Responses of LDL and HDL particle size and distribution to omega-3 fatty acid supplementation and aerobic exercise

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Wooten JS, Biggerstaff KD, Ben-Ezra V. Responses of LDL and HDL particle size and distribution to omega-3 fatty acid supplementation and aerobic exercise. J Appl Physiol 107: 794–800, 2009. First published July 9, 2009; doi:10.1152/japplphysiol.91062.2008.—The purpose of this investigation was to determine the independent and combined effects of aerobic exercise and omega-3 fatty acid (n-3fa) supplementation on lipid and lipoproteins. Sedentary, normoglycemic, nonsmoking men (n = 11) were assigned to perform rest and exercise before and during n-3fa supplementation. Exercise consisted of 3 consecutive days of treadmill walking at 65% maximum O2 consumption for 60 min. Supplementation consisted of 42 days of 4.55 g/day of n-3fa. A two-way factorial ANOVA with repeated measures revealed significant reductions in total cholesterol (P = 0.001, −9.2%) and triglyceride (P = 0.007, −32.4%) concentrations postexercise. In addition, exercise increased LDL peak particle size (P = 0.001) from 26.2 to 26.4 nm, but not HDL size. The n-3fa supplementation resulted in a significant shift in the distribution of cholesterol among LDL and HDL subspecies. In contrast, n-3fa supplementation shifted HDL peak particle size (P = 0.001, 14.2%) and HDL2a (P = 0.001, −22.8%), despite no significant changes in lipoprotein-cholesterol concentrations. The majority of the shift in HDL-C was noted in HDL2b (P = 0.001, 20.9%) and HDL3a (P < 0.001, −31.0%) particles. There were no combined effects of exercise and n-3fa supplementation on lipids and lipoproteins. Three consecutive days of aerobic exercise reduced triglyceride and total cholesterol concentrations with a concomitant increase in LDL peak particle size. In contrast, n-3fa supplementation shifted HDL-C from HDL3 particles to HDL2a particles, despite no significant changes in HDL2a-C and HDL3a-C concentrations. Exercise and n-3fa supplementation do not synergistically improve serum lipids and lipoproteins, but rather independently affect the metabolism of lipids and lipoproteins.

docosahexaenoic acids; eicosapentaenoic acids; lipoprotein-cholesterol

IMPROVEMENTS IN LIPID AND lipoprotein-cholesterol concentrations in active and sedentary men have been reported following as few as a single bout of aerobic exercise (9, 13, 17). Short-term decreases in serum triglyceride concentration 24 and 48 h following a single aerobic exercise session requiring 350–1,500 kcal of energy expenditure have been reported in both trained and untrained men (9, 13, 17). The decrease in triglyceride concentration in these studies was matched by an increase in high-density lipoprotein-cholesterol (HDL-C) concentrations (6.7–26.8%) 24 and 48 h postexercise (9, 13, 17); however, this is not always the case (10). In contrast, the acute effects of aerobic exercise on low-density lipoprotein (LDL) and high-density lipoprotein (HDL) electrophoretic characteristics remain unclear. Investigations reporting responses of lipoprotein particle size following a single bout of aerobic exercise have been limited to exhaustive, prolonged exercise sessions in very active or highly fit participants (19, 36) and have shown equivocal results.

Omega-3 fatty acids (n-3fa) have been investigated as a nonpharmacological approach for the treatment of dyslipidemia. Supplementation with n-3fa containing eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) reduced serum triglyceride concentration up to 30% (4) and altered very-low-density lipoprotein (VLDL), LDL, and HDL particle size and distribution to omega-3 fatty acid supplementation have been investigated by models using a single session of aerobic exercise, as well. Thomas et al. (30) reported a reduced triglyceride concentration after as little as 3 wk of n-3fa supplementation in men. When participants were divided into exercise and no-exercise groups, the statistical difference was no longer present. Thomas et al. (31) conducted a follow-up study reporting that triglyceride concentration was decreased, and HDL-C and HDL2-C concentrations were elevated following 4 wk of n-3fa supplementation. Furthermore, following 60 min of aerobic exercise during n-3fa supplementation, HDL-C concentration was elevated with no change in triglyceride concentration. The data of Thomas et al. (31) are difficult to compare to, because both control and preexercise blood samples were collected ~3 h following the participant’s last meal.

The combined effects of aerobic exercise and n-3fa supplementation have been investigated by models using a single session of aerobic exercise, as well. Thomas et al. (30) reported a reduced triglyceride concentration after as little as 3 wk of n-3fa supplementation in men. When participants were divided into exercise and no-exercise groups, the statistical difference was no longer present. Thomas et al. (31) conducted a follow-up study reporting that triglyceride concentration was decreased, and HDL-C and HDL2-C concentrations were elevated following 4 wk of n-3fa supplementation. Furthermore, following 60 min of aerobic exercise during n-3fa supplementation, HDL-C concentration was elevated with no change in triglyceride concentration. The data of Thomas et al. (31) are difficult to compare to, because both control and preexercise blood samples were collected ~3 h following the participant’s last meal.

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MATERIALS AND METHODS

Participants and study design. Nonsmoking, normoglycemic, sedentary men (n = 11), ages 19–47 yr, were recruited through local media advertisements (Table 1). Sedentary was defined as exercising ≤2 days/wk, with a cumulative exercise time of ≤30 min/wk for the past 3 mo. During interviews, each participant was asked about the frequency and duration of exercise performed each week for the past 3 mo to determine whether the participant met the sedentary inclusion criteria. Volunteers were excluded from the investigation if they were
Table 1. Descriptive characteristics of participants at baseline

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean ± SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>30.6 ± 10.0</td>
<td>19–47</td>
</tr>
<tr>
<td>Height, cm</td>
<td>179.3 ± 10.9</td>
<td>167.0–201.0</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>85.4 ± 11.2</td>
<td>65.0–97.7</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>26.7 ± 3.8</td>
<td>20.1–32.8</td>
</tr>
<tr>
<td>VO₂max, ml·kg⁻¹·min⁻¹</td>
<td>3.4 ± 0.5</td>
<td>2.6–4.3</td>
</tr>
<tr>
<td>VO₂max, ml·kg⁻¹·min⁻¹</td>
<td>40.8 ± 6.5</td>
<td>29.6–50.3</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>90.5 ± 9.4</td>
<td>79.9–106.6</td>
</tr>
</tbody>
</table>

n = 11 subjects. BMI, body mass index; VO₂max, maximum O₂ consumption.

Currently taking any medications for the regulation of lipids and lipoprotein-cholesterol, diabetes, or hypertension. In addition, volunteers were not admitted into the study if they were taking n-3fa supplementation during the past 6 mo. Volunteers were not included on the basis of body mass index or body composition. Each participant was screened and informed of the risks associated with the study. Volunteers with an impaired fasting glucose (≥110 mg/dl) were excluded from the investigation, as they presented a greater risk for diabetes. Volunteers were not screened for serum concentrations of lipids or lipoprotein-cholesterol, because the purpose of the study was to determine whether exercise and n-3fa supplementation would alter these concentrations in an otherwise normal participant pool. Volunteers who met all inclusion criteria signed a written, informed consent, as approved by Texas Woman’s University Institutional Review Board and were admitted to the study as participants. Following the completion of the written, informed consent, a detailed medical history questionnaire was completed. Participants were scheduled for a maximal graded exercise test to determine maximal oxygen consumption (VO₂ max) and subsequent testing protocols outlined in Fig. 1.

**Determination of VO₂ max.** VO₂ max was determined by having each participant complete a graded exercise test on a Quinton Series S65 treadmill (Quinton, Bothell, WA). The graded exercise test consisted of walking at 3.5 mph with stages of increasing intensity (increase of 5.0% grade per 2-min stage) until the participant could no longer keep pace with the treadmill. Respiratory gases were analyzed by a Parvo-Medics TrueMax 2400 metabolic cart (Consentius Technologies, Sandy, UT) following O₂ and CO₂ gas and air flow calibration using Medics Truemax 2400 metabolic cart (Consentius Technologies, Bothell, WA). The exercise session consisted of walking at 3.5 mph on a motorized treadmill for 60 min at a grade that elicited an exercise intensity of 65% of VO₂ max. The speed and grade determined for the first exercise session were later used for the second and third exercise sessions. Exercise sessions were performed 24 h apart between 2:00 and 6:00 PM. During all exercise sessions, participants were allowed to consume water ad libitum. All procedures were repeated for the resting and exercise protocols performed during n-3fa supplementation (Fig. 1).

**n-3fa Supplementation.** Participants consumed seven packets (4.55 g/day) per day of n-3fa (Coromega, European Reference Botanical Laboratories, Carlsbad, CA) for 42 days. Each packet contained 650 mg of n-3fa (350 mg EPA, 230 mg DHA, and 70 mg unreported type of n-3fa). Participants were instructed to consume two to three packets at each meal throughout the day until all seven had been ingested each day. The dosage and duration of n-3fa consumption in this study were based on similar dosages and durations of use reported in previous studies that examined lipid and lipoprotein-cholesterol metabolism (5, 29–31) with the exception of Bell et al. (4), who used 15.8 g/day. Participants continued the consumption of the n-3fa supplement throughout the remaining RS and ES protocols (Fig. 1). Compliance and side effects of n-3fa supplementation were monitored every 2 wk by personal contact when participants arrived at the laboratory to replenish their n-3fa supplement.

**Dietary records and analyses.** Each participant was asked to record all food and beverages consumption for all experimental protocols. Consumption of alcohol was not permitted during the four experimental sessions due to the acute effect of alcohol on triglyceride and HDL-C concentrations (32). Each participant was instructed to record all dietary consumption for 3 days before each blood draw so that four, 3-day food records were recorded by each participant. The food record from the first experimental session was photocopied and returned to the participant so that a similar diet could be consumed during the remaining experimental sessions. In addition, the evening...
before each blood draw, participants consumed the same meal for each trial. The meal was a submarine sandwich prepared by a local restaurant. This meal provided a small amount of control for lipid and lipoprotein fluctuations that may be induced by dietary factors following the most recent meal. Food records were analyzed using Nutritionist V software (First Databank, San Bruno, CA) to determine the participant’s total caloric consumption and percentage of total calories derived from fat, saturated fat, protein, and carbohydrate.

Blood collection. Fasting blood samples were collected by venipuncture into K2-EDTA and serum-separator vacutainer tubes following the week of rest and 14–16 h after the third exercise session (Fig. 1). Participants fasted for at least 10 h prior to all blood collections. Each participant was seated for 20 min before each blood sample to control for the effect of postural changes on plasma volume (20). Hematocrit was measured using whole blood and recorded using the microhematocrit technique (12). Plasma and serum were separated from blood by low-speed centrifugation (1,500 g, 15 min, 10°C), transferred into aliquots, and stored at −17°C until analysis. These procedures were repeated during resting and exercise sessions of the supplementation phase (Fig. 1).

Analytic methods. Hemoglobin was determined using the cyanmethemoglobin technique (H4390, Sigma, St. Louis, MO). Serum was assayed for total cholesterol, HDL-C, and triglyceride concentrations using standard enzymatic colorimetric techniques (kit no. 276–64909 and no. 432–40201, Wako, Richmond, VA). The concentration of HDL-C was measured by the precipitation of apolipoprotein B containing lipoproteins, followed by enzymatic measurement of the remaining cholesterol (kit no. 278–67409, Wako). A second precipitation technique was performed using dextran sulfate and MgCl2 to determine HDL3-C concentration (16, 34). The concentration of HDL2-C was calculated as the difference between HDL-C and HDL3-C concentration. The Friedewald equation was used to estimate the concentration of LDL-cholesterol (LDL-C) (14). All lipid and lipoprotein-cholesterol concentrations were corrected for postexercise plasma volume changes using the Dill and Costill method (11).

A composite non-denaturing 2–31% gradient polyacrylamide gel (Alamo Gels, San Antonio, TX) was used for the simultaneous separation of LDL and HDL subclasses from the same plasma sample as described by Rainwater et al. (25, 26). Gels were calibrated using high molecular weight protein standards (Amersham Biosciences, Piscataway, NJ) and 30-nm carboxylated polystyrene microsphere latex beads (Duke Scientific, Palo Alto, CA). Following electrophoresis, the high molecular weight protein standards and latex beads were stained with Coomassie brilliant blue G-250 (Sigma, St. Louis, MO). Lipids were stained with a 20% Sudan black B solution to resolve the lipoprotein peak particle size and the distribution of cholesterol among the lipoprotein particles, as described by Singh et al. (28). Sudan black B binds neutral lipid in the gel; however, Callais et al. (7) concluded the HDL-C quantification with polycrylamide gel electrophoresis was unaffected by the triglyceride concentration. In addition, several studies have demonstrated that Sudan black B accurately reflects cholesterol distributions among size-resolved lipoproteins (7, 8, 15).

Gels were scanned and subsequently analyzed using ImageJ version 1.34 software (National Institutes of Health, Bethesda, MD). Peak particle diameter for LDL and HDL subtypes 2b, 2a, 3a, and 3b were quantified, as well as the relative distribution of cholesterol among LDL subtypes 1–3 and HDL subtypes 2b, 2a, 3a, and 3b. The relative distribution of cholesterol under each lipoprotein peak was calculated as a percentage of the total area for LDL and HDL subtypes (e.g., %HDL-C in HDL2b = area of HDL2b + total HDL area). In addition, the sum of the areas under HDL2b and HDL2a (HDL2b + 2a) and HDL3a and HDL3b (HDL3a + 3b) were also quantified.

The coefficient of variation was calculated as the percent difference between samples that were analyzed in duplicate. The coefficient of variation was 3.2, 3.9, 2.4, and 6.5% for serum concentrations of HDL-C, HDL3-C, total cholesterol, and triglyceride, respectively. The coefficient of variation for the peak LDL (0.5%), HDL2b (0.8%), HDL2a (0.8%), HDL3a (0.4%), and HDL3b (0.2%) particle sizes were within acceptable ranges. The coefficient of variation for the distribution of LDL-C between LDL2, LDL3, and LDL4 was 15.6, 15.4, and 20.9%, respectively. The coefficient of variation for the distribution of HDL-C between HDL2a, HDL2b, HDL3a, and HDL3b was 13.3, 22.1, 21.9, and 19.6%, respectively.

Statistical analysis. Descriptive data are displayed as means ± SD. A two-way factorial analysis of variance with repeated measures was used to quantify significant main effects of exercise and n-3fa supplementation and significant interactions (SPSS version 12.0, SPSS, Chicago, IL). A Bonferroni post hoc test was used when appropriate to determine the difference between means following significant univariate effects. The criterion reference for statistical significance was set at P < 0.05. Due to the dependent variables being statistically and physiologically related, the experiment-wise error rate may be higher than the P < 0.05 level. Paired t-tests were performed to identify significant changes in plasma volume between RNS and ENS, RNS and RS, and RNS and ES trials. The criterion reference for statistical significance between trials for plasma volume was set at P < 0.05.

RESULTS

Exercise and diet characteristics. Participants exercised at 66.1 ± 0.2% of V\textsubscript{O2 max} during ENS and 65.8 ± 0.1% of V\textsubscript{O2 max} during ES. Energy expenditure was estimated to be 659.9 ± 24.9 and 654.6 ± 25.4 kcal/session for the ENS and ES exercise protocols, respectively.

No significant differences in total caloric or macronutrient intake were observed among the four experimental protocols (Table 2). Only minor inconsistencies (e.g., missing a packet during the day) were discovered regarding the actual intake and expected intake of n-3fa during the supplementation phase. Only one participant dropped out of the study, and this was due to bowel irritation that occurred with n-3fa supplementation. This participant was not included in the analyses.

Lipid and lipoprotein-cholesterol concentrations. When comparing plasma volumes between RNS and ENS, paired t-tests revealed a significantly (P = 0.034) greater plasma volume (8.1%) following the ENS trial. In addition, a significantly (P = 0.044) greater plasma volume (8.9%) was ob-

### Table 2. Total energy and macronutrient intake from 3-day dietary record

<table>
<thead>
<tr>
<th></th>
<th>R</th>
<th>EX</th>
<th>NS</th>
<th>S</th>
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<tbody>
<tr>
<td>Total kcal, kcal/day</td>
<td>2,204±671.2</td>
<td>2,525.9±704.7</td>
<td>2,379.2±730.4</td>
<td>2,250.7±683.7</td>
</tr>
<tr>
<td>Carbohydrates, %</td>
<td>54.0±8.5</td>
<td>51.4±7.8</td>
<td>52.7±9.1</td>
<td>52.7±7.2</td>
</tr>
<tr>
<td>Protein, %</td>
<td>16.9±6.1</td>
<td>16.7±4.7</td>
<td>16.2±4.7</td>
<td>17.4±6.0</td>
</tr>
<tr>
<td>Fat, %</td>
<td>36.4±13.7</td>
<td>32.1±4.3</td>
<td>31.4±5.3</td>
<td>37.2±13.1</td>
</tr>
<tr>
<td>Saturated fat, %</td>
<td>10.0±2.3</td>
<td>10.8±2.1</td>
<td>10.2±2.2</td>
<td>10.5±2.4</td>
</tr>
</tbody>
</table>

Values are means ± SD. R, rest; EX, exercise; NS, without omega-3 fatty acid supplement; S, with omega-3 fatty acid supplement.
erved following the ES trial compared with the RNS trial; however, the change in plasma volume (3.9%) following RS was not significantly ($P = 0.143$) different than that following the RNS trial. Despite the fact that no difference in plasma volume was observed between the RNS and RS trials, lipid and lipoprotein-cholesterol concentrations measured during the ENS, RS, and ES trials were corrected for changes in plasma volume using the RNS trial as the reference time point.

Descriptive data for serum concentrations of lipid and lipoprotein-cholesterol are displayed in Table 3. Analysis of variance revealed a significant ($P = 0.007$) exercise effect was observed for total cholesterol concentration. Following exercise, total cholesterol concentration was 9.2% lower than during rest. No supplement effect or exercise and supplement interaction was observed for total cholesterol concentration. Following exercise, total cholesterol concentration was 31.0% less than during no n-3fa supplementation. Conversely, the percentage of HDL-C carried by HDL2b particles during n-3fa supplementation do not synergistically improve blood lipids. A significant ($P < 0.001$) exercise effect was observed for triglyceride concentration. In addition, no exercise or supplement effect nor supplement and exercise interaction was observed for triglyceride concentration. In addition, no exercise or supplement effect nor supplement and exercise interaction was observed for total cholesterol concentrations measured during the ENS, RS, and ES trials were corrected for changes in plasma volume using the RNS trial as the reference time point.

**Table 3. Lipids and lipoproteins following the experimental trials**

<table>
<thead>
<tr>
<th>R</th>
<th>EX</th>
<th>NS</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol, mg/dl</td>
<td>164.6±31.2</td>
<td>149.5±29.7*</td>
<td>155.0±33.4</td>
</tr>
<tr>
<td>Triglycerides, mg/dl</td>
<td>116.5±74.7</td>
<td>78.7±44.0*</td>
<td>101.9±53.2</td>
</tr>
<tr>
<td>LDL-C, mg/dl</td>
<td>99.1±33.4</td>
<td>93.2±26.8</td>
<td>93.6±32.1</td>
</tr>
<tr>
<td>HDL-C, mg/dl</td>
<td>42.3±6.4</td>
<td>40.6±8.2</td>
<td>41.0±5.9</td>
</tr>
<tr>
<td>HDL2a, %HDL-C</td>
<td>13.3±6.0</td>
<td>12.3±6.1</td>
<td>11.7±4.7</td>
</tr>
<tr>
<td>HDL2b, %HDL-C</td>
<td>28.9±3.6</td>
<td>28.3±4.7</td>
<td>29.3±3.9</td>
</tr>
<tr>
<td>LDL1, %LDL-C</td>
<td>42.9±28.5</td>
<td>45.6±24.2</td>
<td>43.6±25.9</td>
</tr>
<tr>
<td>LDL2, %LDL-C</td>
<td>29.1±14.0</td>
<td>31.6±13.1</td>
<td>30.4±15.3</td>
</tr>
<tr>
<td>HDL2b, %HDL-C</td>
<td>52.0±12.3</td>
<td>53.0±12.3</td>
<td>47.5±11.5</td>
</tr>
<tr>
<td>HDL2a, %HDL-C</td>
<td>14.3±3.1</td>
<td>13.0±3.9</td>
<td>14.3±4.9</td>
</tr>
<tr>
<td>HDL3a, %HDL-C</td>
<td>33.7±9.1</td>
<td>33.9±9.5</td>
<td>38.2±8.5</td>
</tr>
<tr>
<td>HDL3b, %HDL-C</td>
<td>20.4±6.6</td>
<td>20.5±7.1</td>
<td>24.2±6.3</td>
</tr>
<tr>
<td>HDL1, %HDL-C</td>
<td>13.3±4.3</td>
<td>13.4±4.2</td>
<td>14.0±4.2</td>
</tr>
</tbody>
</table>

Values are means ± SD. *Significant omega-3 fatty acid supplement effect.

Descriptive data for serum concentrations of HDL and HDL particle subspecies following experimental trials

**Table 4. Distribution of cholesterol among LDL and HDL particle subspecies following experimental trials**

<table>
<thead>
<tr>
<th>R</th>
<th>EX</th>
<th>NS</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL1, %LDL-C</td>
<td>42.9±28.5</td>
<td>45.6±24.2</td>
<td>43.6±25.9</td>
</tr>
<tr>
<td>LDL2, %LDL-C</td>
<td>29.1±14.0</td>
<td>31.6±13.1</td>
<td>30.4±15.3</td>
</tr>
<tr>
<td>HDL2b, %HDL-C</td>
<td>52.0±12.3</td>
<td>53.0±12.3</td>
<td>47.5±11.5</td>
</tr>
<tr>
<td>HDL2a, %HDL-C</td>
<td>14.3±3.1</td>
<td>13.0±3.9</td>
<td>14.3±4.9</td>
</tr>
<tr>
<td>HDL3a, %HDL-C</td>
<td>33.7±9.1</td>
<td>33.9±9.5</td>
<td>38.2±8.5</td>
</tr>
<tr>
<td>HDL3b, %HDL-C</td>
<td>20.4±6.6</td>
<td>20.5±7.1</td>
<td>24.2±6.3</td>
</tr>
<tr>
<td>HDL1, %HDL-C</td>
<td>13.3±4.3</td>
<td>13.4±4.2</td>
<td>14.0±4.2</td>
</tr>
</tbody>
</table>

Values are means ± SD. *Significant omega-3 fatty acid supplement effect.

Descriptive data for HDL2a, HDL3a, and HDL3b peak particle size and distribution of cholesterol among LDL and HDL subspecies were determined. Exercise significantly reduced serum concentrations of total cholesterol and triglyceride independent of n-3fa supplementation. In addition, exercise independently increased LDL peak particle size from 26.2 to 26.4 nm. The n-3fa supplementation did not alter serum lipid and lipoprotein-cholesterol concentrations; however, n-3fa supplementation appears to cause a shift in HDL-C from smaller, denser HDL3a + 3b to larger, more buoyant HDL2b + 2a subfraction. These results provide evidence that exercise and n-3fa supplementation do not synergistically improve blood lipid and lipoproteins, but rather favorably alter lipids and lipoproteins in different ways.

**DISCUSSION**

This study examined the independent and combined effects of exercise and n-3fa supplementation on lipid and lipoprotein-cholesterol concentrations. Additionally, lipoprotein peak particle size and distribution of cholesterol among LDL and HDL subspecies were determined. Exercise significantly reduced serum concentrations of total cholesterol and triglyceride independent of n-3fa supplementation. In addition, exercise independently increased LDL peak particle size from 26.2 to 26.4 nm. The n-3fa supplementation did not alter serum lipid and lipoprotein-cholesterol concentrations; however, n-3fa supplementation appears to cause a shift in HDL-C from smaller, denser HDL3a + 3b to larger, more buoyant HDL2b + 2a subfraction. These results provide evidence that exercise and n-3fa supplementation do not synergistically improve blood lipid and lipoproteins, but rather favorably alter lipids and lipoproteins in different ways.

**Effect of exercise.** In sedentary men who performed a single bout (9, 13, 17) of aerobic exercise that expended between 350 and 500 kcal of energy, HDL-C concentration increased 6.7–14.3% in the 24 and 48 h postexercise. To our knowledge, Angelopoulos et al. (3) is the only group that has examined the effects of a single and 2 and 3 nonconsecutive days of aerobic exercise separated by 48 h on postexercise responses of lipid and lipoprotein-cholesterol concentrations. Angelopoulos et al. reported that, at 24 h following the last bout of exercise, HDL-C concentration was increased 1.0, 5.4, and 9.8% after one, two, and three sessions of aerobic exercise, respectively. In contrast to both a single bout and multiple bouts of aerobic exercise, HDL-C or HDL-C subfractions in the present study did not respond to the accumulated bouts of aerobic exercise. It is unclear why HDL-C or HDL-C subfractions did not respond to the 3 consecutive days of aerobic exercise, despite the fact that the caloric expenditure in the present study was greater than the caloric expenditure used in previous studies. In addition, the baseline HDL-C concentration of the men in the present study was similar to preexercise HDL-C concentration of the men in these previous investigations (12, 22).
Three consecutive days of aerobic exercise promoted a 9.2% reduction in total cholesterol concentration. Compared with responses after a single session of aerobic exercise, total cholesterol concentration has been observed to be reduced immediately postexercise, followed by a return to preexercise concentrations by 24 h postexercise (9, 17). In contrast to the present study, the participants in these previous studies were both normocholesterolemic and hypercholesterolemic men; however, Grandjean et al. (17) concluded that cholesterol status of the participants did not influence the effects of the exercise session on lipid and lipoprotein metabolism.

Following 3 nonconsecutive days of aerobic exercise, Angelopoulos et al. (3) did not report any changes in total cholesterol concentration 24 or 48 h postexercise. This observation suggests that the duration between exercise sessions may have an influence on the ability of exercise to reduce total cholesterol concentration. Therefore, it may be likely that the accumulation of exercise or caloric expenditure over the 3 consecutive days promoted a transient reduction in total cholesterol concentration that is not typically observed following a single session or nonconsecutive days of aerobic exercise.

Similar to effects of a single exercise session (9, 13, 17), the 3 consecutive days of moderate-intensity exercise promoted a reduction in triglyceride concentration; however, the magnitude of reduction was much greater in the present study. Triglyceride concentration has been reported to be reduced 18.6 and 11.1% following aerobic exercise, expending 350 and 500 kcal, respectively (9, 17). In the present study, the 3 consecutive days of aerobic exercise requiring a net caloric expenditure of ~1,950 kcal resulted in a ~32% postexercise reduction in triglyceride concentration.

Aerobic exercise training has been reported to increase LDL particle size. Altima et al. (1) reported an increase in LDL mean particle size from 271.3 to 272.5 Å following 4 wk of moderate-intensity aerobic exercise that was performed 5 days per week for 30 min per exercise bout. The present study is the first to our knowledge to report an increase in LDL peak particle size from an acute exercise session. The majority of investigations reporting the acute response of lipoprotein size have been limited to exercise that was exhaustive in nature and in participants that were very physically active or highly fit, which is in contrast to the participants in the present investigation. In the present study, post hoc analysis of the association between the percent change in LDL peak particle size and triglyceride concentration postexercise revealed a significant inverse correlation (r = −0.51, P = 0.015). The reduction in triglyceride concentration and increased LDL peak particle size may be best explained by an increase in lipoprotein lipase activity (LPLa). A compositional modification of the LDL particle may occur with increased LPLa by reducing the triglyceride content in the lipid core of LDL, which would lead to the formation of a larger, less dense LDL particle (19). The proposed mechanism is consistent with the reduction of triglyceride concentration measured in this investigation.

Effect of n-3fa supplementation. In contrast to previous investigations (21, 23), no significant changes in triglyceride concentration were observed in the present study as a result of n-3fa supplementation. This observation may be due in part to the normal triglyceride concentration of the participants at rest or baseline. However, Harris (18) reported that healthy, normolipidemic participants mean triglyceride concentration can be reduced by 25% following consumption of 7 g/day or less of n-3fa for at least 2 wk. Evidence suggests that n-3fa may increase LPLa contributing to the hypotriglyceridemic effects of fish oils (22); however, the reduction in triglyceride concentration may be predominantly due to a reduction in the hepatic availability of fatty acids for VLDL synthesis (24). In this study, the dosage or duration of n-3fa consumption may be below the threshold required to either stimulate an increase in LPLa or reduce triglyceride synthesis and VLDL secretion.

In contrast to previous investigations, no significant changes in total cholesterol or LDL-C concentrations were observed in the present investigation. Pownall et al. (23) observed a 16.7% increase in median LDL-C concentrations in hypertriglyceridemic (type IV) and normotriglyceridemic participants fed 4 g/day of n-3fa for 6 wk. In contrast to the current study, Pownall et al. reported a 9.9% reduction in total cholesterol, which was attributed to the large (~29.9%) reduction in VLDL-C. It may be likely that the VLDL-C concentration in the present study was unaffected by the 4.55 g/day of n-3fa supplementation, which would best explain the observed unchanged LDL-C and total cholesterol concentrations postsupplementation. This observation is speculative, given that VLDL-C concentration was not quantified in this study.

In the present study, supplementation with n-3fa did not significantly alter HDL-C, HDL2-C, or HDL3-C concentrations. During n-3fa supplementation, HDL2-C concentration was observed to be 19.7% greater than presupplementation concentrations; however, the change in HDL2-C was not significant (P = 0.158). In contrast, Calabresi et al. (6) reported that, in patients with familial combined hyperlipidemia following 4 g/day of n-3fa supplementation for 8 wk, no significant changes were observed for HDL-C concentration; however, HDL2-C concentration increased significantly by 40%. The increase in HDL2-C was attributed to an increase in HDL2 cholesteryl ester content; however, the increase (2.7%) in lecithin-cholesterol acyl transferase activity, which may explain the increase in HDL2 cholesteryl ester content, was not significant (6).

A 20.9% increase in the percentage of HDL-C carried by HDL2a particle, which contributed to the 14.2% increase in the percentage of HDL-C carried by HDL2b+2c particles, was observed postsupplementation. In contrast, a 31.0% reduction in the percentage of HDL-C carried by the HDL3a particle was observed. The reduction in HDL-C carried by HDL3a may explain the 22.8% reduction in HDL-C transported by HDL3a+3b particles. This shift in the distribution of HDL-C among HDL2 and HDL3 particles may explain the observed nonsignificant increase in HDL2-C concentration.

The shift in HDL-C as a result of the n-3fa supplementation may suggest a reduction in risk for coronary heart disease (CHD). Williams et al. (35) reported that higher areas under the HDL2a curve, as an index of the mass concentration, was associated with reduced risk of CHD, whereas higher areas under the HDL3b curve were suggestive of increased risk for CHD. Based on the observations in the present study, the shift in HDL-C from HDL3a+3b to HDL2b+2c may suggest a potential CHD risk-reducing benefit of a diet supplemented with n-3fa. Despite the shifts in HDL-C between HDL2 and HDL3 subfractions, no significant changes in HDL peak particle size were observed in this cohort postsupplementation. This observation is in agreement with the findings of Calabresi et al. (6),
reporting no significant change in mean HDL-2 and HDL-3 particle size. The mean or peak LDL particle size has been reported to increase following n-3fa supplementation (21); however, this response has not been consistent (27). Mori et al. (21) reported an increase in LDL peak particle size from 25.69 to 25.96 nm following the consumption of 4 g of DHA for 6 wk in overweight men with mild hyperlipidemia. The increase in the LDL size was attributed to the reduction (−32%) in serum triglyceride, which would lower the amount of available triglyceride to be transferred to LDL by cholesteryl ester transfer protein (21). A reduction in cholesteryl ester transfer protein activity, which has been observed following n-3fa supplementation (23), would likely reduce the formation of triglyceride-enriched LDL particles, thereby minimizing the opportunity for LPL to convert large LDL particles to small LDL particles. In this present study, LDL peak particle size and the distribution of LDL-C among LDL subfractions were unaffected by the n-3fa supplementation prescribed. It is likely that the dosage of DHA in the present study (1.6 g/day) was below the threshold to reduce triglyceride concentration and increase LDL size.

Combined effect of exercise and n-3fa supplementation. Few studies have quantified the combined effect of aerobic exercise and n-3fa supplementation on lipid and lipoprotein metabolism (29–31). In the present study, we observed no combined synergetic effects of aerobic exercise and n-3fa supplementation on lipid and lipoprotein-cholesterol concentrations or the electrophoretic characteristics of lipoproteins. These findings are similar to previous observations (30, 31). Thomas et al. (30) reported no changes in fasting and postprandial triglyceride responses in sedentary men after a single session of aerobic exercise following n-3fa supplementation of 4 g/day for 3 wk. Thomas et al. speculated that n-3fa supplementation had an antagonistic action on the exercise-induced increase in LPLa. Interestingly, in the present study, the percent change in triglyceride concentration postexercise was similar between no-supplement and supplement trials, suggesting that several consecutive sessions of aerobic exercise may prevent the proposed inhibition of n-3fa supplementation on LPLa.

Conclusions. In summary, the primary findings in our study in sedentary men were that 3 consecutive days of aerobic exercise expending ~650 kcal per session independently reduced total cholesterol and triglyceride concentrations with a concomitant increase in LDL peak particle size. In addition, n-3fa supplementation of 4.55 g/day for 42 days independently shifted HDL-C from HDL3 to HDL2 particles, despite no significant changes in HDL2-C and HDL3-C concentrations. Practitioners targeting risk reduction for coronary heart or metabolic disease should consider combining aerobic exercise and either n-3fa supplementation or a diet rich in n-3fa. Despite no synergistic effects of aerobic exercise and n-3fa supplementation observed in this study, combining these strategies does provide additional lipid and lipoprotein benefits than either strategy alone.

REFERENCES


