Dyslipidemia is a prominent feature of insulin resistance and type 2 diabetes mellitus, playing an important role in the etiology of cardiovascular disease in these conditions. An important component of the typical dyslipidemia of type 2 diabetes mellitus is elevated triglycerides (TG). We and others have demonstrated in animal models that, in insulin-resistant states, triglyceride-rich lipoprotein (TRL; apolipoprotein [apo] B-100–containing lipoproteins in the liver and apoB-48–containing lipoproteins in the intestine) production is increased. We have also demonstrated in humans that TRL production is regulated by several factors, including circulating free fatty acid (FFA) and pancreatic hormones (insulin and glucagon). Understanding the regulation of TRL production and strategies in ameliorating TRL overproduction in insulin resistance may lead to new therapies to treat dyslipidemia and prevent cardiovascular disease.

The identification and characterization of intestinally derived incretins, including glucagon-like peptide-1 (GLP-1) and gastric inhibitory polypeptide, have led to the development and clinical use of incretin-based therapies for type 2 diabetes mellitus. GLP-1, through its receptor (GLP-1R), executes multiple physiological functions including enhancing glucose-stimulated insulin secretion by pancreatic ß-cells, inducing ß-cell proliferation, and inhibiting ß-cell apoptosis (the latter shown in animal models and in vitro but not directly in humans). GLP-1 also slows gastric emptying, inhibits glucose-dependent glucagon secretion, and promotes satiety. The incretins are rapidly degraded by the enzyme dipeptidyl peptidase-4 (DPP-4). GLP-1 receptor agonists, such as the GLP-1 mimic exenatide, and DPP-4 inhibitors have proven efficacy in treating type 2 diabetes mellitus. Aside from their primary effects on glucose homeostasis, recent clinical studies also suggest their potential role in ameliorating hyperlipidemia. Because of the known effects of GLP-1 on satiety and weight gain, the exact mechanism of GLP-1R modulation on lipid homeostasis remains poorly defined from...
these long-term studies. GLP-1R agonists have been shown to acutely attenuate postprandial lipidemia in healthy subjects or patients with type 2 diabetes mellitus. GLP-1R signaling has also been shown to be important for lipid and intestinal lipoprotein metabolism in animal models, thus GLP-1R agonism or DPP-4 inhibition suppressed while antagonism or genetic deletion of GLP-1R enhanced TRL secretion. Despite these studies, because GLP-1 improves glycemic control in patients with diabetes mellitus, profoundly slows gastric emptying, and stimulates insulin and suppresses glucagon, all of which can independently affect lipoprotein metabolism, the mechanism of GLP-1 receptor agonists on lipoprotein metabolism in humans remains unclear. The current study was designed to examine the mechanism of GLP-1R agonist exenatide on TRL metabolism in healthy humans.

Materials and Methods

Subjects

Fifteen healthy, normolipidemic male subjects participated in this study. Their demographic characteristics and fasting biochemical profiles are shown in Table 1. Subjects had body mass index of 20–27 kg/m² and normal glucose tolerance in response to a 75-g, 2-hour oral glucose tolerance test performed immediately before enrollment in the study. None of the participants had any previous history of cardiovascular disease, gastrointestinal or systemic illness, surgical intervention within 6 months before the studies, or were taking medications. The Research Ethics Board of the University Health Network, University of Toronto approved the study, and all subjects gave written informed consent before their participation.

Experimental Protocol for Lipoprotein Kinetic Studies

TRL kinetics were studied in constant fed state by continuous infusion of a liquid lipid/carbohydrate formula because apoB-48 in plasma and TRL concentrations at fasting are too low to allow accurate quantification of stable isotope enrichment. To circumvent the effects of exenatide on gastric emptying, a nasoduodenal tube was inserted into the duodenum under the guidance of fluoroscopy 1 day before the kinetic study for infusion of the liquid formula. A pancreatic clamp was performed to minimize exenatide’s known effects on secretion of islet hormones (Figure 1A).

Each subject underwent 2 separate lipoprotein kinetic studies in random order, 4 to 6 weeks apart. In each study, after an overnight fast,
Table 2. Mean Plasma and TRL Lipids, TRL-ApoB-100 and ApoB-48 Concentrations, Fractional Catabolic Rates, and Production Rates During the Kinetic Study

<table>
<thead>
<tr>
<th>Variable</th>
<th>Placebo</th>
<th>Exenatide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma FFA, mmol/L</td>
<td>0.13±0.02</td>
<td>0.11±0.01</td>
</tr>
<tr>
<td>Plasma TG, mmol/L</td>
<td>0.93±0.08</td>
<td>0.93±0.11</td>
</tr>
<tr>
<td>Plasma TC, mmol/L</td>
<td>3.74±0.15</td>
<td>3.88±0.14</td>
</tr>
<tr>
<td>TRL-TG, mmol/L</td>
<td>0.59±0.07</td>
<td>0.59±0.10</td>
</tr>
<tr>
<td>TRL-Cholesterol, mmol/L</td>
<td>0.33±0.03</td>
<td>0.38±0.05</td>
</tr>
<tr>
<td>TRL-apoB-100 concentration, mg/L</td>
<td>56.53±6.62</td>
<td>66.27±8.11</td>
</tr>
<tr>
<td>TRL-apoB-100 FCR, pools/day</td>
<td>3.83±0.38</td>
<td>3.03±0.22</td>
</tr>
<tr>
<td>TRL-apoB-100 PR, mg/kg per day</td>
<td>9.88±1.59</td>
<td>7.84±0.71</td>
</tr>
<tr>
<td>TRL-apoB-48 concentration, mg/L</td>
<td>1.83±0.30</td>
<td>1.24±0.19*</td>
</tr>
<tr>
<td>TRL-apoB-48 FCR, pools/day</td>
<td>1.54±0.22</td>
<td>1.36±0.24</td>
</tr>
<tr>
<td>TRL-apoB-48 PR, mg/kg per day</td>
<td>0.12±0.02</td>
<td>0.08±0.02*</td>
</tr>
</tbody>
</table>

ApoB, apolipoprotein B; FCR, fractional catabolic rate; PR, production rate; TC, total cholesterol; TG, triglycerides; TRL, TG-rich lipoprotein; FFA, free fatty acid.

Values are mean±SEM for the duration of the 10-h kinetic study. *P<0.05 vs Placebo.

Kinetic Analysis

Stable isotope enrichment curves for apoB-48 and apoB-100 were fitted to a multicompartmental model using SAAM II software (version 1.2, University of Washington, Seattle, WA) to derive the fractional catabolic rates (FCR) as previously described. The model consisted of the synthesis of TRL apoB from the precursor pool via a delay compartment. Plasma-free leucine tracer-to-tracer ratios were used as a forcing function and individual tracer-to-tracer ratio time-course curves were used to derive kinetic rate constants. Production rates (PR) of each apolipoprotein were calculated as PR=FCR×pool size, where pool size=average plasma concentration (mg/L) over the 10 hours of the kinetic study×plasma volume (estimated as 0.045 L/kg body weight).

Statistics

Results are presented as mean±SEM. Repeated measures ANOVA was used to compare the time course of parameters during the kinetic experiments. Paired t-test was used to compare TG, FFA, apoB-100 and apoB-48 mass, and FCR and PR between the 2 treatments. All statistics were performed with SAS (version 8, Cary, NC). A P value <0.05 was considered significant.

Results

Effects of Exenatide on Plasma and TRL Lipids

Exenatide did not significantly affect plasma total cholesterol, TG, FFA, and TRL-TG and TRL-cholesterol concentrations compared with placebo (Table 2; Figure 1B–D). Plasma and TRL-TG levels increased after intraduodenal infusion of the liquid formula and returned toward baseline before the beginning of the kinetic study. This pattern was reminiscent of our previous study performed in healthy subjects with similar body mass index and fasting plasma TG, in which the same liquid formula was ingested at frequent intervals. The less robust response of plasma and TRL-TG in these studies compared with high-fat meal test studies may be attributable to the constant low rate of delivery of nutrients compared with fat meals. We have previously shown in humans that an acute elevation of plasma FFA stimulates both hepatic and adipose tissue uptake of fatty acids.
intestinal lipoprotein particle production. The similar levels of circulating FFA in the current study suggest that any effect exenatide may have on TRL particle production is not mediated by alterations in plasma FFA.

Effects of Exenatide on TRL ApoB-100 and ApoB-48

ApoB-100 concentrations in the TRL fraction were not significantly different between exenatide and placebo (Table 2; Figure 2A). In contrast, TRL-apoB-48 concentrations were significantly lower with exenatide than placebo (P<0.05) (Table 2; Figure 2C). This decrease was detected within 1 hour after exenatide administration, where TRL-apoB-48 concentrations were 24% lower than in the placebo-treatment study. The difference increased to 37% by the end of the kinetic study, ie, 10 hours after exenatide injection. There was an initial reduction in TRL-apoB-48 concentration in both treatments during the early period of the kinetic study, which returned to slightly above baseline at the end of the study. The reasons for this decrease were not clear, but may be related to the fact that the kinetic studies were performed under pancreatic clamp with intraduodenal infusion of nutrients to circumvent the impact of exenatide on gastric emptying. Such phenomenon was not observed in previous studies where frequent oral ingestion was used to achieve a constant fed state.

Effects of Exenatide on TRL-ApoB-100 and ApoB-48 Production and Clearance

To elucidate the mechanism whereby exenatide decreases TRL-apoB-48 concentration, we examined the turnover of TRL-apoB-48 and apoB-100 using established stable isotope enrichment methodology. Consistent with the lack of effect of exenatide on TRL-apoB-100 concentrations, no significant differences were detected in FCR and PR for TRL-apoB-100, despite trends of reduction with exenatide (Table 2; Figure 2B). Power calculation indicated that, with 80% power and α=0.05%, 46 subjects would have to be studied to detect a significant difference in TRL-apoB-100 PR between exenatide and placebo. TRL-apoB-48 FCR was not significantly different between treatments. However, TRL-apoB-48 PR was decreased by 38% (P<0.05) with exenatide treatment compared with placebo (Table 2; Figure 2D), which accounted for the reduction in TRL-apoB-48 concentration.

Because of the changes in TRL-apoB-48 concentrations, kinetic parameters derived from steady-state modeling may not be accurate. Without a full account of the cause of these changes in TRL-apoB-48 concentrations, certain assumptions and functional dependencies to describe the time-variable processes, which are required for non–steady-state modeling, cannot be adequately substantiated, nor can unique solutions to such non–steady-state modeling be assured. Nevertheless, future reanalysis using non–steady-state modeling with the above requirements fulfilled might yield additional insights into the underlining mechanisms. It is noted that FCR for TRL-apoB-48 and TRL-apoB-100 were derived using the identical model. Because the TRL-apoB-100 levels were constant throughout the kinetic study, estimation of FCR for apoB-100 was not compromised by non–steady-state. TRL-apoB-100 FCR were not significantly different between placebo and exenatide.

Effects of Exenatide on Circulating Levels of Glucose, Insulin, C-Peptide, and Glucagon

Glucose levels were suppressed immediately after exenatide administration and remained lower than placebo for the duration of the 10-hour kinetic study (Figure 3A). To maintain euglycemia, all subjects required a low rate (59.2 ± 8.2 mL/h, 40.3 ± 5.6 kcal/h) IV infusion of 20% dextrose, which typically started within 1 hour of exenatide injection and lasted for 3 to 5 hours. Throughout the study, blood glucose levels were above 4 mmol/L; therefore, no counterregulatory response to glucose levels was expected to occur. Despite constant infusion of somatostatin during the standard pancreatic clamp, circulating insulin and C-peptide concentrations increased immediately after exenatide injection (Figure 3B, C), indicating exenatide-mediated enhancement of glucose-mediated insulin secretion. The increases in insulin and C-peptide lasted for 2 to 3 hours. No significant differences in glucagon levels (Figure 3D) or growth hormone (not shown) were observed between exenatide and placebo arms of the study.

Discussion

Previous studies have reported hypolipidemic effects of short-term treatment with native GLP-1 or GLP-1R agonists. Infusion of synthetic GLP-1 in healthy humans for 390 minutes abolished postprandial lipemia, along with reduced glycemia, elevated insulin, suppressed glucagon and FFA, and slowed gastric emptying. In type 2 diabetic patients, a single dose of SC exenatide also decreased postprandial lipoprotein response. The current study has provided new evidence in humans that exenatide acutely suppresses intestinal lipoprotein particle production,8,9 The similar levels of circulating FFA in the current study suggest that any effect exenatide may have on TRL particle production is not mediated by alterations in plasma FFA.
patients versus healthy subjects) and the experimental conditions (intraduodenal infusion versus meal test, use of pancreatic clamp). In chow-fed hamsters, 2- to 3-week treatment with the DPP-4 inhibitor sitagliptin suppressed TRL-apoB-48 but not TRL-TG secretion. Decreased TRL-apoB-48 but not TG levels suggested secretion of fewer numbers but greater lipid enrichment of lipoprotein particles with exenatide treatment. Alternatively, because TRL-apoB-48 concentrations were low in both treatments as expected in these normolipidemic subjects, suppression of TRL-apoB-48 by exenatide may not be reflected in the concentrations of plasma TG during the kinetic study. No significant effect of exenatide on hepatic lipoprotein production was observed. The differential responses of the liver and intestine to exenatide point to divergent regulatory pathways with regard to TRL secretion in these 2 organs.

Although there are many similarities in TRL metabolism in the liver and intestine, differences between them also exist. One potential mechanism whereby exenatide may affect TRL particle production in these 2 organs may be related to tissue distribution of GLP-1R. Whereas GLP-1R expression has been identified in the small intestine, its expression in the liver is minimal or undetectable in some studies. The current study does not exclude the possibility that, in longer-term studies with long-term exposure to exenatide, hepatic lipoprotein metabolism may be affected via activation of GLP-1R in the liver.

Several factors have been demonstrated to acutely regulate intestinal and hepatic lipoprotein production, including pancreatic hormones and circulating FFA. As exenatide is a potent modulator of pancreatic hormone secretion, we performed lipoprotein turnover studies under conditions of a pancreatic clamp to dissect the mechanisms whereby exenatide affects lipoprotein metabolism with minimal confounding effects of pancreatic hormones. Even under standard pancreatic clamp conditions, exenatide was able to overwhelm the suppressive effects of somatostatin on insulin secretion, resulting in increased insulin levels during the initial part of the kinetic study. Because acute hyperinsulinemia suppresses both hepatic and intestinal TRL production in humans, one might attribute the exenatide-mediated suppression of TRL-apoB-48 production in the present study to exenatide-induced stimulation of insulin secretion. Although suppression of TRL-apoB-48 secretion by insulin cannot be fully excluded, several points argue against this interpretation. First, the increase in insulin levels after exenatide administration was transient and lasted 2 to 3 hours, after which insulin levels returned to baseline and were closely matched between exenatide and placebo studies. In contrast, the reduction in TRL-apoB-48 concentrations in exenatide was sustained throughout the 10-hour kinetic study. In fact, the difference in TRL-apoB-48 concentrations between the 2 treatments tended to increase with progression of the study. Second, hyperinsulinemia inhibits hepatic lipoprotein secretion and such acute suppressive effects of insulin have been demonstrated to affect TRL of both hepatic and intestinal origin in a similar fashion. In the current study, however, only intestinal, but not hepatic, lipoprotein particle secretion was affected by exenatide. Third, there was no close correlation between TRL-apoB-48 PR and incremental area under the insulin curve within the first 3 hours of the kinetic study, in either placebo ($R^2=0.02$) or exenatide ($R^2=0.10$) or combined ($R^2=0.004$). In our previous study, very low-density lipoprotein apoB-48 PR was decreased by $\approx 50\%$ under a hyperinsulinemic clamp that maintained circulating insulin

![Figure 3. Plasma glucose (A), insulin (B), C-peptide (C), and glucagon (D) levels during the study period. Infusion of a 20% dextrose solution was required in all subjects after exenatide administration to maintain blood glucose levels in the euglycemic range.](http://atvb.ahajournals.org/ Downloaded from)
levels at ≈350 pmol/L for 10 hours. The transient increase in insulin secretion in this study thus did not seem to elicit a similar suppressive effect on TRL turnover. Two subjects in the present study had either no or minimal (<10%) increase in insulin and C-peptide, whereas their exenatide-induced suppression of TRL-apoB-48 PR was similar in magnitude to the other subjects. In the current study, glucagon levels were closely matched between placebo and exenatide, and we have recently shown no effect of glucagon on intestinal lipoprotein secretion; therefore, the decreased TRL-apoB-48 PR was not likely attributed to any effect via incretin-mediated glucagon secretion. We conclude, therefore, that the decreased intestinal lipoprotein production with exenatide treatment in the present study cannot be fully accounted for by its effects on pancreatic hormonal secretion. In addition, modulation of FFAs has been shown to occur with attenuation of postprandial lipemia by GLP-1 or exenatide in humans. In previous studies in humans, we have demonstrated that TRL production is acutely stimulated by elevated circulating FFA. In the current study, plasma TG and FFA concentrations were similar between exenatide and placebo, suggesting that the suppression of TRL-apoB-48 production was not because of limited availability of lipid substrate for lipoprotein particle assembly or changes in circulating FFA.

Exenatide treatment resulted in lower glucose levels throughout the kinetic study. After exenatide administration, IV glucose infusion was required to maintain glucose levels in the euglycemic range. It is noted that glucose infusion was also required in the 2 subjects who did not have the transient increase in insulin levels. These results strongly suggest that exenatide induced an increase in glucose disposal or suppression of hepatic glucose production, independent of insulin. Unfortunately, we did not administer isotonically enriched glucose in the present study, and were therefore unable to elucidate whether exenatide suppressed endogenous glucose production, enhanced its clearance, or both. The issue of a direct insulin-sensitizing effect of incretin therapy has been examined extensively in animals and humans and remains controversial. In our previous study in somatostatin-treated mice, the DPP-4 inhibitor sitagliptin acutely and profoundly enhanced hepatic insulin sensitivity, independent of its ability to enhance insulin secretion. In both humans and animals, GLP-1 agonists have been shown to modulate glucose homeostasis independent of islet hormones. As TRL overproduction is associated with insulin resistance and is attenuated with improved insulin sensitivity, improved intestinal insulin sensitivity by exenatide may have contributed to the observed effect of exenatide on TRL-apoB-48 production in this study. Another possibility is that the lower glucose levels in exenatide versus placebo treatment may directly contribute to decreased TRL-apoB-48 production. However, these possibilities are unlikely to play a major role because TRL-apoB-100 secretion was not similarly affected. Direct effects of GLP-1 on modulation of intestinal lipid metabolism have been reported in animal models. In rats, recombinant GLP-1 inhibited lymph flow, lymph TG absorption, and lymph apoB secretion in response to intraduodenal fat infusion. In hamsters and mice, apoB-48 was acutely decreased with exenatide treatment. Exenatide also suppressed apo-48 secretion in isolated hamster enterocytes, indicating direct involvement of GLP-1R signaling in intestinal lipoprotein particle secretion. It is therefore highly likely that exenatide suppressed intestinal TRL production at least partly by directly suppressing chylomicron assembly and secretion in the intestine. Decreased TRL-apoB-48 but not TG levels with exenatide treatment suggests that exenatide suppressed not only apoB-48 synthesis but also chylomicron packaging with neutral lipids, without suppressing microsomal triglyceride transfer protein. Whether exenatide suppressed apoB mRNA translation, apoB intracellular trafficking, and stability in the enterocytes remains unknown, and further studies are required to elucidate the underlying molecular mechanisms.

In conclusion, the results from the present study suggest a possible direct effect of exenatide on intestinal lipoprotein particle production, independent of changes in weight gain and satiety as seen in long-term studies, and independent of changes in gastric emptying. This finding expands our understanding of GLP-1 in metabolic regulation beyond its primary therapeutic role in the regulation of glucose homeostasis. Large, long-term studies are needed to examine the cardiovascular and lipid effects of incretin-based therapies in individuals with type 2 diabetes mellitus.

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Disclosures
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References
GLP-1 Regulation of Lipoprotein Metabolism

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