Altered triglyceride-rich lipoprotein production in Zucker diabetic fatty rats

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Chirieac, Doru V., Heidi L. Collins, Joanne Cianci, Janet D. Sparks, and Charles E. Sparks. Altered triglyceride-rich lipoprotein production in Zucker diabetic fatty rats. Am J Physiol Endocrinol Metab 287: E42–E49, 2004. First published February 17, 2004; 10.1152/ajpendo.00297.2003.—Triglyceride-rich lipoprotein (TRL) production was studied in Zucker diabetic fatty (ZDF) rats, a model of insulin-resistant type 2 diabetes progression. TRL production was measured in vivo by blocking catabolism with Triton WR-1339. Ten-week ZDF rats are hyperinsulinemic with increased TRL production [both triglyceride and apolipoprotein B (apoB)]. Twenty-week ZDF rats are insulinopenic, and TRL production is similar to lean controls. Insulin infusion suppresses glucose and free fatty acids in 10- and 20-wk ZDF rats. Increased TRL production is not reduced by insulin in 10-wk rats; however, at 20 wk, TRL production is suppressed by insulin. In vitro studies with hepatocytes derived from 10-wk ZDF rats showed minimal insulin dose effects on apoB secretion compared with the response and sensitivity of hepatocytes derived from 20-wk ZDF and control lean rats. Hepatic sterol regulatory-binding protein (SREBP)-1c mRNA levels are increased at 10 wk but return to control levels at 20 wk. ApoB mRNA levels are similar to lean controls at 10 and 20 wk. The following two mechanisms for hypertriglyceridemia associated with hyperinsulinemia are suggested: increased TRL synthesis and loss of TRL suppression. Increased triglyceride production in hyperinsulinemic rats likely relates to increased expression of SREBP-1c, whereas increased apoB production involves posttranscriptional processes.

hyperinsulinemic diabetes; insulinopenic diabetes; apolipoprotein B metabolism; triglyceride-rich lipoprotein production

HYPERTRIGLYCERIDEMIA IS A KEY COMPONENT OF METABOLIC SYNDROME AND TYPE 2 DIABETES AND IS STRONGLY CORRELATED WITH INCREASED RISK OF CARDIOVASCULAR DISEASE (12). Insulin increases the intracellular availability of triglyceride (TG) for very low density lipoprotein (VLDL) production (1), possibly through enhanced lipogenesis (23). Pancreatic availability of insulin is diminished as disease progresses, with late-stage diabetes being characterized by insulinopenic hyperglycemia. Previous in vitro studies performed in cultured hepatocytes isolated from fructose-fed, insulin-resistant rats demonstrate blunting of the acute suppressive effects of insulin on VLDL secretion (37). Similar results are observed in hepatocytes from insulin-resistant obese rats (31). Furthermore, insulin fails to suppress hepatic VLDL apolipoprotein B (apoB) production in diabetic patients (16) and in hyperinsulinemic individuals during euglycemic clamp experiments (13, 21). It is known that, during late-stage diabetes, pancreatic insulin release is progressively diminished; however, the effect of insulin on liver VLDL production during this transition from hyperinsulinemia to insulinopenia is not known.

Hepatic expression of key enzymes involved in lipogenesis and cholesterogenesis is regulated by a family of transcription factors known as sterol regulatory element-binding proteins (SREBP; see Ref. 9). SREBP-1c is mainly involved in regulation of TG-synthesizing genes, whereas SREBP-2 has its main effect on cholesterol-synthesizing genes. SREBP-1c has been implicated as a mediator of insulin action in hepatic lipogenesis (19). In insulin-resistant states, hyperinsulinemia increases lipogenesis mediated by increased SREBP expression (9). Two mechanisms are believed to increase production of VLDL in obesity and type 2 diabetes. First, SREBPs stimulate the expression of lipogenic enzymes that increase lipid content of VLDL (8), and, second, resistance to insulin-mediated suppression of apoB production increases the amount of apoB available for packaging of lipids, with the net result of more secretory VLDL particles (14). The current study examines the mechanisms involved in hypertriglyceridemia of type 2 diabetes during the early stage of the disease characterized by hyperinsulinemia compared with the late insulinopenic stage. Progression of type 2 diabetes in the male ZDF rat model has been previously characterized. Beginning at 7 wk of age, ZDF rats become progressively hyperglycemic and are fully diabetic by 10 wk. This stage, characterized by hyperglycemia and hyperinsulinemia, mimics early human type 2 diabetes. As ZDF rats age, there is a progressive loss of pancreatic insulin production, and, by 20 wk of age, ZDF rats become insulinopenic (4, 29).

To measure hepatic triglyceride-rich lipoprotein (TRL) production rates, we employed Triton WR-1339, a nonionic detergent that strips TRL particles of cofactors and prevents them from being catabolized (2). After detergent treatment, TRL particles secreted by the liver accumulate in the plasma in a linear fashion that allows quantitation of TRL production rate in vivo. TRL TG and apoB production rates were determined in 10- and 20-wk ZDF rats, and results were compared with age-matched lean controls. In parallel experiments, hepatic expression of apoB and SREBP mRNAs was evaluated in 10- and 20-wk ZDF rats. The ability of insulin to suppress TRL production in hyperinsulinemic and insulinopenic ZDF rats was assessed by measuring production rates during insulin infusion at levels demonstrated to reduce plasma glucose and free fatty acids (FFA). Our results suggest that the mechanisms responsible for hypertriglyceridemia are substantially different.
in 10-wk compared with 20-wk ZDF rats. The suggested mechanism for increased TG production during the hyperinsulinemic phase is lipogenic induction via SREBP-1c expression, whereas increased apoB production occurs via posttranscriptional mechanisms. Moreover, hyperinsulinemic 10-wk ZDF rats are resistant to the acute inhibitory effects of insulin. Hypertriglyceridemia in 20-wk insulinopenic ZDF rats is most likely the consequence of altered TRL catabolism.

METHODS

Animals. Protocols used in this study were approved by the University Committee on Animal Resources, University of Rochester. Male Zucker diabetic fatty (ZDF/Gmi, fa/fa) rats and their lean controls (fa/+) were obtained from Genetic Models (Indianapolis, IN) at 7 (group 1) and 15 (group 2) wk of age. Rats were housed in single cages on a 12:12-h light-dark cycle with free access to standard rodent chow (Purina 5008) and drinking water until reaching 9–11 wk (group 1) or 19–20 wk (group 2) of age. Body weights of ZDF rats used in the study averaged 339 ± 22 g (group 1) and 383 ± 30 g (group 2). Similarity in weight of 10- and 20-wk ZDF rats may be related to the failure to thrive associated with hyperinsulinemia in 20-wk ZDF rats.

Measurement of lipoprotein production. Rats were fasted for 4 h and then between 1400 and 1600 were anesthetized with ketamine (80 mg/kg) and xylazine (10 mg/kg) and fitted with carotid (polyethylene-10; Clay Adams) and jugular (22-gauge; Intracath) catheters (Becton-Dickinson, Sandy, UT). Anesthesia was maintained throughout the 3-h experimental period. The arterial line was connected to an infusion pump, and saline was infused at a rate of 1.5 ml/h. The venous line was flushed with a small volume of heparinized saline (20 U/ml) and stoppered with a Luer-lock injection site adapter (Baxter HealthCare, Englewood, NJ). Serum apoB was quantitated by monoclonal RIA (27), and results were corrected for protein content per dish.

Measurement of apoB degradation in primary hepatocytes. ApoB pulse-chase studies were carried out in primary cultures of hepatocytes derived from ZDF rats, as previously described (30). Briefly, hepatocytes were prepared and incubated overnight 12–14 h in Waymouth’s medium containing 0.1 nM insulin (basal conditions). Cells were then incubated in media containing either 0 or 100 nM insulin for 3 h. Afterward, monolayers were rinsed three times with methylene-free, cysteine-free, and cystine-free Waymouth’s medium containing 0.2% (wt/vol) BSA (depletion medium) and incubated in depletion medium with and without insulin for 30–45 min. Afterward, 150–300 μCi of 35S label (EXPRE35S35S-Protein labeling mix, specific activity >1,000 Ci/mmol; New England Nuclear, Boston, MA) were added to each dish, and incubation was continued for exactly 15 min. Immediately thereafter, media were aspirated, and monolayers were rinsed two times with cold (4°C) Waymouth’s medium containing 5 mM L-methionine and 2.5 mM L-cysteine (chase medium) and reincubated in chase medium with and without insulin for 15 min to complete elongation of full-length apoB (total apoB synthesis) or for 180 min (recovered apoB). ApoB degradation was calculated by subtracting total 35S-labeled apoB recovered after 180 min of chase from 35S-labeled apoB synthesized from the pulse. Monolayers were collected at each time point and scraped three times in cold (4°C) HBSS and frozen in liquid nitrogen. Media samples were preserved by addition of 1% (vol/vol) Protease Inhibitor Cocktail I (Calbiochem-Novabiochem, La Jolla, CA). Monolayers were scraped in lysis buffer (25 mM Tris-HCl, 20 mM phosphate buffer, pH 7.4), containing 300 mM NaCl, 1% (vol/vol) Nonidet P-40, 0.5% (wt/vol) sodium deoxycholate, 5 mM benzamidine (freshly added), 1.6% (vol/vol) TrasyloL VLE (Miles Laboratories, Kankakee, IL), and 1% (vol/vol) Protease Inhibitor Cocktail I. Cell debris was removed by centrifugation at 4°C for 15 min at 12,000 rpm, and a small aliquot was removed for protein determination (17). 35S-labeled apoB was specifically immunoprecipitated from cell and media samples by addition of rabbit anti-rat apoB and incubation overnight at 4°C with constant mixing. Afterward, protein A-Sepharose (Amersham Pharmacia Biotechnology) was added, and incubation continued for 4 h at 4°C again with constant mixing. Immunoprecipitates were collected by slow-speed centrifugation and washed as described by Lodish and Kong (15). After removal of the last wash, labeled proteins were eluted in Laemmli’s buffer (11) containing freshly added dithiothreitol (final concentration, 10 mM). Labeled proteins were separated by SDS-PAGE using gradient Acrylamide/ acrylamide gels cast on GelBond PAG film (32). After electrophoresis, gels were heat fixed in a convection oven by heating for 45 min at 190°F (33). ApoB radioactivity in heat-fixed gels was quantified by PhosphorImager analysis (Molecular Dynamics, Sunnyvale, CA).

Biochemical analyses. Serum glucose was measured by a colorimetric assay employing glucose oxidase (Sigma Diagnostics). Serum cholesterol was quantitated using a cholesterol oxidase method, and serum TG was measured by assay of glycerol released after lipase treatment, both being determined using commercial kits (Sigma Diagnostics). Serum FFAs were determined by kit (Wako FFA kit, Englewood, NJ). Serum apoB was quantitated by monoclonal RIA employing a monoclonal antibody equally reactive to B100 and B48 (27). The first 30 min after Triton WR-1339 injection are required for detergent equilibration and initiation of lipoprotein accumulation (18). Production rates for individual rats were therefore calculated using the linear increment between 30 min and 3 h. Steady-state TG and apoB levels of 10- and 20-wk ZDF rats have been reported previously (29). Pearson’s linear correlation coefficient (r) for TG, cholesterol, and apoB averaged (mean ± SD) 0.98 ± 0.02, 0.95 ± 0.05, and 0.94 ± 0.04, respectively. Average production rates (n = 3 animals per condition) were compared using Student’s t-test for unpaired samples. The mass of serum B48 and B100 was determined by immunoblotting, as described previously (2). Rabbit anti-rat apoB was the primary anti-
Fig. 1. Triglyceride (A) and apolipoprotein B (apoB; B) production rates in 10- and 20-wk Zucker diabetic fatty (ZDF) rats after Triton WR-1339 injection. Rates were determined by the slope of the linear regression line from 30 min to 3 h after Triton WR-1339 injection. Results are averages ± SD indicated by the nos. above bars. *Significant difference from lean and 20-wk animals (P < 0.05). Nos. inside bars are production rates shown on graphs; n, no. of rats.

Results

TG and apoB production rates in 10- and 20-wk ZDF rats. Hepatic TRL production rates of 10- and 20-wk ZDF rats were determined by measuring TG and apoB accumulation in serum after Triton WR-1339 injection (Fig. 1). TG and apoB production rates of lean 10- and 20-wk rats were similar, and values were averaged. TG and apoB production were increased by 120 and by 74%, respectively, in 10-wk ZDF rats compared with lean rats. In contrast, no significant differences in TG and apoB production rates were observed in 20-wk ZDF rats compared with lean controls.

Effect of insulin infusion on serum glucose and FFA levels in 10- and 20-wk ZDF rats. To study the effect of insulin, we designed experiments so that infusion of insulin achieved significant suppression of both glucose and FFAs (Fig. 2). Insulin infusion produced a progressive decline in serum glucose in 10- (A) and 20 (B)-wk ZDF rats, becoming significantly lower than saline infusion by 60 min in both cases (P < 0.04; Fig. 2). After 3 h of insulin infusion, serum glucose levels were lowered to 41.8 ± 18.9% of initial values in 10-wk ZDF rats.
Serum FFAs fell rapidly during the first 30 min of insulin infusion to 44.8 ± 21.7% of initial values in 10-wk ZDF rats and to 57.2 ± 31.2% in 20-wk ZDF rats (P < 0.03). The progressive decline in serum glucose and rapid suppression of serum FFA by insulin demonstrated that insulin action on glucose and FFA was achieved in vivo during infusion.

**Effect of insulin on TG and apoB production rates in 10- and 20-wk ZDF rats.** TG and total apoB production rates were similar in saline- and insulin-infused 10-wk ZDF rats (Fig. 3). In contrast, TG production in 20-wk ZDF rats was significantly inhibited by insulin by 48% (P < 0.01; Fig. 3). Insulin infusion also significantly depressed the apoB production rate by >95% in 20-wk ZDF rats (P < 0.02; Fig. 3).

**Insulin infusion and B100 and B48 ratios in 10-wk ZDF rats.** Unlike human liver, rat liver secretes TRL particles that contain a molecule of either B100 or B48 (6). Because no change in total apoB was observed with insulin infusion in 10-wk ZDF rats, serum B100 and B48 were measured by immunoblotting to determine whether insulin altered the proportion of B100 vs. B48 particles secreted. Paired serum samples from saline- and insulin-infused 10-wk ZDF rats were coelectrophoresed on the same gel alongside purified rat apoB standards for immunoblotting analysis (Fig. 4). B48 and B100 accumulated in serum over the 3-h time course in both saline- and insulin-infused 10-wk ZDF rats. By the end of 3 h, accumulated B48, B100, and total apoB were similar in saline- and insulin-infused ZDF rats. No difference was observed between the B48-to-B100 ratio in saline-infused vs. insulin-infused 10-wk ZDF rats in three paired blotting studies (4.2 ± 1 vs. 3.1 ± 1.2, P > 0.05).

**Insulin effects on apoB secretion by hepatocytes derived from 10- and 20-wk ZDF rats.** To evaluate specifically the role of liver in insulin-suppressive effects on apoB independent of intestine, we performed studies in primary cultures of hepatocytes derived from ZDF rats using methods similar to those previously described (28, 30, 31). The average percent reduction in secreted apoB from hepatocytes derived from ZDF rats at each insulin dose was plotted against the initial concentration of insulin added to medium (Fig. 5). For comparison, insulin dose-response curves for hepatocytes derived from Zucker obese (fafa) rats and lean Fa? Zucker rats are included (31). The dose-response curve of the inhibitory effect of insulin on apoB secretion was attenuated significantly in hepatocytes derived from 10-wk ZDF rats compared with lean controls, comparable to results obtained from hepatocytes derived from Zucker obese rats. In contrast, the dose-response curve generated from 20-wk ZDF hepatocytes was similar to that obtained from hepatocytes derived from lean rats.

Hepatocytes derived from 10-wk ZDF rats demonstrated a rightward shift in the dose-response curve (decreased insulin
sensitivity) and a significant decrease in the maximum percent apoB inhibition (decreased responsiveness). The insulin concentration at which 50% of the maximum inhibitory effect on apoB secretion is achieved in 10-wk ZDF rats was calculated at 50–60 nM, comparable to the 40-nM value reported for Zucker obese rats. This is significantly greater than the 4- to 5-nM concentration calculated for hepatocytes derived from 20-wk ZDF rats and also greater than the 1-nM concentration reported for hepatocytes derived from lean rats (31). The maximum inhibition of apoB secretion achieved by 1,000 nM insulin in hepatocytes derived from 10-wk ZDF rats was 19.9% and from 20-wk ZDF rats was 40%.

**Effect of insulin on apoB degradation in hepatocytes derived from 10-wk ZDF rats.** The decrease in apoB secretion mediated by insulin in control hepatocytes is partly the result of enhanced intracellular degradation of newly synthesized apoB (30). To examine intracellular apoB degradation in 10-wk ZDF rats, pulse-chase studies were performed in primary cultures of hepatocytes. Hepatocytes were incubated in medium with and without 100 nM insulin for 3 h followed by incubation with $^{35}$S label for 15 min to label nascent apoB (synthesized apoB). After labeling medium was removed, cells were reincubated for 15 and for 180 min in chase medium with and without insulin, and $^{35}$S-labeled apoB in cells and medium was assessed. The difference between apoB that was synthesized from the pulse and that recovered at 180 min after chase represents the percentage of apoB degraded. In two independent experiments using hepatocytes derived from lean rats, B100 degraded in response to insulin averaged 50.4% (57.6 and 43.1%), whereas B48 degraded averaged 23.2% (25.1 and 21.3%). In the corresponding experiments using hepatocytes derived from 10-wk ZDF rats, neither B100 nor B48 was degraded to any great extent in response to insulin incubation. B100 degraded in response to insulin by hepatocytes derived from ZDF rats averaged 3.3% (0.2 and 6.4%), whereas B48 degraded averaged 4.5% (4.7 and 4.3%).

**Hepatic expression of mRNAs for SREBPs and apoB in 10- and 20-wk ZDF rats.** To determine if alterations in SREBP expression were associated with increased TG production in 10-wk ZDF rats, hepatic mRNA levels of SREBP isoforms were measured by RNase protection assay (Fig. 6A and Table 1). SREBP-1c mRNA is the most abundant isoform in lean and ZDF rat livers, in agreement with previous reports for rodent livers (23, 24). Compared with lean rats, hepatic SREBP-2 and SREBP-1a mRNAs were not altered in 10-wk ZDF rats. In contrast, hepatic expression of SREBP-1c was increased significantly by 65% in 10-wk ZDF rats compared with lean rats ($P < 0.04$). To examine the effect of reduced serum insulin levels on hepatic expression of SREBP isoforms, SREBP mRNA levels were also examined in 20-wk ZDF rats. Expression of hepatic SREBP isoform mRNAs in 20-wk ZDF rats was similar to that observed in livers derived from 10- and 20-wk lean rats. The return of expression of SREBP isoforms in 20-wk ZDF rats to lean levels corresponds with the return of hepatic TG production rates to control levels.

To rule out the possibility that the observed increase in apoB output in 10-wk ZDF rats was regulated at the transcriptional level, hepatic SREBP-1a mRNA levels were also examined in 20-wk ZDF rats. The return of expression of SREBP isoforms in 20-wk ZDF rats to lean levels corresponds with the return of hepatic TG production rates to control levels.

**Table 1. Expression of ApoB and SREBP mRNAs in ZDF rats**

<table>
<thead>
<tr>
<th>Animal</th>
<th>Relative to $\beta$-Actin</th>
<th>Relative to Cyclophilin</th>
</tr>
</thead>
<tbody>
<tr>
<td>SREBP-1c</td>
<td>SREBP-1a</td>
<td>SREBP-2</td>
</tr>
<tr>
<td>Lean (n = 6)</td>
<td>1.28±0.39</td>
<td>0.08±0.00</td>
</tr>
<tr>
<td>*ZDF (n = 3)</td>
<td>2.12±0.63*</td>
<td>0.09±0.01</td>
</tr>
<tr>
<td>Lean (n = 3)</td>
<td>1.77±0.22</td>
<td>0.10±0.02</td>
</tr>
<tr>
<td>*ZDF (n = 3)</td>
<td>1.35±0.24</td>
<td>0.06±0.01</td>
</tr>
</tbody>
</table>

Values are means ± SD; n, no. of rats. ApoB, apolipoprotein B; SREBP, sterol regulatory-binding protein; ZDF, Zucker diabetic fatty. *$P < 0.05$ vs. lean control using Student's t-test.
level, hepatic apoB mRNA abundance in ZDF rats was measured. As expected, hepatic apoB mRNA levels in 10- and 20-wk ZDF rats were similar to those in age-matched lean rats (Fig. 6A and Table 1). This finding supports the constitutive nature of hepatic apoB gene expression and further strengthens the hypothesis that the increase in apoB production observed in 10-wk ZDF rats relates to posttranscriptional mechanisms.

DISCUSSION

Hypertriglyceridemia is a component of the changes that occur with metabolic syndrome and type 2 diabetes. The hypertriglyceridemia relates to increased hepatic VLDL output attributed to hyperinsulinemia and increased circulating FFA (12). The role of insulin in stimulating hepatic lipogenesis has been established, and the mechanism is likely mediated through induction of hepatic SREBP-1c (9). The role of insulin in stimulating hepatic apoB production, however, is more controversial, and increases in hepatic apoB output in type 2 diabetes are less firmly established. Recent studies implicate insulin as a direct inhibitor of VLDL-apoB secretion, a process mediated by apoB degradation and reduced apoB synthesis (30). We suggest that hepatic VLDL production, including both TG and apoB, is stimulated by sustained hyperinsulinemia, but the mechanisms occur through distinct pathways. Transcriptional control of lipogenesis by insulin through SREBP-1c contributes to the increased hepatic TG production, whereas stabilization of apoB in the secretory pathway contributes to the increased hepatic apoB production. The combination of increased hepatic VLDL output observed in metabolic syndrome and hyperinsulinemic type 2 diabetes.

Current studies examine mechanisms of hepatic VLDL production in the ZDF rat, a strain that has been used as a model of type 2 diabetes. ZDF rats are derived from the hyperleptinemic Zucker fatty rat (3), and both ZDF and Zucker fatty rats are leptin resistant because of a missense mutation in the leptin receptor blocking leptin signaling (20, 35). The ZDF rat develops hypertriglyceridemia and hyperinsulinemia by 10 wk of age. By 20 wk of age, ZDF rats have progressed from a hyperinsulinemic to an insulinopenic state; however, rats remain hyperglycemic. We have previously characterized the hyperlipoproteinemia in 10- and 20-wk ZDF rats (29) and in the current study have used the same dietary regimen. This is important, since the dyslipidemia in ZDF rats is dependent on dietary fat and gender (4). Both 10- and 20-wk ZDF rats are hyperglycemic (413 and 621 mg/dl, respectively) and hypertriglyceridemic (423 and 585 mg/dl, respectively). Serum insulin levels differ, however. On average, 10-wk ZDF rats have 140% higher insulin levels than lean rats (150 μU/ml), and 20-wk ZDF rats have 50% lower insulin levels than lean rats (35 μU/ml; see Ref. 29). Serum apoB levels are 14 and 23 mg/dl in 10- and 20-wk ZDF rats, respectively, vs. 7 mg/dl in lean control rats. Thus ZDF rats are dyslipidemic and hyperglycemic at both 10 and 20 wk of age, but are hyperinsulinemic at 10 wk, becoming insulinopenic at 20 wk as diabetes progresses.

Results of the current study indicate that, at 10 wk of age, there is increased hepatic production of TG that is associated with increased hepatic SREBP-1c mRNA expression. Considering recent evidence that hepatic TG production is regulated through transcriptional control over lipogenic enzymes mediated by SREBP-1c (9, 19, 23), our data suggest that enhanced lipogenesis increases TG available in the liver for VLDL assembly. Studies with transgenic mice overexpressing SREBP-1c show increased hepatic TG content (22). Hyperinsulinemia is associated with increases in SREBP-1c mRNA in corpulent rats (5) and in fat-fed Sprague-Dawley rats (10). Although SREBP-1c and SREBP-1a are derived from the same gene (9), exclusively SREBP-1c, the dominant hepatic form increases with hyperinsulinemia; however, the reason is not clear (5, 10). A selective increase in SREBP-1c in 10-wk ZDF rats is also observed in the current study. As ZDF rats age and hyperinsulinemia is reduced, TG production rates return to control levels, which correlates with a reduction in hepatic SREBP-1c mRNA. Interestingly, in 20-wk ZDF rats, hepatic TG production is similar to lean rats and yet animals remain severely hypertriglyceridemic. This suggests that there is a catabolic defect in VLDL metabolism in 20-wk ZDF rats, since they become insulinopenic. In severely hyperinsulinemic streptozotocin-induced diabetic rats, hypertriglyceridemia results mainly from defective TRL catabolism (34).

apoB mRNA abundance is not elevated in livers of 10-wk ZDF rats, supporting the constitutive nature of apoB gene expression (34). Therefore, the increased hepatic apoB output that occurs with hyperinsulinemia and insulin resistance of the 10-wk ZDF rat cannot be explained by transcriptional changes in apoB mRNA. Therefore, mechanisms must relate to increased efficiency and utilization of apoB in VLDL formation. In primary cultures of rat hepatocytes, insulin reduces apoB secretion by posttranscriptionally inhibiting synthesis and increasing intracellular degradation. The loss of these insulin-mediated effects observed in 10-wk ZDF rats could explain the net increase in assembly and secretion of VLDL-apoB. Current studies support that hepatocytes derived from 10-wk ZDF rats are in fact resistant to the inhibitory effects of insulin. Moreover, the lack of insulin sensitivity is accompanied by loss of insulin-dependent apoB intracellular degradation. These results support the hypothesis that increased hepatic VLDL-apoB production with insulin resistance results from stabilization of apoB with increased efficiency in assembly of VLDL with available TG. In hepatocytes derived from 20-wk ZDF rats, although the insulin inhibitory effect returns, physiological relevance is unlikely, considering multiple defects in β-cell function and insulin secretion during progression of diabetes (36).

Several mechanisms have been described that alter apoB stability in hepatocytes and favor subsequent apoB degradation (7). These include proteasomal degradation of apoB in response to lipid deficiency, endoplasmic reticulum (ER) degradation of apoB involving the ER 60 protease, and phosphatidylinositol 3-kinase-dependent post-ER degradation. Our data suggest that the increased output of hepatic VLDL-apoB in insulin resistance relates to mechanisms involving posttranscriptional regulation. In 10-wk ZDF rats, insulin resistance results in corresponding increases in VLDL-apoB and TG production; however, VLDL-TG and -apoB do not always increase coincidentally. Recent studies using LXR, a member of the nuclear receptor superfamily of transcription factors, and peroxisome proliferator-activated receptor (PPAR)-α agonists suggest there is independent regulation of hepatic lipogenesis and apoB biosynthesis. Studies in vivo and in vitro using LXR agonists demon-
strate that increased VLDL-TG production is related to increased de novo lipogenesis via induction of SREBP-1c gene expression. Larger VLDL particles with increased TG content are produced without affecting VLDL-apoB secretion rates (8). In contrast, activation of PPAR-α increases the secretion of B100 with higher secreted particle density, suggesting the secretion of increased numbers of apoB particles at the same level of hepatic TG production (14). Thus LXR and PPAR-α agonist studies suggest there are separate inputs of TG and apoB in VLDL particle formation with the possibility for asynchronous regulation.

Hepatic VLDL production is increased in hyperinsulinemic ZDF rats, and we suggest that VLDL-TG secretion is facilitated through induction of hepatic lipogenesis, whereas VLDL-apoB secretion is increased by the loss of destabilizing effects of insulin, making more apoB available for VLDL formation. The increased VLDL production contributes to the dyslipidemia observed in insulin-resistant states. In later stages of diabetes modeled in the 20-wk ZDF rat, the decline in serum insulin is associated with normalization of SREBP-1c mRNA expression and return of VLDL production to control levels. Because older ZDF rats remain severely hypertriglyceridemic, even as hepatic VLDL production declines, it is likely that altered VLDL catabolism significantly contributes to the dyslipidemia in older rats. Current studies emphasize the complexity of insulin regulation of distinct pathways involved in hepatic VLDL production. Further studies are needed to dissect out individual mechanisms involved in the multiple pathways implicated in hyperlipidemia associated with metabolic syndrome and type 2 diabetes.

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