Postprandial effect of n–3 polyunsaturated fatty acids on apolipoprotein B–containing lipoproteins and vascular reactivity in type 2 diabetes\textsuperscript{1–3}

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ABSTRACT

Background: Plasma lipoproteins may be classified by their apolipoprotein composition. The lipoprotein subclass containing apolipoproteins B and C (LpB:C) is considered the most atherogenic.

Objective: We evaluated the acute effects of individual fatty acids on apolipoprotein B (apo B)–containing lipoproteins in adults with type 2 diabetes (n = 15).

Design: We administered 3 meals in a randomized, double-blind, crossover design. Treatments contained skim milk and 50 g fat from high–oleic acid safflower and canola oils (monounsaturated fatty acid; MUFA), MUFA + 3.5 g α-linolenic acid (ALA; MUFA + ALA) from high-ALA canola oil, or MUFA + 4.0 g both eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA; MUFA + EPA/DHA) from sardine oil. Apo B, LpB, LpB:C, LpB:E, LpB:C:E, and LpA-II:B:C:D:E were measured at baseline and 2 and 4 h after the meal. Flow-mediated dilation was measured at baseline and 4 h after the meal.

Results: The treatments significantly increased apo B and LpB postprandially (P < 0.03 for both), but the magnitude of the changes did not differ significantly between the treatments. The postprandial change in LpB:C was 23% lower after MUFA + EPA/DHA than after MUFA (treatment \times time interaction, P < 0.0001). MUFA + ALA attenuated the increase in LpA-II:B:C:D:E in those with high triacylglycerols (≥1.69 mmol/L) but was the only treatment to significantly increase this particle in those with low triacylglycerols (treatment \times group interaction, P < 0.0001). Examination of change scores did not reveal the source of the interaction of treatment and time (P < 0.007) for LpB:E + LpB:C:E. Furthermore, the subjects with the largest increases in LpB:C exhibited the largest impairment in endothelial function.

Conclusions: The results suggest that unsaturated fatty acids differentially affect concentrations of apo B–containing lipoprotein subclasses. A rise in LpB:C adversely affects endothelial function. Meals containing MUFA + EPA/DHA attenuated the postprandial rise in LpB:C and the impairment of endothelial function.

KEY WORDS Lipoproteins, apolipoprotein B, type 2 diabetes, hypertriglyceridemia, n–3 fatty acids, monounsaturated fatty acids, lipoproteins LpB:C, postprandial apolipoproteins

INTRODUCTION

The categorization of discrete lipoprotein families based on apolipoprotein composition instead of size and density provides a new way of describing plasma lipoproteins (1, 2). According to this novel classification system (1, 2), there are 2 principal classes of lipoproteins, one of which is characterized by apolipoprotein A (apo A; apo A-I + apo A-II) and the other by apolipoprotein B (apo B) as the major apolipoprotein constituents. The apo A–containing lipoproteins include 3 major subclasses [lipoprotein A-I (LpA-I), lipoprotein A-I and All (LpA-I:A-II), and lipoprotein A-II (LpA-II)], and the apo B–containing lipoproteins consist of 5 major subclasses [lipoprotein B (LpB); lipoprotein B and C (LpB:C); lipoprotein B and E (LpB:E); lipoprotein B, C, and E (LpB:C:E); and lipoprotein A-II, B, C, D, and E (LpA-II:B:C:D:E)]. Each of these apo A– and apo B–containing lipoproteins is a polydisperse system of particles heterogeneous with respect to physical properties (ie, density and size) and lipid-to-protein ratios but homogeneous with regard to the qualitative apolipoprotein composition. As presented in Figure 1, polydisperse apo A–containing lipoproteins overlap within the HDL-density range, and the apo B–containing lipoproteins overlap within the VLDL-, LDL-, and LDL-density ranges. Apo A– and apo B–containing lipoprotein subclasses differ from each other not only by their apolipoprotein composition, but also by their specific metabolic properties (1, 2). Although this area of research is relatively new, several studies have shown that individual apo A–containing lipoprotein subclasses differ in their relative antiatherogenic capacities and that apo B–containing lipoprotein subclasses differ in their atherogenic capacities (1–7). The relative atherogenic potential of the LpB:C subclass may be more pronounced than that of other apo B–containing lipoproteins (1, 2).

It has been shown that various dyslipoproteinemias are characterized by different concentration profiles of apolipoprotein–defined lipoprotein subclasses that can be modified by pharmacologic interventions (2, 8, 9). Although diet plays a crucial role...
in modulating lipid metabolism, little is known of its effects on apolipoprotein-defined lipoprotein subclasses (10, 11). Although there is evidence that dietary fatty acids influence plasma apo B concentrations (12–16), the present study provides the first description of the effects of unsaturated fatty acids on individual apo B–containing lipoprotein subclasses in the postprandial state. We focused on the apo B–containing lipoproteins because of their well-documented atherogenic potential and their increased concentrations in triacylglycerol-rich lipoproteins in the postprandial state (17–19).

We previously reported, on the same subject cohort, that only subjects with high fasting triacylglycerol had improved endothelial function when 7–8% of the oleic acid fat blend was replaced with n-3 fatty acids from canola or sardine oil (20). Thus, a goal of the present study was to examine whether changes in apo B–containing lipoprotein subclasses were associated with significant changes in vascular endothelial function and triacylglycerol status. This is important, because endothelial dysfunction is common in patients with type 2 diabetes and is considered a key initiating step in the development of atherosclerosis. Therefore, studies are needed to clarify the underlying biological mechanisms that adversely affect vascular reactivity in the postprandial state.

SUBJECTS AND METHODS

Plasma concentrations of lipids and apo B–containing lipoproteins were measured in 10 men and 5 women with type 2 diabetes mellitus who participated in a postprandial study designed to examine the acute effects of 3 monounsaturated fatty acid (MUFA)–rich test meals on flow-mediated dilation (FMD) of the brachial artery and postprandial triacylglycerol. Details of this randomized, double-blind, 3-phase crossover study were reported previously (20).

Subjects

All subjects had type 2 diabetes and were treated with diet or oral hypoglycemic agents only (glycated hemoglobin <8%). All subjects were nonsmokers and had no history of cardiovascular disease (CVD) or diabetes-related retinopathy, neuropathy, or nephropathy. Other exclusion criteria included fasting glucose >16.7 mmol/L, triacylglycerols >4.5 mmol/L, systolic blood pressure ≥160 mm Hg, diastolic blood pressure ≥95 mm Hg, body mass index (in kg/m²) >35, and use of dietary supplements and lipid- or blood pressure–lowering medications. The protocol was approved by the Biomedical Committee of the Institutional Review Board at The Pennsylvania State University, and written informed consent was obtained from all subjects.

Study design

All subjects completed 3 treatment sessions that were separated by ≥1 wk. The subjects were required to consume 1 of 3 test meals during each treatment session in random order. Of the 5 women in the study, 1 was premenopausal, and she was assessed during the early follicular phase (days 1–7) of her menstrual cycle on all 3 visits. All subjects were asked to abstain from alcohol for 48 h and to discontinue all medications the evening before each test. Blood samples for endpoint determinations were collected after a 12-h fast (baseline) and 2 h and 4 h after each test meal.

Test meals

The 3 isocaloric, isovolumetric test meals were high in MUFAs (ranging from 46–49% kcal) and similar in saturated fat (7% kcal), but differed in the amount and type of n–3 fatty acids they contained (Table 1). The test meals, given in the form of milkshakes, contained 473 mL skim milk, 50 g fat from 1 of 3 blends of unsaturated fatty acids, ice, and flavorings that masked...
the characteristic aroma and flavors of the oils (Ross Products Division, Abbott Laboratories, Columbus, OH). The subjects were required to consume freshly prepared test meals within 15 min. The control meal (MUFA) was prepared with high oleic safflower oil (90%) and canola oil (10%). It contained 0.5 g ALA, 1.2 g EPA (20:5n–3), 1.2 g DHA (22:6n–3), and 0.2 g ALA. Treatments were matched for protein (20 g), carbohydrate (24 g), and saturated fat (4 g).

### Biochemical assays

Lipids, lipoproteins, HbA1c, glucose, and insulin were measured using conventional methods, as described previously (20). Total apo B and individual apo B-containing subclasses (LpB, LpB:C, LpB:E + LpB:C:E and LpA-II:B:C:D:E) were measured at baseline (time = 0) and 2 and 4 h postprandially (Lipid and Lipoprotein Laboratory, Oklahoma Medical Research Foundation, Oklahoma City, OK). As previously described (21), the quantitative determination of individual apo B-containing subclasses was performed in 3 separate steps based on sequential immunoprecipitation of whole plasma by polyclonal antiserum to apo A-II [immunoglobulin G (IgG) fraction] and incubated overnight at 4 °C. After low-speed centrifugation (10 000 rpm; 4500 × g) for 30 min at 4 °C, the supernatant fraction was removed, and its apo B concentration was quantified by electroimmunoassay. The precipitate contained LpA-II:B:C:D:E particles, whereas the supernatant fraction contained LpB, LpB:E, LpB:C:E, and LpB:E subclasses [anti–apo A-II supernatant (anti–apo A-II-S)]. The concentration of LpA-II:B:C:D:E was calculated as the difference between the concentration of apo B in WP and that of apo B in the anti–apo A-II-S fraction.

In the second step, an aliquot of WP (100 μL) was treated with a mixture of polyclonal antisera to apo A-II and apolipoprotein E (apo E; IgG fractions), as described in the first step. Precipitated lipoprotein subclasses consisted of LpA-II:B:C:D:E, LpB:E, and LpB:C:E and the remaining soluble lipoproteins of LpB:C and LpB (anti–apo A-II + anti–apo E-S). The concentrations of LpB:E + LpB:C:E particles were calculated as the difference between the apo B concentration of anti–apo A-II-S and apo B concentration of anti–apo A-II + anti–apo E-S.

In the last step, the anti–apo A-II + anti–apo E-S–soluble lipoproteins (LpB + LpB:C) were treated with a polyclonal antiserum to apolipoprotein C-III (apo C-III; IgG fraction). The precipitate consisted of LpB:C and the supernate of LpB subclasses. Concentrations of the LpB:C subclass were calculated as the difference between the apo B concentrations of anti–apo A-II + anti–apo E-S lipoproteins and those of the supernate, which consisted of the LpB subclass. Alternatively, anti–apo A-II + anti–apo E–soluble lipoprotein (LpB + LpB:C) solution was placed on an anti–apo C-III immunosorber and incubated for 12 h. The unretracted fraction (LpB) was eluted with a running buffer, and the retained fraction (LpB:C) was eluted with 3 mol NaSCN/L. After dialysis and concentration to a smaller volume, both fractions were analyzed for apo B contents. The preparation of the anti–apo C-III–immunosorber and a detailed description of immunoaffinity chromatography was previously reported (24).

The concentrations of the LpB, LpB:C, LpB:E + LpB:C:E, and LpA-II:B:C:D:E subclasses are expressed in terms of their apo B contents. The between-assay CVs for immunoprecipitation with antisera to apo A-II or antisera to apo A-II + apo E were 2–3%, and the corresponding CV for immunoprecipitation with an antisera to apo C-III was 6–7%.

### Assessment of vascular reactivity

The ultrasound protocol to measure FMD of the brachial artery before the meals and 4 h postprandially was described in detail previously (20). Arterial diameter was measured by using an Acuson 128XP imaging system (Siemens Medical Solutions, Malvern, PA) with a 10-MHz linear array transducer. Images were collected during quiet rest (1 min), arterial occlusion via inflation of a cuff on the forearm (distal to the target artery) to 200 mm Hg (5 min), and reactive hyperemia (2 min). Frames for analysis were sampled at end diastole and diameters were measured continuously by using automated edge-detection software (Brachial Analyzer; Medical Imaging Applications, Iowa City, IA), with manual review of arterial boundaries by a trained technician. FMD was measured as the percentage change (Δ%) in arterial diameter from resting to the postischemia peak.

### Statistical analysis

The subjects were categorized as having high (≥ 1.69 mmol/L; n = 6) or low (< 1.69 mmol/L; n = 9) fasting triacylglycerols,
which was determined by the average of 4 fasting values from screening and the 3 test visits. Independent t tests were used to test whether the high and low triacylglycerol groups differed on any of the demographic or cardiovascular health variables at study entry.

PROC GLM (SAS version 9.1; SAS Institute, Cary, NC) was used to test whether the main effects of time, treatment, triacylglycerol group, or their interactions were significant. This provided a test of whether change over time was significantly different across the 3 treatments and the 2 groups. When the effect of time or the interaction of time and treatment was significant, we examined the effect of treatment on change scores (the 2-h level minus fasting baseline, and the 4-h level minus fasting baseline), using a mixed-models approach (SAS PROC MIXED). Models included treatment, time, triacylglycerol group, visit number, and treatment order as fixed effects and subject as a random effect, nested within triacylglycerol group. The dfs were adjusted for unequal group variance by Satterthwaite’s approximation. Tukey-Kramer adjusted P values were used to examine the source of significant effects. Values are presented as least squares means ± SEMs, and P values ≤ 0.05 were considered statistically significant.

Stepwise regression analysis was used to examine the strength of the relation between the postprandial changes in apo B–containing lipoproteins and changes in FMD. The model included the absolute magnitude of the change in FMD at 4 h after the meal as the dependent variable, and the independent variables included treatment, basal arterial diameter, age, total apo B, and LpB:C. A significant increase in $R^2$ ($P < 0.05$) with the addition of a variable was considered significant in the regression equation. Correlation analyses examined the relation between LpB:C and FMD.

RESULTS

Subject characteristics at baseline

Comparisons between the subjects with high fasting triacylglycerol concentrations and those with low fasting triacylglycerols on demographic and lipid and lipoprotein subclass variables are shown in Table 2. The groups did not differ significantly in age, weight, or cholesterol concentrations, except for the predetermined difference in triacylglycerol concentrations. The subjects in the high triacylglycerol group had 12% higher concentrations of total apo B ($P < 0.007$) and 25% higher concentrations of LpB:E + LpB:C:E ($P < 0.02$) than did their low triacylglycerol counterparts. LpB:C was 17% higher in the high than in the low triacylglycerol group; however, this was not statistically significant ($P < 0.07$). No significant differences in the other apo B–containing particles were observed between the groups.
Effects of treatment over time

In the PROC GLM analysis, we found significant interactions of treatment and time for LpB:C and LpB:E + LpB:C:E and significant effects of time for total apo B, LpB, LpB:C, LpB:E + LpB:C:E, and LpA-II:B:C:D:E. Subsequent analyses of group and treatment effects were conducted on postprandial change scores. No significant differences in postprandial changes in total apo B or LpB were observed between the 3 treatments (Table 3). All treatments increased total apo B at 2 h and 4 h compared with baseline, and the magnitude of the increases did not differ significantly between the treatments. In addition, LpB was increased at 2 h and there were no significant differences between the treatments (Table 3).

The treatments differed in their effects on the postprandial change in LpB:C (treatment-by-time interaction, P < 0.0001; Table 3). Both the MUFA treatment (P < 0.005) and MUFA + ALA treatment (P < 0.03) significantly increased LpB:C concentrations at 2 h and 4 h, but the MUFA + EPA/DHA treatment did not (P = 0.35) (Table 3). The changes in LpB:C at 2 h and 4 h were not significantly different. Furthermore, the postprandial change in LpB:C after the MUFA + EPA/DHA treatment was significantly lower than after the MUFA treatment (Tukey P = 0.02). In addition, examination of change scores did not reveal the source of the interaction of treatment and time (P < 0.007) for LpB:E + LpB:C:E.

We also found a significant treatment-by-triacylglycerol group interaction for change in LpA-II:B:C:D:E (P < 0.0001; Figure 3). In the high triacylglycerol group only, the MUFA and MUFA + EPA/DHA treatments significantly increased LpA-II:B:C:D:E compared with baseline (32% at 2 h and 36% at 4 h, P < 0.0005; and 45% at 2 h and 47% at 4 h, P < 0.0007, respectively), and the changes were not significantly different in magnitude. The MUFA + ALA treatment had a different effect and significantly increased LpA-II:B:C:D:E by 37% at 2 h and 30% at 4 h in the low triacylglycerol subjects only (P < 0.05). In addition, the MUFA + EPA/DHA treatment elicited a significantly greater increase in LpA-II:B:C:D:E in the high than in the low triacylglycerol group (46% compared with 19%, P < 0.04). No significant differences in changes in LpA-II:B:C:D:E were observed between 2 and 4 h.

Predictors of postprandial change in FMD

As reported previously (20), FMD of the brachial artery was 17% higher 4 h after the meal than at fasting baseline (mean FMD = 5.16 ± 0.51 and 6.04 ± 0.51 at 0 h and 4 h, respectively, P = 0.01). To examine whether apo B–containing subclasses were independent predictors of the postprandial change in FMD, we used stepwise multiple regression. The best predictor of the change in FMD was basal arterial diameter, which explained 21% of the variance in response to the treatments (Table 4). Therefore, the subjects with smaller diameters at baseline showed the greatest increase in FMD 4 h after the meal. The postprandial change in LpB:C explained an additional 13% of the variance in FMD response to the fat loads. The postprandial increase in LpB:C adversely affected FMD response; for each 1-mg/dL increase in LpB:C, FMD would be expected to decrease by 0.3% (% FMD units). The final parameter to enter the model was the postprandial change in total apo B, which explained an additional 8% of the variance. Overall, this 3-predictor model for the change in FMD had a cumulative R² of 0.42 (P < 0.0001).

Within-subject correlations support the results of the regression analysis. The postprandial changes in LpB:C were inversely associated with postprandial changes in FMD (Figure 4). Therefore, the subjects with the smallest postprandial increases in LpB:C showed the largest improvements in endothelium-dependent vasodilation.

DISCUSSION

The present study is the first study to evaluate the acute effects of different dietary fatty acid blends on concentrations of apo
B–containing lipoprotein subclasses in humans. These results show that all treatments had similar effects on total apo B but different effects on individual apo B–containing particles in adults with type 2 diabetes. We found that a meal containing 2.8 g EPA + 1.2 g DHA attenuated the postprandial rise in LpB:C seen with the control treatment. Furthermore, the postprandial change in LpB:C was shown to be a significant predictor of the change in FMD after the meal. The subjects with the largest postprandial increases in LpB:C exhibited the smallest improvements in endothelium-dependent vasodilation. This finding has provided additional evidence for the potentially marked atherogenic character of the LpB:C subclass. According to density-defined lipoproteins, cholesterol-rich LDL are generally considered to have the highest atherogenic capacity, followed, in decreasing order of atherogenicity, by intact or partially delipidized triacylglycerol-rich LDL, small VLDL, and large VLDL. Although cholesterol-rich LpB and LpB:E subclasses occur mainly, but not exclusively, in LDL density ranges and triacylglycerol-rich LpB:C, LpB:C:E, and LpA-II:B:C:D:E in VLDL and IDL density ranges, these 2 groups of lipoprotein subclasses overlap to varying degrees in most, if not all, segments of the density gradient (Figure 1).

Major lipoprotein density classes are mixtures of individual apo B–containing lipoprotein subclasses, and their atherogenicity cannot be directly compared with that of individual apolipoprotein-defined subclasses. It has been shown in several clinical trials (1–7) that intact or partially delipidized triacylglycerol-rich lipoproteins, measured as a sum of LpB:C, LpB:C:E, and LpA-II:B:C:D:E or as individual subclasses, may have an atherogenic capacity similar, if not greater, to that of cholesterol-rich LpB and LpB:E. In an ancillary project within the Cholesterol and Recurrent Events trial, Sacks et al (5, 25) showed that, on the basis of equimolar apo B contents, the LpB:C subclass occurring in IDL and LDL had several-fold greater predictive power for recurring coronary events than did the LpB subclass. Results of an ancillary study within the HDL-Atherosclerosis Treatment Study (26) showed that LpB:C particles were significantly associated with the progression of proximal stenosis in subjects with type 2 diabetes or impaired glucose tolerance and that increased concentrations of LpB:C may serve as a marker, or as a mediator, for the accelerated atherosclerosis of diabetes (27). Additional evidence for the atherogenic potential of LpB:C subclass has been provided by results of another study showing that the apo C-III content of apo B–containing lipoproteins is crucial for their binding to vascular proteoglycan biglycan, a process considered to be a critical step in pathogenesis of atherosclerosis (28). Finally, there are several studies showing that apo C-III and especially apo C-III bound to apo B–containing lipoproteins are strongly associated with coronary heart disease (29–33). The finding that LpB:C response is closely tied to vascular endothelial response suggests a potential mechanism for previous reports of the atherogenic effects of LpB:C.

Distinguishing between discrete apolipoprotein-defined lipoprotein subclasses provides a new dimension in characterizing plasma lipoproteins and provides a basis to evaluate their effects.
on atherosclerosis initiation and progression. A better understanding of the lipid transport system is important for developing interventions that favorably affect atherogenic lipoprotein subclasses. Although the contribution of specific apo B–containing lipoprotein (i.e., LpB:C) to the risk of CVD has yet to be determined, these preliminary findings suggest that changing the fatty acid profile of a meal has significant acute effects on specific apo B–containing lipoprotein metabolism, similar to the effects observed with cholesterol-lowering drugs (1, 2). Because of the extent of time spent in the postabsorptive state, changes that occur in apo B–containing lipoprotein (i.e., LpB:C) in the postprandial period are important. Further studies will be necessary to identify the diet that is most effective postprandially, as well as chronically, with respect to CVD risk reduction. Although much is known about the effects of the habitual diet on CVD risk, the present study showed that there are opportunities to target the postprandial period to further reduce heart disease, especially in persons with diabetes.

We thank our sonographer Paul Wagner for his services and expertise in measuring FMD. KFH was responsible for the statistical analysis, the interpretation of results, and the writing of the manuscript. SGW, PMK-E, and KDH were responsible for the original concept of the study, the study design, and development of dietary challenges. KDH oversaw and carried out the recruiting and data collection, and she scored vascular ultrasounds. PA developed the methodology for measuring apo B–containing lipoprotein subclasses and NMS conducted the assays. Planning, interpreting and analyzing the results, and writing the manuscript were done under general supervision of SGW, PMK-E, and PA, each of whom contributed to the writing. None of the authors had any conflicts of interest.

REFERENCES


TABLE 4

Stepwise regression models relating apolipoprotein B (apo B–)–containing lipoprotein to flow-mediated dilation (FMD)

<table>
<thead>
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<th>Dependent variable</th>
<th>Predictor variable</th>
<th>Parameter estimate</th>
<th>Cumulative $R^2$</th>
<th>$P$ of step</th>
<th>$P$ of equation</th>
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<td>0.0014</td>
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<td>Step 3</td>
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<td>0.0202</td>
<td>0.0001</td>
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</table>

*Stepwise regression was performed with SAS STEPWISE procedure. The parameter estimates are included in the order of the predictor variables entered into the model. Age and treatment were not significant predictors and did not enter the model. LpB:C, lipoprotein B and C.*


