Increased Hepatic Secretion of Very-Low-Density Lipoprotein Apolipoprotein B-100 in Cholesteryl Ester Storage Disease

Michael H. Cummings¹ and Gerald F. Watts²

Using a stable isotope method, we measured the hepatic secretion rate of very-low-density lipoprotein apolipoprotein B-100 (VLDL apoB) in a 26-year-old woman who had dyslipidemia due to cholesteryl ester storage disease (CESD) and in five normolipidemic subjects. [1-¹³C]Leucine was administered by a primed constant intravenous infusion and the enrichment of VLDL apoB was determined by gas chromatography–mass spectrometry. The absolute secretion rate (ASR) of VLDL apoB in the patient was more than twice the mean ASR of the normolipidemic group (17.1 vs 8.0 ± 0.8 mg/kg body wt. per day). The plasma mevalonic acid concentration, a measure of intrahepatic cholesterol synthesis, was also greater in the patient than in the normolipidemic subjects (8.3 vs 4.4 ± 1.8 µg/L). The findings are consistent with the hypothesis that in CESD increased intrahepatic synthesis of cholesterol stimulates hepatic secretion of VLDL apoB and this may partly account for the dyslipidemia.

Indexing Terms: dyslipidemia/stable isotope analysis/metabolism/mevalonic acid/GC-MS

Cholesteryl ester storage disease (CESD) is a rare, autosomal recessive disorder characterized by deficiency of the lysosomal enzyme, cholesteryl ester hydrolase (1). In this condition, the plasma concentrations of both cholesterol and triglyceride may be elevated, but the mechanism by which this occurs is not fully established. Cholesteryl ester hydrolase deficiency reduces the intrahepatic conversion of cholesteryl ester to free cholesterol, which stimulates the rate of cholesterol biosynthesis (2). Persuaded rat liver experiments (3) and clinical studies (4) with inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A have suggested that intrahepatic cholesterol synthesis is a regulator of the secretion of very-low-density lipoprotein (VLDL) apolipoprotein B-100 (apoB), the transport protein for plasma lipids (5) and a risk factor for coronary artery disease (6). It is possible, therefore, that in CESD, increased intrahepatic cholesterol synthesis stimulates the hepatic secretion of VLDL apoB. In a previous report (2), Ginsberg et al. did not make clear whether the hepatic VLDL apoB secretion rate is increased in CESD; those authors did not refer to a normolipidemic control group (2) and they used a radioactive isotope method subject to methodological errors (7,8).

A relatively new approach for examining apoB metabolism involves stable isotopes (9, 10), which overcome many of the potential disadvantages of radioactive isotope methods (9). We report here our use of a stable isotope method to examine whether VLDL apoB secretion is increased in CESD.

Clinical Report and Laboratory Methods

We studied a 26-year-old woman with CESD, who was receiving a fat-modified diet (equivalent to the American Heart Association Step 1 diet) without medication, and five normolipidemic control subjects consuming an ad libitum diet. The patient had presented at age 9 years with abdominal pain and on examination at that time was noted to have hepatosplenomegaly. Her plasma total cholesterol concentration was 11.8 mmol/L (418 mg/dL); triglyceride, 2.71 mmol/L (240 mg/dL); and high-density lipoprotein (HDL) cholesterol, 0.85 mmol/L (30 mg/dL). Hepatic ultrasound revealed an echogenic liver consistent with fatty infiltration. A liver biopsy demonstrated accumulation of excessive cholesteryl esters, and CESD was diagnosed by the absence of cholesteryl esterase activity in mononuclear blood cells and liver tissue (1). The patient did not have a family history of premature coronary artery disease, and both parents and a sister were normolipidemic—thereby excluding primary hyperlipidemia. The physical and biochemical characteristics of the patient and control subjects are shown in Table 1. Neither the patient nor the controls had diabetes mellitus, proteinuria, hypothyroidism, or abnormal liver enzymes or were taking drugs known to affect lipid metabolism. All subjects provided informed written consent and the study was approved by West Lambeth Health Authority Ethics Committee.

The subjects were admitted to a metabolic ward at 0900 after a 12-h fast. They remained in a semirecumbent position and were allowed only water during the study. A cannula was placed in a superficial vein in the antecubital fossa of each arm, and through one of these...
Table 1. Physical characteristics and kinetic parameters of VLDL apoB metabolism of a patient with cholesteryl ester storage disease (CESD) and of five normolipidemic control subjects.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age, years</th>
<th>Sex</th>
<th>Weight, kg</th>
<th>Cholesterol, mg/dL*</th>
<th>Triglyceride, mg/dL*</th>
<th>HDL cholesterol, mg/dL*</th>
<th>Mevalonic acid, μg/L</th>
<th>VLDL apoB pool size, mg/day</th>
<th>Secretion rate of VLDL apo B</th>
</tr>
</thead>
<tbody>
<tr>
<td>CESD patient</td>
<td>26</td>
<td>F</td>
<td>60</td>
<td>277</td>
<td>222</td>
<td>15</td>
<td>8.3</td>
<td>109</td>
<td>9.4</td>
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<tr>
<td>Controls</td>
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<tr>
<td>1</td>
<td>28</td>
<td>F</td>
<td>66</td>
<td>156</td>
<td>36</td>
<td>57</td>
<td>3.3</td>
<td>23</td>
<td>24.1</td>
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<tr>
<td>2</td>
<td>42</td>
<td>M</td>
<td>74</td>
<td>190</td>
<td>125</td>
<td>34</td>
<td>2.3</td>
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<td>F</td>
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<td>68</td>
<td>7.4</td>
<td>17</td>
<td>26.6</td>
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<tr>
<td>5</td>
<td>49</td>
<td>M</td>
<td>72</td>
<td>201</td>
<td>80</td>
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<td>3.7</td>
<td>116</td>
<td>5.5</td>
</tr>
<tr>
<td>Mean</td>
<td>37.4</td>
<td>63.2</td>
<td>163.4</td>
<td>62.3</td>
<td>53.2</td>
<td>4.4</td>
<td>42</td>
<td>17.9</td>
<td>8.0</td>
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<tr>
<td>SD</td>
<td>(7.3)</td>
<td>(9.5)</td>
<td>(26.6)</td>
<td>(8.9)</td>
<td>(11.4)</td>
<td>(1.8)</td>
<td>(37)</td>
<td>(7.5)</td>
<td>(0.8)</td>
</tr>
</tbody>
</table>

FSR, fractional secretion rate.

* To convert to mmol/L, multiply cholesterol and HDL cholesterol × 0.02586, triglyceride × 0.01129.

[1-13C]leucine (Tracer Technologies, Somerville, MA) was administered by primed (1 mg/kg), constant (1 mg/kg per hour) infusion for 8 h. At 30-min intervals between 0 and 6 h, and at 7 and 8 h, 10 mL of blood was collected into K$_2$EDTA (0.34 mol/L) to determine isotopic enrichment of VLDL apoB. Blood samples (5 mL) were also collected into lithium heparin (15 IU/mL blood) every 2 h between 0 and 8 h to measure isotopic enrichment of α-ketoisocaproic acid (KIC), the deamination product of leucine, which provides a measure of intracellular leucine enrichment (11). At the beginning of the study, 10 mL of blood was collected into lithium heparin-containing tubes to measure plasma concentrations of lipid, lipoprotein, and mevalonic acid (MVA).

VLDL was isolated by preparative ultracentrifugation at a density of 1.006 kg/L. ApoB was precipitated with tetramethylurea and hydrolyzed. Leucine was then extracted from the hydrolysate by cation-exchange chromatography and derivatized with N-methyl-N-(tert-butyl)dimethylsilyl trifluoroacetamide (Aldrich, Dorset, UK). Isotopic enrichment of VLDL apoB and KIC was determined by positive-ion electron-impact ionization and selected-ion monitoring at m/z 303 and 302 (VLDL apoB) and at m/z 233 and 232 (KIC) by gas chromatography–mass spectrometry (TRIO-2, VG Biotech, Altrincham, UK; and MSD Model 5970A, Hewlett-Packard, Bracknell, UK, respectively). The interassay CV for VLDL apoB and KIC enrichment was <8%. Isotopic enrichment was expressed at atom percent excess, as described by Cobelli et al. (12). VLDL samples were pooled and VLDL apoB concentration was measured by the Lowry method (Sigma kit, Sigma Diagnostics, St. Louis, MO; and Phillips PU8700 Series UV/Visible spectrophotometer, Pye Unicam, Cambridge, UK). Kinetic parameters were derived by using a modified monoeponential function (11): E(t) = F[1 - e^(-kt + d)], where E(t) is the isotopic enrichment at time t, F is the precursor pool (KIC) enrichment, k is the fractional secretion rate of VLDL apoB, and d is the intrahepatic delay time. The standard errors of the parameter estimates, expressed as a percentage of the calculated values, were <15%.

The absolute secretion rate (ASR) of VLDL apoB in mg/kg per day was calculated as the product of the fractional secretion rate and the VLDL apoB pool size, where VLDL apoB pool size = mean VLDL apoB concentration × 4.5% body weight.

Plasma MVA concentration was measured by capillary gas chromatography–electron capture mass spectrometry as described elsewhere (13) (Sigma, Dorset, UK) with a 30-m DB5 capillary column (Jones Chromatography, Reading, UK), with an interassay CV <7.7%. Plasma cholesterol and triglyceride concentrations (mmol/L) were measured by enzymatic methods (Boehringer Mannheim, Mannheim, Germany) with a Cobas centrifugal analyzer (Roche, Welwyn Garden City, Hampshire, UK). HDL cholesterol was measured enzymatically (Boehringer Mannheim) after precipitating the apoB-containing proteins with dextran sulfate/magnesium chloride.

Results

Table 1 shows that the patient had high cholesterol and triglyceride concentrations and a lower HDL cholesterol concentration than the normolipidemic group. Fig. 1 shows the enrichment of the patient's VLDL apoB
and KIC with $^{13}$C-leucine. Steady-state isotopic enrichment of VLDL apoB was reached within 5 h; steady-state enrichment of KIC was achieved within 4 h and remained constant throughout the study. Table 1 shows that the VLDL apoB pool size and the ASR in the patient were more than twice the mean values of the normolipidemic group. The patient’s plasma MVA concentration was almost twice that of the control group.

Discussion

This is the first study in which a stable isotope and gas chromatography–mass spectrometry were used to demonstrate that VLDL apoB secretion and plasma MVA are increased in CESD. This finding supports our hypothesis that increased intrahepatic cholesterol synthesis stimulates VLDL apoB secretion. Our conclusion is consistent with evidence from both experimental and clinical studies (2–4).

In contrast to our finding, however, Ginsberg et al. (2) reported that the hepatic secretion of VLDL apoB in a 9-year-old girl with CESD was 12.2 mg/kg per day, reported within the normal adult reference range used by these authors. Their relatively lower VLDL apoB secretion rate may have been due to differences in patient characteristics or tracer methodology. Their patient was younger than ours and was below the 10th percentile for weight, factors that may influence VLDL apoB secretion rate (14). In addition, liver biopsy of their patient revealed evidence of cirrhosis, which may have impaired her liver function and decreased protein synthesis. Furthermore, they used a radioactive technique involving exogenous labeling, which might have given rise to methodological errors (7, 8)—e.g., chemical modification during harvesting of VLDL that could alter its metabolism in vivo; inability to achieve uniform labeling of all subspecies of VLDL, resulting in lower estimates of apoB transport; and indirect estimation of VLDL secretion from fractional catabolic rates. Although we are confident that our CESD patient did not have familial combined hyperlipidemia, her VLDL apoB flux rate was comparable with that seen in patients with the latter disorder (15). This suggests that increased intrahepatic synthesis of cholesterol may form a metabolic basis for familial combined hyperlipidemia.

Although stable isotopes overcome many of the disadvantages associated with exogenous radioactive labeling, certain assumptions are made when interpreting the isotopic enrichment data. By using the enrichment of plasma KIC as a measure of intracellular leucine enrichment, we assumed that plasma KIC is in equilibrium with intrahepatic KIC and that plasma KIC (and hence intrahepatic KIC) reflects the enrichment of hepatic leucine amino acyl tRNA, the direct precursor of apoB. These assumptions are, however, supported by experimental work demonstrating that in dogs there is equivalent enrichment of $^{13}$C in leucine from hepatic tissue and from plasma KIC (16). In addition, in vivo studies (15, 17) have shown that the steady-state isotopic enrichments of plasma KIC and VLDL apoB are highly correlated. To model the enrichment data, we used a modified monoexponential function that has been reported elsewhere (10, 18). In contrast, linear regression analysis has no physiological basis and underestimates lipoprotein fluxes (10). Multicompartamental modeling, which gives results comparable with those of monoexponential analysis (10), would have been appropriate had we measured the transport rates of other lipoprotein species. VLDL is the major apoB-containing lipoprotein secreted by the liver (5, 6) and, as stated in our original hypothesis, we were solely interested in measuring its hepatic secretion rate in CESD.

We have demonstrated that intrahepatic cholesterol synthesis, as determined by plasma MVA concentration (13), was greater in our patient than in our normolipidemic controls. This is consistent with the findings of Ginsberg et al. (2), who found an increased urinary MVA concentration in their patient with CESD. In CESD, deficiency of cholesteryl ester hydrolase results means less conversion cholesteryl ester to free cholesterol (1), thereby reducing the intrahepatic pool of free cholesterol. Consequently, there is a reciprocal increase in intrahepatic cholesterol synthesis. Given previous experimental and clinical studies (2–4), we hypothesized that this would stimulate secretion of VLDL apoB. Of particular relevance is evidence that the pharmacological inhibition of in vivo cholesterol synthesis in a patient with CESD decreased the hepatic secretion of VLDL apoB (2). We cannot rule out, however, that the availability of intrahepatic cholesteryl ester also contributed to the increased rate of VLDL apoB secretion. Inhibition of acyl CoA:cholesterol acyltransferase achieves parallel reductions of cholesteryl ester synthesis and of VLDL apoB secretion in HepG2 cells (19). We have also recently demonstrated increased hepatic secretion rates of VLDL apoB in patients with heterozygous familial hypercholesterolemia (20), who are known to have normal rates of cholesterol synthesis but increased hepatic uptake of cholesteryl esters via the receptor-independent pathway. We have not examined the production rate of LDL apoB, but we would expect this to be increased, given that at least two-thirds of low-density lipoprotein (LDL) apoB is derived from VLDL apoB in CESD (2). Increased direct hepatic secretion of LDL apoB has also been shown in CESD (2), consistent with our original hypothesis. However, evidence for de novo production of LDL apoB by the liver in human subjects has recently been challenged (21). As in other reports, our patient had a low concentration of HDL cholesterol. The mechanism by which this occurs is not fully understood but may be a consequence of hypertriglyceridemia or increased activity of hepatic lipase (1).

In conclusion, secretion of VLDL apoB is increased in CESD. This increase may contribute to the greater plasma lipid concentrations and, consequently, the greater risks of coronary artery disease observed in this condition (22). Our findings are also consistent with the hypothesis that apoB secretion is constitutively expressed and that its rate of hepatic secretion is determined by substrate availability (15). The hepatic avail-
ability of free cholesterol (2, 4), however, may be as important a rate determinant of apoB secretion as is the availability of cholesteryl esters (19, 20).

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References


