Apoprotein C-III deficiency markedly stimulates triglyceride secretion in vivo: comparison with apoprotein E

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Hirano, Tsutomu, Toshihiro Takahashi, Shigenobu Saito, Hiroto Tajima, Tetsu Ebara, and Mitsuru Adachi. Apoprotein (apo) C-III deficiency markedly stimulates triglyceride secretion in vivo: comparison with apoprotein E. Am J Physiol Endocrinol Metab 281: E665–E669, 2001.—Apoprotein (apo) C-III plays an important role in the development of hypertriglyceridemia by inhibiting triglyceride (TG) removal. However, the effect of apo C-III on TG production remains unclear. We measured TG secretion rate (TGSR) in apo C-III gene-disrupted (apo C-III-null) mice to investigate the influence of this protein on TG turnover. TGSR measured by the Triton WR-1339 method was increased twofold in these mice compared with wild-type (WT) mice. Obesity was induced by the injection of gold-thioglucose (GTG), which made the WT mice hypertriglyceridemic due to a threefold increase of TGSR. However, TG-induced obesity failed to increase TG in apo C-III-null mice, although TGSR was increased 10-fold, suggesting substantial stimulation of TG removal. Apo E-null mice were severely hypercholesterolemic but were not hypertriglyceridemic, and TGSR was rather decreased. GTG-induced obesity made these mice hypertriglyceridemic because of TG overproduction to an extent similar to that seen in WT mice. These results suggest that apo C-III deficiency potently enhances TG turnover, especially when TG production is stimulated, and that apo E deficiency is not always rate limiting for TG production.

Apoprotein C-III; apoprotein E; triglyceride secretion; mice

MATERIALS AND METHODS

Mice. We used homozygous apo C-III-deficient mutants originally established by Maeda et al. (18) and homozygous apo E-deficient mutants originally established by Breslow’s laboratory [see Ito et al. (12)]. Apo C-III-null and apo E-null mice (both are C57 BL/6J background) and WT (C57 B/6J) mice were purchased from Jackson Laboratory (Bar Harbor, ME), and the animals were mated in our laboratory. In this study, we used male mice only to avoid the effect of sex differences on lipoprotein metabolism. GTG (500 mg/kg body wt) was injected intraperitoneally into mice at the age of 6 wk, and obese mice were used 14 wk after the injection. All mice were kept on a rotating 12:12-h light-dark cycle and given free access to food and water. The mice were fed ad libitum standard rat chow (Oriental Food Tokyo, Japan).

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which contained 60% vegetable starch, 11% corn oil, and 29% animal protein. Food was withdrawn at 9:00 AM on the day of the experiments, and all experiments were carried out after a 5-h fast. Animals were anesthetized with methoxyflurane for retroorbital phlebotomy and intravenous femoral injection.

**Triglyceride secretion rate.** The triglyceride secretion rate (TGSR) was determined by measuring the increase in plasma TG concentration after an intravenous injection of Triton WR-1339 (Sigma Chemical, St. Louis, MO; 500 mg/kg body wt, 25% solution in saline). Mice were anesthetized with pentobarbital sodium (0.4 mg/100 g body wt; Nembutal, Dainabot, Osaka, Japan), and blood was collected immediately before Triton WR-1339 injection and at 30, 60, and 90 min thereafter. Plasma TG concentration was found to increase linearly ($r = 0.98$) over the 90-min period in each mouse. TGSR was calculated from the increment in TG concentration per minute multiplied by the plasma volume of mouse (estimated by 0.035% of body wt in g) and expressed in milligrams per minute. The validity of the Triton method for estimating TGSR has been described elsewhere (8, 15, 22, 25). A major- 

**Measurements.** Plasma TG concentration was determined by the enzyme method with a commercially available kit (Triglyceride-G test, Wako Pure Pharmaceutical, Osaka, Japan). Plasma glucose levels were determined by the glucose oxidase method (Glucose B-test, Wako Pure Pharmaceutical). Immuno-reactive insulin (IRI) concentrations were determined by ELISA assay kit (Morinaga, Tokyo, Japan) standardized against mouse insulin.

**Statistics.** Data are expressed as means ± SD. Statistical analyses were performed with the Stat View J-4.5 for the Macintosh system. Statistical significance was assessed by one-way ANOVA, and $P < 0.05$ was accepted as a significant difference.

**RESULTS**

Table 1 shows the body weight and the plasma levels of total cholesterol, glucose, and insulin. GTG injection induced a similar degree of weight gain in WT, apo C-III-null, and apo E-null mice. The total cholesterol level increased significantly in WT mice when obesity developed. In contrast, this was not observed in apo C-III-null mice, so total cholesterol levels were similar in lean and obese mice lacking apo C-III. Apo E-null mice showed marked hypercholesterolemia, with a cholesterol level that was 10 times higher than in WT mice. GTG-induced obesity caused even more pronounced hypercholesterolemia in apo E-null mice, with cholesterol levels that were 20- and 10-fold higher than those in lean or obese WT mice, respectively. Plasma glucose levels were comparable among WT, apo C-III-null, and apo E-null mice, and experimental obesity did not induce significant hyperglycemia in any group of mice. Plasma IRI levels were increased threefold in obese mice over lean mice, regardless of the group.

![Fig. 1. Plasma triglyceride (TG) levels in wild type (WT), apoprotein (apo) C-III-null, and apo E-null mice with (obese) and without obesity (lean). Mice were made obese by the ip injection of gold-thioglu- cose (GTG; 500 mg/kg body wt) and were used 14 wk after the injection. Data represent means ± SD. $P < 0.05$. *Obese vs. lean; †vs. WT; ‡vs. apo C-III-null mice.](http://ajpendo.physiology.org/)

**Table 1. Characteristics of wild type, apo C-III-null, and apo E-null mice with and without obesity**

<table>
<thead>
<tr>
<th></th>
<th>Wild Type</th>
<th>Apo C-III-Null</th>
<th>Apo E-Null</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lean ($n = 13$)</td>
<td>Obese ($n = 6$)</td>
<td>Lean ($n = 14$)</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>26 ± 3</td>
<td>44 ± 9*</td>
<td>26 ± 1</td>
</tr>
<tr>
<td>Total cholesterol, mg/100 ml</td>
<td>44 ± 18</td>
<td>73 ± 21*</td>
<td>43 ± 11</td>
</tr>
<tr>
<td>Glucose, mg/100 ml</td>
<td>147 ± 27</td>
<td>154 ± 75</td>
<td>107 ± 42</td>
</tr>
<tr>
<td>IRI, μU/ml</td>
<td>4.3 ± 3.9</td>
<td>11.9 ± 2.3*</td>
<td>3.6 ± 2.6</td>
</tr>
</tbody>
</table>

Data represent means ± SD; $n$, no. in group. Apo C-III-null or apo E-null, apoprotein C-III or E gene disrupted; IRI, immunoreactive insulin. $P < 0.05$: *obese vs. lean; †vs. wild type; ‡vs. apo C-III-null.
1). In contrast to apo C-III-null mice, apo E-null mice became severely hypertriglyceridemic after obesity was induced, and the TG levels in obese apo E-null mice were comparable to those in obese WT mice (145 ± 42 vs. 106 ± 27 mg/100 ml).

TGSR data obtained by the Triton WR-1339 method are shown in Fig. 2. GTG-induced obesity substantially increased the TGSR in WT mice, with the TGSR of obese WT mice being 6.5-fold greater than that of lean WT mice (0.072 ± 0.018 vs. 0.011 ± 0.008 mg/min, P < 0.0001). Apo C-III-null mice had a twofold higher TGSR than in WT mice (0.022 ± 0.013 vs. 0.011 ± 0.008 mg/min, P < 0.02). Similar to WT mice, there was a dramatic increase of the TGSR in obese apo C-III-null mice until it was 6.5-fold greater than that in lean apo C-III-null mice (0.143 ± 0.047 vs. 0.022 ± 0.013 mg/min, P < 0.0001). In contrast, apo E-null mice had a lower TGSR than WT mice, although this difference did not attain statistical significance (0.006 ± 0.003 vs 0.011 ± 0.008 mg/min, P = 0.07). Obesity substantially elevated the TGSR of apo E-null mice until it was 14 times greater than in their lean counterparts (0.087 ± 0.063 vs 0.006 ± 0.003 mg/min, P < 0.0001).

FCR data estimated from the TGSR and the TG pool size are shown in Fig. 3. FCR was 2.7-fold greater in obese WT mice than in lean WT mice (0.046 ± 0.013 vs. 0.017 ± 0.009 min⁻¹, P < 0.0001). Apo C-III-null mice had a fivefold higher FCR than WT mice (0.085 ± 0.096 vs. 0.017 ± 0.009 min⁻¹, P < 0.02). Obesity doubled the FCR in apo C-III-null mice (0.197 ± 0.040 min⁻¹, P < 0.05). Thus the FCR of obese apo C-III-null mice was 11.6-fold greater than that of lean WT mice. The FCR of apo E-null mice was similar to that of WT mice (0.014 ± 0.007 vs. 0.017 ± 0.009 min⁻¹). Obesity caused the FCR to increase by twofold in apo E-null mice (0.034 ± 0.016 vs. 0.014 ± 0.007 min⁻¹, P < 0.01), as it did in WT mice or apo C-III-null mice.

**DISCUSSION**

Maeda et al. (18) first established apo C-III-null mice and studied TG metabolism in these animals. They reported that the plasma clearance of radiolabeled chylomicron TG was significantly faster in apo C-III-null mice than in WT mice. They also demonstrated that the postprandial increase of TG after a fat load was substantially suppressed in apo C-III-null mice. Conversely, some studies have shown that human apo C-III transgenic mice develop severe hypertriglyceridemia due to a defect in TG removal (1, 3, 4). These results suggest that apo C-III may play a critical role in TG clearance from the plasma. Aalto-Setala et al. (1) reported that TG production determined by the Triton WR-1339 method is increased by about twofold in transgenic mice with high expression of human apo C-III. However, the modest increase of TG production cannot explain the 6- to 20-fold higher plasma TG level in these mice, suggesting again that defect in TG removal is the main reason for their hypertriglyceridemia. On the other hand, the influence of apo C-III deficiency on TG production remains unknown. If apo C-III itself has the power to increase TG production, as demonstrated in apo C-III transgenic mice, the lack of this apoprotein might reduce TG production. Contrary to this speculation, we found that the TGSR was remarkably increased in apo C-III-null mice. How does apo C-III deficiency stimulate TG production? We can
suggest the following hypothesis: because apo C-III inhibits the uptake of TGRL particles by the liver (23), the lack of this apoprotein may increase hepatic TG influx and subsequently stimulate VLDL secretion to prevent excess accumulation of TG in the liver. Because exogenous VL DL directly stimulates nascent VLDL production by hepatocytes (24), it is not surprising that increased influx of VLDL into the liver can stimulate VLDL production in vivo.

A number of studies have demonstrated that VLDL production is significantly stimulated by obesity (5, 11, 15, 21, 22). We previously examined TG kinetics in ventromedial hypothalamic (VMH)-lesioned rats, a representative experimental animal model of obesity (22). We found that the TGSR is markedly elevated in these obese animals without any defect of TG removal. Similar to VMH lesions, GTG injection causes obesity by destroying the satiety center in the hypothalamus (7). We explored the effect of apo C-III deficiency on TG production when it was stimulated by the development of obesity. As expected, obese mice had higher plasma TG levels and a severalfold increase of TGSR compared with their lean controls. In contrast to WT mice, plasma TG and cholesterol levels were not increased in apo C-III-null mice when obesity was induced. These findings suggest that apo C-III plays an important role in the development of hyperlipidemia associated with obesity. Although plasma TG remained lower in obese apo C-III-null mice, TG production was markedly stimulated. These results may support our finding that apo C-III deficiency stimulates TGSR in vivo.

Recent gene-targeting animal studies have revealed that apo E plays a significant role in VLDL production, because the TGSR increases along with the expression of apo E-III in human apo E-III transgenic mice or rabbits (9, 10). Studies of apo E-null mouse hepatocytes have demonstrated that absence of hepatic apo E expression is associated with a 50% reduction of VLDL production (16). Similar to previous reports (20), we found that the TGSR of apo E-null mice was lower than that of WT mice, although this difference was not significant. However, TGSR was markedly increased in apo E-null mice after obesity was induced, suggesting that apo E deficiency lost its suppressive effect on VLDL production when such production was strongly stimulated. This may imply that apo E does not always have a rate-limiting role in VLDL synthesis.

The plasma TG level reflects the steady-state rate of TG turnover. Because TG removal from the circulation was blocked by Triton WR-1339, it was not possible to determine both the entry and removal of TG in the same mice. However, estimation of the FCR for plasma TG is possible from the plasma TG pool size and TGSR (2). In support of this, the capacity for VL DL-TG removal is similar when estimated by the Triton method and by a tracer method using radiolabeled glycerol or fatty acids (2, 8). As expected, apo C-III-null mice had a substantially higher FCR than WT mice. Our apo E-null mice were slightly hypotriglyceridemic due to a decrease of TGSR, so the FCR was virtually normal. It remains unknown why apo E deficiency does not impair TGRL removal in these mice. Obesity caused FCR to double in all groups of mice. Although we did not measure the activity of lipoprotein lipase, a key enzyme in TG catabolism, Inoue et al. (11) reported that its activity is significantly increased in VMH-lesioned obese rats. Because GTG-induced obesity is a model of hypothalamic-lesioned obesity similar to that of VMH-lesioned rats, lipoprotein lipase activity should also be increased in these mice.

The present study demonstrated that apo C-III-null mice have high TG production and plasma clearance rates, and this was more clearly observed when VLDL production was stimulated by the induction of obesity. These results suggest that apo C-III strongly regulates the overall speed of TG turnover. Physiologically, apo C-III may play an important role in suppressing futile TG cycling from liver and back to liver, thereby providing more TG to peripheral tissues. Apo E may play a significant role in VLDL production, as demonstrated elsewhere, but our results suggest that apo E is not always essential for VLDL production. Therefore, we conclude that apo C-III deficiency rather than apo E deficiency has a stronger effect on TG production in vivo.

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REFERENCES


