Glucose-stimulated insulin secretion suppresses hepatic triglyceride-rich lipoprotein and apoB production

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Chirieac, Doru V., Lucian R. Chirieac, James P. Corsetti, Joanne Cianci, Charles E. Sparks, and Janet D. Sparks. Glucose-stimulated insulin secretion suppresses hepatic triglyceride-rich lipoprotein and apoB production. Am J Physiol Endocrinol Metab 279: E1003–E1011, 2000.—The current study assessed in vivo the effect of insulin on triglyceride-rich lipoprotein (TRL) production by rat liver. Hepatic triglyceride and apolipoprotein B (apoB) production were measured in anesthetized, fasted rats injected intravenously with Triton WR-1339 (400 mg/kg). After intravascular catabolism was blocked by detergent treatment, glucose (500 mg/kg) was injected to elicit insulin secretion, and serum triglyceride and apoB accumulation were monitored over the next 3 h. In glucose-injected rats, triglyceride secretion averaged 22.5 ± 2.1 μg·mL⁻¹·min⁻¹, which was significantly less by 30% than that observed in saline-injected rats, which averaged 32.1 ± 1.4 μg·mL⁻¹·min⁻¹. ApoB secretion was also significantly reduced by 66% in glucose-injected rats. ApoB immunoblotting indicated that both B100 and B48 production were significantly reduced after glucose injection. Results support the conclusion that insulin acts in vivo to suppress hepatic very low density lipoprotein (VLDL) triglyceride and apoB secretion and strengthen the concept of a regulatory role for insulin in VLDL metabolism postprandially.

In the current study, we were interested in determining whether the ability of insulin to suppress hepatic VLDL production could be documented in vivo. Our experimental approach was to follow hepatic triglyceride and apoB production rates after bolus injection of glucose to elicit insulin secretion while simultaneously preventing lipoprotein catabolism by using the nonionic detergent Triton WR-1339 (3, 13, 15, 28, 29). Fasted rats were employed to minimize the contribution by the intestine to apoB and triglyceride production rates. As shown in the current study, bolus glucose injection resulted in the rapid release of insulin in the circulation, which led to the significant suppression of hepatic triglyceride and apoB production. This result supports the concept that in vivo insulin action limits rather than stimulates hepatic secretion of VLDL. We conclude that insulin acts in vivo and in vitro to regulate VLDL and apoB secretion by liver.

MATERIALS AND METHODS

Animals and surgery. The protocol used in this study was approved by the University Committee on Animal Resources,

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University of Rochester. Male Sprague-Dawley [Crl:CD (SD) BR] rats weighing 200–300 g were housed on a 12:12-h light-dark cycle with free access to standard rodent chow and drinking water for at least 7 days before experimentation.

**Primary rat hepatocyte experiments.** Collagenase perfusions of rat liver were performed to prepare hepatocytes between 0800 and 1000, and viable hepatocytes were isolated using Percoll (Pharmacia Fine Chemicals, Piscataway, NJ; see Ref. 41). Hepatocytes were centrifuged for 2 min at 50 g and were resuspended in Waymouth’s medium at containing 0.2% (wt/vol) BSA, hereafter referred to as Waymouth’s medium. Hepatocytes were seeded on sterile 60-mm culture dishes precoated with rat tail collagen, and dishes were then incubated at 37°C in an atmosphere of 95% air-5% CO₂. For insulin dose-response studies, hepatocytes were incubated for 14–16 h in Waymouth’s medium containing various concentrations of porcine pancreas insulin (Sigma Chemical, St. Louis, MO). In several experiments, in addition to insulin, other serum factors were added to medium, including dexamethasone (1 μM), glucagon (300 nM), or oleic acid (0.2% [wt/vol] BSA complexes (0.75 mM). Oleic acid/BSA complexes were prepared at a molar ratio of fatty acid to albumin of approximately four to one, as previously described (49). At the end of the incubation period, media were collected, cells were washed three times in Hanks’ balanced salt solution, and cell lysates were prepared in 0.5% (vol/vol) Triton X-100, as previously described (43). Cellular protein of each dish was measured by a modification of the Lowry procedure (26).

**In vivo experiments.** Rats were fasted for 16–18 h the night before experiments were carried out. Rats were anesthetized with ketamine (80 mg/kg) and xylazine (10 mg/kg) and were fitted with carotid (polyethylene 10; Clay Adams) and jugular (22 gauge; Intracath) catheters (Becton-Dickinson, Sandy, UT). Anesthesia was maintained throughout the 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 was stopped with a Luer-lock injection site adapter (Baxter Healthcare, Deerfield, IL). Catheters were secured using several silk sutures (5-0), and rats were allowed 30 min to acclimatize. Tyloxapol (Triton WR-1339) diluted in saline (300 mg/ml) and warmed to 37°C was slowly injected (1–3 min) via the jugular line (400 mg/kg body wt). Immediately thereafter, glucose dissolved in saline [45% (wt/wt)] or an equivalent volume of saline alone was injected intravenously (500 mg/kg body wt). Blood was sampled at 0, 30, 60, 90, 120, 150, and 180 min thereafter and was collected in microfuge tubes. Blood was allowed to clot, and serum was prepared by centrifugation. In control studies using intravenous L-arginine injection, the dosage of arginine used was 2.1 mmol/kg to elicit insulin secretion (9). Blood was collected at 0 and 60 min after arginine injection, and serum was prepared.

**Experimental procedures.** Serum glucose was measured by a colorimetric assay employing glucose oxidase (Sigma Diagnostics). Serum cholesterol was quantitated using a cholesterol oxidase method (1), and serum triglycerides were measured by assay of glycerol released after lipase treatment (27). Serum FFAs were determined by a kit (Wako FFA kit, Englewood, NJ). Serum insulin was measured by RIA using a commercial kit (Linco Research, St. Charles, MO) and modified rat insulin standards. Rat insulin standards were reconstituted in buffer containing 0.8% (vol/vol) Triton WR-1339. Cell and media apoB concentrations were assayed by monoclonal immunoassay (40), and results were normalized to protein content per dish. For apoB immunoblotting, 25 μl of freshly prepared serum were mixed with 225 μl of 0.1 M Tris-HCl buffer, pH 7.0, containing 10% (wt/vol) SDS, 100 mM dithiothreitol, 10% (vol/vol) 2-mercaptoethanol, and 2% (vol/vol) glycerol. Samples were mixed thoroughly, heated to 100°C for 15 min, and centrifuged to remove nonsolubilized material. Samples and authentic rat B100 and rat B48 standards were loaded on Acrylaide/acylamide gradient gels, and proteins were separated by SDS-PAGE (46). To control for potential variability in gel separation and in electrophoretic transfer, timed serum samples from saline-injected rats were electrophoresed on the same slab gel as those from glucose-injected rats. Separated proteins were electrophoretically transferred to polyvinylidene difluoride (PVDF) membranes (46), and nonspecific binding sites were blocked by overnight incubation at 4°C in 0.1 M Tris-HCl buffer, pH 7.0, containing 0.05% (wt/vol) Tween 20 and 5% (vol/vol) milk. Membranes were incubated with rabbit anti-rat apoB in blocking buffer overnight at 4°C, and then membranes were washed three times in 0.1 M Tris-HCl buffer, pH 7.0, containing 0.05% (vol/vol) Tween 20 (wash buffer). Antibody binding was detected by final incubation for 4 h at room temperature with 125I-labeled goat anti-rabbit IgG (Amer sham Pharmacia Biotech) in blocking buffer. After thorough rinsing of membranes in wash buffer, membranes were air-dried, and labeled antibody bound was visualized by PhosphorImager detection.

**RESULTS**

**Inhibition of apoB secretion by insulin in hepatocytes derived from fasted rats.** Previous studies of insulin effects on apoB secretion in primary hepatocyte cultures used cells derived from fed rats (2, 38, 41, 43, 44). In our in vivo strategy, we evaluated the suppressive effects on apoB secretion in primary hepatocyte cultures derived from fed rats (2, 38, 41, 43, 44). In vivo experiments.

Our in vivo strategy necessitated the use of fasted rats to minimize the intestinal contribution to lipoprotein production; therefore, we first evaluated the effect of fasting on the ability of insulin to inhibit apoB secretion. This evaluation involved the comparison of insulin dose effects on apoB secretion by hepatocytes derived from fed and fasted rats (Fig. 1). In hepatocytes derived from fasted rats, a slightly higher level of inhibition of apoB secretion was attained, averaging 46 ± 3.6% (mean ± SE; n = 11 livers). The comparable maximum inhibition of apoB secretion elicited by insulin in hepatocytes derived from fed rats averaged 40 ± 2.5% (n = 14 livers). The initial medium insulin concentration at which 50% of the maximum inhibitory effect on apoB secretion is achieved was 0.9 nM in hepatocytes derived from fed rats and 1.7 nM in hepatocytes derived from fed rats. These values are similar to that previously reported of 1 nM insulin observed in hepatocytes derived from lean Zucker rats (44).

**Effects of dexamethasone, oleic acid, and glucagon on hepatocyte apoB secretion.** To further support the use of our in vivo strategy, we evaluated the suppressive effect of insulin on apoB secretion by hepatocytes derived from fasted rats in coincubation studies employing rat insulin standards. Rat insulin standards were reconstituted in buffer containing 0.8% (vol/vol) Triton WR-1339. Cell and media apoB concentrations were assayed by monoclonal immunoassay (40), and results were normalized to protein content per dish. For apoB immunoblotting, 25 μl of freshly prepared serum were mixed with 225 μl of 0.1 M Tris-HCl buffer, pH 7.0, containing 10% (wt/vol) SDS, 100 mM dithiothreitol, 10% (vol/vol) 2-mercaptoethanol, and 20% (vol/vol) glycerol. Samples were mixed thoroughly, heated to 100°C for 15 min, and centrifuged to remove nonsolubilized material. Samples and authentic rat B100 and rat B48 standards were loaded on Acrylaide/acylamide gradient gels, and proteins were separated by SDS-PAGE (46). To control for potential variability in gel separation and in electrophoretic transfer, timed serum samples from saline-injected rats were electrophoresed on the same slab gel as those from glucose-injected rats. Separated proteins were electrophoretically transferred to polyvinylidene difluoride (PVDF) membranes (46), and nonspecific binding sites were blocked by overnight incubation at 4°C in 0.1 M Tris-HCl buffer, pH 7.0, containing 0.05% (wt/vol) Tween 20 and 5% (vol/vol) milk. Membranes were incubated with rabbit anti-rat apoB in blocking buffer overnight at 4°C, and then membranes were washed three times in 0.1 M Tris-HCl buffer, pH 7.0, containing 0.05% (vol/vol) Tween 20 (wash buffer). Antibody binding was detected by final incubation for 4 h at room temperature with 125I-labeled goat anti-rabbit IgG (Amer sham Pharmacia Biotech) in blocking buffer. After thorough rinsing of membranes in wash buffer, membranes were air-dried, and labeled antibody bound was visualized by PhosphorImager detection.
coincubated with 1 μM dexamethasone, 0.75 mM oleic acid, or 100 nM glucagon.

**Glucose dosage effects on serum glucose levels.** After the use of the fasted rat model was validated, the optimal concentration of glucose for bolus injections was determined. To avoid possible effects of sustained hyperglycemia, the dosage of glucose was determined that allowed for the return of serum glucose to preinjection values. Rats were fasted for 16–18 h, after which they were injected intravenously with Triton WR-1339 detergent. Three groups of rats were then injected (3 rats/group) with glucose at dosages of 500, 1,000, or 2,000 mg/kg body wt. The immediate serum glucose concentration after injection was calculated by dividing the glucose dosage by the blood volume, which was assumed to be 5% of the body weight, and this value was plotted as the peak glucose concentration at 1 min. Serum glucose values at 30 min were dependent on the dosage of glucose injected (Fig. 3). The highest serum glucose concentration at 30 min occurred after injection of 2,000 mg/kg, whereas the lowest serum glucose concentration occurred after injection of 500 mg/kg. The decline in glucose toward fasting levels was prolonged in groups receiving either 1,000 or 2,000 mg/kg glucose. In the group of rats injected with 500 mg/kg glucose, serum glucose returned to baseline levels shortly after 1 h. This latter dose of 500 mg glucose/kg body wt was chosen for bolus injection in subsequent experiments.

**Glucose-stimulated insulin secretion.** To document that insulin was indeed secreted in response to glucose injection, serum insulin was assayed (Fig. 4). In glucose-injected rats, serum insulin rose fourfold; 1 min after glucose injection and fell rapidly to fasting levels by 1 h after injection. Saline-injected, control rats maintained fasting insulin levels (data not shown). The acute, incremental rise in insulin concentration between 0 and 5 min in response to glucose injection is similar to that observed in fasted rats upon initiation of a hyperglycemic clamp (48).
Effect of glucose-stimulated insulin secretion on hepatic triglyceride production. After injection of Triton WR-1339, both hepatic and peripheral clearance of TRL are blocked (3, 13–15, 28, 29). Under these conditions, triglyceride accumulates at a rate equivalent to its production rate. In fasted rats, the majority of the triglyceride that accumulates in plasma originates in the liver. Both glucose-injected and saline-injected rats demonstrated a linear accumulation of serum triglycerides over the time course studied (Fig. 5A). Triglyceride secretion rates over 3 h averaged \( (n = 9) \) 32.1 ± 1.4 (SD) \( \mu \text{g triglyceride} \cdot \text{ml}^{-1} \cdot \text{min}^{-1} \) in saline-injected rats. The triglyceride secretion rate in glucose-injected rats was significantly less \( (P < 0.002) \) and averaged \( (n = 9) \) 22.5 ± 2.1 (SD) \( \mu \text{g triglyceride} \cdot \text{ml}^{-1} \cdot \text{min}^{-1} \). Hence, triglyceride accumulation in serum over the 3-h time course was reduced by 30% after glucose injection vs. saline injection. These results indicate that hepatic triglyceride production is significantly suppressed after glucose-stimulated insulin secretion. In control studies, L-arginine (2.1 mmol/kg) was injected intravenously to elicit insulin secretion, and serum glucose and triglyceride were measured 0 and 60 min after injection. Serum glucose averaged \( (n = 3) \) 11.5 ± 0.39 (SD) mM at 0 min and 13.1 ± 0.78 mM at 60 min in saline-injected rats. Serum glucose results were similar in arginine-injected rats and averaged \( (n = 3) \) 12.1 ± 1.17 (SD) mM at 0 min and 13.4 ± 0.33 mM at 60 min. Initial serum triglycerides (0 min) in saline-injected and arginine-injected rats were comparable, averaging \( (n = 3) \) 0.98 ± 0.15 (SD) and 0.94 ± 0.28 mM, respectively. At 60 min, serum triglyceride production was significantly lower by 19% \( (P < 0.02) \) in arginine-injected vs. saline-injected rats, averaging \( (n = 3) \) 3.14 ± 0.25 vs. 3.88 ± 0.11 (SD) mM, respectively. These results indicate that arginine injection significantly suppressed triglyceride production by 60 min without major changes in serum glucose.

In control studies, two groups of detergent-injected rats (4 rats/group) were exsanguinated (one group at 30 min and one group at 150 min after saline or glucose injection). Sera were prepared, and VLDL (density < 1.006 g/ml) and low-density lipoprotein (1.006 g/ml < density < 1.063 g/ml) were isolated by sequential density ultracentrifugation. Of the triglycerides present in sera, most were recovered in the VLDL fraction. For the saline-injected group, an average of 97 ± 1.0 and 98 ± 0.5% were recovered in the VLDL fraction at 30 and 150 min, respectively. For the glucose-injected group, an average of 97 ± 1.9 and 96 ± 4.9% were recovered in the VLDL fraction at 30 and 150 min, respectively. Because >95% of serum triglycerides are present in VLDL in both groups, the 30% reduction in serum triglyceride secretion that was observed in glucose-injected rats vs. saline-injected rats supports the conclusion that there is reduced secretion of VLDL in glucose-injected rats.

Cholesterol levels remained relatively constant in both glucose-injected and saline-injected rats (Fig. 5B), suggesting no major differences in blood volumes of...
glucose- vs. saline-injected animals. Sera from detergent-treated rats were also analyzed for changes in FFAs (Fig. 6). No significant differences were observed in serum FFAs in detergent-treated rats after glucose or saline injection.

Effect of glucose-stimulated insulin secretion on apoB production rates. Because each lipoprotein particle contains a single apoB molecule (11), VLDL particle production rates can be estimated by measuring the accumulation of apoB after WR-1339 injection (Fig. 7; see Ref. 16). In saline-injected rats, the average apoB production rate (µg·ml⁻¹·h⁻¹) was (n = 6) 140 ± 39.0 vs. 46 ± 11.8 observed in glucose-injected rats. These results indicate that hepatic apoB production is significantly reduced by glucose-stimulated insulin secretion (P < 0.03). Overall, apoB secretion was reduced by an average of 66% in glucose-injected rats compared with saline-injected rats. In control experiments using arginine injection, apoB production rates (µg·ml⁻¹·h⁻¹) at 60 min were significantly reduced by 47% (P < 0.01) compared with saline injection, averaging (n = 3) 170 ± 18 vs. 91 ± 26 µg·ml⁻¹·h⁻¹ (n = 3).

The presence of significant apoB aggregation prevented us from being able to accurately assess levels of VLDL B100 and B48 in delipidated (density <1.006 g/ml) lipoproteins by standard gel electrophoresis methods. As an alternative, we employed immunoblotting analysis of serum B100 and B48. Serum proteins derived from detergent-treated rats were denatured by heating with excess SDS, 2-mercaptoethanol, and dithiothreitol. After SDS-PAGE, separated proteins were transferred to PVDF membranes. Timed samples from saline-injected rats and from glucose-injected rats were electrophoresed in pairs on the same membrane along with authentic rat B100 and B48 standards to control for variability of protein separations and electrophoretic transfers. Compared with saline-injected rats, the accumulation of B48 and B100 in sera of glucose-injected rats was markedly suppressed (Fig. 8). This result is consistent with the reduced accumulation of total apoB observed in glucose-injected rats as quantified by RIA (Fig. 7).

DISCUSSION

Results from the current in vivo study support previous in vitro experiments demonstrating that insulin inhibits the hepatic secretion of VLDL and apoB (2, 10, 30, 38, 43). Considering that there is one molecule of apoB per lipoprotein particle (11), the decreased production of total apoB, B100, and B48 that we observed supports the conclusion that insulin reduced the hepatic secretion of VLDL particles. Although the liver is the major site of B100 synthesis in the particle, both liver and intestine are able to synthesize and secrete B48 (17, 37) due to expression of the apoB mRNA editing enzyme APOBEC-1 in these tissues (7). The use of fasted rats in the current study, however, minimizes the intestinal contribution of B48 and associated triglyceride-enriched particles.

A short burst of insulin secretion had a suppressive effect on lipoprotein production for 3 h after acute hyperinsulinemia subsided. It is possible that the initial release of insulin by the pancreas via the portal vein, and substantial clearance of insulin by liver during first passage, produces a “priming” effect. This “signal” to liver dictates the retention of hepatic triglyceride destined for secretion during intestinal lipid absorption, with triglyceride levels maximizing ~3–4 h after ingestion of food. We have shown previously that only 5 min of insulin exposure leads to the translocation of cytosolic PI 3-kinase to the rough endoplasmic reticulum (RER), a location where VLDL assembly takes place (32). We speculate that translocated PI 3-kinase may be retained on RER membranes until enzyme activity subsides and dislocation of the enzyme occurs. PI 3-kinase lipid products accumulate in primary hepatocytes over 60 min after a transient 5-min exposure to insulin (data not shown). Hence, down-
stream effects of PI 3-kinase activation are likely to be maintained as long as phospholipid products are present in membranes. These data suggest that a sustained insulin signal may not be required to observe the inhibitory effect on triglyceride and apoB secretion. Because apoB suppression is greater than triglyceride suppression after glucose injection, the net effect is to increase the triglyceride-to-apoB ratio and increase particle size. Further studies need to be performed, including secretory kinetics of B48 and B100 relative to triglyceride, to clearly make this point, since, although there was suppression of both B48 and B100, accurate quantitation was not possible. In addition, earlier time points reflect the smaller apoB-containing remnants present in fasting compared with longer time points that reflect larger, nascent secretory particles. We have therefore been circumspect about drawing conclusions related to changes in particle composition after glucose injection.

In the current study, serum cholesterol levels remained unchanged over the 3-h time period. Because cholesterol in rats is transported primarily in high-density lipoprotein (HDL) and because HDL has a relatively long half-life in serum (39), our results suggest that neither glucose injection nor Triton WR-1339 treatment had a major effect on HDL cholesterol metabolism. However, because the fractional catabolic rate of HDL and its associated apolipoproteins is long compared with the time course studied, no definitive conclusions should be drawn with respect to glucose-stimulated insulin secretion on overall HDL metabolism in detergent-treated rats.

As demonstrated in the current study, time course changes in serum FFAs in glucose- and saline-injected rats were similar. Because Triton WR-1339 suppresses intravascular lipolysis, the release of FFAs derived from TRL lipolysis would be minimal. However, in fasted rats, insulin secretion should inhibit adipose tissue lipolysis. Hence, a decline in serum FFAs might be anticipated due to a combination of tissue uptake of FFAs and reduced entry of FFAs into plasma. As recently shown in fasted rats, plasma FFAs showed a dramatic decline within 15 min of initiating hyperglycemic clamp conditions (48). The major difference between the two studies is that, during the hyperglycemic clamp, insulin levels remained significantly elevated compared with fasting levels, whereas, in the current study, serum insulin levels peaked within minutes of glucose injection but rapidly returned to fasting levels. It is therefore possible that a single pulse of insulin elicited by bolus glucose injection was not able to prevent adipose tissue lipolysis present in the fasting state. Alternatively, because FFAs were unchanged in both glucose- and saline-injected rats and both groups were treated with Triton WR-1339, detergent treatment itself may have had an effect on either FFA release or FFA uptake.

Control studies using primary hepatocytes were performed to validate the use of fasted rats for in vivo studies. These experiments were done because our previous studies of insulin action were conducted in hepatocytes derived from fed rats (2, 12, 32, 41, 43, 44), and the current study necessitated the use of fasted rats to minimize the intestinal contribution to lipoprotein production. In hepatocytes derived from fasted rats, the ability of insulin to inhibit apoB secretion was accentuated compared with hepatocytes derived from fed rats, and this is consistent with increased insulin receptor signaling observed during fasting (35). We also performed experiments to test conditions that may alter VLDL-apoB secretion in the presence of insulin. Studies by Wang et al. (51, 52) indicate that dexamethasone stimulates VLDL apoB secretion. Dexamethasone did not affect apoB secretion in our studies, which employed hepatocytes derived from fasted rats and medium containing basal concentrations of insulin (0.1 Fig. 8. Time course changes in serum B100 and B48 after intravenous injection of glucose in Triton WR-1339-treated rats. Blood was sampled at 0, 1, 2, and 3 h after injection of saline or glucose. Serum was prepared, and proteins were denatured by heating in SDS buffer. After protein separation by SDS-PAGE and electrophoretic transfer, apoB was analyzed by immunoblotting. Antibody binding was detected using a 125I-labeled anti-rabbit IgG secondary antibody followed by autoradiography. Each membrane (A or B) contained paired samples derived from saline- and glucose-injected rats along with rat B100 and B48 standards.
nM). Moreover, the inhibitory effect of 100 nM insulin in the presence of dexamethasone was not altered. Fatty acids, specifically 0.75 mM oleic acid, also did not affect apoB secretion in the presence of basal or high levels of insulin, nor did the presence of oleic acid affect the maximum inhibition of apoB secretion attained in medium containing 100 nM insulin. These studies using primary cultures contrast to studies in transformed cells lines (e.g., Hep G2) where fatty acids stimulate the secretion of B100 (8). As we have reported previously (2), glucagon inhibits apoB secretion by rat hepatocytes. The current results demonstrate that this is also true in hepatocytes derived from fasted rats. Importantly, however, is the finding that the maximum inhibition of apoB secretion by insulin is not altered by coinucbuation with glucagon.

Previous studies have shown that intravenous glucose injection in 4-h-fasted rats results in an almost immediate 5-fold increase in plasma glucose and a 10-fold increase in plasma insulin followed by a sustained two times increase in plasma norepinephrine (NE) 60 min after injection (18). Because NE has been shown to decrease triglyceride and apoB production by perfused liver (54), it is possible that NE released after intravenous glucose might be responsible. Four lines of evidence support that it is insulin release and not NE release that is responsible for the suppression of triglyceride and apoB production. First, in control studies, intravenous arginine injection significantly inhibits triglyceride and apoB production without a major change in blood glucose. Because elevated blood glucose is the major effector for stimulation of NE release, it is unlikely that NE would be released after arginine injection. Second, levels of plasma NE produced after glucose injection are relatively modest, rising from 250 pg/ml (1.5 nM) to ~500 pg/ml (3 nM; see Ref. 18). The liver perfusion studies of Yamauchi et al. (54) involved high levels of pulsatile NE given at 10-min intervals over the course of 2 h of perfusion at a level in the perfusate of 1 μM. These concentrations represent a 300-fold greater concentration than that produced in vivo after bolus glucose injection. Third, the intravenous glucose dose used in current studies (500 mg/kg) did not lead to an increase in plasma NE in previous studies (19). Fourth, although NE was shown to inhibit B48 secretion by liver, Yamauchi et al. (54) failed to demonstrate a significant effect on B100 secretion. These results differ from the current study showing effects on both B100 and B48.

The role of insulin in the regulation of VLDL secretion is complex. Acute hyperinsulinemia inhibits the production of VLDL triglyceride (2, 10, 30, 38) and apoB (30, 38, 43) by rat hepatocytes and apoB by perfused rat livers (47). The current study demonstrates that the suppressive effect of insulin on VLDL apoB can be demonstrated in vivo. In humans, insulin acutely inhibits the hepatic production of VLDL triglyceride (20, 22–24, 36, 50) and apoB (20, 23–25), and the effect is independent of FFA availability (23). We suggest that the inhibition of hepatic VLDL apoB secretion by insulin prevents the secretion of TRL particles by the liver that would compete with the clearance of intestinal TRL. By inhibiting hepatic VLDL release, intestinal TRL are allowed to be rapidly cleared from the circulation during the postprandial period. The suppression of hepatic TRL secretion also allows hepatic triglyceride stores to expand, and, when insulin levels fall during the postabsorbptive period, hepatically stored triglyceride is available to be assembled with apoB to form VLDL for secretion. By regulating hepatic VLDL secretion during the postprandial period, insulin allows for a smooth transition from the fasting state to the fed state in terms of serum triglyceride response. This regulatory pathway may prevent exaggerated hypertriglyceridemia after feeding that might occur if both intestinal and hepatic TRLs were secreted simultaneously and competed for common clearance pathways.

Resistance to insulin-stimulated glucose uptake is a prevalent condition in the human population that is frequently associated with hypertriglyceridemia (reviewed in Ref. 34). The compensatory rise in plasma insulin necessary to normalize blood glucose leads to the enhanced secretion of hepatic VLDL triglyceride and ultimately hypertriglyceridemia. The mechanism for the enhanced secretion of hepatic VLDL in insulin resistance states remains controversial. The mechanism, however, is unlikely to be an exaggerated insulin effect and is more likely to be the result of failure of insulin action. As shown in the current study and in numerous reports, insulin action, however, is also associated with hyperinsulinemia persists in its ability to suppress the hepatic secretion of VLDL apoB (5).

In conclusion, insulin acts in vivo to suppress hepatic VLDL triglyceride and apoB secretion, and these results strengthen the concept of a regulatory role for insulin in VLDL metabolism postprandially.

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