Postprandial lipemia in subjects with the threonine 54 variant of the fatty acid–binding protein 2 gene is dependent on the type of fat ingested\textsuperscript{1–4}

Paula DN Dworatzek, Robert A Hegele, and Thomas MS Wolever

ABSTRACT

Background: The alanine-for-threonine substitution at codon 54 (A54T polymorphism) in the fatty acid–binding protein 2 gene (FABP2) has been associated with hypertriglyceridemia and insulin resistance. Obese and diabetic T54 carriers have greater postprandial lipemia than do A54 homozygotes. The T54 protein isoform is also associated with increased triacylglycerol secretion in vitro.

Objective: We investigated diet-gene interactions by measuring postprandial lipids, glucose, insulin, and C-peptide in healthy, nonobese A54 homozygotes and T54 carriers after ingestion of 3 different fats.

Design: Eleven A54 homozygotes and 11 T54 carriers were given 3 oral-fat-tolerance tests (butter, safflower oil, and olive oil). Cholesterol and triacylglycerol were measured in plasma and in chylomicron fractions.

Results: There was no main effect of FABP2 genotype for chylomicron triacylglycerol, glucose, or C-peptide. The area under the insulin curve and the ratio of insulin to C-peptide were lower in T54 carriers than in A54 homozygotes [312 ± 29 (\(\bar{x} \pm \text{SEM}\)) compared with 425 ± 31 mmol · h/L (P = 0.05) and 0.23 ± 0.03 compared with 0.40 ± 0.05 (P = 0.04), respectively], which suggests greater hepatic insulin clearance in T54 carriers. An association between genotype and chylomicron cholesterol was seen only after olive oil: values were higher (P = 0.02) in T54 carriers (0.087 ± 0.006 mmol · h/L) than in A54 homozygotes (0.058 ± 0.004 mmol · h/L). The main effect of fat was significant for the areas under the chylomicron cholesterol and chylomicron triacylglycerol curves [higher values for safflower (0.635 ± 0.053 and 2.48 ± 0.30 mmol · h/L, respectively) and olive (0.592 ± 0.052 and 2.48 ± 0.32 mmol · h/L, respectively) oils than for butter (0.425 ± 0.043 and 1.69 ± 0.20 mmol · h/L, respectively); P < 0.05].

Conclusions: The A54T polymorphism results in a diet-gene interaction: the T54 group had increased chylomicron cholesterol after olive oil only. Nevertheless, the greater hepatic insulin clearance in T54 carriers suggests that the polymorphism may not be deleterious in nonobese subjects. Am J Clin Nutr 2004;79:1110–7.

KEY WORDS Fatty acid–binding protein 2 gene, intestinal fatty acid–binding protein, postprandial lipids, dietary fat, chylomicrons, glucose, insulin, C-peptide

INTRODUCTION

The fatty acid–binding protein 2 gene (FABP2) encodes intestinal fatty acid–binding protein (I-FABP), which is found in enterocytes and is involved in the intracellular transport of long-chain fatty acids via a collisional mechanism (1). A common alanine for threonine substitution at FABP2 codon 54 (the A54T polymorphism) has been associated with hypertriglyceridemia, increased body mass index (BMI; in kg/m\(^2\)), hyperinsulinemia, and insulin resistance (2–4). The T54 variant has been shown to have a two-fold greater binding affinity to the long-chain fatty acids oleate and arachidonate than does the A54 isoform (3). The T54 variant is associated with increased transport and secretion of triacylglycerol in in vitro enterocyte and organ explant models (5, 6), and in humans, the T54 allele is associated with increased postprandial lipemia in obese (7) and diabetic (8) subjects. The T54 variant appears to affect the entry of long-chain fatty acids into the interior of the clam-shaped protein (9, 10). Taken together, the above results support the hypothesis that the T54 variant results in greater binding to long-chain fatty acids, increased transport and secretion of triacylglycerol in chylomicrons, and concomitant increases in percentage of body fat, hypertriglyceridemia (2), and insulin resistance (3, 4). However, not all studies have supported the associations with postprandial lipemia (11), hyperinsulinemia, and insulin resistance (12, 13). We hypothesized that if the sole mechanism for the phenotype associations with T54 was increased binding and trafficking of fatty acids, then we should see increased postprandial lipemia in healthy, nonobese subjects. However, we suspected that the association might depend on the type of fat ingested, because olive oil results in higher postprandial lipemia than does butter (14, 15) in genetically heterogeneous populations. In addition, A54-containing I-FABP has increased binding affinity for fatty acids with increasing chain length and saturation
TABLE 1
Characteristics of the subjects at screening

<table>
<thead>
<tr>
<th>FABP2 codon 54 genotype</th>
<th>T54 (n = 6 F, 5 M)</th>
<th>A54 (n = 6 F, 5 M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>33.2 ± 3.2</td>
<td>27.3 ± 2.4</td>
</tr>
<tr>
<td>BMI (kg/m^2)</td>
<td>24.4 ± 1.2</td>
<td>23.6 ± 1.0</td>
</tr>
<tr>
<td>APOE genotype</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E3/3</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>E2/2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>E2/4</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>E3/4 or E4/4</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>4.51 ± 0.30</td>
<td>4.02 ± 0.10</td>
</tr>
<tr>
<td>Triacylglycerol (mmol/L)</td>
<td>1.12 ± 0.16</td>
<td>1.15 ± 0.08</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>2.65 ± 0.28</td>
<td>2.14 ± 0.13</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.35 ± 0.08</td>
<td>1.36 ± 0.11</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>4.45 ± 0.12</td>
<td>4.56 ± 0.16</td>
</tr>
<tr>
<td>Glycated hemoglobin (%)</td>
<td>5.2 ± 0.1</td>
<td>5.3 ± 0.1</td>
</tr>
<tr>
<td>Insulin (pmol/L)</td>
<td>40.8 ± 4.8</td>
<td>48.8 ± 5.8</td>
</tr>
<tr>
<td>Free fatty acids (mmol/L)</td>
<td>0.357 ± 0.055</td>
<td>0.465 ± 0.080</td>
</tr>
</tbody>
</table>

1 The subjects had fasted for ≥12 h when the measurements were taken. FABP2, fatty acid–binding protein 2 gene; T54, heterozygous or homozygous for threonine at FABP2 codon 54; A54, homozygous for alanine at FABP2 codon 54; APOE, apolipoprotein E. There were no significant differences between the groups (t test).

SUBJECTS AND METHODS

Subjects

Fifty-two healthy adults (24 men and 28 women) who had no history of endocrine or gastrointestinal disorders and who had a self-reported BMI < 30 had their FABP2 genotype determined (17). Subjects were excluded from the study if they had a fasting glucose concentration > 5.6 mmol/L, a glycated hemoglobin concentration > 5.9%, a total cholesterol or triacylglycerol concentration above the 95th percentile for age and sex (18), or any abnormal biochemistry results. Eleven subjects carrying the T54 allele (9 heterozygotes and 2 homozygotes), hereafter called the T54 group, and eleven homozygous A54 subjects, hereafter called the A54 group, participated in the study. The A54 group was selected so that their mean plasma triacylglycerol concentrations and apolipoprotein E (APOE) genotype distribution did not differ significantly from those of the T54 group (Table 1), because fasting triacylglycerol concentrations are strongly correlated with the magnitude of postprandial triacylglycerol response (19), and APOE genotype has been shown to affect chylomicron clearance (20). All subjects gave written informed consent, and the study was approved by the Ethics Committee of the University of Toronto.

Experimental design

In a single-blind, randomized crossover design, each subject underwent three 8-h oral-fat-tolerance tests featuring safflower oil, olive oil, or butter (Table 2). The subjects were asked not to participate in any strenuous physical activity for 24 h before the study days and were provided with a standardized low-fat meal on the evening before the study. The subjects arrived at the Clinical Nutrition and Risk Factor Modification Centre at St Michael’s Hospital, Toronto, after a 12-h overnight fast. The oral fat load was prepared as a milk shake that contained the following (per kg body wt): 0.5 g fat, 0.6 g glucose, 1.14 g skim milk powder (providing 0.4 g protein and 0.6 g carbohydrate), 7 mL water, and 0.14 mL vanilla. The milk shake provided 11 kcal/kg with 41% of energy from fat, 15% from protein, and 44% from carbohydrate. The subjects had their heights and weights measured on the first visit. The subjects subsequently had a forearm vein cannulated for collection of a fasting blood sample (at ≈0800), after which they had 15 min to consume the oral fat drink. Blood was drawn every 30 min for the first 2 h and then hourly until 8 h after consumption of the oral fat drink. The subjects were required to stay within the study area throughout the sample collection, 250 mL water was provided at 4 h, and a meal was provided at the end of the study period. All the subjects completed the 3 oral-fat-tolerance tests.

Laboratory methods

Whole blood was centrifuged at 600 × g for 10 min at 4 °C. Established procedures were used to extract leukocyte DNA and determine the genotypes of FABP2 codon 54 (3) and APOE (21), with known genotypic controls run as standards for each electrophoresis. The chylomicron fraction (S, > 400; density < 1.006 kg/L) was separated by salt gradient ultracentrifugation at 26 000 × g for 30 min at 22 °C (50.3 Ti rotor; Beckman, Palo Alto, CA) (22). Cholesterol and triacylglycerol were determined in whole plasma and lipoprotein fractions at the J Alick Little Lipid Laboratory, St Michael’s Hospital, Toronto, which is certified by the National Heart, Lung, and Blood Institute–Centers for Disease Control and Prevention Lipid Standardization Program (Lipid Research Clinics Program, 1982). The Technicon RA 1000 and Technicon enzymatic reagents were used to determine total cholesterol (Technicon method SM4-0139G86) and triacylglycerol

TABLE 2
Fatty acid composition of the 3 fats used for separate oral-fat-tolerance tests

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Safflower oil</th>
<th>Olive oil</th>
<th>Butter</th>
</tr>
</thead>
<tbody>
<tr>
<td>SFA</td>
<td>9.90</td>
<td>15.42</td>
<td>60.86</td>
</tr>
<tr>
<td>MUFA</td>
<td>17.00</td>
<td>68.81</td>
<td>21.92</td>
</tr>
<tr>
<td>PUFA</td>
<td>67.84</td>
<td>12.00</td>
<td>2.70</td>
</tr>
</tbody>
</table>

1 SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.
(Technicon method SM4-0173G90 with triacylglycerol blank reagent no. T01-2013-01) (Technicon-Miles, Mississauga, Canada) (23). Frozen plasma samples were analyzed for glucose (glucose oxidase method, Synchron CX7/CX3; Beckman Coulter, Mississauga, Canada), insulin (radioimmunoassay; Pharmacia Upjohn, Uppsala, Sweden), and C-peptide (radioimmunoassay; Diagnostic Products Corporation, Los Angeles).

Statistical analyses

SAS version 8.01 (SAS Institute Inc, Cary, NC) was used for all statistical comparisons. Univariate analyses were performed for all quantitative variables to assess normality. Logarithmic transformations were performed for plasma triacylglycerol, chyomicron triacylglycerol, and insulin, after which the data showed less skew and kurtosis and no considerable deviation from a normal distribution. Baseline differences (at screening) between the genotype groups were assessed by two-tailed t test. Repeated-measures analysis of variance (RMANOVA) with general linear modeling was conducted for 3 factors [genotype (2 groups: A54 and T54), fat, and time] and their interactions, with repeated measures on fat and time and with subject nested within genotype. When there was a significant interaction term, within-factor analyses were conducted, except for baseline (time = 0), which was assessed for genotype differences regardless of a significant interaction between genotype and time. The analyses of plasma cholesterol and triacylglycerol and chyomicron cholesterol and triacylglycerol were corrected for baseline values by using an analysis of covariance model. Analyses were also performed on summary measures, ie, area under the curve (AUC) calculated by using the trapezoid rule and peak-rise (peak minus baseline) for all variables except free fatty acids, for which peak minus nadir was used. For chyomicron cholesterol and triacylglycerol, the total area under the curve was used because fasting chyomicron concentrations are very close to zero; however, we corrected for baseline because even insignificant differences in fasted concentrations may be correlated with postprandial concentrations. One day profile (olive oil, A54 group) was removed from the analysis because the AUC was > 5 SDs from the mean for plasma and chyomicron cholesterol and triacylglycerol. For glucose, insulin, and C-peptide, the incremental area under the curve up to the time point at which the concentration was not significantly different from that at baseline was used (ie, 2, 3, and 5 h for glucose, insulin, and C-peptide, respectively). Data for 9 of the 66 C-peptide day profiles (and their corresponding insulin values) were omitted because samples were presumably spoiled (C-peptide was not detectable in the presence of a detectable insulin). Main effects that were found to be significant in the RMANOVA were analyzed for pairwise comparisons by using Tukey’s studentized range test to control for type I error. Results are considered significant at P ≤ 0.05 and are expressed as means ± SEMs.

RESULTS

Subjects

In total, 52 persons were screened to identify potential subjects with the T54 variant for further study. There were no significant differences in the screening variables (age, sex, BMI, cholesterol, triacylglycerol, HDL cholesterol, LDL cholesterol, glucose, glycated hemoglobin, insulin, and free fatty acids) between the T54 group (2 homozygotes and 19 heterozygotes) and the A54 group (31 homozygotes) as previously reported (17). The frequency of the FABP2 T54 allele in this sample was 0.22 (95% CI: 0.14, 0.30), and there was no deviation from the Hardy-Weinberg law. We purposely chose our 2 genotype groups of 11 subjects each to have no significant differences in plasma triacylglycerol or APOE genotype (Table 1), but there were also no significant differences in BMI, age, sex, plasma cholesterol, HDL cholesterol, LDL cholesterol, free fatty acids, glucose, glycated hemoglobin, or insulin between the 2 groups. The T54 group consisted of 9 heterozygotes and 2 homozygotes, whereas the wild-type group had 11 A54 homozygotes. The mean age and BMI of the 22 subjects were 30.2 ± 2.0 y and 24.0 ± 0.8, respectively.

Baseline values

The mean fasting concentrations of plasma cholesterol and triacylglycerol, chyomicron cholesterol and triacylglycerol, glucose, insulin, C-peptide, and free fatty acids in the T54 and A54 groups over the 3 oral-fat-tolerance tests are presented in Table 3. The only variable to differ significantly between the genotype groups was chyomicron cholesterol; the concentration in the T54 group was higher than that in the A54 group. However, the difference between the 2 groups in chyomicron triacylglycerol concentration was nearly significant (P = 0.068).

Cholesterol, triacylglycerol, and free fatty acids

The results of the RMANOVA (3-way with genotype, fat, and time) showed no significant main effect of genotype on plasma cholesterol or triacylglycerol or on chyomicron cholesterol or triacylglycerol (Table 4, Figure 1). There was a significant main effect of fat on plasma cholesterol and triacylglycerol (P = 0.03 for both); however, this effect did not remain significant after correction for baseline values (Table 4). There was no significant effect of fat on free fatty acids (data not shown). There was a significant main effect of fat on chyomicron cholesterol and chyomicron triacylglycerol: values obtained after ingestion of safflower and olive oils were higher than those obtained after ingestion of butter (Table 4, Figure 1). The fat-by-time interaction was also significant for chyomicron triacylglycerol (P <

TABLE 3

<table>
<thead>
<tr>
<th>FABP2 codon 54 genotype</th>
<th>T54</th>
<th>A54</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma cholesterol (mmol/L)</td>
<td>4.51 ± 0.14</td>
<td>4.03 ± 0.11</td>
</tr>
<tr>
<td>Plasma triacylglycerol (mmol/L)^2</td>
<td>1.32 ± 0.12</td>
<td>1.22 ± 0.09</td>
</tr>
<tr>
<td>Chyomicron cholesterol (mmol/L)^2</td>
<td>0.058 ± 0.007</td>
<td>0.039 ± 0.004</td>
</tr>
<tr>
<td>Chyomicron triacylglycerol (mmol/L)^2</td>
<td>0.110 ± 0.015</td>
<td>0.082 ± 0.013</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>5.24 ± 0.06</td>
<td>5.27 ± 0.09</td>
</tr>
<tr>
<td>Insulin (pmol/L)^2</td>
<td>36.8 ± 2.2</td>
<td>33.9 ± 2.0</td>
</tr>
<tr>
<td>C-peptide (pmol/L)</td>
<td>187 ± 21</td>
<td>131 ± 16</td>
</tr>
<tr>
<td>Free fatty acids (mmol/L)</td>
<td>0.401 ± 0.030</td>
<td>0.461 ± 0.026</td>
</tr>
</tbody>
</table>

All values are \( \bar{x} \pm \text{SEM} \). The subjects had fasted for \( \geq 12 \) h when the measurements were taken. FABP2, fatty acid–binding protein 2 gene; T54, heterozygous or homozygous for threonine at FABP2 codon 54; A54, homozygous for alanine at FABP2 codon 54. Log transformed for statistical analyses. Significantly different from A54, \( P = 0.02 \) (repeated-measures ANOVA).
Safflower oil | Olive oil | Butter | $P^{2}$ | FABP2 $\times$ fat
---|---|---|---|---
**Plasma (mmol/L)**
- **0-8 h Cholesterol** $^f$ | 4.03 ± 0.06 | 4.03 ± 0.06 | 4.03 ± 0.06 | NS | NS | NS
- **0-8 h Triacylglycerol** $^f$ | 1.56 ± 0.08 | 1.56 ± 0.08 | 1.56 ± 0.08 | NS | NS | NS
- **FFA, peak minus nadir** | 0.38 ± 0.025 | 0.38 ± 0.025 | 0.38 ± 0.025 | NS | NS | NS
**Chylomicron**
- **0-8 h Cholesterol (mmol/L)$^f$** | 0.077 ± 0.005 | 0.078 ± 0.005 | 0.078 ± 0.005 | NS | <0.0001 | 0.05
- **Cholesterol AUC (mmol $\cdot$ h/L)$^f$** | 0.628 ± 0.064 | 0.642 ± 0.089 | 0.642 ± 0.089 | NS | 0.0001 | 0.05
- **0-8 h Triacylglycerol (mmol/L)$^f$** | 0.282 ± 0.022 | 0.307 ± 0.030 | 0.307 ± 0.030 | NS | 0.02 | NS
- **Triacylglycerol AUC (mmol $\cdot$ h/L)$^f$** | 2.37 ± 0.29 | 2.59 ± 0.55 | 2.59 ± 0.55 | NS | 0.01 | NS
**Plasma**
- **0-8 h Glucose (mmol/L)** | 5.50 ± 0.10 | 5.69 ± 0.10 | 5.69 ± 0.10 | NS | 0.04 | NS
- **Glucose AUC (mmol $\cdot$ h/L)$^f$** | 1.92 ± 0.55 | 2.62 ± 0.42 | 2.62 ± 0.42 | NS | NS | NS
- **0-8 h Insulin (pmol/L)$^f$** | 87.3 ± 8.6 | 103.4 ± 9.8 | 103.4 ± 9.8 | NS | NS | NS
- **Insulin AUC (pmol $\cdot$ h/L)$^f$** | 293 ± 52 | 384 ± 37 | 384 ± 37 | NS | 0.05 | NS
- **0-8 h C-peptide (pmol/L)** | 463 ± 37 | 374 ± 31 | 374 ± 31 | NS | NS | NS
- **C-peptide peak time (h)** | 1.1 ± 0.2 | 1.0 ± 0.1 | 1.0 ± 0.1 | NS | 0.04 | NS

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$^f$ All values are $\bar{x} \pm$ SEM. $T54$, heterozygous or homozygous for threonine at FABP2 codon 54; $A54$, homozygous for alanine at FABP2 codon 54; FFA, free fatty acids; AUC, area under the curve.

$^f$ For $F$ value from repeated-measures ANOVA.

$^f$ $P$ values are baseline corrected.

$^f$ Log transformed for statistical analysis.

$^f$ Two-hour incremental AUC.

$^f$ Three-hour incremental AUC.

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0.0001). Analyses by time showed that chylomicron triacylglycerol concentrations after ingestion of safflower and olive oils were significantly higher than those after ingestion of butter at 5, 7, and 8 h (Figure 1). There was a significant interaction between genotype and fat for chylomicron cholesterol (Table 4), and analyses by fat showed a significant effect of genotype on olive oil only (Figure 2). Time as a main effect was significant for all variables ($P < 0.0001$).

For AUC, there was no significant main effect of genotype on plasma cholesterol or triacylglycerol (data not shown) or on chylomicron cholesterol or triacylglycerol. There was a significant main effect of fat on plasma cholesterol and triacylglycerol total AUC ($P = 0.02$ and 0.03, respectively), however, this effect did not remain significant after correction for baseline values (data not shown). The main effect of fat was significant for chylomicron cholesterol and triacylglycerol AUC: values after ingestion of safflower and olive oils were higher than those after ingestion of butter (Table 4, Figure 1).

For peak-rise, there was significant no effect of genotype or fat on plasma triacylglycerol (data not shown). Similarly, there was no significant effect of genotype on chylomicron cholesterol or triacylglycerol peak-rise (data not shown). However, there was a significant main effect of fat on chylomicron cholesterol peak-rise ($P = 0.03$): values after ingestion of olive oil were higher than those after ingestion of butter (0.11 ± 0.02 compared with 0.06 ± 0.01 mmol/L; $P < 0.05$, Tukey’s test). A significant main effect of fat was also observed for chylomicron triacylglycerol peak-rise ($P = 0.004$): values after ingestion of olive and safflower oils were higher than those after ingestion of butter (0.52 ± 0.06 and 0.52 ± 0.07, respectively, compared with 0.35 ± 0.04 mmol/L; $P < 0.05$, Tukey’s test). There were no significant differences between any of the groups for any of the other variables.

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FIGURE 1. Mean ($\bar{x} \pm$ SEM) chylomicron cholesterol and triacylglycerol values for concentration and area under the curve (AUC) in 11 subjects with an alanine for threonine substitution at codon 54 in the fatty acid–binding protein 2 gene (either homozygotes or heterozygotes, T54 group) and in 11 subjects who were homozygous for alanine (A54 group) during 8 h after 3 oral-fat-tolerance tests (safflower oil, SO; olive oil, OO; and butter, B). Significantly different from A54, $P < 0.05$. **Significantly different from butter, $P < 0.05$. Means or bars with different letters are significantly different, $P < 0.05$ (Tukey’s test). To improve legibility, error bars are not shown if they are smaller than the symbol or if they overlap with other error bars. All results were corrected for baseline values. AUC units are mmol $\cdot$ h/L.
significant main effects of genotype or fat on free fatty acid peak-rise (Table 4).

**Glucose, insulin, and C-peptide**

The results of the RMANOVA (3-way with genotype, fat, and time) showed no significant main effect of genotype on glucose, insulin, or C-peptide (Table 4, Figure 3); however, there was a significant interaction between genotype and time for insulin ($P < 0.0001$), and analysis by time showed that insulin concentrations in the T54 group were significantly lower than those in the A54 group at 1 and 1.5 h (Figure 3).

There was a significant main effect of fat on glucose: concentrations after ingestion of butter were higher than those after ingestion of safflower oil (Table 4, Figure 3). There was also a significant fat-by-time interaction for glucose ($P = 0.01$), and analyses by time showed a significant effect of fat at 1.5 h (again, concentrations after ingestion of butter were higher than those after ingestion of safflower oil). There was no significant effect of fat on insulin or C-peptide (Table 4). There was a significant fat-by-time interaction for C-peptide ($P = 0.04$), but analyses by time did not show significant differences at any time point. However, time to C-peptide peak was delayed for butter compared with safflower oil (Table 4). Time as a main effect was significant for all variables ($P < 0.0001$).

For AUC, there was no significant main effect of genotype on glucose or C-peptide. However, insulin AUC in the T54 group was significantly lower than that in the A54 group (Table 4, Figure 3), and the ratio of insulin to C-peptide was significantly lower in the T54 group than in the A54 group (Figure 4). There was no significant main effect of fat on insulin or C-peptide AUC; however, glucose AUC showed a trend ($P = 0.057$; Table 4). There was no significant effect of genotype or fat on peak-rise for glucose, insulin, or C-peptide (data not shown).

**DISCUSSION**

In the present study, we investigated the effects of 3 fat sources (safflower oil, olive oil, and butter) on postprandial lipids, glucose, insulin, and C-peptide in subjects with or without the threonine variant at codon 54 of the *FABP2* gene. The results suggested a diet-gene interaction in which the T54 group had increased chylomicron cholesterol after the olive oil–containing diet.
A similar trend was seen with chylomicron triacylglycerol ($P = 0.11$); although this trend was not significant, note that a larger sample size or the ability to implement more stringent controls on lifestyle habits may have resulted in a significant effect (24).

Few studies have evaluated interindividual differences in response to more than one fatty acid, so it is difficult to directly compare our results with those in the literature. However, our data are generally consistent with the notion that the T54 variant results in increased binding to long-chain fatty acids, which causes increased trafficking of fatty acids to chylomicron assembly. In addition, we showed that this effect may depend on the type of fat ingested, which may help to explain some of the discrepancies in the literature. In vitro studies showing increased binding of I-FABP to long-chain fatty acids (3) and increased triacylglycerol secretion in a cellular enterocyte model (5) or in human intestinal explants (6) used oleic acid but did not report results with other fatty acids. Tahvanainen et al (11) reported no association of the T54 allele with whole plasma postprandial responses to an oral-fat-tolerance test in healthy young men. However, the postprandial lipid responses were examined only in whole plasma and not in the chylomicron fraction. In the present study, we found a significant effect of genotype on chylomicron cholesterol responses to olive oil, but this effect was not reflected in a significant difference in whole plasma. In addition, Tahvanainen et al used a test meal containing saturated fat, which we found had no effect. Another study showing no effect used radio-labelled oleic acid; however, the researchers in that study added free oleic acid to a corn oil mixture (25), and nonemulsified free fatty acids may be less able than are emulsified free fatty acids to form micelles within the lumen (26), which may result in lower affinity to I-FABP and reduced ability to follow the chylomicron production pathway. Human studies that showed increased postprandial lipids in T54 subjects used a mixed-fat meal (7) or corn oil (8). Although these studies did not use a high-oleic acid oral fat load, all the subjects were homozygous for the T54 allele and were either obese (7) or obese with diabetes (8), and thus the studies may have had increased sensitivity to detect an effect.

We observed a lower plasma insulin response in the T54 group than in the A54 group. In previous studies, the postprandial insulin response was either not reported (8) or not significantly different between the A54 ($3 \pm 21.2 \pm 15.1 \text{ mU/L}$) and T54 ($19.7 \pm 9.5 \text{ mU/L}$) groups (7). The reduced plasma insulin concentration that we observed could have been due to lower insulin secretion or greater hepatic insulin extraction. Although there was no significant difference in C-peptide, the T54 group tended to have a higher C-peptide response, which suggests that reduced insulin secretion alone could not explain the reduced plasma insulin response. Therefore, increased hepatic extraction (27) is a more likely explanation and is supported by the significantly reduced ratio of insulin to C-peptide in the T54 group. We hypothesize that if the T54 protein product, I-FABP, results in increased binding affinity to fatty acids, then a larger proportion of absorbed fat may have been packaged into chylomicrons and less fat may have been absorbed into the portal vein in the T54 group than in the A54 group. A lower concentration of portal free fatty acids in the T54 group may, in turn, have resulted in increased hepatic insulin extraction, because it is known that free fatty acids can impede hepatic insulin clearance (28–30). Although long-chain fatty acids have traditionally been thought to be taken up into the lymphatic system, there is a growing body of evidence to suggest that at least some are absorbed portally (31–33).

In the present study, when subjects consumed safflower or olive oil, they had higher chylomicron cholesterol and triacylglycerol than they did after they consumed butter, whereas glucose was higher after the intake of butter than after the intake of safflower oil. Thus, various fats have different effects on postprandial lipids, which is consistent with the results of previous studies in which olive oil resulted in higher triacylglycerol than did milk fat 4 h after consumption (15); similarly chylomicron triacylglycerol AUC was higher after consumption of olive oil and sunflower oil than after consumption of butter (14). In the latter study, the authors did not observe any differences in postprandial glucose. In contrast, one group reported higher postprandial lipids after consumption of butter than after consumption of olive oil in healthy subjects (34) and overweight subjects with diabetes (35). It is difficult to explain these opposing results; however, the differences could be due to differences in administered fat loads (ie, either fixed or variable), the total quantity of fat ingested, the nature of the fat, or the macronutrient composition of the test meals.

Several potential explanations of why the butter-containing oral fat load produced a lower chylomicron response than did the other oral fat loads are available. We cannot rule out the possibility that the chylomicrons that were formed after ingestion of butter were smaller, dense lipoproteins that were not captured in the fraction with $S_i > 400$ (14, 36). Nevertheless, short- and medium-chain fatty acids, as found in butter, may be absorbed directly into the portal circulation, and this may account, at least in part, for the decreased chylomicron triacylglycerol after the butter-containing oral-fat-tolerance test. Proportionally, butter had 24% short- and medium-chain fatty acids, but the chylomicron triacylglycerol AUC after ingestion of butter was 32% lower than that after ingestion of safflower or olive oil. This accounts for most, but not all, of the observed differences. In addition, the long-chain saturated fatty acid stearic acid has been shown to be
absorbed less than are other fatty acids; however, when stearic acid was consumed in a quantity equivalent to that in the butter in the present study, the apparent digestibility was only \( \approx 2-3\% \) lower than that of other fatty acids (37), which would account for only a very small part of the 32\% decrease in AUC observed with butter. However, a greater proportion of saturated than unsaturated long-chain fatty acids may be taken up into the portal circulation. Specifically, glycerol tristearate was shown to have less absorbed lipid appearing in the lymph than did glycerol trioleate (32.9\% compared with 61.4\%) (33), and as much as 20\% of infused radiolabelled palmitic acid was absorbed portally in rats (32). Thus, we believe that increased portal absorption of short- and medium-chain fatty acids and saturated long-chain fatty acids could explain the decreased chylomicron formation and triacylglycerol concentrations that we observed after the butter-containing oral-fat-tolerance test. Furthermore, our data showing increased postprandial glucose after the ingestion of butter may support the theory of increased portal absorption, because increased free fatty acids reaching the liver may stimulate hepatic glucose production (38) or impede the uptake of glucose (28, 39, 40).

The time required for C-peptide to reach a peak after the butter treatment was greater than that after the safflower oil treatment. This is of interest because, in comparison with monounsaturated fatty acids, butter has been shown to result in a blunt incretin response, specifically a blunted glucagon-like peptide 1 response (35), and the glucagon-like peptide 1 response did not differ between monounsaturated and polyunsaturated fatty acids (41).

We did not measure fecal fat, but it seems unlikely that the T54 variant would result in differences in total fat absorbed or that butter would be absorbed to a lesser extent than would olive oil. Persons with healthy gastrointestinal tracts have \( >93\% \) fat absorption (42), and diets in the range of fatty acid profiles that we used in the present study have been shown to have 96–99\% absorption (37); therefore, small changes within this range would not account for the magnitude of difference in chylomicron cholesterol and triacylglycerol response that we observed. In addition, according to in vitro and animal models in which fat absorption still occurred in cells (43) and mice (44) that did not express I-FABP, I-FABP does not appear to be an absolute requirement for fat absorption.

Overall, these results suggest that the FABP2 T54 variant may be beneficial by effectivley trafficking fatty acids within enterocytes and thereby increasing the absorption of fats via chylomirons and the lymphatics. If handled by increased adipocyte uptake, increased chylomicron formation may not be the hazard. Rather, an inability of adipocytes to trap fatty acids, as in a lipoprotein lipase deficiency (45) or a defective acylation-stimulating protein receptor (46), which results in excess fatty acids reaching the liver and concomitant hyperinsulinemia and insulin resistance, is the real hazard (38).

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TMSW designed the study, obtained funding, interpreted the data, and reviewed and edited the manuscript. PDND recruited and screened subjects, managed the oral-fat-tolerance test study days, conducted laboratory analyses, performed the statistical analyses, interpreted the data, and wrote the manuscript. RAH conducted the genotype analyses, and reviewed and edited the manuscript. TMSW has served on the grants review committee and the expert committee for the revision of clinical practice guidelines and chaired the national nutrition committee as an unpaid volunteer for the Canadian Diabetes Association (CDA). PDND received the CDA Brain Dufton Memorial Manuscript Award (2003) for publication of a manuscript pertaining to the screening data from this study and has accepted a position as Senior Research Associate at CDA, effective fall 2003. RAH held an operating grant from the CDA (no. 992), in honor of Hazel E Kerr, during the period in which the present experiments were performed.

REFERENCES

EFFECT OF FAT AND FABP2 ON POSTPRANDIAL LIPIDS


