ATP-Binding Cassette Transporter G8 Gene As a Determinant of Apolipoprotein B-100 Kinetics in Overweight Men

D.C. Chan, G.F. Watts, P.H.R. Barrett, A.J. Whitfield, F.M. van Bockxmeer

Objective—We examined the influence of genetic variation of the ATP-binding cassette (ABC) transporter G8 on apolipoprotein B (apoB) kinetics in overweight/obese men.

Methods and Results—Very low–density lipoprotein (VLDL) and low-density lipoprotein (LDL) apoB kinetics were determined in 47 men (body mass index 32±3 kg/m²) using stable isotope and multicompartmental modeling to estimate production rate (PR), fractional catabolic rate (FCR), and VLDL to LDL–apoB conversion. Relative to the wild-type (400TT), subjects carrying the ABCG8 400K allele had significantly decreased plasma concentrations of triglycerides, sitosterol, and campesterol, lower PR of VLDL–apoB, and higher VLDL to LDL–apoB conversion (P<0.05). The PR and FCR of LDL–apoB were also significantly higher with 400K allele (P<0.05). No association was found with ABCG8 D19H. Compared with APOE2 or APOE3, APOE4 carriers had significantly higher plasma LDL-cholesterol concentrations and lower LDL–apoB FCR. During multiple regression analysis including age, homeostasis model assessment score, plasma concentrations of sitosterol, and lathosterol, ABCG8 and apoE genotypes were independent determinants of VLDL–apoB PR and LDL–apoB FCR, respectively (P<0.05).

Conclusions—Variation in the ABC transporter G8 appears to independently influence the metabolism of apoB-containing lipoproteins in overweight/obese subjects. This may have therapeutic implications for the management of dyslipidemia in these subjects. (Arterioscler Thromb Vasc Biol. 2004;24:2188-2191.)

Key Words: ATP binding cassette transporter ▪ lipoprotein metabolism ▪ obesity ▪ cardiovascular disease

Obesity induces dyslipidemia, and this may in large measure account for the associated increased risk of cardiovascular disease (CVD). Although the precise mechanisms whereby obesity results in dyslipidemia have not been fully established, experimental and human data suggest that the increased availability of cholesterol in the liver may increase the secretion of very low–density lipoprotein (VLDL) apolipoprotein B-100 (apoB-100) and decrease the expression of low-density lipoprotein (LDL) receptors. The kinetics of apoB metabolism in vivo, and specifically in obesity, may also depend on allelic variations in genes, such as apoE and apoB signal peptide, that regulate neutral lipid supply to the liver, the intrahepatic processing of apoB, and the clearance of apoB-containing lipoproteins from plasma.

Cholesterol homeostasis is a complex process that involves coordination of intestinal absorption, hepatic synthesis, and biliary excretion of cholesterol. Recent evidence has indicated that cholesterol absorption in humans has a major heritable component. Moreover, subjects who are high-cholesterol absorbers may be at increased risk of coronary disease. The recent identification of the ATP-binding cassette (ABC) G5 and G8 transporters has greatly advanced our understanding of molecular events in sterol absorption and transport. ABCG5 and G8 are hemitransporters that selectively limit intestinal absorption and promote biliary excretion of neutral sterols. Mutations in the genes encoding for ABCG5/G8 have been identified and linked to sitosterolemia. Allelic variations of the ABC transporter G8 may therefore control the availability of cholesterol in the liver and, by implication, the kinetics of apoB-containing lipoproteins in plasma. In this study, we hypothesized that ABCG8 gene polymorphisms would have independent effects on apoB kinetics in overweight/obese subjects.

Methods

Subjects
We studied 47 nonsmoking overweight/obese men selected from the community with body mass index (BMI) ranging from 25 to 40 kg/m². None had diabetes mellitus, apoE2/E2 or E4/E4 genotype, macroproteinuria, creatinemia (>120 μmol/L), hypothyroidism, abnormal liver enzymes, or consumed >30 g of alcohol per day. None reported a history of CVD or were taking agents affecting lipid...
metabolism. The study was approved by the ethics committee of Royal Perth Hospital.

Clinical Protocols
All subjects were admitted to a metabolic ward in the morning after a 14-hour fast. Venous blood was collected for measurements of biochemical analyses. A single bolus of $d_0$-leucine (5 mg/kg body weight) was administered intravenously, and blood samples were taken at baseline and after isotope injection at 5, 10, 20, 30, and 40 minutes, and 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, and 10 hours. Additional fasting blood samples were collected in the morning on the following 4 days of the same week. Diets were assessed for energy and major nutrients using at least 2 24-hour dietary diaries.

ApoB Kinetics Measurement
Laboratory methods for isolation and measurement of isotopic enrichment apoB have been described fully. Briefly, apoB in the VLDL and LDL fractions were separated by sequential ultracentrifugation, precipitated by isopropanol, delipidated, hydrolyzed, and derivatized. Isotopic enrichment was determined by ion monitoring of derivatized samples at a mass to charge ratio of 305 and 302. Tracer/tracer ratios were derived for each sample. Production, fractional catabolic rate (FCR), and percentage conversion of apoB were derived using multicompartmental modeling (SAAM-II).

Quantification of ApoB and Other Analyses
ApoB in VLDL and LDL fractions from the pooled plasma samples was isolated and determined by a modified Lowry method. Laboratory methods for measurements of lipids, lipoproteins, and other biochemical analyses have been detailed previously. Insulin resistance was estimated using the homeostasis model assessment (HOMA) score. Plasma lathosterol, sitosterol, and campesterol concentrations were measured by gas-liquid chromatography and expressed in mmol/L.$^{10}$ per mol/L cholesterol.$^{13,14}$

ABCG8 (T400K, D19H) and ApoE Genotyping
ABCG8 (exon 1 D19H, exon 8 T400K) genotypes were determined by polymerase chain reaction amplification using as forward primer 5’ AGG AAA CAG AGT GAA GAC ACT GG 3’ and as reverse primer 5’ AGA AAG GTT TGA TTT CTC CTA CCC 3’ (T400K); and for D19H forward primer 5’ ACA CCT GTG TGG AAA GGT AAG GT 3’ and reverse primer 5’ GCC GGT trichloroacetic acid GTA ATA AAA TGA CAG 3’ as described by Huback et al. ApoE genotype was determined as described by Hixson and Vernier.$^{15}$

Statistical Analysis
All analyses were performed using SPSS 10.1 (SPSS). Data were expressed as mean±SD or SEM. Group characteristics were compared by $t$ tests, after logarithmic transformation of skewed variables where appropriate. Normalized linkage disequilibrium coefficients (D’) were calculated as described previously. Associations were examined by multiple regression methods. Binary variables were used to describe ABCG8 genotype (ie, 0 for ABCG8 TT and 1 for ABCG8 TK alleles) and apoE genotype (ie, 0 for apoE2/3 and apoE3/3 and 1 for apoE3/4). Statistical significance was defined at the 5% level using a 2-tailed test.

Results
Table 1 shows the clinical and biochemical characteristics of the 47 men studied. On average, they were middle-aged, centrally obese, normotensive, insulin resistant, and dyslipidemic. With the exception of indices of lipid metabolism, there were no significant group differences in these characteristics according to ABCG8 genotype (Table 1). Average daily energy intake or the proportion of energy intake from carbohydrate, fat, protein, and alcohol did not differ between ABCG8 genotypes (data not shown). Allele frequencies of ABCG8 T400K were 0.86 (wild-type) and 0.14 (variant) and D19H were 0.92 (wild-type) and 0.08 (variant). Significant linkage disequilibrium was not found between T400K and D19H (D’=0.86; $P$=0.14). These data were comparable and consistent with other reports for white populations.$^{5,17}$

Table 1 also shows the plasma lipids, lipoproteins, apolipoproteins, and noncholesterol sterols in subjects according to ABCG8 genotype. No significant influence of the ABCG8 D19H polymorphism on any lipid or anthropometric parameter was found. Compared with those homozygous for the 400T allele (wild-type), TK individuals had significantly lower plasma triglyceride concentration and VLDL–apoB pool sizes ($P<0.01$). Intestinal absorption of cholesterol, as reflected by plasma campesterol and sitosterol to cholesterol ratio, was also significantly lower in the TK than in TT subjects. These differences remained significant after adjusting for age, BMI, and dietary fat intake ($P<0.05$). As shown in the Figure, heterozygous ABCG8 TK individuals had significantly lower VLDL–apoB production rate (PR) than TT homozygotes ($P=0.005$). The PR and FCR of LDL–apoB were significantly higher in the TK than in the TT group ($P<0.05$). However, the VLDL–apoB FCR did not differ between the groups ($P=0.459$). Conversion of VLDL–apoB to LDL–apoB was significantly higher in the TK than TT group (58±8 versus 33±3%; $P=0.001$).

Compared with non-apoE4 carriers, carriers of apoE4 allele had significantly higher plasma LDL cholesterol concentration ($P=0.005$) and LDL–apoB pool sizes ($P=0.004$). ApoE4 allele carriers had significantly lower LDL–apoB

<table>
<thead>
<tr>
<th>TABLE 1. Clinical and Biochemical Characteristics of the 47 Subjects</th>
<th>ABCG8</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TT</td>
</tr>
<tr>
<td>No. of subjects</td>
<td>37</td>
</tr>
<tr>
<td>Age (years)</td>
<td>54±7</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>110±1.5</td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>32±3</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>134±16</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>80±10</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>5.4±0.7</td>
</tr>
<tr>
<td>Insulin (IU/L)</td>
<td>32±11</td>
</tr>
<tr>
<td>HOMA score</td>
<td>7.9±3.5</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>5.8±0.9</td>
</tr>
<tr>
<td>Total triglyceride (mmol/L)</td>
<td>1.8±0.7</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/L)</td>
<td>1.0±0.2</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/L)</td>
<td>3.7±0.7</td>
</tr>
<tr>
<td>VLDL-apoB pool size (mg)</td>
<td>371±139</td>
</tr>
<tr>
<td>LDL-apoB pool size (mg)</td>
<td>2178±595</td>
</tr>
<tr>
<td>Campesterol†</td>
<td>266±118</td>
</tr>
<tr>
<td>Sitosterol†</td>
<td>133±89</td>
</tr>
<tr>
<td>Lathosterol†</td>
<td>250±86</td>
</tr>
</tbody>
</table>

Mean±SEM.  †Expressed as 10²×mmol/mol of cholesterol. HDL indicates high-density lipoprotein.
FCR than the non-apoE4 carriers (0.23±0.12 versus 0.29±0.12 pools per day; \( P=0.03 \)), with no significant difference in the PRs of both VLDL–apoB and LDL–apoB (14.8±5.8 versus 14.1±7.7 mg/kg per day and 6.0±2.6 versus 5.9±2.7 mg/kg per day, respectively; \( P>0.05 \)). The FCR of VLDL–apoB was 18% higher in the apoE4 than non-apoE4 carriers, but the difference failed to reach statistical significance (4.5±1.5 versus 3.8±1.1 pools per day; \( P=0.07 \)).

In multiple regression analysis including age, HOMA score and plasma concentrations of sitosterol and lathosterol, the ABCG8 TK and apoE3/E4 genotypes were independent and significant predictors of a lower hepatic secretion of VLDL–apoB (Table 2 model A; \( R^2=17\%; \ P=0.033 \)) and lower fractional catabolism of LDL–apoB (Table 2 model B; \( R^2=16\%; \ P=0.042 \)), respectively. Including BMI as an independent variable in both these models did not alter these findings (data not shown).

**Discussion**

This is the first study to demonstrate that ABCG8 (T400K) genotype may determine the metabolism of apoB in overweight/obese subjects. Our major finding was that compared with TT, ABCG8 TK individuals had lower VLDL–apoB PR and higher LDL production and FCRs. We also found that the ABCG8 genotype was a significant predictor of the PR of VLDL–apoB independent of age, apoE genotype, HOMA score, and plasma concentrations of sitosterol and lathosterol.

Previous studies of ABCG8 polymorphisms have examined their effect on plasma levels of sterols, insulin sensitivity, and plasma lipid response to statin therapy.\(^{5,6,17,18}\) We extend these reports by examining the influence of ABC transporter G8 polymorphisms on apoB kinetics. The precise molecular mechanisms whereby ABCG8 contributes to apoB metabolism are unclear. Polymorphisms in ABCG8 could contribute to variations in apoB metabolism by controlling liver cholesterol content. Yu et al have shown previously that overexpression of ABC transporter G8 promotes biliary cholesterol secretion and reduces fractional absorption of dietary cholesterol.\(^{19}\) The ABCG8 K variant may result in structural and physiochemical changes of the transporter that alters its physiological function, such as ATP binding or dimerization. We hypothesize that the ABCG8 K variant lowers intestinal cholesterol absorption and increases biliary cholesterol excretion. This would in turn decrease the cholesterol content in the liver with 2 potential consequences: first, a reduced secretion of VLDL–apoB particles, and second, upregulation of LDL receptor expression. As we have demonstrated here, these effects would accordingly decrease the secretion of VLDL–apoB and increase the removal of LDL–apoB from plasma. Consistent with these kinetic observations, we also found that compared with TT subjects, plasma triglyceride concentration and VLDL–apoB pool size were lower in TK individuals. However, the PR of LDL–apoB was higher in TK individuals, probably because of a preferential conversion of VLDL to LDL. Berge et al reported that there were no significant differences in plasma cholesterol between TT and TK/KK genotypes,\(^3\) and our findings (Figure) suggest that this may be attributable to compensatory changes in LDL production and catabolism.

We also confirm previous findings that carriers of apoE4 allele have significantly lower LDL–apoB FCR than non-
apoE4 carriers.20–22 In Hep G2 cells, apoE4 is associated with an increased affinity for LDL receptor.23 Hence, it is possible that this mechanism could enhance uptake of VLDL particles in apoE4 subjects, thereby increasing delivery of cholesterol to the liver and consequently downregulating the hepatic LDL receptor. This is consistent with our observation that apoE3/4 subjects tended to have higher VLDL FCR compared with non-apoE4 subjects. Our demonstration that apoE and ABCG8 polymorphisms could independently regulate apoB kinetics by apparently different molecular mechanisms is a new finding that requires further investigation, especially in relation to other genes that regulate lipid substrate supply to the liver and the intrahepatic processing of apoB.3,4

A high rate of cholesterol absorption influences dyslipidemia and risk of CVD.7 Our study provides a kinetic base for the role of ABCG8 genes in regulating lipoprotein metabolism in overweight or obese subject. Whether these genotypic associations determine the response of apoB metabolism to lifestyle changes (eg, weight loss, plant sterol, or fish oil supplementation) or pharmacotherapeutic interventions (eg, Ezetimibe or statins) in overweight/obese or other patient groups also merits examination. Further studies to examine the combined effects of genetic variation in ABC transporters G5 and G8 on intestinal absorption and biliary secretion of sterols and the corresponding relationships with lipoprotein kinetics, in particular HDL metabolism, are warranted.

Acknowledgments
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References
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