

# Factorial study of the effect of n-3 fatty acid supplementation and atorvastatin on the kinetics of HDL apolipoproteins A-I and A-II in men with abdominal obesity<sup>1-3</sup>

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## ABSTRACT

**Background:** Disturbed HDL metabolism in insulin-resistant, obese subjects may account for an increased risk of cardiovascular disease. Fish oils and atorvastatin increase plasma HDL cholesterol, but the underlying mechanisms responsible for this change are not fully understood.

**Objective:** We studied the independent and combined effects of fish oils and atorvastatin on the metabolism of HDL apolipoprotein A-I (apo A-I) and HDL apo A-II in obese men.

**Design:** We conducted a 6-wk randomized, placebo-controlled, 2 × 2 factorial intervention study of the effects of fish oils (4 g/d) and atorvastatin (40 mg/d) on the kinetics of HDL apo A-I and HDL apo A-II in 48 obese men with dyslipidemia with intravenous administration of [<sup>3</sup>H]-leucine. Isotopic enrichments of apo A-I and apo A-II were measured with gas chromatography-mass spectrometry with kinetic parameters derived from a multicompartmental model (SAAM II).

**Results:** Fish oils and atorvastatin significantly decreased plasma triacylglycerols and increased HDL cholesterol and HDL<sub>2</sub> cholesterol ( $P < 0.05$  for main effects). A significant ( $P < 0.02$ ) main effect of fish oils was observed in decreasing the fractional catabolic rate of HDL apo A-I and HDL apo A-II. This was coupled with a significant decrease in the corresponding production rates, accounting for a lack of treatment effect on plasma concentrations of apo A-I and apo A-II. Atorvastatin did not significantly alter the concentrations or kinetic parameters of HDL apo A-I and HDL apo A-II. None of the treatments altered insulin resistance.

**Conclusions:** Fish oils, but not atorvastatin, influence HDL metabolism chiefly by decreasing both the catabolism and production of HDL apo A-I and HDL apo A-II in insulin-resistant obese men. Addition of atorvastatin to treatment with fish oils had no additional effect on HDL kinetics compared with fish oils alone. *Am J Clin Nutr* 2006;84:37-43.

**KEY WORDS** Cardiovascular disease, n-3 fatty acids, 3-hydroxy-3-methylglutaryl coenzyme A reductase, HMG CoA reductase inhibitor, HDL, lipoprotein metabolism

## INTRODUCTION

Obesity, an important risk factor for cardiovascular disease and type 2 diabetes, is a growing problem in developed and developing populations (1). Abdominal obesity is typically seen

in overweight men and is strongly associated with hypertriglyceridemia and low HDL cholesterol, which may chiefly account for the increased risk of atherosclerosis in these men (2).

With the use of stable isotopes and multicompartmental modeling, we previously showed that in men with insulin resistance the dysregulation of apolipoprotein (apo) B metabolism may be caused by a combination of overproduction of VLDL apo B and decreased catabolism of apo B-containing particles (3). Apo A-I and apo A-II are the main apolipoproteins of HDL. Compelling evidence supports the antiatherogenic role of apo A-I in preventing cardiovascular disease, with less consistent data available for apo A-II (4, 5). Expansion of the VLDL-triacylglycerol pool in insulin resistance enhances cholesteryl ester transfer protein (CETP)-mediated heteroexchange of neutral lipids among lipoproteins, thereby increasing HDL-triacylglycerol content. Subsequent hydrolysis by hepatic lipase results in a thermodynamically unstable HDL particle that is catabolized rapidly by the liver and kidney (6). Consistent with this, we and others previously showed an increased catabolism of HDL-apo A-I and HDL-apo A-II particles in insulin-resistant subjects (7-10).

Effective management of dyslipidemia in abdominal obesity often requires lipid-regulating pharmacotherapy (11). Data from several clinical trials support the use of fish oils (rich source of n-3 fatty acids) and 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins) to treat dyslipidemia in insulin resistance and obesity (12-15). Combination of n-3 fatty acids with a statin may also constitute a safe and effective treatment for obesity-related dyslipidemia (16, 17). We have previously

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reported that both n-3 fatty acids and atorvastatin effectively lowered plasma triacylglycerol and increased HDL-cholesterol concentrations (17). Our kinetic data suggest that n-3 fatty acids decrease VLDL-apo B secretion (18), whereas atorvastatin increases hepatic clearance of all apo B-containing lipoproteins (19). By decreasing plasma triacylglycerols, n-3 fatty acids and statins may alter the metabolic fate of HDL particles. However, the kinetic effects of n-3 fatty acids and atorvastatin, used alone or in combination, on HDL-apo A-I and HDL-apo A-II kinetics in abdominally obese subjects have not yet been investigated.

In the present study, we examined the independent and combined effects of n-3 fatty acid supplementation and atorvastatin on HDL-apo A-I and HDL-apo A-II kinetics in insulin-resistant men with abdominal obesity. We hypothesized that n-3 fatty acids and atorvastatin would decrease the fractional catabolic rate (FCR) of both HDL apo A-I and HDL apo A-II.

## SUBJECTS AND METHODS

### Subjects

Forty-eight obese men were recruited (17). Abdominal obesity was defined as waist circumference >100 cm. All subjects had plasma triacylglycerol >1.2 mmol/L and cholesterol >5.2 mmol/L. None of the subjects had diabetes mellitus, apolipoprotein E2/E2 genotype, macroproteinuria, creatinemia, hypothyroidism, or abnormal liver enzymes. Subjects consumed <1 fish meal/wk, <30 g alcohol/d, and no fish-oil supplements. None reported a history of cardiovascular disease or was taking agents that affect lipid metabolism. All provided written consent, and the study was approved by the Royal Perth Hospital Ethics Committee.

### Study design

This study is a component of a large placebo-controlled 2 × 2 factorial-designed intervention trial with fish-oil supplementation and atorvastatin on lipoprotein metabolism that commenced in 1998 (17–20). Volunteers entered a randomized, double-blind, placebo-controlled intervention trial that involved a 3-wk run-in period with body weight variations of <2%. After the run-in period they were randomly assigned to 1 of 4 treatment groups for 6 wk: atorvastatin (40 mg/d), fish oils [4 Omacor capsules (Provona Biocare, Oslo, Norway), consisting of 45% eicosapentaenoic acid and 39% docosahexaenoic acid, taken orally at night, ie, 4 g/d], atorvastatin plus fish oils, or atorvastatin placebo plus 4 g/d placebo corn oil. Compliance with fish oils and atorvastatin were checked by tablet count at weeks 3 and 6.

### Clinical protocols

Subjects were admitted to the metabolic ward after a 14-h fast. They were studied in a semirecumbent position and allowed only water for 10 h. Venous blood was collected for biochemical analyses. Plasma volume was determined by multiplying body weight by 0.045 (in L/kg), with adjustment for decrease in relative plasma volume associated with obesity as described by Riches et al (21). Dietary intake was assessed for energy and major nutrients by using at least two 24-h dietary diaries at the beginning and end of the study. Diets were analyzed with the use of DIET 4 NUTRIENT CALCULATION SOFTWARE (Xyris

Software, Highgate Hill, Queensland, Australia). Physical activity was assessed by a 7-d recall questionnaire as described by Blair (22).

A single bolus of [ $d_3$ ]-leucine (5 mg/kg body weight) was administered intravenously into an antecubital vein by a 21G butterfly needle (3). Blood samples were taken at baseline; at 5, 10, 20, 30, 40 min; and at 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, and 10 h after isotope injection. Additional fasting blood samples were collected in the morning on the following 4 d (24, 48, 72, and 96 h). All procedures were repeated after 6 wk.

### Biochemical analyses

Laboratory methods for measurements of lipid and lipoprotein were previously detailed (7). HDL<sub>2</sub> (1.063–1.125 g/L) and HDL<sub>3</sub> (1.125–1.21 g/L) were isolated by ultracentrifugation (200 000 × g for 24 h at 20 °C) from 0.5 mL plasma, and cholesterol concentrations were measured. Plasma nonesterified fatty acids were measured with a kit (Randox, CO Antrim, United Kingdom). Plasma insulin was measured by radioimmunoassay (DiaSorini, Saluggia, Italy). Plasma glucose concentration was measured by the hexokinase method. Plasma lathosterol concentration was assayed by using gas chromatography–mass spectrometry. Insulin resistance was estimated by using the homeostasis model assessment formula (23). Apolipoprotein E genotype was determined as previously described (3).

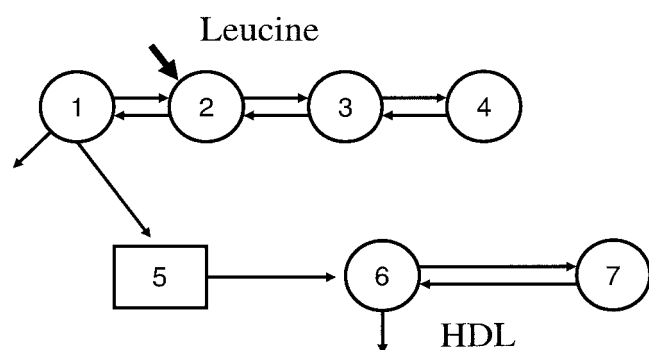
### Isolation and measurement of isotopic enrichment of HDL apo A-I and HDL apo A-II

Laboratory methods for isolation and measurement of isotopic enrichment were previously detailed (7, 8). Briefly, apo B was precipitated from 250 μL plasma by using heparin (25 μL) and 12.5 μL 2.0 M MnCl<sub>2</sub>. A volume of 60 μL 64% CsCl was added to 200 μL heparin and manganese-treated plasma to adjust the density to 1.21 g/mL. HDL was subsequently isolated from 230 μL of this sample by ultracentrifugation (200 000 × g for 24 h at 20 °C; Optima XL-100K; Beckman Coulter, Fullerton, Australia). Apo A-I and apo A-II were isolated by using polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride membrane. The apo A-I and apo A-II bands were excised from the membrane, hydrolyzed with 200 μL 6 M HCL at 110 °C for 16 h, and dried for derivatization by using the oxazolinone method as previously described (7). Apo A-I and apo A-II isotopic enrichment was measured with gas chromatography–mass spectrometry.

### Model of apo A-I and apo A-II metabolism and calculation of kinetic parameters

The multicompartamental model used to describe HDL-apo A-I leucine tracer-to-tracee ratios is shown in **Figure 1**. The SAAM II program (SAAM Institute, Seattle, WA) was used to fit the model to the observed tracer data. The model includes a 4-compartment subsystem (compartments 1–4) that describe plasma leucine kinetics. This subsystem is connected to an intrahepatic delay compartment 5 that accounts for the time required for the synthesis and secretion of apo A-I into plasma. Compartments 6 and 7 describe the kinetics of apo A-I in the plasma HDL fraction and in a nonplasma compartment, respectively. HDL-apo A-I metabolic markers, including production rate and FCR, were derived after a fit of the model to the plasma leucine and apolipoprotein AI tracer:tracee data. The same





**FIGURE 1.** Compartment model describing the tracer kinetics of HDL apolipoprotein A-I (apo A-I) and HDL apo A-II. The model includes a 4-compartment subsystem (compartments 1–4) that describes plasma leucine kinetics. This subsystem is connected to an intrahepatic delay compartment (compartment 5), which accounts for the time required for the synthesis and secretion of apo A-I into plasma. Compartments 6 and 7 describe the kinetics of apo A-I (or apo A-II) in the plasma HDL fraction and in a nonplasma compartment, respectively.

model and calculation of kinetic parameters was used for HDL apo A-II.

### Statistical analysis

All analyses were done by using SPSS 11.5 (SPSS Software, Chicago, IL). Group characteristics were compared by analysis of variance with Bonferroni adjustments. Associations were examined by simple linear regression method. General linear modeling was used to assess the main and interactive effects of the interventions.

## RESULTS

### Subject characteristics

The pretreatment clinical and biochemical characteristics of the obese subjects randomly assigned to the 4 treatment groups are shown in **Table 1**. On average, they were middle-aged, centrally obese, normotensive, and insulin resistant (3). No significant group differences were observed in any of the variables. Of the 48 obese subjects, 31 were *E3/E3* homozygotes, 2 were *E2/E3* heterozygotes, 13 were *E3/E4* heterozygotes, and 2 were *E4/E4* homozygotes. No statistically significant differences were observed in the frequency distribution of *E* alleles among

the groups. Tablet and capsule counts confirmed that compliance with random assignment to active intervention or placebo was >95%.

The effects of interventions on plasma lipid, lipoprotein, and apolipoprotein concentrations are shown in **Table 2**. No significant interactions were observed between fish oils and atorvastatin treatment for any of the variables. In a factorial analysis conducted with general linear modeling, a significant ( $P < 0.05$ ) main effect was observed for fish oils in decreasing plasma triacylglycerol concentrations and increasing HDL-cholesterol and HDL<sub>2</sub>-cholesterol concentrations. A significant ( $P < 0.01$ ) main effect was also observed for atorvastatin in lowering plasma triacylglycerol, total cholesterol, LDL-cholesterol, apo B, and lathosterol concentrations and in raising plasma HDL-cholesterol and HDL<sub>2</sub>-cholesterol concentrations. Neither agent had a significant effect on plasma concentrations of apo A-I and apo A-II. No significant within-group differences were observed in body weight, waist circumference, waist-to-hip ratio, blood pressure, plasma nonesterified fatty acids, glucose, and homeostasis model assessment score (data not shown). Nutrient and alcohol intake and exercise did not differ significantly among groups at baseline or during the study (data not shown).

The effects of the interventions on HDL-apo A-I and HDL-apo A-II metabolism are shown in **Table 3**. No significant interactions between fish-oil and atorvastatin treatments were observed for any of the variables shown in this table. A significant ( $P < 0.02$ ) main effect was observed for fish oils on decreasing HDL-apo A-I and HDL-apo A-II FCRs. This was coupled to a significant decrease ( $P < 0.05$ ) in the corresponding production rates, accounting for a lack of treatment effect in plasma apo A-I and apo A-II pool sizes. Atorvastatin did not significantly alter the pool sizes and kinetic parameters of HDL apo A-I and HDL apo A-II.

In univariate analysis, baseline plasma concentrations of apo A-I and apo A-II were directly and significantly associated with their corresponding production rates ( $r = 0.549$  and  $r = 0.362$ , respectively;  $P < 0.02$  for both). With the use of apo B kinetic data from the same subjects (3), the FCRs of apo A-I and apo A-II were significantly associated with VLDL-apo B secretion rate ( $r = 0.290$  and  $r = 0.310$ , respectively,  $P < 0.05$  for both). In subgroup analysis, percentage changes in the FCRs of apo A-I and apo A-II were significantly associated ( $P < 0.01$  for both) with corresponding changes in production rates in the fish-oil ( $r = 0.932$  and  $r = 0.949$ , respectively) and combined treatment

**TABLE 1**  
Pretreatment clinical and biochemical characteristics in 48 obese subjects<sup>1</sup>

|                                    | Placebo<br>(n = 12) | Fish oils<br>(n = 12) | Atorvastatin<br>(n = 13) | Fish oil + atorvastatin<br>(n = 11) |
|------------------------------------|---------------------|-----------------------|--------------------------|-------------------------------------|
| Age (y)                            | 51 ± 9              | 58 ± 8                | 52 ± 10                  | 54 ± 8                              |
| Body weight (kg)                   | 100 ± 8             | 106 ± 18              | 107 ± 18                 | 101 ± 15                            |
| BMI (kg/m <sup>2</sup> )           | 32 ± 3              | 35 ± 4                | 35 ± 5                   | 33 ± 3                              |
| Waist circumference (cm)           | 110 ± 6             | 115 ± 9               | 118 ± 13                 | 110 ± 8                             |
| Systolic blood pressure (mm Hg)    | 128 ± 11            | 132 ± 13              | 132 ± 19                 | 138 ± 17                            |
| Diastolic blood pressure (mm Hg)   | 78 ± 10             | 74 ± 10               | 79 ± 12                  | 83 ± 8                              |
| Nonesterified fatty acids (mmol/L) | 0.26 ± 0.10         | 0.36 ± 0.19           | 0.29 ± 0.13              | 0.27 ± 0.06                         |
| Fasting glucose (mmol/L)           | 5.4 ± 0.5           | 5.7 ± 1.0             | 5.6 ± 0.6                | 5.3 ± 0.7                           |
| Fasting insulin (mU/L)             | 32 ± 9              | 41 ± 12               | 33 ± 13                  | 33 ± 7                              |
| Insulin resistance (HOMA score)    | 7.7 ± 2.6           | 10 ± 5                | 8.1 ± 3.6                | 7.8 ± 2.4                           |

<sup>1</sup> All values are  $\bar{x} \pm SD$ . HOMA, homeostasis model assessment. ANOVA with Bonferroni adjustments was used to compare group difference. There were no significant group differences in any of the variables,  $P > 0.05$ .

**TABLE 2**Plasma concentrations of lipid, lipoprotein, apolipoprotein (apo), and lathosterol in the subjects at baseline and at week 6<sup>1</sup>

|                                       | Placebo<br>(n = 12) | Fish oils<br>(n = 12) | Atorvastatin<br>(n = 13) | Fish oil +<br>atorvastatin<br>(n = 11) | Main effects        |                     |
|---------------------------------------|---------------------|-----------------------|--------------------------|--|---------------------|---------------------|
|                                       |                     |                       |                          |  | Fish oils           | Atorvastatin        |
| Triacylglycerol (mmol/L)              |                     |                       |                          |  |                     |                     |
| Baseline                              | 1.7 ± 0.2           | 2.0 ± 0.3             | 1.9 ± 0.1                | 2.0 ± 0.2                              |                     |                     |
| Week 6                                | 1.6 ± 0.2           | 1.5 ± 0.2             | 1.4 ± 0.1                | 1.2 ± 0.2                              | -0.3 ± 0.1 (0.001)  | -0.3 ± 0.1 (0.001)  |
| Total cholesterol (mmol/L)            |                     |                       |                          |  |                     |                     |
| Baseline                              | 5.8 ± 0.1           | 5.9 ± 0.2             | 5.8 ± 0.1                | 6.3 ± 0.3                              |                     |                     |
| Week 6                                | 5.6 ± 0.1           | 5.5 ± 0.2             | 3.6 ± 0.1                | 3.9 ± 0.3                              | -0.04 ± 0.17 (NS)   | -1.8 ± 0.2 (0.001)  |
| HDL cholesterol (mmol/L)              |                     |                       |                          |  |                     |                     |
| Baseline                              | 1.05 ± 0.06         | 0.99 ± 0.06           | 1.00 ± 0.05              | 1.10 ± 0.09                            |                     |                     |
| Week 6                                | 1.03 ± 0.06         | 1.00 ± 0.04           | 1.04 ± 0.05              | 1.25 ± 0.09                            | 0.07 ± 0.04 (0.041) | 0.10 ± 0.04 (0.007) |
| HDL <sub>2</sub> cholesterol (mmol/L) |                     |                       |                          |  |                     |                     |
| Baseline                              | 0.37 ± 0.03         | 0.31 ± 0.03           | 0.39 ± 0.05              | 0.47 ± 0.06                            |                     |                     |
| Week 6                                | 0.36 ± 0.03         | 0.36 ± 0.02           | 0.43 ± 0.02              | 0.57 ± 0.06                            | 0.07 ± 0.03 (0.013) | 0.07 ± 0.03 (0.008) |
| HDL <sub>3</sub> cholesterol (mmol/L) |                     |                       |                          |  |                     |                     |
| Baseline                              | 0.68 ± 0.05         | 0.68 ± 0.05           | 0.61 ± 0.05              | 0.63 ± 0.06                            |                     |                     |
| Week 6                                | 0.67 ± 0.06         | 0.64 ± 0.04           | 0.61 ± 0.04              | 0.68 ± 0.07                            | 0.01 ± 0.03 (NS)    | 0.04 ± 0.03 (NS)    |
| LDL cholesterol (mmol/L)              |                     |                       |                          |  |                     |                     |
| Baseline                              | 3.8 ± 0.1           | 3.9 ± 0.2             | 3.8 ± 0.2                | 4.0 ± 0.3                              |                     |                     |
| Week 6                                | 3.8 ± 0.1           | 3.7 ± 0.2             | 1.8 ± 0.1                | 2.2 ± 0.2                              | 0.02 ± 0.14 (NS)    | -1.8 ± 0.1 (0.001)  |
| Apo A-I (mg/dL)                       |                     |                       |                          |  |                     |                     |
| Baseline                              | 128 ± 5             | 118 ± 4               | 119 ± 5                  | 128 ± 6                                |                     |                     |
| Week 6                                | 126 ± 4             | 121 ± 4               | 123 ± 4                  | 135 ± 9                                | 5 ± 4 (NS)          | 5 ± 4 (NS)          |
| Apo A-II (mg/dL)                      |                     |                       |                          |  |                     |                     |
| Baseline                              | 35 ± 1              | 30 ± 1                | 30 ± 1                   | 33 ± 1                                 |                     |                     |
| Week 6                                | 33 ± 1              | 26 ± 1                | 29 ± 2                   | 31 ± 2                                 | -0.8 ± 1.1 (NS)     | 1.7 ± 1.1 (NS)      |
| Apo B (mg/dL)                         |                     |                       |                          |  |                     |                     |
| Baseline                              | 129 ± 4             | 128 ± 6               | 122 ± 6                  | 134 ± 6                                |                     |                     |
| Week 6                                | 123 ± 3             | 118 ± 6               | 69 ± 3                   | 73 ± 5                                 | -2 ± 4 (NS)         | -49 ± 4 (0.001)     |
| Lathosterol (μmol/L)                  |                     |                       |                          |  |                     |                     |
| Baseline                              | 9.3 ± 1.0           | 11.2 ± 1.5            | 10.8 ± 1.2               | 13.0 ± 1.0                             |                     |                     |
| Week 6                                | 9.6 ± 0.7           | 11.2 ± 1.1            | 1.9 ± 0.3                | 3.8 ± 1.1                              | 1.5 ± 0.8 (NS)      | -7.8 ± 0.9 (0.001)  |

<sup>1</sup> All values are  $\bar{x} \pm \text{SEM}$ ; *P* values in parentheses. General linear modeling was used to assess the main and interactive effects of fish-oil and atorvastatin treatments. There were no significant interactive effects between the treatments.

( $r = 0.833$  and  $r = 0.872$ , respectively) groups. Changes in the kinetic parameters of apo A-I and apo A-II, as a result of treatment, were not associated with changes in apo B kinetic parameters.

## DISCUSSION

This is the first study to examine the independent and combined effects of n-3 fatty acid supplementation and a statin on HDL-apo A-I and HDL-apo A-II metabolism in nondiabetic, insulin-resistant obese subjects. We provide new evidence that n-3 fatty acids influence the kinetics of HDL by decreasing both catabolism and production of apo A-I and apo A-II without significantly altering in their pool sizes. These effects were achieved with no significant alteration in insulin resistance, body weight, or dietary intake. In contrast, atorvastatin did not significantly alter synthesis or the turnover of HDL apo A-I and HDL apo A-II. Combination treatment did not provide an additional effect on HDL metabolism compared with n-3 fatty acids alone.

### Effect of fish-oil supplementation on HDL metabolism

By using stable isotope techniques, only one study has examined the effects of n-3 fatty acid supplementation on HDL-apo A-I metabolism (24). Frenais et al (24) reported that in 5 diabetic

patients HDL-apo A-I FCR and production rate were significantly decreased after treatment with n-3 fatty acid supplementation. The concentration of HDL apo A-I was not significantly changed. Our data extend the previous study by using a significantly larger sample size and examining the effect of n-3 fatty acid supplementation on both HDL-apo A-I and HDL-apo A-II metabolism. We report a new finding that, similar to HDL-apo A-I kinetics, n-3 fatty acids decreased the FCR and production of HDL apo A-II. Although n-3 fatty acids did not alter pool sizes of apo A-I or apo A-II in plasma, our data clearly show that n-3 fatty acids exhibit significant effects on HDL turnover. We have previously shown that n-3 fatty acids lower plasma triacylglycerols by a reduction in VLDL-apo B production (18). As described earlier, this in turn decreases CETP-mediated heteroexchange of neutral lipids among lipoproteins, thereby delaying the uptake of HDL by the liver (6). This notion is consistent with our present findings. The precise reason for decreased production of apo A-I and apo A-II with fish oils in abdominal obesity remains unclear. Given that n-3 fatty acids are peroxisome proliferator-activated receptor- $\alpha$  agonists (25), one would expect fish oils to increase apo A-I and apo A-II production. However, note that n-3 fatty acids are only "weak" agonists, and their effect on the gene expression of apo A-I and apo A-II might be

TABLE 3

Effect of interventions on HDL apolipoprotein (apo) A-I and apo A-II metabolism<sup>1</sup>

|  | Placebo<br>(n = 12) | Fish oils<br>(n = 12) | Atorvastatin<br>(n = 13) | Fish oil +<br>atorvastatin<br>(n = 11) | Main effects         |                   |
|--|---------------------|-----------------------|--------------------------|--|----------------------|-------------------|
|  |                     |                       |                          |  | Fish oils            | Atorvastatin      |
| Pool size (mg)   |                     |                       |                          |  |                      |                   |
| Apo A-I  |                     |                       |                          |  |                      |                   |
| Baseline   | 5051 ± 158          | 4491 ± 202            | 4707 ± 255               | 5132 ± 237                             |                      |                   |
| Week 6   | 4942 ± 130          | 4597 ± 195            | 4844 ± 220               | 5400 ± 312                             | 145 ± 137 (NS)       | 249 ± 138 (NS)    |
| Apo A-II   |                     |                       |                          |  |                      |                   |
| Baseline   | 1389 ± 56           | 1118 ± 39             | 1174 ± 63                | 1307 ± 59                              |                      |                   |
| Week 6   | 1282 ± 46           | 982 ± 34              | 1141 ± 77                | 1247 ± 70                              | -41 ± 40 (NS)        | 73 ± 39 (NS)      |
| Production rate (mg · kg <sup>-1</sup> · d <sup>-1</sup> ) |                     |                       |                          |  |                      |                   |
| Apo A-I  |                     |                       |                          |  |                      |                   |
| Baseline   | 14.6 ± 1.4          | 14.0 ± 1.3            | 11.8 ± 1.1               | 15.9 ± 2.9                             |                      |                   |
| Week 6   | 13.6 ± 1.2          | 10.8 ± 0.8            | 11.8 ± 1.0               | 13.3 ± 2.0                             | -1.6 ± 0.7 (0.034)   | 0.5 ± 0.7 (NS)    |
| Apo A-II   |                     |                       |                          |  |                      |                   |
| Baseline   | 3.9 ± 0.3           | 3.1 ± 0.3             | 2.7 ± 0.3                | 3.4 ± 0.5                              |                      |                   |
| Week 6   | 3.4 ± 0.3           | 2.2 ± 0.1             | 2.6 ± 0.2                | 2.9 ± 0.6                              | -0.4 ± 0.2 (0.013)   | 0.3 ± 0.2 (NS)    |
| Fractional catabolic rate (pools/d)                        |                     |                       |                          |  |                      |                   |
| Apo A-I  |                     |                       |                          |  |                      |                   |
| Baseline   | 0.27 ± 0.02         | 0.31 ± 0.03           | 0.23 ± 0.02              | 0.25 ± 0.02                            |                      |                   |
| Week 6   | 0.26 ± 0.02         | 0.24 ± 0.01           | 0.24 ± 0.01              | 0.22 ± 0.02                            | -0.04 ± 0.02 (0.012) | -0.01 ± 0.02 (NS) |
| Apo A-II   |                     |                       |                          |  |                      |                   |
| Baseline   | 0.24 ± 0.02         | 0.26 ± 0.02           | 0.19 ± 0.02              | 0.21 ± 0.02                            |                      |                   |
| Week 6   | 0.21 ± 0.02         | 0.20 ± 0.01           | 0.20 ± 0.02              | 0.19 ± 0.02                            | -0.04 ± 0.01 (0.014) | 0.01 ± 0.02 (NS)  |

<sup>1</sup> All values are  $\bar{x} \pm \text{SEM}$ ; *P* values in parentheses. General linear modeling was used to assess the main and interactive effects of fish-oil and atorvastatin treatments. There were no significant interactive effects between the treatments.

limited (26). We have previously shown that subjects with insulin resistance without diabetes have accelerated catabolism of HDL apo A-I with elevated production rates of apo A-I compared with lean control subjects (7). Consistent with this, we found that changes in the FCRs of apo A-I and apo A-II were significantly associated with their corresponding changes in production rates in the fish-oil and the combined treatment groups. Taken together, it is likely that fish oils decreased the FCRs of apo A-I and apo A-II and that a decreased production rate may be a "balancing feedback" mechanism of reduced uptake of HDL particles, but this requires further investigation.

### Effect of atorvastatin on HDL metabolism

Previous studies have only examined the effect of statins on HDL-apo A-I metabolism (7, 27, 28). Consistent with the present findings, all have consistently failed to show significant changes in the production and catabolic rates of HDL apo A-I. By decreasing plasma triacylglycerol concentrations, statins may, indirectly, decrease CETP-mediated heteroexchange of neutral lipids and subsequently delay the uptake of HDL by the liver (6). However, atorvastatin could also possibly enhance hepatic HDL receptors for the uptake of larger HDL particles in plasma (29). Such a mechanism may therefore account for the lack of treatment effect in the FCRs of apo A-I and apo A-II particles but this requires further investigation.

In contrast to fish oils, atorvastatin treatment did not alter HDL-apo A-I and HDL-apo A-II production. Although statin-induced inhibition of the Rho-signaling pathway may increase the expression and production of apo A-I in vitro (30, 31), no in vivo effect on production was seen in this study. A recent study by Mauger et al (32) compared the effect of simvastatin (80 mg) and atorvastatin (40 mg) on HDL-apo A-I kinetics in overweight

persons with dyslipidemia. They found that simvastatin significantly increased HDL-apo A-I production compared with atorvastatin, provocatively suggesting statin-specific or dose effects on HDL-apo A-I metabolism. Although several studies have examined the effects of statin on HDL-apo A-I kinetics, no studies have investigated its effect on the flux of HDL apo A-II. Given that both apo A-I and apo A-II share similar metabolic pathways, the lack of effect of atorvastatin on HDL apo A-II was not unexpected.


### Effect of fish oils plus atorvastatin on HDL metabolism

Our previous findings showed that the combination of n-3 fatty acids and atorvastatin effectively normalized the kinetic abnormalities in apo B metabolism by decreasing VLDL-apo B production and increasing the FCR of VLDL, intermediate-density lipoprotein, and LDL apo B (20). In this study, we further explore the potential effect of the combination treatment on HDL metabolism. The present data suggest that the combination treatment did not provide additional effect on HDL metabolism compared with fish oils alone.

Fish oils plus atorvastatin did not alter plasma HDL-apo AI or HDL-apo AII concentrations; however, the positive and additive effect of each agent on HDL cholesterol, specifically the HDL<sub>2</sub> subfraction, was observed. Although the basis for this effect is unclear, it may be attributable to the greater reductions in plasma triacylglycerols and VLDL-apo B pool sizes with combination treatment than with either of the monotherapies that result in less CETP-mediated exchange of neutral lipids between lipoproteins, as discussed earlier.

The study has limitations. Our study was restricted to men, and, given the sex differences in HDL metabolism, our results may not apply to women; however, this requires investigation.

HDL particles are subjected to modifications by several lipase and lipid transfer proteins, particularly, lipoprotein lipase, hepatic lipase, CETP, and phospholipid transfer protein (33). Measurements of their activities in plasma may help to formally corroborate our findings. Moreover, examination of the effect of both agents on the kinetics of HDL<sub>2</sub>, HDL<sub>3</sub>, LpA-I, LpA-I:LpA-II, and pre- $\beta$  HDL may also be important to elucidate their precise mechanisms of action on HDL metabolism.

Elevated triacylglycerols and low HDL cholesterol are significant risk factors for coronary disease (34, 35). Correction of these abnormalities with n-3 fatty acids and atorvastatin may have clinical significance, consistent with evidence that these agents decrease the risk of coronary disease. Taken together with our previous findings on apo B metabolism, we provide a kinetic basis for changes in lipids and lipoproteins with n-3 fatty acids and atorvastatin. Despite this, it appears that such changes in apo B and HDL metabolism are largely independent of each other. With the exception of the associations between VLDL-apo B secretion and apo A-I and apo A-II FCRs, apo B metabolic limits were not associated with HDL apoprotein metabolism. The results of the present study indicate that in obese subjects with insulin resistance and dyslipidemia, n-3 fatty acids influence the metabolism of HDL metabolism by decreasing both catabolism and production of apo A-I and apo A-II. Combination treatment of fish oils with other therapeutic agents that selectively increase apo A-I and apo A-II production may be particularly important in the prevention of cardiovascular disease in this group of patients. Given that statins regulate lipoprotein metabolism chiefly by decreasing hepatic cholesterol biosynthesis, their impact on HDL metabolism might be limited. Despite this, our previous data clearly show its favorable effect on the catabolism of apo B lipoproteins. Hence, further studies should also examine the additive effect of fibrates, insulin sensitizers, or CETP inhibitors together with fish oils or a statin regimen. 

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DCC, GFW, and PHRB contributed to the conception and design of the study. GFW and DCC supervised the experiment and the data collection. DCC and MNN performed the statistical analysis. PHRB and MNN performed the modeling of the data. All authors contributed to the writing of the manuscript. None of the authors had a conflict of interest with any aspect of this research.

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