Journal of Nutrition & Food Sciences

Thomas et al., J Nutr Food Sci 2013, 3:5
DOI: 10.4172/2155-9600.1000221

Research Article | Open Access

Two Weeks of Low Dose Fish Oil Supplementation Followed By A Single Bout of Exercise Increases High Density Lipoprotein Cholesterol in College-Aged and Middle-Aged Men

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Abstract

There is a significant inverse relationship between high density lipoprotein cholesterol (HDL-C) and cardiovascular events. Previous studies have reported increased HDL-C in response to 4 weeks of pharmacological doses of omega-3 fatty acids (n-3 FA) (3-5g/day). Our hypothesis was that a low dose of n-3 FA (eicosapentaenoic and docosahexaenoic acid), one achievable in the diet, ingested in the form of one fish oil capsule three times per day by college-aged and middle-aged men, over a short period of 2 weeks, preceded and followed by a single bout of exercise, would increase HDL-C levels. We determined serum lipid profile (triglyceride, VLDL-C, LDL-C, HDL-C, total cholesterol) in response to a single bout of exercise (60 min, 55-60% HR_{max}) after consuming a low dose of 0.9 g n-3 FA/day for 14 days in eight middle-aged men (age 48.9 ± 1.4) and seven college-aged men (age 21 ± 2.5). There were no significant changes with blood lipids except in HDL-C. In middle-aged and college-aged men HDL-C significantly increased ($P<0.005$). The college-aged men's TC/HDL-C ratio significantly decreased ($P<0.05$). Our findings suggest that two weeks of ingesting dietary achievable doses of n-3 FA followed by a single bout of exercise increase HDL-C, which is associated with a decreased risk for coronary heart disease (CHD).

Keywords: Cholesterol; HDL; Omega 3 Fatty Acids; Exercise; Lipid Profile; Diet; Men

Abbreviations: HDL-C: High Density Lipoprotein Cholesterol; n-3 FA: Omega-3 Fatty Acids; EPA: Eicosapentaenoic Acid; DHA; Docosahexaenoic Acid; TG: Triglycerides; VLDL-C: Very Low Density Lipoproteins; LDL-C: Low-Density Lipoprotein Cholesterol; TC: Total Cholesterol; CHD: Coronary Heart Disease; BMI: Body Mass Index; LPL: Lipoprotein Lipase; LPLa; Lipoprotein Lipase Activity; PPARγ: Peroxisome Proliferator-Activated Receptor γ

Introduction

According to scientific literature, fish and fish oil rich in omega-3 fatty acids (n-3 FA) are considered beneficial in the prevention of coronary heart disease (CHD) [1-6]. Supplementation of the biologically active n-3 FA eicosapentaenoic acid (EPA) (20:5n-3) and docosahexaenoic acid (DHA) (22:6n-3) has consistently been shown to reduce the concentration of triglycerides (TG) and very low density lipoprotein cholesterol (VLDL-C) in the plasma by inhibition of hepatic TG synthesis, which leads to reduced synthesis and secretion of VLDL-C [7-9]. Reduction of VLDL-C leads to reduced formation of low-density lipoprotein cholesterol (LDL-C), a major risk factor for CHD [10]. High doses of n-3 FA increase high-density lipoprotein cholesterol (HDL-C), which is inversely related to incidence of CHD [11]. This effect is significant, beneficial, yet small [8,9]. The results of a study of 6928 people (age 55 ± 12) over a 12 year period identified low HDL-C level as the third best indicator for a major coronary event behind prior coronary events (1st) and age (2nd) [12]. Results from the National Health Examination Survey 2009-10 indicate that 31% of men had low levels of HDL-C [13].

Exercise has been shown to have an inverse relationship with incidence of CHD [14]. More specifically, exercise favorably affects lipids and lipoproteins [15,16], thereby reducing the risk for cardiovascular disease. Regular exercise, sustained over weeks or months, significantly reduces TG and increases HDL-C, with beneficial changes in total cholesterol (TC) and LDL-C [15-17]. In a single, acute bout of exercise, no changes are typically observed in TC, TG, VLDL-C, and LDL-C concentrations [16]. The effect of one, acute bout of exercise on HDL-C concentrations is not clearly defined, and in some cases increases HDL-C and in other cases causes no change [16,18].

Only a few studies have investigated the combined effects of n-3 FA and exercise [19-23], and even fewer used shorter supplementation time periods to observe changes in HDL-C [20,21]. There have been no studies that have determined lipid levels in response to the combined effects of a short duration (2 weeks) of low dose n-3 FA (0.9 g/day) supplementation followed by a single bout of exercise. Thomas et al. investigated the HDL-C response of 4 weeks of high dose (4 g n-3 FA) supplementation bookended with an acute bout of exercise running on a treadmill at 60% VO_2 max for 60 minutes [20]. They found that both supplementation and exercise increase HDL-C.

Modeling our study after Thomas et al. [20], we were interested in determining if similar HDL lowering effects could be achieved using a much lower dose of n-3 fatty acids (0.9 g n-3 FA), one achievable in the diet, and over a shorter treatment period of 2 weeks. The subjects were recruited from Wheaton College and the surrounding area in the Chicago suburbs. We hypothesized that a low dose of n-3 FA (Eicosapentaenoic and Docosahexaenoic acid) ingested in the form of one fish oil capsule three times per day over a short period of 2 weeks preceded and followed by a single bout of exercise, would increase

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Received May 29, 2013; **Accepted** July 22, 2013; **Published** July 24, 2013

Citation: Thomas RM, Meadors E, Pilgrim C, Johnson H, Smith M, et al. (2013) Two Weeks of Low Dose Fish Oil Supplementation Followed By A Single Bout of Exercise Increases High Density Lipoprotein Cholesterol in College-Aged and Middle-Aged Men. J Nutr Food Sci 3: 221. doi: 10.4172/2155-9600.1000221

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HDL-C levels in college-aged and middle-aged men with normal, but low HDL levels.

Methods and Materials

Subjects

Volunteers were recruited from Wheaton College and the surrounding area of Wheaton, Illinois, a Chicago suburb. Men were chosen for the study because of their higher risk for cardiovascular disease due to lower HDL levels than women. We conducted two experiments: one with white middle-aged men and one with white college-aged men. The middle-aged men were 7 normoglycemic, nonsmoking, overweight or obese men. Subjects had a mean age 48.9 ± 1.4 and body mass index (BMI) 30.2 ± 2.1 kg/m^2 (Table 1), and exercised for 0-2 hours per week. In the second experiment were 8 college-aged men, age 21 ± 2.5 with a BMI 24.1 ± 0.95 kg/m^2 (Table 1).

Volunteers were excluded from the study if they were taking n-3 FA supplementation, if they smoked, or if their fasting blood glucose levels were greater than 100 mg/dL. All subjects signed a written, informed consent, as approved by the Wheaton College Institutional Review Board, and completed a Physical Activity Readiness Questionnaire to ensure physical activity clearance.

Design

The study design was modeled after that of Thomas et al. which involved 2 bouts of exercise separated by 4 weeks of 4 g n-3 FA supplementation [20], our design reduced the supplementation period from 4 weeks to 2 weeks and the n-3 FA dose from 4g/day to 0.9 g/day. Fasting levels of blood lipids and lipoproteins were determined before and after both exercise sessions.

Exercise session

The exercise treatment was similar to the previously published study of Thomas et al. [20]. Subjects were instructed to adhere to their normal exercise and diet routine throughout the study. Before blood collection, subjects did not exercise for 24 hours and fasted for 12 hours. For the exercise session, subjects performed cycle ergometry on a Life Fitness 93C bicycle (Schiller Park, IL, USA) for 60 minute at 55-60% of estimated maximum heart rate (220-age), with a 5 minute warm up and cool down period. Heart rate was measured throughout exercise by an Acumen Heart Rate Monitor (Sterling, WA, USA).

n-3 FA supplementation

While exercising, all subjects were read a standard protocol to be followed for the fish oil diet. All subjects were instructed to take one Nature's Bounty (Bohemia, NY, USA) cholesterol free n-3 FA fish oil capsule for 14 days, 3 times per day, beginning on the date of the first exercise bout. Each capsule contained 1 g of fish oil with 180 mg EPA, 120 mg DHA, and providing 0.9 g of n-3 FA per day. Subjects were instructed to take the fish oil with food in order to reduce possible side effects. They were also provided with a calendar to note each time they took the capsules, in order to document compliance.

Middle-aged men	
Age (years)*	48.9 ± 1.4
BMI (kg/m2)**	30.1 ± 2.1
College-aged Men	
Age (years)	21.0 ± 2.5
BMI (kb/m2)	24.1 ± 0.9

[α]The subjects were recruited from Wheaton College, Wheaton, IL or the surrounding area.
*Data reported as means + SE. *BMI, body mass index

Table 1: Subject characteristics of middle-aged men at baseline[α].

Lipid Analyses

Blood was collected before and after exercise with a finger prick by a registered nurse. Subsequently, the blood lipid profile ratio was measured using the Cholestech LDX Analyzer (Hayward, CA, USA). The same blood collection procedure was carried out before and after exercise.

As others have measured blood volume changes due to exercise [24,25], hematocrit samples were taken by a registered nurse and run in a Clay Adams® TRIAC® centrifuge (Becton Dickinson Company, Franklin Lakes, NJ). Hematocrit was read by the same researcher each time.

Statistical analyses

All data are presented as means ± SE. Within each value of the lipid profile, differences over time were tested by using 2-way analysis of variance (ANOVA) with repeated measures according to previously published procedures [20]. The level of significance was $P<0.05$. Follow-up protected t tests were performed as a post-hoc analysis to determine the location of significant differences within treatments with specifically determined error terms.

Results

Hematocrit changes were minor (<4%). Adjusting the results for estimated changes in plasma volume did not alter the data or the statistical results; therefore, the actual values are reported in this article. According to the accountability calendars given to each participant, 98.3% ± 1.7 of the supplement pills were taken during the entire study.

Middle-aged Men

There were no significant differences in blood lipids except in HDL-C. Data for serum concentrations of lipids and lipoprotein cholesterol in middle-aged men over the two week treatment period are displayed in Table 2. Analysis of variance revealed a significant (P=0.0005) fish oil supplementation main effect for HDL-C concentration. The mean value of the HDL-C post-supplement values (pre and post exercise) was significantly higher ($P<0.05$) than the combined mean values of the HDL-C pre-supplement values (pre and post exercise). In addition, HDL-C concentration was significantly

	Pre-supplementation		Post-supplementation	
	Pre-exercise	Post-exercise	Pre-exercise	Post-exercise
TC (mg/dL)*	193 ± 14	188 ± 16	208 ± 17	190 ± 13
TG (md/dL)	138 ± 23	135 ± 21	138 ± 22	128 ± 20
VLDL-C (md/dL)	28 ± 5	27 ± 4	28 ± 4	26 ± 4
LDL-C (mg/dL)	126 ± 11	121 ±12	135 ± 12	132 ± 11
HDL-C (mg/dL)	40 ± 3[a]	40 ± 3[a]	45 ± 2[ab]	47 ± 3[b]
TC/HDL-C Ratio#	5.0 ± 0.5	4.8 ± 0.4	4.7 ± 0.4	4.4 ± 0.3

The subjects exercised for 60 minutes at 55-60 maximal heart rate followed by 2 weeks fish oil supplementation(0.9g n-3 FA), followed by the same amount and intensity as the initial bout of exercise. Blood lipid values were determined before and after each bout of exercise.
*Data are reported as means + SE, n=7. Means in same row with different letters are significantly different from each other at P<0.001. # There is a trend for an decreased TC/HDL-C ratio from pre-exercise:pre-supplementation to post-exercise:post-supplementation at P=0.072.

Table 2: Lipids and lipoprotein profiles of middle-aged men.

higher for post-exercise: post-supplementation when compared with pre-exercise:pre-supplementation (17.1% increases) and post-exercise: pre-supplementation (18.4% increase) (Table 2 and Figure 1). There was no exercise only effect in HDL-C, nor was there a supplementation + exercise interaction effect.

Despite no significant main effect for TC/HDL-C ratio, there was a trend (*P*=0.072) toward decreased TC/HDL-C ratio from pre-exercise: pre-supplementation to post-exercise: post-supplementation (11% decrease) and from post-exercise: pre-supplementation to post-exercise: post-supplementation (8.6% decrease); with the significant n-3 FA supplementation increase in HDL-C accounting for most of the decrease.

College-aged Men

In the College-aged men, similar blood lipid results were noted as we had observed for the middle-aged men (Table 3 and Figure 1). Over the two weeks, a significant main effect for supplementation

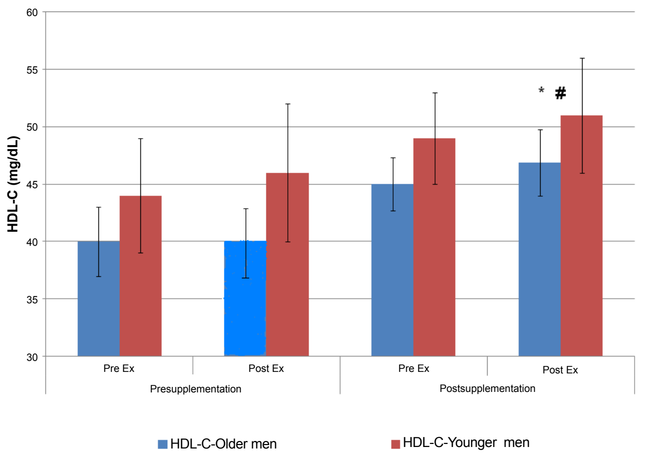

Figure 1: Comparison of HDL-C in college-aged and middle-aged men in response to n-3 FA supplementation and bouts of exercise. The subjects exercised for 60 minutes at 55-60 maximal heart rate followed by 2 weeks fish oil supplementation (0.9g n-3 FA), followed by the same amount and intensity as the initial bout of exercise. HDL-C values were determined before and after each bout of exercise. Baseline values are indicated as PreEx. Data are reported as means ± SE, n=7 for older middle-aged men and n=8 for younger college-aged men.
* Significantly different from pre-exercise:pre-supplementation in respective group, P<0.001 for middle-aged men; P<0.02 for college-aged men).
\# Only in the middle-aged men significantly different from post-exercise:pre-supplementation (P<0.001).

	Pre-supplementation		Post-supplementation	
	Pre-exercise	Post-exercise	Pre-exercise	Post-exercise
TC (mg/dL)*	139 ± 7	139 ± 7	135 ± 11	141 ± 8
TG (md/dL)	73 ± 13	73 ± 13	121 ± 23	84 ± 11
VLDL-C (md/dL)	15 ± 3	15 ± 3	20 ± 5	17 ± 2
LDL-C (mg/dL)	80 ± 9	80 ± 9	78 ± 11	74 ± 10
HDL-C (mg/dL)	44 ± 5[a]	44 ± 5[a]	49 ± 4[ab]	51 ± 5[b]
TC/HDL-C Ratio[#]	3.3 ± 0.3[a]	3.2 ± 0.3[a]	3.2 ± 0. 4[ab]	2.9 ± 0.3[b]

The subjects exercised for 60 minutes at 55-60 maximal heart rate followed by 2 weeks fish oil supplementation(0.9g n-3 FA), followed by the same amount and intensity as the initial bout of exercise. Blood lipid values were determined before and after each bout of exercise.
*Data are reported as means + SE, n = 8. Means in same row with different letters are significantly different at P<0.02.

Table 3: Lipids and Lipoprotein profiles of college-aged men.

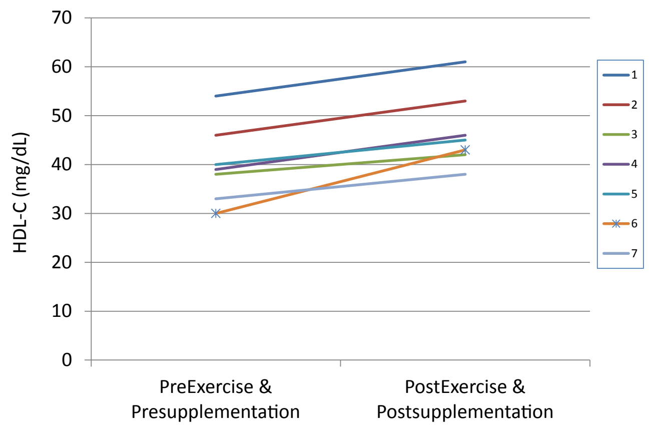

Figure 2: Individual HDL-C responses to n-3 FA supplementation and exercise for middle-aged men. The subjects exercised for 60 minutes at 55-60 maximal heart rate followed by 2 weeks fish oil supplementation (0.9g n-3 FA), followed by the same amount and intensity as the initial bout of exercise. HDL-C values reported are the baseline values compared to the values after n-3 FA supplementation and both bouts of exercise. Data are reported as means ± SE, n=7 for older middle-aged men. There was a significant increase of 17.1% (P<0.001) from pre-exercise: pre-supplementation to post-exercise:post-supplementation. Each subject's HDL-C levels increased due to the supplementation and exercise.
* signifies the hyper-responder who increased his HDL-C by 43%.

was observed in the HDL-C concentrations and TC/HDL-C Ratio (*P*<.05). For combined treatments, HDL-C increased 16% from pre-exercise: pre-supplementation to post-exercise: post-supplementation (*P*<.02), and TC/HDL-C decreased by 13% from pre-exercise: pre-supplementation to post-exercise: post-supplementation (*P*<.02).

Individual responses of middle-aged men

When the individual responses were evaluated for middle-aged men, the HDL-C in every subject was found to have increased over the course of the study. It is interesting to note that in one subject with the lowest baseline HDL-C increased his HDL-C by 43% from pre-exercise: pre-supplementation to post-exercise: post-supplementation (Figure 2).

Discussion

The purpose of this study was to determine whether HDL lowering effects as observed in a previous study by Thomas et al. [20] could be achieved using a similar design, but a substantially lower dose of n-3 fatty acids, achievable in the diet, and over a shorter length of time. Our research hypothesis was accepted, that two weeks of n-3 FA supplementation at a dose of 0.9 g/d, which is achievable in the diet, coupled with exercise, can beneficially alter lipids and lipoproteins in the plasma, specifically by increasing HDL-C. Also, exercise seems to enhance the beneficial lipoprotein-altering effects of n-3 FA.

In both middle-aged and college-aged men n-3 FA supplementation followed by an acute bout of exercise significantly increased HDL-C concentrations. To our knowledge, no one has reported significant increases in HDL-C concentration in response to a low dose of 0.9 g/d of n-3 FA supplementation within a two week period. These results are consistent with the findings of others, that n-3 FA supplementation, combined with exercise, increases HDL-C [20,21,23]; however, these other studies used longer time periods of supplementation and doses of n-3 FA 3-5x that used in the present study. With a similar study design, Thomas et al. [20] reported increased HDL-C after 4 weeks of a much larger dose of n-3 FA (4 g/d) bookended by exercise. HDL-C significantly increased due to a main effect of supplementation, but only when it was combined with the exercise bout, similar to our study.

Other studies, using only supplementation, have measured the effects of larger n-3 FA doses on lipids and lipoproteins over the 2 week period. Sanders et al. [27] measured lipid and lipoprotein levels in healthy, normolipoprotein volunteers who took 3 g/d n-3 FA for 2 weeks. After the 2 weeks, HDL-C significantly increased 7.4%. Also, Singer et al. [28] reported a 17% increase in HDL-C from 2 weeks of n-FA (5 g/d from 2 cans/d mackerel) in men with mild hypertension. In the present study, we observed a 16% increase in HDL-C in college-aged men and a 17% increase in middle aged men. This could be achieved using a low dose of n-3 FA (0.9 g/d) when combined with exercise. Please see the omega-3 fatty acid content of a number of food sources listed in Table 4. As noted from the table a variety of food sources could provide 0.9 g/d.

Fish	EPA, *mg*	DHA, *mg*	EPA+DHA, *mg*
Orange roughy	5	21	26
Tilapia	4	111	115
Mahi-mahi (dolphin)	22	96	118
Cod	3	131	134
Catfish (farmed)	42	109	151
Catfish (wild)	85	116	201
Light chunk tuna (canned)	40	190	230
Yellowfin Tuna	40	197	237
Clams	117	124	241
Mixed shrimp	145	122	267
Skipjack tuna	77	20	278
Scallops	141	169	310
Dungeness crab	239	96	335
Walleye	93	245	338
King crab	251	100	351
Oysters (farmed)	195	179	374
Halibut	77	318	395
Blue crab	207	196	403
Flat fish (flounder/sole)	207	219	426
Pollock	77	383	460
Sea bass	175	473	648
Swordfish	117	579	696
Shark (raw)	267	444	711
White tuna (canned)	198	535	733
Sardines (canned)	402	433	835
Coho salmon (wild)	341	559	900
Rainbow trout (farmed)	284	697	981
Chum salmon (canned)	402	597	999
Mackerel (canned)	369	677	1046
Sockeye salmon (wild)	451	595	1046
Coho salmon (farmed)	347	740	1087
Pink salmon (wild)	456	638	1094
Bluefin tuna	309	970	1279
Atlantic salmon (wild)	349	1215	1564
Atlantic herring	773	939	1712
Pacific herring	1056	751	1807
Atlantic salmon(farmed)	587	1238	1825

*Per 3-oz (85 g) serving. †Cooked with dry heat unless otherwise noted. DHA—docosahexaenoic acid; EPA—eicosapentaenoic acid.
Table used with permission from Harris et al. [26]

Table 4: Content of EPA and DHA in 37 commonly consumed types of fish*†.

The increased HDL-C induced by the n-3 FA and exercise was observed in each of the college-aged men (data not shown) and the middle-aged men. One of the middle-aged men, in particular, appeared to be a hyper-responder to n-3 FA supplementation and exercise, increasing his HDL-C by 43% with about half of the increase due to supplementation and the other half due to exercise. This subject had the lowest baseline level of HDL-C and therefore had the highest risk for CHD. Future studies in this high risk population validating this finding could be particularly beneficial in helping reduce risk for CHD in the subset of the population with low HDL, the third best indicator for CHD and the best indicator over which individuals have any control [12].

In the present study, we observed increased HDL-C immediately after the second bout of exercise, which is consistent with some studies [25,29,30]. Angelopoulos et al. [25] found HDL-C to increase immediately post-exercise (30 minutes 65% VO_2 max on treadmill); HDL-C levels returned to pre-exercise levels 48 hours after exercise. The increased HDL-C peaking-times could be due varying factors such as intensity of exercise, the length of exercise, or dietary influences [29,31]. Other studies found that HDL-C increases 24 or 48 hours after exercise [29,31,32]. Because we did not measure HDL-C 24 hours after exercise, these measurements will be helpful for future experiments to determine the maximal response as well as how long the effect can be sustained.

HDL-C consists of sub fractions which are affected by n-3 FA. HDL_2-C is increased by n-3 FA [20,33-35], while HDL_3-C is decreased [33] or unchanged [34,35]. There appears to be a shift in HDL-C sub fractions, from smaller, denser HDL_3-C to larger, more buoyant HDL_2-C particles [22]. Thomas et al. found that HDL_2-C was significantly increased in response to n-3 FA [20]. Because we modeled our study similar to that of Thomas et al., it is likely that HDL_2-C also increased in our own study. Future studies should be done to establish this. An increase in HDL_2-C is beneficial, because this particular subfraction of HDL-C has been found to be associated with lower risk for development of atherosclerosis [36].

The precise mechanism of how n-3 FA increase HDL_2-C is not known but could be through increasing metabolism of chylomicrons, TGs, and VLDL-C via increased lipoprotein lipase (LPL) activity (LPLa) [37]. The increased LPLa is directly related to increase HDL-C production [38]. It is interesting to note that the hyper-responder in Figure 2 that had a 43% increase in HDL-C also had a 26% decrease in both VLDL-C and TG (data not reported). The increased catabolism of VLDL-C and TG could be explained by increased LPLa which could account for the increase in HDL-C.

In contrast to what is typically observed n-3 FA supplementation did not significantly decrease plasma TG concentration, possibly because of this study's low dose of n-3 FA supplementation and short supplementation time period. It has been well established that n-3 FA significantly reduce serum triglycerides [8], ranging from a 20-50% decrease, depending on baseline values [39]. In a review article, Harris [39] found the average effective n-3 FA dose to induce hypotriglyceridemia to be about 3-4 g/d with average decreases in triglycerides 25-34%. It seems that this study's n-3 FA dosage of 0.9 g/d was not sufficient to decrease TGs.

There were some limitations to the present study. The sample size was small and the subjects were from a select population that was relatively normal with regard to HDL-C levels. These finding, however, provides some preliminary work as a basis for further research using a more clinically diverse populations in which the patients have abnormally low HDL-C.

In summary we found that a low-dose n-3 FA supplementation for a short period of 2 weeks followed by acute, aerobic exercise can significantly increase HDL-C. The major contributor was n-3 FA supplementation, but the increase did not reach statistical significance until right after exercise. The precise mechanism of how n-3 FA increases HDL-C is unknown, but could be via increased LPLa. Our findings suggest that regular exercise and dietary intake of n-3 FA are important in HDL-C metabolism and in lowering one's risk for cardiovascular disease.

Acknowledgements

We thank Sigrid Ianuzzo and Danette Farrell for their nursing expertise and assistance in the laboratory. We also thank Emily Bellfi for her technical assistance. Thanks to Provost Stanton L. Jones and Dean Dorothy F. Chappell for their financial support enabling student research. We thank Judy Kawakami for her technical assistance with certain aspects of the project. This work also was financially supported by Wheaton College with its motto to do all things "for Christ and His Kingdom".

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Citation: Thomas RM, Meadors E, Pilgrim C, Johnson H, Smith M, et al. (2013) Two Weeks of Low Dose Fish Oil Supplementation Followed By A Single Bout of Exercise Increases High Density Lipoprotein Cholesterol in College-Aged and Middle-Aged Men. J Nutr Food Sci 3: 221. doi: 10.4172/2155-9600.1000221