Postprandial Plasma ApoB-48 Levels Are Influenced by a Polymorphism in the Promoter of the Microsomal Triglyceride Transfer Protein Gene

Björn Lundahl, Anders Hamsten, Fredrik Karpe

Abstract—The microsomal triglyceride transfer protein (MTP) plays a key role in the secretion of apolipoprotein B (apoB)-containing lipoproteins. The rare variant of a functional polymorphism in the promoter region of the MTP gene has been associated with elevated transcriptional activity of the gene in vitro (MTP−493T/G). With use of a “recruit-by-genotype” approach, we investigated one of the potentially complex phenotypes of this polymorphism, the appearance in plasma of apoB-48 after a meal intake. A total of 12 homozygous carriers of the rare MTP−493T variant were identified from a population-based screening of 50-year-old healthy white men. All subjects were of the apoE3/3 genotype. Along with 48 baseline well-matched heterozygotes (n=24) plus homozygotes (n=24) for the common variant, they were given a standardized oral fat meal. Postprandial plasma concentrations of apoB-48 were determined by the combination of density gradient ultracentrifugation and analytical SDS-PAGE. The postprandial plasma concentrations of triglyceriderids did not differ between the groups, but homozygous carriers of the rare MTP−493T variant showed a >100% greater increase in apoB-48 in the smallest (Svedberg flotation rate constant 20 to 60) triglyceride-rich lipoprotein fraction (P=0.005). These data support the notion that elevated transcriptional activity of MTP leads to an increased generation of the smallest triglyceride-rich lipoprotein from the intestine. (Arterioscler Thromb Vasc Biol. 2002;22:289-293.)

Key Words: genetic variability ■ DNA bank ■ genetic screening ■ complex phenotype ■ alimentary lipemia

Several studies have suggested that there is a heritable component determining the magnitude of postprandial lipemia.¹ In part, this can be attributed to functional polymorphisms in genes coding for proteins involved in regulatory steps in the pathways for postprandial lipid and lipoprotein metabolism. For this purpose, a number of candidate genes have been investigated in relation to postprandial lipemia; most of these genes are involved in the catabolic pathways, such as apoE, which is the ligand for receptor-mediated removal of remnant lipoproteins, and lipoprotein lipase (LPL), which hydrolyzes triglycerides from chylomicrons and VLDLs. The effect on postprandial lipemia exerted by the common ε2/3/4 polymorphism in the apoE gene has been observed in several studies in which carriers of the ε2 allele have shown consistently delayed clearance of triglyceride-rich lipoprotein (TRL) remnants.²⁻⁴ In addition, it has been suggested that carriers of the ε4 allele have increased plasma levels of endogenous TRL in the postprandial state.⁵

The LPL gene is the obvious candidate for studies of postprandial lipemia, inasmuch as it codes for the single-protein—hydrolyzing triglycerides from chylomicrons and large VLDLs. Talmud et al⁶ studied the interaction of the functional variants involving the LPL−93T/G promoter polymorphism and the LPL D9N substitution. Carriers of the haplotype constituting the rare LPL−93T variant (presumably higher transcriptional activity) and the common LPL9N variant (presumably secretion-defective LPL protein) exhibit higher plasma triglyceride levels after meal intake than do carriers of other haplotypes.⁷ The LPL A291S residue variant affects the specific activity of the enzyme.⁸ Two studies show that carriers of this variant have significantly higher postprandial triglyceridemia.⁸,⁹

Hepatic lipase (HL) has been implicated in the removal of remnant lipoproteins. The promoter of the HL gene contains several single nucleotide polymorphisms.¹⁰ The rare variant of the −480C/T (also called −514C/T) polymorphism has been associated with lower HL activity. This does not seem to affect total postprandial triglyceridemia but does affect the retention of a specific lipoprotein subspecies in the postprandial state, the LpCIII:B particles, which are likely to reflect remnant lipoproteins.¹¹

So far, polymorphisms in candidate genes involved in the secretion of apoB-containing lipoproteins have given less convincing evidence of influencing postprandial lipemia. The non-cutting variant of the APOB XbaI polymorphism, which has been linked to neither a functional effect on the gene nor the...
gene product, has been associated with a small but statistically significant elevation of postprandial plasma retinyl palmitate and apoB-48 concentrations in healthy subjects. Of note, this effect was independent of apoE carrier status and the fasting plasma triglyceride level.

Intestinal absorption of triglycerides and intestinal handling of fatty acids might be influenced by a single nucleotide polymorphism leading to amino acid exchange in the intestinal fatty acid binding protein (the FABP-2 Ala54Thr polymorphism). Thr54 carrier status has been associated with higher postprandial plasma triglycerides, but these findings could not be reproduced in a much larger cohort.

In summary, there is substantial evidence that functional changes in genes involved in catabolic pathways of postprandial lipoproteins have physiological and potentially pathophysiological consequences, whereas the corresponding evidence for genes implicated in the production of postprandial lipoproteins is sparse, with the potential exception being an as-yet-uncharacterized apoB polymorphism. Therefore, we set out to study the effects of a recently detected functional polymorphism in the promoter region of the gene encoding the microsomal triglyceride transfer protein (MTP). MTP plays a role in the formation of VLDL in the liver and of chylomicrons in the intestine by transferring core lipids to the apoB molecule. The rare variant of the MTP—493G/T transition has been shown to confer higher transcriptional activity in vitro; therefore, we hypothesized that this might result in increased production of apoB-48—containing lipoproteins in the postprandial state. To test this hypothesis, we selected matched healthy carriers of either genetic variant from a well-characterized and homogeneous population and challenged them with an oral fat load to stimulate apoB-48 lipoprotein secretion.

Methods

Subjects, Recruitment, and Baseline Matching

Sixty healthy 50-year-old white men participated in the present study. They were recruited through an ongoing population survey of 50-year-old residents in the greater Stockholm area. The present study consisted of the assessment of postprandial lipoprotein metabolism. Because of the relative infrequency of homozygous carriers of the rare MTP—493T variant (approximate frequency 5%), these men were mostly recruited by genotype for the second visit. Four of the 12 subjects were part of a previously reported study, whereas the remaining 8 subjects were recruited specifically for the present study.

Statistical Analysis

Conventional methods were used for the calculation of mean±SD values. Coefficients of skewness and kurtosis were calculated to test deviations from a normal distribution. Logarithmic transformation was performed on the individual values of skewed variables, and a normal distribution of transformed values was confirmed before statistical computations and significance testing. The statistical method used was ANOVA, with the Scheffé post hoc test. Statview 5.0 for Windows 97 was used for statistical analysis.

Results

Fasting plasma lipid and major lipoprotein lipid concentrations and BMI are shown in Table 1. Average BMI and fasting plasma triglycerides were almost identical as a consequence of the matching procedure. There were no statistically significant differences in total plasma or major cholesterol concentrations according to MTP genotype. We have previously reported that the homozygous carriers of the
TABLE 1. BMI and Fasting Plasma Lipid and Lipoprotein Values are mean ± SD.

<table>
<thead>
<tr>
<th></th>
<th>GG (n=24)</th>
<th>GT (n=24)</th>
<th>TT (n=12)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI, kg/m²</td>
<td>25.3 ± 3.1</td>
<td>25.2 ± 1.7</td>
<td>25.5 ± 2.1</td>
<td>0.93</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>5.24 ± 0.77</td>
<td>5.63 ± 1.19</td>
<td>5.16 ± 0.71</td>
<td>0.27</td>
</tr>
<tr>
<td>VLDL</td>
<td>0.37 ± 0.21</td>
<td>0.45 ± 0.26</td>
<td>0.40 ± 0.26</td>
<td>0.50</td>
</tr>
<tr>
<td>LDL</td>
<td>3.58 ± 0.75</td>
<td>3.84 ± 0.96</td>
<td>3.46 ± 0.67</td>
<td>0.38</td>
</tr>
<tr>
<td>HDL</td>
<td>1.21 ± 0.27</td>
<td>1.16 ± 0.32</td>
<td>1.19 ± 0.33</td>
<td>0.87</td>
</tr>
<tr>
<td>TGs, mmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1.34 ± 0.63</td>
<td>1.43 ± 0.63</td>
<td>1.46 ± 0.84</td>
<td>0.85</td>
</tr>
<tr>
<td>VLDL</td>
<td>0.94 ± 0.56</td>
<td>0.99 ± 0.51</td>
<td>1.08 ± 0.88</td>
<td>0.81</td>
</tr>
<tr>
<td>LDL</td>
<td>0.28 ± 0.09</td>
<td>0.31 ± 0.11</td>
<td>0.27 ± 0.04</td>
<td>0.55</td>
</tr>
<tr>
<td>HDL</td>
<td>0.11 ± 0.03</td>
<td>0.11 ± 0.03</td>
<td>0.12 ± 0.03</td>
<td>0.44</td>
</tr>
</tbody>
</table>

GG indicates homozygotes for the common MTP–493G allele; GT, heterozygotes; TT, homozygotes for the rare MTP–493T allele; and TGs, triglycerides. Values are mean ± SD.

*P values indicate overall significance of ANOVA between the 3 groups.

MTP–493T variant have significantly lower LDL cholesterol levels, and this could be reproduced in the cohort from which the present subjects were matched and recruited (N=418; LDL cholesterol for MTP–493GG [n=231], 3.53 ± 0.85 mmol/L; LDL cholesterol for MTP–493GT [n=161], 3.59 ± 0.85 mmol/L; and LDL cholesterol for MTP–493TT [n=26], 3.09 ± 0.73 mmol/L; P=0.02 by ANOVA). Therefore, the lack of difference between the genotypes for LDL cholesterol in the present study groups is likely to depend on the matching procedure, which focused on fasting plasma triglycerides, BMI, and apoE genotype to eliminate major confounders for the study of postprandial TRL metabolism.

Fasting plasma apoB-100 and apoB-48 concentrations in major subfractions of TRLs were also similar (Table 2). In most subjects, the amounts of apoB-100 and apoB-48 contained in the Svedberg flotation rate constant (Sf) >400 fractions in the fasting state were below the detection limit, whereas almost all subjects had detectable levels of apoB-48 in the Sf 60 to 400 and Sf 20 to 60 fractions. The ratio between apoB-48 and apoB-100 in fasting TRL fractions varied between 3% and 4%, reflecting the relationship between chylomicron remnants and VLDL particle number (calculating the particle number assumes that there is 1 apoB per particle). This ratio did not differ between genotype.

Plasma triglycerides peaked at ≈3 hours after meal intake in all groups (Figure 1). Overall, postprandial triglyceride levels did not differ between the genotype groups, either when they were calculated as the area under the curve or when they were assessed as the incremental area under the curve (P=0.34 and P=0.29, respectively, by ANOVA; Table 3).

Plasma concentrations of apoB-48 in TRLs increased after fat intake (Figure 2). The largest increase was seen in the Sf 60 to 400 fraction. However, there was no genotype-specific effect (Table 3). This was also true for the Sf >400 fraction. In contrast, homozygous carriers of the MTP–493T allele showed a markedly greater elevation of apoB-48 in the Sf 20 to 60 fraction in response to fat intake. The increase was >2-fold greater than that of the other genotype groups, but the apoB-48 level returned to baseline after 6 hours (Table 3).

The apoB-100 concentrations in TRLs did not differ according to MTP genotype (Figure 3).

### Discussion

The present study investigated the physiological consequences of a common polymorphism in the MTP gene in relation to the generation of chylomicrons and chylomicron remnants in the plasma after the intake of a fatty meal. Homozygous carriers of the rare MTP–493T variant, which is associated with higher transcriptional activity of the gene in vitro, showed markedly elevated accumulation of small apoB-48–containing lipoproteins in the postprandial state. In contrast, the postprandial plasma triglyceride concentrations did not differ according to MTP genotype.

Why would increased transcriptional activity of the MTP gene be associated with increased postprandial plasma levels of small apoB-48–containing particles? There is considerable evidence that the intestine secretes apoB-48 particles of a wide range of sizes. When large amounts of lipid substrate become available, as is the case after oral intake of fat, some of these particles are recruited to be particularly filled with triglycerides, and they are then secreted as “chylomicon.” Simultaneously, there is continued secretion of smaller-sized apoB-48 particles, and our results indicate that the rate at which these particles are filled with triglycerides is likely to be determined by the MTP activity. This finding is in accordance with the 2-step model for chylomicron assembly in which the availability of primordial...
apoB particles would be enhanced by higher MTP activity. In addition, small and relatively lipid-poor chylomicrons are likely to compete less well for LPL because of the presence of larger chylomicrons and large VLDLs in the postprandial state. This is likely to further enhance the accumulation of small apoB-48–containing particles. We do not believe that the postprandial accumulation of the small apoB-48–containing particles is the consequence of a higher secretion rate of larger particles for 2 reasons. First, there was no sign of higher apoB-48 concentrations in the chylomicron (Sf >400) and the large TRL (Sf 60–400) fraction in the postprandial state; ie, there is hardly any input of apoB-48 into the Sf 20–60 fraction from the more triglyceride-rich fractions. Examples of common functional polymorphisms regulating postprandial plasma apoB-48 concentrations. Lopez-Miranda et al have studied the effect of the common apoB XhoI polymorphism in the postprandial state in a rather large cohort of well-matched subjects and found that carriers of the cutting variant had significantly lower plasma apoB-48 concentrations than did carriers of the noncutting variant. However, the functional significance of this polymorphism is unknown. The apoB signal peptide SP24/27 variant has been implicated to play a role in determining the magnitude of postprandial lipoproteinemia, but the effect is likely to be due to a genotype-specific effect on fasting plasma triglycerides. In addition, in a large cohort of 274 healthy subjects given an oral fat meal, there was no effect at all by the apoB SP24/27 variant on the postprandial elevation of plasma triglycerides. The present study involved a “recruit-by-genotype” approach. Because postprandial lipemia and lipoproteinemia are very much dependent on the fasting plasma triglyceride concentration, it is of utmost importance to eliminate that factor in the study of more subtle aspects of regulation of postprandial lipemia. The obvious benefit of the approach is that it enables matching for baseline factors that may interfere with an intermediary phenotype determined after a provocation test, such as the interference between fasting plasma triglycerides and the

| Table 3. Plasma TG AUC and Baseline to Peak Increment of ApoB-48 in Sf >400, Sf 60–400, and Sf 20–60 TRL Fractions |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|
|                                | GG (n=24)       | GT (n=24)       | TT (n=12)       | P*              |
| TG, (mmol/L) · h               |                 |                 |                 |                 |
| AUC                            | 11.1 (6.7–23.6) | 13.1 (5.4–26.0) | 10.5 (7.9–38.9) | 0.34            |
| iAUC                           | 4.4 (2.2–10.6)  | 5.8 (1.8–12.7)  | 4.4 (0.2–18.2)  | 0.29            |
| ApoB-48, mg/L                  |                 |                 |                 |                 |
| 0–3 h change at Sf >400        | 0.19 (0.26)     | 0.25 (0.34)     | 0.16 (0.28)     | 0.32            |
| 0–3 h change at Sf 60–400      | 0.81 (0.74)     | 1.25 (1.01)     | 0.92 (0.67)     | 0.17            |
| 0–3 h change at Sf 20–60       | 0.27 (0.26)     | 0.16 (0.30)     | 0.52 (0.36)     | 0.005           |

AUC indicates area under the curve; iAUC, incremental AUC. Values are median and (range) for the TG values and median and (interquartile range) for change in apoB-48 values.

*p values indicate overall significance of ANOVA between the 3 groups.
postprandial rise of TRLs in plasma. However, a limitation of this approach is that the matching-recruiting procedure may restrict the expression of the phenotype by “overmatching.”

The question arises of whether the greater postprandial increase of S, 20 to 60 apoB-48 containing lipoproteins exhibited by homozygous carriers of the MTP–493T allele is proatherogenic. We have previously reported on an association between the accumulation of apoB-48 in postprandial plasma and the progression of coronary atherosclerosis in young survivors of myocardial infarction; this association fits well with the notion that excessive postprandial lipemia might be a risk factor for coronary heart disease. However, the pattern of postprandial plasma apoB-48 concentrations observed in those studies is somewhat different from the pattern encountered in the present study. In the present study, the rare MTP–493T variant exhibited increased postprandial plasma apoB-48 concentrations at 3 hours, whereas the concentration had returned completely to baseline at 6 hours; ie, there was no sign of a delayed removal of those lipoprotein particles. Therefore, it might not be justifiable to compare this pattern with the delayed catabolism of S, 20 to 60 apoB-48 particles that has been associated with coronary progression of coronary lesions in patients with premature coronary atherosclerosis. However, the total vessel wall exposure of small chylomicron remnants is certainly greater in the MTP–493T/T carriers, and this could potentially have an atherogenic effect. Furthermore, the postprandial lipoprotein phenotype of this polymorphism on a hyperlipidemic background remains to be established.

Acknowledgments

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References

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