sudden death ever occurred in a parent or sibling? **Y / N**
Do you smoke? **Y / N** If Yes, how much? _____
You are an/a African-American ___ Asian___ Caucasian___ Hispanic___ Native American___ Pacific Islander___ Other___

Have you ever taken medication for? Medication and Dosage Date

Y / N High Blood Pressure: _____
Y / N Low Blood Pressure: _____
Y / N Heart Disease: _____
Y / N Diabetes: _____
Y / N Thyroid Disease: _____
Y / N Lung Disease: _____
Y / N Arthritis: _____

Do you participate in a regular exercise program? **Y / N**
Times per week: _____
For how many years? _____

What activity? _____

If you are a woman, is there any chance you could be pregnant?

Medical Questionnaire:

1. Have you ever been advised by a physician to avoid exercise? **Y / N**
2. Do you ever have shortness of breath during or after exercise? **Y / N**
3. Have you ever experienced fainting or dizzy spells? **Y / N**
4. Have you ever experienced pain or discomfort in the chest? **Y / N**
5. Have you ever experienced back, jaw or left arm pain or recurrent indigestion? **Y / N**
6. Have you ever experienced swollen ankles (excluding sprains)? **Y / N**
7. Have you recently experienced heart palpitations (rapid heart beat) at rest? **Y / N**
8. Have you ever experienced claudication (pain in the calf, thigh, or buttocks with walking)? **Y / N**
9. Is there any other health condition that might limit your participation in exercise programs (e.g., bone or joint disorders, pregnancy, etc.)? **Y / N** If Yes, please explain:_____
10. Are you taking any medication not listed above? **Y / N**
    If Yes, please list:_____
11. Have you had a medical exam in the last 12 month? **Y / N**
    If Yes, please list date:_____

Signature:_____

Date:_____

University of Missouri-Columbia
Exercise Physiology Lab

Follow-up Medical History Questions

8. Have you had any surgeries? Please list

10. Did you have any medical conditions for which you received treatment during the past year?

Do you take any vitamins or dietary supplements? Please list

University of Missouri-Columbia
Exercise Physiology Lab

Additional Medical Questionnaire
(For Women only)

1. Do you have a menstrual cycle? Yes No
   If Yes, go to question #2
   If No, go to question #5

2. What is the frequency of your menstrual cycle? _____ times/year

3. How many days does each cycle last? _____ days.

4. When was your last menstrual period?

5. Are you taking birth control pills? Yes No

5a. If Yes, please record the brand name_____,
the amount_____.

6. Are you on estrogen therapy? Yes No

7. If Yes, please list the name (s), and frequency.

Name	Frequency (i.e. 1 per day)

Additional comments:

University of Missouri-Columbia
Exercise Physiology Lab

Activity Questionnaire

(Please circle appropriate response and elaborate when necessary)

Name: _____ Date: _____ Weight: _____

Gender: Male Female Age: _____

1. Do you usually engage in some form of planned regular or semi-regular exercise?

   Yes No (If no, please go to the last question)

2. Are you currently exercising?

   Yes No (If Yes, please answer Question #3 in detail)

3. Please complete the following table. Please give your best estimate!

Mode of exercise (jog, bike, swim, etc.)	Days per week	Duration (minutes)	Pace (mph)	Intensity (mild/ mod/vigor)	History (# of months)

4. Are you presently, or have you recently trained for a competitive event?

   Yes No (If yes, what event_____ and duration of training_____)

5. Do you have any comments about your exercise program that you feel we should know about?

   Yes No (If yes, Please explain in space provided below)

   _____

   _____

   _____

6. Does your occupation or daily routine involve a considerable amount of activity? For example, walking, stair climbing, lifting, etc.?

   Yes No (If yes, Please explain in the space provided below)

   _____

   _____

   _____

   _____

Dietary record

Date: Day of week:

Time of Day	Food/Drink	Brand	Amount (tsp, cup, oz)	Condiments	Location/Place
BREAKFAST					
MORNING SNACK					
LUNCH					

Time of Day	Food/Drink	Brand	Amount (tsp, cup, oz)	Condiments	Location/Place
AFTERNOON SNACK					
DINNER					
EVENING SNACK					

Exercise training form

Max HR______
Target HR (75% HRmax)______

SUBJECT #______
week #______week date______ -______
group ______

GET FIT NOW IN-LAB TRAINING LOG

Day_____
Date_____
Session____
Initials_____
Body wt.____
Rest HR_____

	1 min	2 min	3 min		min 1	min 2	min 3	min 4	min 5
WARM-UP HR				COOL DOWN HR					
TIME (after warmup)	5 min	10 min	15 min	20 min	25 min	30 min	35 min	40 min	45 min
HR									
SPEED									

Day_____
Date_____
Session_____
Initials______
Body wt.____
Rest HR_____

	1 min	2 min	3 min		min 1	min 2	min 3	min 4	min 5
WARM-UP HR				COOL DOWN HR					
TIME (after warmup)	5 min	10 min	15 min	20 min	25 min	30 min	35 min	40 min	45 min
HR									
SPEED									

Day_____
Date_____
Session_____
Initials______
Body wt.____
Rest HR_____

	1 min	2 min	3 min		min 1	min 2	min 3	min 4	min 5
WARM-UP HR				COOL DOWN HR					
TIME (after warmup)	5 min	10 min	15 min	20 min	25 min	30 min	35 min	40 min	45 min
HR									
SPEED									

Day_____
Date_____
Session_____
Initials______
Body wt._____
Rest HR_____

	1 min	2 min	3 min		min 1	min 2	min 3	min 4	min 5
WARM-UP HR				COOL DOWN HR					
TIME (after warmup)	5 min	10 min	15 min	20 min	25 min	30 min	35 min	40 min	45 min
HR									
SPEED									

Day_____
Date_____
Session_____
Initials_____
Body wt.____
Rest HR____

	1 min	2 min	3 min		min 1	min 2	min 3	min 4	min 5
WARM-UP HR				COOL DOWN HR					
TIME (after warmup)	5 min	10 min	15 min	20 min	25 min	30 min	35 min	40 min	45 min
HR									
SPEED									

******COOL DOWN TO 110 BPM*****

APPENDIX D

STATISTICAL RESULTS

Two-way repeated measures ANOVA

Statistical results: TG AUC_T in PPL (mg/dl* 8hr)

Source of Variation	df	F	P-value
Treatment	1	0.902	0.353
Time	1	5.596	0.028
Treatment x Time	1	1.807	0.194

Statistical results: TG AUC_I in PPL (mg/dl* 8hr)

Source of Variation	df	F	P-value
Treatment	1	0.314	0.581
Time	1	0.208	0.653
Treatment x Time	1	2.119	0.161

Statistical results: TG $Peak_T$ in PPL (mg/dl)

Source of Variation	df	F	P-value
Treatment	1	0.303	0.588
Time	1	2.23	0.151
Treatment x Time	1	3.101	0.094

Statistical results: TG $Peak_I$ in PPL (mg/dl)

Source of Variation	df	F	P-value
Treatment	1	0.00007	0.993
Time	1	0.046	0.832
Treatment x Time	1	3.461	0.078

Statistical results: TG PPL 0h (mg/dl)

Source of Variation	df	F	P-value
Treatment	1	1.305	0.267
Time	1	27.642	<0.001
Treatment x Time	1	0.0614	0.807

Statistical results: TG PPL 2h (mg/dl)

Source of Variation	df	F	P-value
Treatment	1	0.134	0.718
Time	1	4.311	0.051
Treatment x Time	1	1.89	0.184

Statistical results: TG PPL 4h (mg/dl)

Source of Variation	df	F	P-value
Treatment	1	1.032	0.322
Time	1	5.629	0.028
Treatment x Time	1	2.2	0.153

Statistical results: TG PPL 6h (mg/dl)

Source of Variation	df	F	P-value
Treatment	1	1.183	0.29
Time	1	1.593	0.221
Treatment x Time	1	1.236	0.28

Statistical results: TG PPL 8h (mg/dl)

Source of Variation	df	F	P-value
Treatment	1	1.477	0.238
Time	1	7.686	0.012
Treatment x Time	1	0.102	0.752

Statistical results: TG AUC_T in Ex-PPL (mg/dl* 8hr)

Source of Variation	df	F	P-value
Treatment	1	0.271	0.608
Time	1	12.681	0.002
Treatment x Time	1	0.151	0.702

Statistical results: TG AUC_I in Ex-PPL (mg/dl* 8hr)

Source of Variation	df	F	P-value
Treatment	1	0.0104	0.92
Time	1	1.625	0.217
Treatment x Time	1	0.0296	0.865

Statistical results: TG $Peak_T$ in Ex-PPL (mg/dl)

Source of Variation	df	F	P-value
Treatment	1	0.005	0.944
Time	1	9.097	0.007
Treatment x Time	1	0.594	0.45

Statistical results: TG $Peak_I$ in Ex-PPL (mg/dl)

Source of Variation	df	F	P-value
Treatment	1	0.642	0.433
Time	1	1.885	0.185
Treatment x Time	1	0.333	0.57

Statistical results: TG Ex-PPL 0h (mg/dl)

Source of Variation	df	F	P-value
Treatment	1	0.588	0.452
Time	1	21.725	<0.001
Treatment x Time	1	0.716	0.407

Statistical results: TG Ex-PPL 2h (mg/dl)

Source of Variation	df	F	P-value
Treatment	1	0.0059	0.94
Time	1	7.386	0.013
Treatment x Time	1	0.896	0.355

Statistical results: TG Ex-PPL 4h (mg/dl)

Source of Variation	df	F	P-value
Treatment	1	0.0593	0.81
Time	1	10.321	0.004
Treatment x Time	1	0.506	0.485

Statistical results: TG Ex-PPL 6h (mg/dl)

Source of Variation	df	F	P-value
Treatment	1	0.473	0.5
Time	1	11.589	0.003
Treatment x Time	1	0.551	0.467

Statistical results: TG Ex-PPL 8h (mg/dl)

Source of Variation	df	F	P-value
Treatment	1	2.096	0.163
Time	1	8.001	0.01
Treatment x Time	1	0.0485	0.828

Statistical results: HDL-C in fasting samples (mg/dl)

Source of Variation	df	F	P-value
Treatment	1	0.475	0.499
Time	1	6.725	0.017
Treatment x Time	1	1.246	0.278

Statistical results: HDL3-C in fasting samples (mg/dl)

Source of Variation	df	F	P-value
Treatment	1	0.153	0.7
Time	1	15.062	<0.001
Treatment x Time	1	0.783	0.387

Statistical results: HDL2-C in fasting samples (mg/dl)

Source of Variation	df	F	P-value
Treatment	1	1.124	0.302
Time	1	31.793	<0.001
Treatment x Time	1	3.564	0.074

Statistical results: HDL-C in exercise samples (mg/dl)

Source of Variation	df	F	P-value
Treatment	1	0.0824	0.777
Time	1	1.803	0.194
Treatment x Time	1	0.464	0.503

Statistical results: HDL3-C in exercise samples (mg/dl)

Source of Variation	df	F	P-value
Treatment	1	0.079	0.782
Time	1	9.466	0.006
Treatment x Time	1	0.813	0.378

Statistical results: HDL2-C in exercise samples (mg/dl)

Source of Variation	df	F	P-value
Treatment	1	0.344	0.564
Time	1	27.695	<0.001
Treatment x Time	1	3.45	0.078

Statistical results: LDL (mg/dl)

Source of Variation	df	F	P-value
Treatment	1	0.626	0.438
Time	1	0.229	0.637
Treatment x Time	1	1.577	0.224

Statistical results: CRP in fasting samples (mg/L)

Source of Variation	df	F	P-value
Treatment	1	0.435	0.517
Time	1	0.0134	0.909
Treatment x Time	1	1.998	0.173

Statistical results: CRP in exercise samples (mg/L)

Source of Variation	df	F	P-value
Treatment	1	0.757	0.395
Time	1	2.685	0.117
Treatment x Time	1	2.422	0.135

Statistical results: IL-6 (pg/ml)

Source of Variation	df	F	P-value
Treatment	1	0.0173	0.897
Time	1	2.246	0.158
Treatment x Time	1	0.998	0.336

Statistical results: sICAM-1 (ng/ml)

Source of Variation	df	F	P-value
Treatment	1	0.69	0.416
Time	1	2.414	0.136
Treatment x Time	1	0.457	0.507

One-way ANOVA

Characteristics of subjects between groups before treatment

	df	F	P value
Age (yr)	1	0.071	0.793
Body weight (kg)	1	0.593	0.45
BMI (kg/m2)	1	0.195	0.664
Waist/hip ratio	1	0.018	0.894
% fat	1	0.167	0.687
VO2max (L/min)	1	0.0497	0.826
max HR (beat/min)	1	0.821	0.376

One-way repeated measures ANOVA

Characteristics of subjects within FO group pre vs. post treatment

	df	F	P value
BMI (kg/m2)	1	0.144	0.713
Waist/hip ratio	1	4.125	0.073
% fat	1	3.78	0.084
VO2max (L/min)	1	0.546	0.481
max HR (beat/min)	1	2.333	0.161

Characteristics of subjects within FO+ExTr group pre vs. post treatment

	df	F	P value
BMI (kg/m2)	1	4.678	0.053
Waist/hip ratio	1	0.262	0.619
% fat	1	33.667	<0.001
VO2max (L/min)	1	17.667	0.001
max HR (beat/min)	1	0.353	0.565

FO group PPL TG pre vs. post

	df	F	P value
AUCT (mg/dl*8hr)	1	4.716	0.058
AUCI (mg/dl*8hr)	1	1.247	0.293
PeakT (mg/dl)	1	4.215	0.07
PeakI (mg/dl)	1	1.855	0.206
0h (mg/dl)	1	18.042	0.002
2h (mg/dl)	1	3.558	0.092
4h (mg/dl)	1	9.836	0.012
6h (mg/dl)	1	1.867	0.205
8h (mg/dl)	1	2.813	0.128

FO+ExTr group PPL TG pre vs. post

	df	F	P value
AUCT (mg/dl*8hr)	1	1.212	0.295
AUCI (mg/dl*8hr)	1	0.542	0.477
PeakT (mg/dl)	1	0.0059	0.94
PeakI (mg/dl)	1	1.372	0.266
0h (mg/dl)	1	14.737	0.003
2h	1	0.746	0.406
4h (mg/dl)	1	0.556	0.472
6h (mg/dl)	1	0.746	0.79
8h (mg/dl)	1	8.068	0.016

FO group Ex-PPL TG pre vs. post

	df	F	P value
AUCT (mg/dl*8hr)	1	3.992	0.077
AUCI (mg/dl*8hr)	1	1.643	0.232
PeakT (mg/dl)	1	1.581	0.24
PeakI (mg/dl)	1	0.229	0.644
0h (mg/dl)	1	5.93	0.038
2h	1	1.092	0.323
4h (mg/dl)	1	4.926	0.054
6h (mg/dl)	1	5.121	0.05
8h (mg/dl)	1	4.503	0.063

FO+ExTr group Ex-PPL TG pre vs. post

	df	F	P value
AUCT (mg/dl*8hr)	1	9.831	0.009
AUCI (mg/dl*8hr)	1	0.499	0.495
PeakT (mg/dl)	1	12.674	0.004
PeakI (mg/dl)	1	2.684	0.13
0h (mg/dl)	1	18.577	0.001
2h	1	9.966	0.009
4h (mg/dl)	1	5.327	0.041
6h (mg/dl)	1	7.277	0.021
8h (mg/dl)	1	4.085	0.068

FO group HDL-C and subfractions in fasting sample pre vs. post

	df	F	P value
HDL-C (mg/dl)	1	1.275	0.288
HDL3-C (mg/dl)	1	4.26	0.069
HDL2-C (mg/dl)	1	6.692	0.029

FO+ExTr group HDL-C and subfractions in fasting sample pre vs. post

	df	F	P value
HDL-C (mg/dl)	1	6.432	0.028
HDL3-C (mg/dl)	1	12.152	0.005
HDL2-C (mg/dl)	1	30.25	0.001

FO group CRP fasting vs. exercise (mg/L)

	df	F	P value
pre	1	0.269	0.617
post	1	1.505	0.245

FO+ExTr group CRP fasting vs. exercise (mg/L)

	df	F	P value
pre	1	0.045	0.836
post	1	0.729	0.415

APPENDIX E

RAW DATA

PPL TG values and parameters

Sub#	Trial	AUCT	AUCI	PeakT	PeakI	0 hr	2hr	4 hr	6 hr	8hr
1	pre-P	1344.8	108.8	205.1	50.6	154.5	205.1	175	153.4	123.3
1	pre-E	1447.6	255.6	225	76.0	149.0	201.7	164.7	225	115.8
1	post-P	1807.9	534.3	279.8	120.6	159.2	245.9	230.5	279.8	136.3
1	post-E	1233.6	370.4	204.8	96.9	107.9	204.8	176.4	134.9	93.5
4	pre-P	641.4	111.0	92.6	26.3	66.3	91.5	92.6	74.4	58.1
4	pre-E	822.8	8.4	118.7	16.9	101.8	118.7	102.6	90.5	97.4
4	post-P	831.2	271.2	140.4	70.4	70.0	103	93.7	140.4	87
4	post-E	1277.1	294.7	206.9	84.1	122.8	206.9	166.6	139	129.3
5	pre-P	901.5	92.7	159.5	58.4	101.1	159.5	115.2	79.2	92.6
5	pre-E	1301.5	444.7	211.9	104.8	107.1	157.6	211.9	166.9	121.6
5	post-P	906.2	237.4	136.4	52.8	83.6	111.5	106.7	136.4	113.4
5	post-E	823.0	210.2	129.4	52.8	76.6	115.6	87.7	129.4	81
6	pre-P	1494.1	496.5	270.5	145.8	124.7	270.5	213.7	148.3	104.4
6	pre-E	1137.9	561.9	195.9	123.9	72.0	195.9	181.5	120.7	69.7
6	pre-P	946.8	129.2	175.6	73.4	102.2	175.6	126.2	77.9	85.2
6	pre-E	1201.6	620.0	231.4	158.7	72.7	231.4	183.4	104.1	91.1
7	pre-P	1989.2	808.4	302.1	154.5	147.6	232.8	302.1	268.3	235.2
7	pre-E	2203.3	445.7	335.9	116.2	219.7	335.9	295.9	239.3	241.4
7	post-P	1398.4	278.4	195.2	55.2	140.0	195.2	185.9	155.5	185.2
7	post-E	1581.6	337.6	220	64.5	155.5	213.4	220	196.2	166.9
8	pre-P	1110.6	419.4	182.8	96.4	86.4	135.3	182.8	146.9	94.2
8	pre-E	1308.5	312.5	179.1	54.6	124.5	171.6	179.1	176.8	129
8	post-P	761.8	244.2	120	55.3	64.7	104.3	120	88.2	72.1
8	post-E	816.2	74.6	127.9	35.2	92.7	127.9	101.7	98.7	66.9
9	pre-P	1607.7	602.1	235.3	109.6	125.7	188.4	241.7	235.3	151.2
9	pre-E	1414.3	540.7	219.4	110.2	109.2	191.9	219.4	194.9	92.7
9	post-P	1069.6	439.2	164.4	85.6	78.8	138.5	153.5	164.4	78
9	post-E	1138.6	350.6	176.6	78.1	98.5	165.2	176.6	127.5	101.5
11	pre-P	996.4	199.6	138.7	39.1	99.6	138.7	136.6	136.9	72.4
11	pre-E	789.2	100.4	132.5	46.4	86.1	132.5	94.2	85.7	78.3
11	pre-P	792.3	258.7	116.8	50.1	66.7	116.8	109.3	97.8	77.8
11	pre-E	782.7	203.5	106.5	34.1	72.4	106.1	106.5	103.2	78.7
12	pre-P	2652.5	1297.3	418.1	248.7	169.4	362.4	339.1	418.1	243.9
12	pre-E	2243.9	694.3	346.1	152.4	193.7	282.3	346.1	304.8	183.8
12	post-P	1286.7	209.1	187.5	52.8	134.7	187.5	185.6	154.2	97.4
12	post-E	1578.8	536.4	252.8	122.5	130.3	209.6	252.8	202.6	118.5
13	pre-P	918.5	92.1	138.4	35.1	103.3	138.4	133.6	85.6	100
13	pre-E	1128.7	208.7	179.4	64.4	115.0	179.4	145	120.8	123.3
13	post-P	1024.0	250.4	154.2	57.5	96.7	154.2	147.6	105.2	113.3
13	post-E	1124.6	222.2	215.9	103.1	112.8	215.9	158	87.5	89
14	pre-P	1324.5	338.9	183.9	60.7	123.2	169.7	162	177	183.9
14	pre-E	1739.7	432.5	253	89.6	163.4	225.6	193.1	253	232.9
14	post-P	1276.9	335.3	178.8	61.1	117.7	178.1	163.1	178.8	119.2
14	post-E	1231.1	350.3	179.9	69.8	110.1	157.2	179.9	154.3	138.2
16	pre-P	1221.6	194.4	181.5	53.1	128.4	153.5	181.5	153.1	117
16	pre-E	1228.1	47.3	193.4	45.8	147.6	156.1	193.4	135.4	110.7
16	pre-P	1296.1	460.9	200	95.6	104.4	200	187.5	150.6	115.5
16	pre-E	1007.9	275.9	147.6	56.1	91.5	146.9	147.6	120.7	86

Note: pre-P, PPL pre testing; pre-E, Ex-PPL pre testing; post-P, PPL post testing; post-E, Ex-PPL post testing.

PPL TG values and parameters (Cont.)

Sub#	Trial	AUCT	AUCI	PeakT	PeakI	0 hr	2hr	4 hr	6 hr	8hr
18	pre-P	1374.3	219.9	196.7	52.4	144.3	196.7	195.6	160.5	124.4
18	pre-E	1049.5	284.7	148	52.4	95.6	147.6	148	109.6	143.5
18	post-P	1089.7	422.5	186	102.6	83.4	186	152.4	124	81.5
18	post-E	1037.1	308.3	150.6	59.5	91.1	129.2	150.6	110	166.4
19	pre-P	1155.4	77.0	159.3	24.5	134.8	149.3	159.3	143.4	116.6
19	pre-E	1372.8	73.6	219	56.6	162.4	219	176.2	153.1	113.8
19	post-P	1166.7	225.9	204.8	87.2	117.6	204.8	134.8	131	107.9
19	post-E	1277.1	294.7	206.9	84.1	122.8	206.9	166.6	139	129.3
21	pre-P	1219.5	149.1	176.6	42.8	133.8	176.6	165.9	145.2	110.3
21	pre-E	1149.5	117.5	164.5	35.5	129.0	161.4	164.5	128.3	112.1
21	post-P	1049.8	161.8	159.7	48.7	111.0	159.7	140.7	117.6	102.8
21	post-E	1153.9	155.5	166.2	41.4	124.8	164.5	166.2	132.8	102.1
24	pre-P	1071.1	430.3	177	96.9	80.1	177	159.8	104.6	108.2
24	pre-E	1250.4	425.6	204	100.9	103.1	184.3	204	146.6	77.5
24	pre-P	984.1	489.7	184.3	122.5	61.8	184.3	134.6	109.7	65.1
24	pre-E	747.3	208.9	118.1	50.8	67.3	118.1	91.8	101.6	57
25	pre-P	1678.2	444.6	278.4	124.2	154.2	157.5	234.4	278.4	183.4
25	pre-E	875.9	298.3	124.5	52.3	72.2	116.4	124.5	121.9	78.1
25	post-P	1262.3	475.9	186.7	88.4	98.3	181.5	148.2	186.7	131.2
25	post-E	801.5	276.7	126.3	60.7	65.6	99.1	103.9	126.3	77.3
27	pre-P	3223.0	953.4	463.6	179.9	283.7	400.4	463.6	455.3	300.7
27	pre-E	3345.6	1216.0	468	201.8	266.2	415.3	463.9	468	385
27	post-P	3664.3	1493.1	622.4	351.0	271.4	374.2	622.4	556.3	287.1
27	post-E	2265.8	435.4	327.5	98.7	228.8	285.2	327.5	267.3	277
28	pre-P	1050.2	246.2	156.6	56.1	100.5	139.2	156.6	126.6	104.9
28	pre-E	813.1	197.1	114.1	37.1	77.0	114.1	111.2	102.6	80.3
28	post-P	752.3	147.5	116.8	41.2	75.6	85.4	116.8	96.2	79.9
28	post-E	867.2	136.0	120.1	28.7	91.4	120.1	118.2	103.7	91.8
29	pre-P	1504.3	375.5	246.8	105.7	141.1	246.8	203.7	162	138.2
29	pre-E	1996.5	370.1	335.6	132.3	203.3	335.6	277.1	211.7	144.4
29	pre-P	1077.8	83.4	185.4	61.1	124.3	185.4	124.3	117.7	98.7
29	pre-E	1251.4	131.4	200	60.0	140.0	200	150.6	152.8	104.6
34	pre-P	849.0	168.2	119.3	34.2	85.1	119.3	117.8	99.3	91.1
34	pre-E	1136.2	237.8	163.6	51.3	112.3	163.6	139.4	155.8	106.3
34	post-P	819.0	188.6	127.9	49.1	78.8	89.2	101.9	127.9	102.2
34	post-E	546.5	97.7	77	20.9	56.1	62.1	71.7	72.9	77
37	pre-P	1131.2	257.2	153.1	43.9	109.2	152.7	153.1	137.0	136.3
37	pre-E	949.3	100.0	142.8	36.6	106.2	142.8	109.9	115.4	106.8
37	post-P	1122.6	240.4	152.7	42.4	110.3	141.8	145.5	152.7	132.2
37	post-E	873.6	120.2	120.9	26.7	94.2	117.1	105.5	120.9	92.5

Note: pre-P, PPL pre testing; pre-E, Ex-PPL pre testing; post-P, PPL post testing; post-E, Ex-PPL post testing.

Characteristics of subjects before treatment

Subject	Weight (kg)	BMI (kg*m2)	W:H	% fat	VO2max (l/min)	max HR
1	81.2	24.9	0.8	16.87	3.28	183
7	84.9	26.2	0.898	21.99	3.34	191
9	95.1	30.52	0.97	24.4	2.98	155
11	66.4	24.8	0.75	33.4	2.43	187
14	78.1	28.86	0.787	38.16	2.87	210
16	65.3	24.13	0.73	32.24	2.11	179
18	80.1	25.42	0.76	20.57	2.9	179
19	71.7	23.95	0.68	33.2	2.54	197
27	88.6	27.16	0.907	15.75	3.41	204
28	77.3	28.91	0.747	34.38	1.49	165
34	68.8	26.22	0.773	25.9	2.6	177
37	78	26.06	0.864	17.49	3.3	189
4	73	29.9	0.74	33.2	2.24	206
5	84	29.93	0.83	41.6	2.7	192
6	83.5	25.07	0.814	17.12	4.23	196
8	78.1	26.7	0.86	14.07	2.86	204
12	94.9	26.99	0.901	17.81	3.78	171
13	84.3	29.17	0.767	43.2	2.23	194
21	65.5	23.77	0.725	29.12	2.05	188
24	67.5	22.8	0.81	9.26	3.53	201
25	79.9	27.3	0.732	32.59	n/a	175
29	72.3	26.88	0.833	41.92	1.93	175

Note: W:H, waist/hip ratio; max HR, maximal heart rate (beat/min).

Characteristics of subjects after treatment

Subject	Weight (kg)	BMI (kg*m2)	w:h	% fat	VO2max(l/min)	max HR
1	81.61	25.04	0.854	14.69	3.77	175
7	86	26.54	0.896	20.48	3.36	181
9	94.8	30.43	0.94	22.43	3.15	150
11	66.9	25.02	0.7448	31.34	2.54	191
14	78.75	29.1	0.792	34.6	2.85	201
16	65.45	24.18	0.747	28.07	2.43	188
18	82.1	26.05	0.788	17.49	3.01	172
19	73.72	24.63	0.675	31.12	2.58	198
27	88.45	27.14	0.871	14.58	3.82	200
28	78.1	29.21	0.731	31.57	1.8	167
34	68.4	26.06	0.785	24.8	2.8	182
37	77.4	25.86	0.887	18.05	3.46	197
4	75.05	30.83	0.743	38.87	2.15	199
5	82.6	29.4	0.842	37.26	2.66	188
6	82.4	24.74	0.806	15.56	4.27	198
8	78.51	26.85	0.866	10.97	3	199
12	91.8	26.12	0.926	16.36	3.74	171
13	83.1	28.75	0.761	39.58	2.08	189
21	66.2	24.02	0.741	27.06	2.14	196
24	70.52	23.83	0.867	7.09	3.85	194
25	82.1	28.07	0.765	31.4	n/a	175
29	71.8	26.69	0.827	38.54	1.97	170

Note: W:H, waist/hip ratio; max HR, maximal heart rate (beat/min).

HDL-C and subfractions before treatment in fasting samples(mg/dl)

Sub	Time	HDL-C	HDL3-C	HDL2-C
27	pre	37.1	24.8	12.3
28	pre	41.4	25.3	16.1
34	pre	61.8	36.2	25.6
18	pre	47.8	32.1	15.7
19	pre	98.9	47.5	51.5
14	pre	53.9	35.1	18.8
16	pre	58.2	32	26.2
7	pre	40.2	29	11.2
9	pre	42.6	37.8	4.8
1	pre	44.1	33.9	10.2
11	pre	46.3	28.2	18.1
37	pre	72.5	39.2	33.3
25	pre	81.5	42.4	39.1
29	pre	49.8	34.1	15.8
21	pre	45.8	33.1	12.7
13	pre	48.6	34.8	13.8
8	pre	46.2	33.8	12.4
4	pre	44.5	30.3	14.3
6	pre	50.5	38.5	12
5	pre	44.6	29.1	15.5
12	pre	50.4	30.9	19.5
24	pre	40.7	30	10.6

HDL-C and subfractions after treatment in fasting samples (mg/dl)

Sub	Time	HDL-C	HDL3-C	HDL2-C
27	post	37.1	24.5	12.7
28	post	47.2	24.9	22.3
34	post	61.1	27.9	33.2
18	post	55	31.2	23.8
19	post	103.2	41.2	62
14	post	65.9	33.5	32.5
16	post	65.3	31.9	33.4
7	post	40.1	25.1	15
9	post	45.1	33.2	11.9
1	post	39.2	29.2	10.1
11	post	56.4	28.6	27.7
37	post	72.9	36.3	36.5
25	post	86.2	36.6	49.6
29	post	48.3	30.3	18
21	post	42.1	30.2	11.9
13	post	49.1	34.7	14.5
8	post	45.6	29.9	15.8
4	post	44.9	27.9	17
6	post	55	36.7	18.3
5	post	51.4	29	22.4
12	post	46.7	30.2	16.5
24	post	47.8	33.9	13.8

HDL-C and subfractions before treatment in exercise samples (mg/dl)

Sub #	Time	HDL-C	HDL3-C	HDL2-C
27	pre	33.3	27.6	5.7
28	pre	43.1	30.7	12.4
34	pre	62.9	43.8	19.1
18	pre	51.3	40.7	10.5
19	pre	94.8	56.8	38
14	pre	50.7	38.2	12.5
16	pre	58.5	38.9	19.6
7	pre	35.7	28.4	7.3
9	pre	44.4	38.5	5.9
1	pre	44	37.9	6
11	pre	45.9	37.6	8.2
37	pre	67.3	43.5	23.7
25	pre	93.4	55.1	38.3
29	pre	49	38.1	10.9
21	pre	42.1	35.9	6.2
13	pre	50.2	42.4	7.8
8	pre	41	31.7	9.3
4	pre	44	33.5	10.5
6	pre	53.6	39.6	14
5	pre	48.4	35.7	12.7
12	pre	45.2	35.5	9.7
24	pre	46.3	36.3	9.9

HDL-C and subfractions after treatment in exercise samples (mg/dl)

Sub #	Time	HDL-C	HDL3-C	HDL2-C
27	post	35	29	6
28	post	44.5	29.3	15.2
34	post	65.6	31.5	34.1
18	post	47.7	34.7	13
19	post	98.9	48.5	50.5
14	post	61.5	44.5	17
16	post	58.3	32.7	25.6
7	post	37.8	25.1	12.7
9	post	43.6	35.6	8
1	post	42.2	34.7	7.5
11	post	52.9	33.2	19.7
37	post	65.2	36.6	28.6
25	post	88.5	44.5	44
29	post	44.8	33.3	11.5
21	post	49.7	37.5	12.2
13	post	46.7	36.6	10.2
8	post	46.6	36.4	10.1
4	post	46.6	35.5	11.2
6	post	54.4	39.3	15.1
5	post	48.1	31.7	16.4
12	post	44.9	32.8	12.1
24	post	48.7	34.7	14

CRP values in fasting samples

Sub #	CRP pre (mg/l)	CRP post (mg/l)
1	1.7	0.5
4	2.3	3.2
5	0.8	5.5
6	1.4	2.3
7	0.9	1.2
8	2.8	1.2
9	4.2	6.2
11	2.4	1.1
12	1.5	9.2
13	9.5	9.2
14	1.0	0.8
16	0.8	1.4
18	2.7	2.6
19	28.8	17.6
21	9.1	10.3
24	0.5	1.0
25	0.2	1.6
27	1.7	0.8
28	3.3	3.4
29	18.2	13.7
34	0.2	0.4
37	0.4	1.0

CRP values in exercise samples

Sub #	CRP pre (mg/L)	CRP post (mg/L)
1	0.2	0.1
4	1.2	2.0
5	1.7	7.9
6	1.4	2.1
7	0.9	0.7
8	1.2	2.5
9	3.5	4.7
11	1.0	0.8
12	1.6	0.5
13	6.0	8.5
14	0.2	5.2
16	1.0	3.9
18	3.2	3.3
19	20.3	15.5
21	12.7	13.6
24	0.4	0.5
25	1.6	1.0
27	1.8	1.1
28	4.4	2.6
29	9.6	27.9
34	0.1	0.1
37	0.8	0.3

IL-6 values in Ex-PPL 2h samples

Subject	IL-6 pre (pg/ml)	IL-6 post (pg/ml)
1	5	4.8
4	2.7	NA
5	3.2	2.6
6	2.5	2.7
7	21.6	20.2
8	2.7	3
9	NA	2.7
11	2.5	2.7
12	2.5	2.8
13	2.8	2.9
14	NA	2.5
16	2.4	2.5
18	NA	NA
19	2.5	2.5
21	2.3	2.7
24	3.9	4.3
25	2.2	2.4
27	2.5	2.6
28	NA	2.2
29	2.4	2.6
34	NA	2.6
37	2.7	2.7

Note: NA undetectable values.

sICAM-1 values

Subject	sICAM-1 pre (ng/ml)	sICAM-1 post (ng/ml)
1	104.7	94.2
4	83.7	84.4
5	94.8	85.8
6	90.3	83.2
7	68.6	66.0
8	74.0	53.5
9	58.9	64.2
11	68.2	59.8
12	80.9	80.6
13	48.2	48.0
14	61.9	66.9
16	23.5	19.7
18	114.8	99.0
19	32.2	16.0
21	33.2	35.4
24	30.9	24.0
25	36.2	40.3
27	10.3	25.6
28	19.2	23.1
29	36.4	30.4
34	12.1	7.5
37	11.7	23.8