A high-fat diet and the threonine-encoding allele (Thr54) polymorphism of fatty acid–binding protein 2 reduce plasma triglyceride–rich lipoproteins

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Abstract

The threonine-encoding allele (Thr54) of the fatty acid–binding protein 2 (FABP2) DNA polymorphism is associated with increased triglyceride (TG)-rich lipoproteins (TRL). We hypothesized that the TRL response to diets of varied fat content is affected by the FABP2 A54T polymorphism, specifically that a high-fat diet would reduce TRL and that the Thr54 allele would have an enhanced response. Sixteen healthy, postmenopausal women completed a crossover dietary intervention that included three 8-week, isoenergetic diet treatments. The treatments consisted of high fat (40% of energy as fat), low fat (20% of energy), and low fat + n-3 fatty acids (20% of energy plus 3% as n-3 fatty acids). Eight subjects were homozygous for the wild type (Ala54/Ala54) of the FABP2 polymorphism, whereas 8 subjects had at least 1 Thr54 allele (7, Ala54/Thr54; 1, Thr54/Thr54). High-fat diet showed significantly reduced plasma TGs, chylomicron TG, and very low-density lipoprotein TG from baseline in all participants. Although carriers of the Thr54 allele of the FABP2 polymorphism had significantly reduced TRL, there is no evidence of an interaction, which does not support our hypothesis. The alanine-encoding allele did not influence the dietary effects on the plasma lipids.

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Abbreviations: Ala54, alanine-encoding allele; BMI, body mass index; CHO, carbohydrate; CR, chylomicron remnant; FABP2, fatty acid–binding protein 2; HF, high fat; LF, low fat; LFn-3, low fat + n-3 fatty acids; n-3, ω-3; SFA, saturated fatty acid; TG, triglycerides; Thr54, threonine-encoding allele; TRL, triglyceride-rich lipoprotein; VLDL, very low-density lipoprotein.

1. Introduction

Higher concentrations of triglycerides (TGs) and TG-rich lipoproteins (TRLs) such as chylomicrons, very low-density lipoproteins (VLDLs), and their respective remnants are known cardiovascular risk factors [1-3]. The current cardiovascular disease primary prevention plan from the National Cholesterol Education Program is to reduce dietary total and saturated fat [4]. Studies have shown that individual response to the recommended National Cholesterol Education Program low-fat diet varies significantly, although the group mean may show a beneficial plasma lipid response [5,6]. Schaefer et al [5] attributed the interindividual differences to genetics because other variables such as diet, body weight, compliance, and lifestyle were well controlled.
Genetic variation in fatty acid–binding protein 2 (FABP2) has been associated with alterations in lipid metabolism. Fatty acid–binding protein 2 is expressed in the enterocytes of the intestine and aids in the absorption and transportation of long-chain fatty acids. A single-nucleotide polymorphism occurs at codon 54 of the FABP2 gene resulting in an alanine (wild type) to threonine (mutated type) substitution (A54T) in the protein [7]. The threonine-encoding allele (Thr54) has a greater affinity for long-chain fatty acids than the Ala54 allele and results in a greater flux of fatty acids across the enterocytes and into the plasma [8]. The FABP2 polymorphism has been associated with dyslipidemia [9] and an increase in plasma TRL [10]. Recently, Zhao et al [11] found that carriers of Thr54 have higher fasting plasma TG than noncarriers in a meta-analysis.

In addition to the role of total fat intake in TG response, the type of fat consumed affects TRL concentrations. Polyunsaturated fatty acids, especially ω-3 (n-3) fatty acids, are recommended to help lower plasma TGs [4,12]. The mechanism by which n-3 lowers TGs is unclear; however, research suggests that n-3 binds to nuclear receptors, such as peroxisome proliferator activator receptors, and decreases hepatic TG and VLDL synthesis and secretion [13].

In this study, we hypothesized that the TRL response to diets of varied fat content is affected by the FABP2 A54T polymorphism, specifically that a high-fat diet would reduce TRL and that the Thr54 allele would have an enhanced response. Our aims were to examine TRL response to 3 whole-food diets with varying amounts and types of dietary fat (high fat, low fat, and low fat supplemented with n-3 fatty acids) and to investigate the role of DNA polymorphisms in FABP2 in modifying the TRL response.

2. Methods and materials

2.1. Study design

Seventeen healthy, postmenopausal women were recruited from the Minneapolis-St Paul, Minnesota, area and enrolled in a randomized, crossover-designed feeding trial at the University of Minnesota to evaluate the effects of the diets on sex hormone concentrations [14]. Postmenopausal status was confirmed by subjects being at least 1 year postmenses, which was also confirmed by follicle-stimulating hormone levels in women 55 years or younger. Inclusion criteria included age 45 to 70 years, postmenopausal and not taking hormone replacement therapy, healthy, weight stable for the last 6 months, a body mass index (BMI) between 19 and 32 kg/m², and consumption of a “typical” American diet with no special dietary practices such as vegetarianism. Subjects were excluded if they used prescription medications or over-the-counter nonsteroidal anti-inflammatory medications, smoked, or had a known chronic disease. One subject was not included in the final analysis because of a laboratory equipment error that resulted in unusable TG data.

2.2. Biochemical analyses

Chylomicrons as well as chylomicron remnants (CRs) and VLDL were isolated by salt density gradient ultracentrifugation, as previously described by Tsai et al [15]. Collection of 2 subfractions, Svedberg units of flotation greater than 400 containing chylomicrons and Svedberg units of flotation 20 to 400 containing VLDL and CR, were obtained from baseline and 8-week plasma samples.

The TG analysis is based on the work of McGowan et al [16] and Fossati and Prencipe [17] using Infinity Triglyceride Liquid Stable Reagent (Thermo Electron Corp, Waltham, Mass). Samples were run in duplicate and with internal controls. Plasma TG intra-assay and interassay coefficients of variation were 5.4% and 20.0%, respectively. Analysis of the FABP2 polymorphism was conducted using an amplification and restriction enzyme digestion technique initially described by Baier et al [7].

2.3. Statistical analysis

All statistical analyses were conducted using Proc Mixed in SAS statistical software version 9.2 (SAS Institute Inc,
Cary, NC). Repeated-measures analysis of variance was conducted to determine the effect of the different diets and the DNA polymorphisms had on plasma TGs. The TG end points had baseline values subtracted for analysis. The general linear model for repeated-measures procedures was used to test the main effects of genotype and diet and gene × diet interactions. Because of the small sample size and few degrees of freedom, we could only analyze the interaction of FABP2 and diet. Pairwise comparisons between treatments were based on the least squares means from the mixed model. The treatment randomization groups are unbalanced, and therefore, treatment order was added to the model for statistical analysis. Differences were considered statistically significant at \( P < .05 \). All data in the text and tables are given as means ± SE.

### 3. Results

The baseline characteristics of the study subjects separated into FABP2 genotype groups are presented in Table 2. The mean age of all the subjects was 56.3 ± 1.5 years. The mean BMI of the subjects was 27.7 ± 1.0 kg/m². Fifteen subjects were white, and 1 was African American. No carryover effect of the diets was detected, and the order in which the diets were given was not significant for any end points.

Eight of our subjects were homozygous for the FABP2 wild type, Ala54/Ala54 (50% of our population); 7 subjects were heterozygotes, exhibiting Ala54/Thr54 (44%); and 1 subject was homozygous for the mutation, Thr54/Thr54 (6%). Because there was a single Thr54/Thr54 subject and no repeated measures of analysis of variance calculations could be performed on a single subject, it was included in the Ala54/Thr54 group for statistical analysis. These results are consistent with previous studies of whites, stating the prevalence of wild-type homozygotes to be 50%, heterozygotes to be 45%, and mutation homozygotes to be 5% [10,18]. There were no statistically significant differences in age, BMI, plasma TG, chylomicron TG, and VLDL TG between the FABP2 genotype groups at baseline.

Table 3 illustrates the change from baseline for TG concentrations in response to dietary treatments. Analysis of mean fasting plasma TGs in response to diet resulted in a statistically significant reduction from baseline with the HF diet \((P = .012)\) but not with the LF or LFn-3 diets, although there were no observed differences between treatments. Plasma TGs were also significantly reduced from baseline in carriers of the Thr54 allele after completion of the HF diet \((P = .003)\), but not after consuming the LF or LFn-3 diets, shown in Table 4 and illustrated in Fig. 1. The plasma TG response was not statistically significantly different between the diets or the FABP2 genotype groups.

### Table 3

<table>
<thead>
<tr>
<th>Diet</th>
<th>Plasma TG (mg/dL)</th>
<th>Chylomicron TG (mg/dL)</th>
<th>VLDL and CR TG (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HF</td>
<td>−35.3 ± 13.1 *</td>
<td>−8.4 ± 3.6 *</td>
<td>−20.1 ± 7.5 *</td>
</tr>
<tr>
<td>LF</td>
<td>−11.5 ± 12.9</td>
<td>−8.5 ± 3.5 *</td>
<td>−2.8 ± 7.4 *</td>
</tr>
<tr>
<td>LFn-3</td>
<td>−4.1 ± 13.1</td>
<td>−5.1 ± 3.6</td>
<td>−1.3 ± 7.5</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± SE. Effects of the study diets on change in TG concentration were analyzed by repeated-measures analysis of variance. Means in a column with different superscript letters indicate that they are significantly different \((P < .05)\).

* Significant change from baseline value, \( P < .05 \).

### Table 2

Baseline anthropometric characteristics and plasma lipid, glucose, and insulin concentrations according to FABP2 group

<table>
<thead>
<tr>
<th>FABP2 groups</th>
<th>Total (n = 16)</th>
<th>Ala54/Ala54 (n = 8)</th>
<th>Ala54/Thr54, Thr54/Thr54 (n = 8)</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>56.3 ± 1.5</td>
<td>55.4 ± 1.4</td>
<td>57.3 ± 2.7</td>
<td>.50</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.7 ± 1.0</td>
<td>27.7 ± 1.6</td>
<td>27.7 ± 1.3</td>
<td>.98</td>
</tr>
<tr>
<td>Plasma TG (mg/dl)</td>
<td>93.5 ± 16.5</td>
<td>102.9 ± 29.1</td>
<td>84.1 ± 17.3</td>
<td>.64</td>
</tr>
<tr>
<td>Chylomicron TG (mg/dl)</td>
<td>12.0 ± 4.3</td>
<td>13.2 ± 7.6</td>
<td>10.8 ± 4.5</td>
<td>.81</td>
</tr>
<tr>
<td>VLDL and CR TG (mg/dl)</td>
<td>40.6 ± 10.2</td>
<td>47.4 ± 17.3</td>
<td>33.7 ± 11.7</td>
<td>.58</td>
</tr>
<tr>
<td>Fasting plasma glucose (mg/dl)</td>
<td>107.7 ± 4.2</td>
<td>115.8 ± 6.1</td>
<td>99.6 ± 4.5</td>
<td>.10</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± SE of subjects at screening. The Thr54/Thr54 group had an \( n = 1 \); thus, this subject was included in the Ala54/Thr54 group for statistical analysis. There were no significant differences between the genotype groups by \( t \) test, \( P < .05 \).
Fasting chylomicron subfraction TG was significantly reduced from baseline with both the HF (P = .027) and LF diets (P = .023), but not with the LFn-3 diet, shown in Table 3. Carriers of the Thr54 allele had significantly reduced chylomicron TG after consuming the HF diet (P = .008), shown in Table 4 and Fig. 2. No difference was observed in chylomicron TG between the diets or by genotype groups.

Very low-density lipoprotein and CR subfraction TG were significantly reduced from baseline after consuming the HF diet (P = .010), and the reduction was statistically significantly different from the LF diet (P = .009) and LFn-3 diet (P = .006), shown in Table 3. Carriers of the Thr54 allele exhibited significantly reduced VLDL and CR TG (P = .006), shown in Table 4 and Fig. 3, compared with baseline in response to the HF diet but not the LF or LFn-3 diet.

The main effects of diet, FABP2, and diet × FABP2 were nonsignificant covariates for any of the end points, with their P values shown in Table 4.

### 4. Discussion

In this study, we evaluated the effects of dietary fat and fatty acid intake and FABP2 genotype on TRL response. Our findings show that carriers of the Thr54 allele of the FABP2 polymorphism had significantly decreased plasma TG, chylomicron TG, and VLDL and CR TG after consuming the HF diet. Homozygotes of the Ala54 allele, alone or in combination with the diets, did not show significant changes from baseline with any diet.

A meta-analysis of 60 controlled feeding studies confirmed that replacing carbohydrates with dietary fat will decrease fasting serum TG [19]. High-fat, low-carbohydrate (CHO) diets, with and without n-3 supplementation, have previously been shown to decrease fasting and postprandial TGs [20,21]. Our results suggest that an elevated n-3 intake with an LF, high-CHO diet does not play a large role in decreasing fasting TGs in postmenopausal women.

Volek et al [22] observed decreased fasting and postprandial TG in response to an HF, low-CHO diet in 10 healthy normolipidemic, postmenopausal women and suggested a mechanism for the decrease in fasting TGs. They propose that an HF, low-CHO diet would induce an increase in lipoprotein lipase activity, presumably to allow fatty acids to play a larger role in the energy supply to peripheral cells.
The authors also state that a decrease in the rate of VLDL production contributes to a reduced postprandial lipemia because a larger VLDL-TG pool competes with dietary TG for removal during postprandial periods. Therefore, a decrease in TG is expected in response to an HF diet because of an increase in lipoprotein lipase activity, which would increase the rate of removal of TG from the plasma.

Parks et al [23] found that fasting TG concentrations increased by 60% after consuming an LF, high-CHO diet and concluded that an LF, high-CHO whole-food diet reduced VLDL clearance. They proposed that the reduced VLDL clearance could be the result of competition between VLDL and CR for TG hydrolysis. Mittendorfer and Sidossis [24] found that fasting VLDL was significantly higher after a high-CHO diet than after an HF diet. They proposed that an increased rate of VLDL secretion resulted in an elevated plasma TG. Thus, HF and LF diets play a role in fasting TG metabolism.

The LFn-3 diet treatment did not produce any change in response variables. Previous studies produced mixed results but gave reason to hypothesize that a significant intake of n-3 would influence TG concentrations. A study of postmenopausal women given 2.8 g/d of docosahexaenoic acid (DHA) for 4 weeks showed a decrease in TG concentrations [25]. A systematic review of n-3 and cardiovascular disease risk factors showed a significant reduction in TG in response to n-3 given upward to 5.9 g/d in the reviewed study diets and factors showed a significant reduction in TG in response to a systemic review of n-3 and cardiovascular disease risk factors. Because the HF diet significantly decreased the same end points in Ala54 allele homozygotes after completing any of the 3 diets. Marin et al [28] found a similar lack of change in plasma-free fatty acid concentration after consuming a high-SFA diet, LF diet, and high–monounsaturated fatty acid diet in Ala54 homozgyotes.

One limitation of our study is that we analyzed fasting samples. It is possible that increased VLDL secretion caused by a high-CHO diet may not be observed in fasting samples [29]. The use of fasting samples decreased the range of concentration for chylomicrons. Chylomicrons are numerous postprandially because of the influx of dietary lipids, but concentrations are much lower after fasting. Thus, the dynamic range of chylomicrons is decreased in a fasting sample. We were unable to evaluate changes in chylomicron concentrations because we used fasting rather than postprandial plasma samples. The other limitations of our study include our small sample size and the inclusion criteria, which consisted of healthy women not on lipid-lowering medication. Such a small sample may not allow adequate power needed to detect differences in our end points. In addition, the low number of subjects hindered our ability to draw a genotypically diverse group from which we could better analyze the DNA polymorphisms.

Our novel findings from this study include a reduction from baseline in fasting plasma TG, chylomicron TG, and VLDL and CR TG in carriers of the Thr54 allele of the FABP2 polymorphism after completion of an 8-week controlled whole-food, HF diet. The Ala54 allele did not significantly alter any TG concentrations after completion of the HF, LF, or LFn-3 diet. Few studies have examined the interaction of diet and DNA polymorphisms with a crossover design controlled-feeding study of similar length involving varying amounts and types of dietary fat.

In conclusion, our results indicate that consumption of an HF diet was associated with significant reductions in plasma TG, chylomicron TG, and VLDL and CR TG. Carriers of the Thr54 allele of FABP2 in particular demonstrated significant reductions in these lipid parameters in response to HF, although there is no evidence of an interaction between the diet and the polymorphism. Consumption of the HF, LF, or LFn-3 diet did not significantly change TG, chylomicron TG, or VLDL and CR TG in Ala54 homozygotes of the FABP2 polymorphism.
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