Long-term effect of soluble-fiber foods on postprandial fat metabolism in dyslipidemic subjects with apo E3 and apo E4 genotypes\textsuperscript{1-3}

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ABSTRACT To determine the long-term effect of soluble fiber on postprandial fat metabolism, we studied 33 dyslipidemic subjects, 16 with apolipoprotein (apo) E3/3 (E3) and 17 with E3/4 or E4/4 (E4) genotypes. They ate preweighed low-fat (20% of energy), high-fiber (> 5.7 g/MJ) diets for two 4-mo periods separated by a 2-mo washout period according to a randomized, crossover design. One diet contained foods rich in insoluble fiber and the other foods rich in soluble fiber. On 1 d during the last 2 wk of each diet, subjects ingested a standard, fiber-free, fatty liquid meal containing retinyl palmitate as a marker of intestinally derived lipoproteins. Plasma samples were obtained at hourly intervals for 10 h. Compared with the insoluble-fiber diet, soluble fiber reduced fasting plasma total cholesterol in both E3 (6.6 ± 2.1%, \(P = 0.007\)) and E4 subjects (5.6 ± 2.1%, \(P = 0.017\)). Soluble fiber increased fecal total bile acid output in both E3 (76 ± 18%, \(P < 0.001\)) and E4 subjects (85 ± 19%, \(P < 0.001\)). The incremental area under the chylomycin triacylglycerol response curve was significantly greater after soluble fiber than after insoluble fiber in E3 (3.56 ± 0.56 compared with 2.87 ± 0.38 mmol·h/L, respectively, \(P = 0.046\)) but not in E4 subjects (5.19 ± 0.78 compared with 4.92 ± 0.81 mmol·h/L). Kinetic analysis suggested an increase in retinyl palmitate absorption in E3 subjects after soluble fiber, but no difference in E4 subjects. These results suggest that a long-term increase in dietary soluble fiber has no effect on postprandial fat metabolism in subjects with an apo E3/4 or E4/4 genotype. However, soluble fiber enhances apparent fat absorption in E3 subjects, which could be due to an increased bile acid pool and increased micelle formation. Am J Clin Nutr 1997; 66:584–90.

INTRODUCTION

Purified soluble fibers such as guar (1), pectin (2), and psyllium (3, 4) and foods rich in soluble fiber such as legumes (5), oats (6), and barley (7) have been shown to reduce serum cholesterol in humans. The mechanism for the cholesterol-lowering effect of fiber is not clear, but at least four different mechanisms have been suggested: \(I\) binding of bile acids in the small intestine (8) leading to increased fecal bile acid excretion and increased primary bile acid synthesis (9, 10) and an increase in the bile acid pool (11), \(2\) reduced fat and cholesterol absorption (12, 13), \(3\) a reduced rate of carbohydrate absorption leading to lower serum insulin concentrations and hence less stimulus to cholesterol and lipoprotein synthesis (14), and \(4\) inhibition of cholesterol synthesis by short-chain fatty acids generated during the colonic fermentation of soluble fiber (15). In addition, it has been suggested that fiber may reduce serum cholesterol simply by displacing fat from the diet (16).

To address the issue of whether soluble fiber has an additional effect on serum cholesterol when the amount of dietary fat is low and to study the possible mechanisms by which soluble fiber lowers serum cholesterol, we conducted a long-term dietary trial in which moderately dyslipidemic subjects were given metabolically controlled diets for two 4-mo periods separated by a 2-mo washout period according to a randomized, crossover design. The main results of the study on fasting serum lipids and lipoproteins and fecal bile acid excretion were reported elsewhere (17). The main purpose of this paper was to report, in subjects with apolipoprotein (apo) E3/3 (E3) and E3/4 and E4/4 (E4) genotypes, the results of fat-tolerance tests done as part of this study to examine the long-term effect of soluble fiber on apparent fat absorption and postprandial triacylglycerol-rich lipoprotein metabolism.

SUBJECTS AND METHODS

Subjects

We studied 43 healthy subjects (15 men and 28 postmenopausal women) with a mean (± SEM) age of 58 ± 3 y (107 ± 4% of ideal body weight) who had previously been found to have mild to severe hyperlipidemia. After following a National Cholesterol Education Program (NCEP) Step 2 diet (18) for

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  \item \textsuperscript{2} Supported by grants from the Heart, Lung and Blood Institute, National Institutes of Health (RO1 HL 39689); Kellogg Canada Inc; and Loblaw Companies Limited.
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  \item Accepted for publication April 19, 1997.
\end{itemize}

Methods

The methods are reported in detail elsewhere (17). The subjects were randomly assigned to two metabolically controlled experimental diets, each for 4 mo, separated by a 2-mo return to an ad libitum NCEP Step 2 diet (total fat < 30% of energy, saturated fat < 7% of energy, and cholesterol < 200 mg/d). One of the experimental diets was high in soluble fiber and the other high in insoluble fiber. Two-week repeating menus were planned to suit individual tastes. The two metabolic diets shared a common core, to which foods high in soluble (barley, dried lentils, peas and beans, oat bran, and psyllium-enriched breakfast cereal) or insoluble (wheat-bran breakfast cereal, high-fiber crackers, and high-fiber bread) fiber were added. The goal of providing ≤ 20% of energy as fat, 20% as protein, ≥ 60% as available carbohydrate, and < 50 mg cholesterol/d were achieved (17). Average dietary fiber intakes with the soluble- and insoluble-fiber diets, respectively, were as follows: total fiber, 5.9 ± 0.1 and 7.1 ± 0.1 g/MJ; soluble fiber, 1.9 ± 0.02 and 1.2 ± 0.05 g/MJ; and insoluble fiber, 4.0 ± 0.07 and 5.8 ± 0.1 g/MJ.

After 15 wk of each diet, the subjects came to the clinical nutrition center after a 12-h overnight fast for a fat-tolerance test, which lasted for 10 h. After a fasting blood sample was obtained, subjects were given a “milkshake” containing (per kg body wt) 5 mL water, 0.5 g corn oil, 1.38 g skimmed-milk powder (containing 0.5 g protein), and 0.5 g glucose (37% of energy as fat, 17% as protein, and 46% as carbohydrate) to which a total of 50 000 IU retinyl palmitate (Hoffmann-La-Roche Ltd, Etobicoke, Canada) was added. Additional blood samples were obtained at hourly intervals for 10 h for retinyl palmitate and lipid measurements. Thirty-six of the 43 subjects underwent the fat-tolerance test (5 subjects refused the test and for 2 subjects there was a lack of venous access). Apo E genotype was determined after the study had been completed (19). Of the 36 subjects completing the fat-tolerance test, 3 were heterozygous for the apo E2 genotype and their results are not reported. We report the results of the remaining 33 subjects, 17 E4 subjects and 16 who were homozygous for the apo E3/3 genotype (E3).

At the end of week 15 of both diet periods, 3-d fecal samples were collected on an outpatient basis. Fecal samples were passed into plastic bags, placed on frozen carbon dioxide in a polystyrene container, and returned by courier to the laboratory, where the samples were weighed and stored at −20 °C before freeze-drying. One subject whose results are reported here did not collect his feces.

Blood samples were drawn into 7-mL EDTA-containing Vacutainer tubes (Becton Dickinson, Rutherford, NJ) and protected from light during transport, processing, and retinyl palmitate analysis by wrapping or covering the tubes with aluminum foil and handling of plasma in a dimly lit room. Plasma was removed from cells by centrifugation at 600 × g for 10 min. The chylomicron fraction (S_{t} > 400) was separated by centrifugation at 26 000 × g for 30 min at 5 °C (rotor 50.3; Beckman, Palo Alto, CA). The fraction with S_{t} > 400 and a density < 1.006 kg/L (termed chylomicron remnants) and the fraction with a density > 1.006 kg/L were separated by ultracentrifugal flotation (20). Cholesterol and triacylglycerol were measured in whole plasma and lipoprotein fractions at the J Alick Little Lipid Laboratory, St Michael’s Hospital, University of Toronto, which is certified by the National Heart, Lung and Blood Institute–Centers for Disease Control Lipid Standardization Program (20). The Technicon RA1000 and Technicon enzymatic reagents were used to determine cholesterol (method SM4–0139G86) and triacylglycerols (method SM4–0173G90 with triacylglycerol blank reagent no. T01–2013-01) (Technicon-Miles, Mississauga, Canada) (21). Retinyl palmitate in whole plasma and retinyl palmitate in the lipoprotein fractions (chylomicrons and chylomicron remnants) were measured in duplicate by HPLC (22). The mean (± SD) recoveries of retinyl palmitate added to whole plasma at concentrations of 53, 263, 1050, and 5256 nmol/L were 97 ± 18%, 109 ± 17%, 101 ± 9%, and 104 ± 12%, respectively. The between-run CVs for repeated analyses of pooled plasma containing 101, 222, and 3216 nmol retinyl palmitate/L were 18%, 6.3%, and 5.1%, respectively. The limit of detection was 20 nmol/L.

Fecal acidic and neutral sterols were measured in finely ground freeze-dried feces from the 3-d collections after extraction, thin-layer chromatography, methylation, and trimethylsilylation of bile acids and neutral sterols followed by gas-liquid chromatography with a DB-1 column (J&W Scientific, Folsom, CA), with 5β-cholic acid and 5α-cholestanol, respectively, as internal standards (23).

Results are expressed as means ± SEMs. The net incremental areas under the curves of cholesterol, triacylglycerol, and retinyl palmitate in the various plasma fractions were calculated geometrically (24). Kinetic analysis of retinyl palmitate was performed with the DOS (386/486) version of CONSAM (Resource Facility for Kinetic Analysis, Seattle) by using the kinetic model of Cortner et al (25) modified by Le (personal communication, 1995) (Figure 1). First, all the parameters for the model for each subject were determined in a preliminary analysis by using the average of the retinyl palmitate values for the soluble- and insoluble-fiber diets. Then, for each subject, random file names were assigned to two decks containing the retinyl palmitate results for the soluble- and insoluble-fiber diets and the parameters obtained from the preliminary analysis. With the program operator blinded to treatment, kinetic analysis of the retinyl palmitate concentrations after each test diet was done by changing the absorption parameters [P_{i}(31), P(1), P(12), L(3, 2), P(2), L(13, 12), and P(15)] and clearance parameters [P_{65}, P(6), P(60), and P(7)] first, with other parameters being altered only if good fits were not obtained.
The significance of differences within the E3 and E4 groups was assessed by paired t test. The criterion for significance was taken to be \( P < 0.05 \) (two-tailed).

**RESULTS**

After 15 wk, fasting plasma total cholesterol was significantly lower with the soluble-fiber diet than with the insoluble-fiber diet in E3 subjects (by \( -6.6 \pm 2.1\% \), \( P = 0.007 \) and E4 subjects (by \( -5.6 \pm 2.1\% \), \( P = 0.017 \) (Table 1). The difference in fasting plasma cholesterol with the soluble- and insoluble-fiber diets was maintained throughout the day in both E3 and E4 subjects (Figure 2). There was no significant effect of the soluble-fiber diet compared with the insoluble-fiber diet on the area under the chylomicron cholesterol curve (Figure 3).

Fasting (Table 1) and postprandial (Figure 2) plasma triacylglycerol concentrations with the soluble-fiber diet did not differ significantly from those with the insoluble-fiber diets in either group of subjects. However, the incremental area under the chylomicron triacylglycerol curve in E3 subjects was significantly greater with the soluble-fiber diet than with the insoluble-fiber diet (3.56 \( \pm \) 0.56 and 2.87 \( \pm \) 0.38 mol \( \cdot \) h/L, \( P = 0.046 \)), but there was no effect in E4 subjects (Figure 3 and Figure 4).

There was no significant effect of diet on any of the kinetic parameters, except for \( P(1) \) and \( P(2) \), representing the rates of fat absorption and chylomicron synthesis, respectively. In E3 subjects, \( P(1) \) and \( P(2) \) were higher after the soluble- than after the insoluble-fiber diet \( [P(1): 3.43 \pm 0.52 \) and 2.65 \( \pm \) 0.52, \( P = 0.029 \); \( P(2): 2.98 \pm 0.43 \) and 2.23 \( \pm \) 0.36, \( P = 0.036 \) but the effect was not significant in E4 subjects \( [P(1): 2.71 \pm 0.43 \) and 2.77 \( \pm \) 0.28; \( P(2): 2.71 \pm 0.43 \) and 2.77 \( \pm \) 0.28].

In E3 subjects there was a significant relation between the value of parameter \( P(1) \) and the incremental area under the chylomicron triacylglycerol curve (\( r = 0.379, P = 0.032 \); however, this relation was not significant in E4 subjects (Figure 4).

**TABLE 1**

<table>
<thead>
<tr>
<th></th>
<th>Apo E3 ( n = 16 )</th>
<th>Apo E4 ( n = 17 )</th>
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<tbody>
<tr>
<td></td>
<td>Insoluble-fiber diet</td>
<td>Soluble-fiber diet</td>
</tr>
<tr>
<td></td>
<td>mmol/L</td>
<td>mmol/L</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>6.07 ( \pm ) 0.22</td>
<td>5.70 ( \pm ) 0.28( ^2 )</td>
</tr>
<tr>
<td>Triacylglycerol</td>
<td>1.68 ( \pm ) 0.10</td>
<td>1.65 ( \pm ) 0.15</td>
</tr>
</tbody>
</table>

\( ^1 \bar{x} \pm \) SEM.

\( ^2, ^4 \) Significantly different from insoluble-fiber diet: \( ^2 P = 0.009, ^4 P = 0.012 \).
Fecal TBA excretion was significantly related to the area under the chylomicron triacylglycerol response in E3 (r = 0.550, P = 0.001) but not in E4 subjects (Figure 7). The change in TBA excretion was not related to changes in chylomicron triacylglycerol responses and there was no significant relation between fecal TBA excretion and any of the parameters in the kinetic model.

**DISCUSSION**

The results show that the effects of a soluble-fiber diet in reducing fasting plasma lipids and increasing fecal bile acid excretion were, in general, similar in E3 and E4 subjects. The diet high in soluble fiber resulted in a small increase in postprandial chylomicron triacylglycerol responses after a fiber-free fatty meal, but this was only significant in E3 subjects. Results of the kinetic analysis of retinyl palmitate concentrations in lipoprotein fractions after ultracentrifugation were consistent with the increased chylomicron triacylglycerol responses in suggesting, only in E3 subjects, that there was an increase in the rate of fat absorption, the rate of chylomicron synthesis, or both.
These studies were done to explore one mechanism by which soluble fiber has been suggested to reduce serum cholesterol concentrations, namely, altering the absorption of fat or the postprandial metabolism of triacylglycerol-rich lipoproteins. The acute effect of dietary fiber on postprandial fat absorption of triacylglycerols has been studied in animals (26–28) and humans (29–31). However, in the present study we looked at the chronic effect of diets high in soluble and insoluble fiber on the metabolism of a fiber-free fatty test meal. Thus, any effect of soluble fiber on fat absorption would be due to a chronic effect of soluble fiber on the structure or function of the gastrointestinal tract. Previous studies suggest that soluble fibers interfere with fat absorption by binding bile acids (8), reducing intraluminal diffusion, and reducing the activity of pancreatic lipase (32, 33). In an attempt to maintain homeostasis with long-term, soluble-fiber intakes, the organism responds by increasing the size of the absorptive surface of the small intestine (34) and increasing bile acid and lipase secretion (35). These effects would be expected to result in an increase in the ability of the organism to absorb a fiber-free fat load.

Apo E is located on the surface of chylomicron remnant particles and is recognized by LDL and chylomicron remnant receptors in the liver. The apo E2 and E4 isoforms are considered to be variant, with E3 being normal. Because the frequency of the apo E2 genotype in the population is only ≈10%, we were unable to find a sufficient number of subjects with an apo E2 genotype for this study. An apo E4 genotype is considered to enhance remnant clearance because of increased affinity for remnant receptors. However, studies that have compared postprandial triacylglycerol responses between subjects with apo E3 and E4 genotypes have produced conflicting results. Weintraub et al (36) found that 10 apo E3 subjects and 8 apo E4 subjects had similar chylomicron retinyl palmitate responses, but that the apo E4 subjects had significantly lower nonchylomicron retinyl palmitate responses than the apo E3 subjects. Brown and Roberts (37) found that 5 apo E4 subjects had lower incremental total plasma triacylglycerol responses after a fatty test meal than did 14 apo E3 subjects. Nevertheless, the apo E4 subjects had significantly higher chylomicron and nonchylomicron retinyl palmitate responses than the E3 subjects. These studies differ from ours in that their subjects had normal plasma cholesterol and triacylglycerol concentrations, whereas our subjects had abnormal blood lipid concentrations. Therefore, we did not necessarily expect to find the same results as others, and, indeed, our results did differ in that the E4 subjects appeared to have greater plasma chylomicron and remnant triacylglycerol and retinyl palmitate responses to the fatty test meal than did E3 subjects.

The APOE*4 allele has been suggested to be associated with high cholesterol absorption and low synthesis of bile acids and cholesterol (38, 39). However, when switched to a low-fat diet,
TABLE 2
Fecal bile acid and neutral sterol excretion in subjects with apo E3 or apo E4 genotypes

<table>
<thead>
<tr>
<th></th>
<th>Insoluble-fiber diet</th>
<th>Soluble-fiber diet</th>
<th>Apo E3 (n = 16)</th>
<th>Apo E4 (n = 16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fecal bile acids</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lithocholic acid</td>
<td>74 ± 13</td>
<td>104 ± 17</td>
<td>55 ± 8</td>
<td>79 ± 13</td>
</tr>
<tr>
<td>Deoxycholic acid</td>
<td>85 ± 20</td>
<td>136 ± 27</td>
<td>58 ± 22</td>
<td>121 ± 22</td>
</tr>
<tr>
<td>Chenodeoxycholic acid</td>
<td>5 ± 1</td>
<td>8 ± 3</td>
<td>3 ± 1</td>
<td>7 ± 2</td>
</tr>
<tr>
<td>Cholic acid</td>
<td>7 ± 2</td>
<td>13 ± 4</td>
<td>5 ± 1</td>
<td>8 ± 3</td>
</tr>
<tr>
<td>Ursodeoxycholic acid</td>
<td>4 ± 1</td>
<td>5 ± 1</td>
<td>4 ± 1</td>
<td>5 ± 1</td>
</tr>
<tr>
<td>Total bile acids</td>
<td>175 ± 34</td>
<td>266 ± 49</td>
<td>126 ± 18</td>
<td>220 ± 37</td>
</tr>
<tr>
<td>Neutral sterols</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coprostanol</td>
<td>312 ± 57</td>
<td>292 ± 48</td>
<td>257 ± 36</td>
<td>262 ± 46</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>117 ± 18</td>
<td>97 ± 16</td>
<td>94 ± 19</td>
<td>151 ± 42</td>
</tr>
<tr>
<td>Coprostanone</td>
<td>49 ± 13</td>
<td>35 ± 9</td>
<td>48 ± 11</td>
<td>47 ± 14</td>
</tr>
<tr>
<td>Total Neutral Sterols</td>
<td>478 ± 67</td>
<td>425 ± 51</td>
<td>399 ± 43</td>
<td>459 ± 65</td>
</tr>
</tbody>
</table>

1 x ± SEM.  
2,3 Significantly different from insoluble-fiber diet; 2 P < 0.01, 3 P < 0.05.

apo E4 subjects tend to have a greater decrease in cholesterol absorption and a greater increase in LDL fractional catabolic rate than apo E3 subjects (39). Reduced cholesterol absorption may explain why apo E4 subjects were found previously to have a greater cholesterol-lowering response to a low-fat diet than were apo E3 subjects (40). In the present study all subjects were on a very-low-fat diet, and E3 and E4 subjects had a similar decrease in serum cholesterol in response to the soluble-fiber diet.

Soluble fiber may have increased chylomicron triacylglycerol responses in E3 subjects because of increased fat absorption secondary, at least in part, to an increased intestinal bile acid pool (11). If this were the case, it would be expected that soluble fiber would increase fecal bile acids in E3 but not in E4 subjects because soluble fiber had no effect on chylomicron triacylglycerol responses in E4 subjects. However, soluble fiber increased fecal bile acid excretion significantly in both E3 and E4 subjects. Why was increased bile acid excretion not associated with increased fat absorption or chylomicron triacylglycerol responses in E4 subjects? One possibility is that increased bile acid excretion is not associated with an increased bile acid pool or increased fat absorption in apo E4 subjects. Another possibility is that the rate of fat absorption does not influence the magnitude of postprandial chylomicron triacylglycerol responses in apo E4 subjects. Both of these suggestions are supported by our data, which show that fecal bile acid excretion was positively related to the area under the chylomicron triacylglycerol curve in E3 but not in E4 subjects. In addition, the correlation between the values of the absorption parameter P(1) and the incremental area under the chylomicron triacylglycerol curve in E3 subjects was positive and significant, but negative and nonsignificant in E4 subjects. Thus, the different effects of soluble fiber on postprandial fat metabolism in subjects with apo E3 and apo E4 genotypes may be due to differences in fat absorption or metabolism caused by differences related to the APOE allele other than altered bile acid metabolism.

It is concluded that a long-term increase in dietary soluble fiber has no effect on postprandial fat metabolism in subjects with an apo E4 genotype. However, soluble fiber enhances apparent fat absorption in subjects with an apo E3 genotype, which could be due to an increased bile acid pool and increased micelle formation.

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