Intravenous Glucose Acutely Stimulates Intestinal Lipoprotein Secretion in Healthy Humans

Changting Xiao,* Satya Dash,* Cecilia Morgantini, Gary F. Lewis

Objective.—Increased production of intestinal triglyceride-rich lipoproteins (TRLs) contributes to dyslipidemia and increased risk of atherosclerotic cardiovascular disease in insulin resistance and type 2 diabetes. We have previously demonstrated that enteral glucose enhances lipid-stimulated intestinal lipoprotein particle secretion. Here, we assessed whether glucose delivered systemically by intravenous infusion also enhances intestinal lipoprotein particle secretion in humans.

Approach and Results.—On 2 occasions, 4 to 6 weeks apart and in random order, 10 healthy men received a constant 15-hour intravenous infusion of either 20% glucose to induce hyperglycemia or normal saline as control. Production of TRL–apolipoprotein B48 (apoB48, primary outcomes) and apoB100 (secondary outcomes) was assessed during hourly liquid-mixed macronutrient formula ingestion with stable isotope enrichment and multicompartmental modeling, under pancreatic clamp conditions to limit perturbations in pancreatic hormones (insulin and glucagon) and growth hormone. Compared with saline infusion, glucose infusion induced both hyperglycemia and hyperinsulinemia, increased plasma triglyceride levels, and increased TRL-apoB48 concentration and production rate (P<0.05), without affecting TRL-apoB100 fractional catabolic rate. No significant effect of hyperglycemia on TRL-apoB100 concentration and kinetic parameters was observed.

Conclusions.—Short-term intravenous infusion of glucose stimulates intestinal lipoprotein production. Hyperglycemia may contribute to intestinal lipoprotein overproduction in type 2 diabetes.

Clinical Trial Registration—URL: http://www.clinicaltrials.gov. Unique identifier: NCT02607839.

Key Words: glucose • humans • intestines • kinetics

It has long been recognized that high carbohydrate diets and refined sugars exacerbate postprandial lipid responses and promote metabolic abnormalities.12–15 High dietary carbohydrates promote fasting and postprandial hypertriglyceridemia during chronic and some, but not all, acute studies.15 Such effects were mostly attributed to increased VLDL secretion, with increased triglyceride and in some studies increased apoB production, along with impaired clearance.15 However, recent evidence suggests that TRL secretion from the intestine also responds to carbohydrates. Furthermore, a single glucose drink is able to release intestinal lipid storage originating from an earlier meal.16 In previous studies, we have demonstrated that confusion of monosaccharides (either glucose or fructose) with lipid emulsion directly into the duodenum promotes TRL particle production during 10-hour kinetic studies.17 Although enteral sources of simple sugars may directly stimulate chylomicron particle production in intestinal enterocytes, they also elevated circulating levels of glucose. In this current experiment, we tested the hypothesis that intravenous glucose infusion, with its associated elevation in circulating glucose level, is capable of stimulating intestinal (and possibly) hepatic lipoprotein particle production in humans.
Materials and Methods

Intravenous Infusion of Glucose Achieved Hyperglycemia

Constant intravenous infusion of glucose led to higher plasma glucose levels compared with infusion of normal saline during the first 3 hours, that is before the pancreatic clamp (glucose=6.77±0.37 versus saline=5.91±0.18 mmol/L, P<0.05; Figure 1B). After the initiation of the clamp, glucose infusion elevated plasma glucose levels to ≈13 mmol/L at the start of the lipoprotein kinetic study. Plasma glucose gradually declined thereafter to ≈8.5 mmol/L at the end of the study. Plasma glucose increased to ≈9 mmol/L with saline infusion during the first 2 hours of the initiation of the pancreatic clamp with consequent change in insulin/glucose ratio. They were in the range of 6.5 to 8 mmol/L during the lipoprotein kinetic study. As a result, the experimental design achieved a difference in plasma glucose levels of 2 to 5 mmol/L (P<0.05) for the study duration. Incremental area under the curve for plasma glucose was significantly higher in glucose than saline (P<0.01). Plasma insulin concentrations were elevated from basal in both treatments during the pancreatic clamp, with mean insulin concentration higher with glucose infusion than with saline infusion (glucose=73.2±8.1 versus saline=43.3±3.9 pmol/L, P<0.05; Figure 1C). This was likely because of hyperglycemia-induced stimulation of insulin secretion, as indicated by increased C-peptide concentrations with glucose (Figure I in the online-only Data Supplement), overcoming the somatostatin-induced suppression of insulin secretion. Plasma glucagon concentrations were not significantly different between treatments (P=NS; Figure 1D).

Intravenous Infusion of Glucose Increased Plasma Triglyceride and Decreased Plasma FFA

Plasma triglyceride was higher with glucose infusion compared with saline during the study period (glucose=1.20±0.15 versus saline=1.08±0.18 mmol/L, P<0.05; Figure 2A). The difference in plasma triglyceride between treatments became evident starting at 4 hours and remained relatively small, likely because of the moderate degree of hyperglycemia achieved with intravenous glucose infusion. Plasma FFA levels transiently increased before the kinetic study and declined thereafter in both treatments, possibly because of the changes in insulin levels as a result of combined glucose infusion and pancreatic clamp. Plasma FFA levels were significantly lower with glucose infusion compared with saline (P<0.01; Figure 2B), likely because of suppression of adipose lipolysis by the hyperinsulinemia with glucose infusion. TRL-triglyceride tended to be higher with glucose infusion than with saline but did not reach statistical significance because of interindividual variations (P=0.17; Figure 2C). Because triglyceride levels in both plasma and TRL seemed to diverge between treatments after 4 hours, TRL from t=4 to 10 hours were separated into chylomicrons and VLDL by ultracentrifugation. Mean triglyceride in both chylomicrons (glucose=0.05±0.01 versus saline=0.04±0.01 mmol/L, P<0.05) and VLDL (glucose=0.23±0.04 versus saline=0.18±0.04 mmol/L, P<0.05) during this study period were higher with glucose infusion than with saline. No significant differences in TRL-triglyceride fractional catabolic rates (FCRs) or production rates (P=NS for both) were observed between treatments (Figure 2D).

Hyperglycemia With Hyperinsulinemia Did Not Significantly Affect TRL-apoB100 Concentrations, Fractional Catabolic Rate, or Production Rate

TRL-apoB100 concentrations during the study were not significantly different between glucose and saline treatments (Figure 3A). TRL-apoB100 FCR were calculated from stable isotopic enrichment time course curves (Figure IIA in the online-only Data Supplement). Both FCR (glucose=3.96±0.50 versus saline=3.94±0.26 pools/d, P=NS) and production rate (glucose=10.04±1.95 versus saline=9.66±1.48 mg/kg/d, P=NS) were not statistically significant between glucose and saline treatments (Figure 3B).

Hyperglycemia Elevated TRL-apoB48 Concentrations by Increasing Production Rate

TRL-apoB48 concentrations increased from fasting levels with constant feeding before the start of the kinetic study (t=0), and declined thereafter in both treatments, likely because of the infusion of somatostatin. Because the experimental conditions (including use of pancreatic clamp and constant feeding) were identical in the 2 treatments arms, any potential influence on TRL-apoB48 is expected to be the same for both treatment arms; therefore, the difference in TRL-apoB48 levels is attributed to the treatments, that is intravenous infusion of glucose versus normal saline. Despite the gradual decline with saline infusion, TRL-apoB48 levels were not below fasting levels even at the end of the study (t=10 hours) with hourly ingestion of the liquid formula. Mean TRL-apoB48 concentrations during the kinetic study period were higher with glucose infusion when compared with saline (glucose=5.80±1.78 versus saline=4.03±1.50 mg/L, P<0.05; Figure 3C). The difference between treatments was more apparent during the last 5 hours of the study, such that at the end of the infusion TRL-apoB48 concentrations were ≈2-fold higher in glucose than in saline. TRL-apoB48 FCR, calculated from stable isotopic enrichment time course curves (Figure IIB in the online-only Data Supplement) were similar in both groups (glucose=1.74±0.24 versus saline=1.78±0.32 pools/d, P=NS). TRL-apoB48 production rates, the primary outcomes, were 47% higher with glucose infusion compared with saline (glucose=0.46±0.16 versus saline=0.32±0.13 mg/kg per day) with 7 of the 10 subjects exhibiting increased

Nonstandard Abbreviations and Acronyms

<table>
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<th>Abbreviation</th>
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<tr>
<td>apoB48</td>
<td>apolipoprotein B48</td>
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<tr>
<td>FCR</td>
<td>fractional catabolic rate</td>
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<tr>
<td>FFA</td>
<td>free fatty acid</td>
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<td>TRL</td>
<td>triglyceride-rich lipoprotein</td>
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<tr>
<td>VLDL</td>
<td>very low-density lipoprotein</td>
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production, which despite individual variation reached statistical significance by paired t test (P<0.05; Figure 3D).

Discussion
In previous studies, we have shown that glucose delivered into the duodenum enhances lipid-stimulated chylomicron secretion. This study provides direct experimental evidence that intravenous glucose and its associated hyperglycemia, despite concurrent hyperinsulinemia, acutely stimulates intestinal lipoprotein particle secretion in humans, adding a further dimension to intestinal lipoprotein secretion in response to glucose.

The magnitude of enhanced apoB48 production by hyperglycemia (44% increase compared with saline control), the primary outcome, in this study was less than that seen previously with enteral glucose (=6-fold). One possible interpretation is that stimulation by glucose is stronger from the luminal side than from the basolateral side of the enterocyte.
There are, however, several factors that should be considered in interpreting these results. In the present experimental setting, besides the difference in glycemic levels between treatment arms, glucose infusion also led to higher plasma insulin and lower plasma FFA concentrations, likely because of glucose-stimulated insulin secretion and insulin suppression of adipose tissue lipolysis. The resulting hyperinsulinemia (≈80 pmol/L) with glucose infusion was not quantitatively large. Nevertheless, both insulin and FFA are important regulators of TRL production, where insulin acutely inhibits while FFA stimulates TRL particle production.\(^{10,18}\) Despite the rise in insulin and the suppression in FFA levels, both of which would be expected to reduce intestinal TRL particle secretion,\(^{10,18}\) thereby having an opposite and offsetting effect on glucose-stimulated TRL-apoB48 secretion, the net effect of intravenous glucose infusion on intestinal particle production was positive. Had insulin and FFA concentrations been identical between treatments, the magnitude of the intravenous glucose-induced rise in TRL-apoB48 production should have been larger. These results therefore exclude the possibility that circulating glucose stimulates TRL-apoB48 production via modulating insulin and FFA levels.

Secretion of preformed chylomicron has been shown to occur following various stimuli, such as oral glucose,\(^{16}\) sham feeding,\(^{19}\) mixed meal,\(^{20}\) and in our own study glucagon-like peptide (GLP)-2.\(^{21}\) Release of preformed chylomicron would cause lower stable isotopic enrichment of TRL-apoB48. In this study, TRL-apoB48 stable isotopic enrichments were similar between treatments, suggesting that release of preformed chylomicron by intravenous glucose, if any, was not greater than by saline during the kinetic study. Gut hormones GLP-1 and GLP-2 are known to affect intestinal lipoprotein secretion\(^{22}\) and GLP-2 rapidly promotes the release of preformed chylomicron.\(^{21}\) Because GLP-2 cosecretes with GLP-1 on a 1:1 molar ratio\(^{23}\) and GLP-1 secretion is stimulated by luminal but not vascular glucose,\(^{24}\) it is unlikely that GLP-1 or GLP-2 played a significant role in this study. An additional consideration is that in this study subjects ingested a liquid formula that contained both lipid and carbohydrates, whereas in the previous enteral glucose study Intralipid (containing no carbohydrate) was delivered. Inclusion of carbohydrates in the hourly ingested liquid formula may have contributed to the minor elevation in blood glucose in the control arm and the relatively small difference in glycemic levels between treatments (2–4 mmol/L between 3 and 10 hours). This may have diminished the confidence in effects on apoB48 production between treatments. Furthermore, the difference in apoB48 concentration occurs late and yet plasma glucose level with intravenous glucose infusion was only modestly higher than with normal saline infusion. An additional consideration is that in this study subjects ingested a liquid formula that contained both lipid and carbohydrates, whereas in the previous enteral glucose study Intralipid (containing no carbohydrate) was delivered. Inclusion of carbohydrates in the hourly ingested liquid formula may have contributed to the minor elevation in blood glucose in the control arm and the relatively small difference in glycemic levels between treatments (2–4 mmol/L between 3 and 10 hours). This may have diminished the confidence in effects on apoB48 production between treatments. Furthermore, the difference in apoB48 concentration occurs late and yet plasma glucose level with intravenous glucose infusion was only modestly higher than with normal saline infusion during the last few hours of the study. This indicates that TRL-apoB48 production responds to elevation in plasma glucose but the response may not be directly proportional to plasma glucose level. Alternatively, the stimulation in TRL-apoB48 production by intravenous glucose is a relatively slow process that takes time to manifest. A slow process implies that glucose may indirectly affect chylomicron production through mechanisms beyond lipoprotein biosynthesis in the enterocytes, arguing against a direct role of glucose on intracellular lipoprotein biosynthesis and assembly.

No stimulation of hepatic TRL-apoB100 particle production was observed in this study. Adiels et al\(^{25}\) demonstrated a correlation between VLDL1-apoB and hyperglycemia in patients with T2D in the fasted state. Lipoprotein production from the intestinal source was not examined in this study. This study examined the effects of a short-term (15 hours) elevation of glucose levels in the fed state, which may have different
mechanisms from correlations in cross-sectional observations where hyperglycemia may have been present for many years. VLDL1-apoB production also correlated with other metabolic parameters, such as insulin resistance (homeostatic model assessment–insulin resistance), in T2D patients with dyslipidemia, whereas in our study participants were healthy, non-diabetic, and normolipidemic (Table). Increased insulin and decreased FFA levels in this study also might have contributed to the lack of effects on TRL-apoB100. Chronic consumption of high carbohydrate diets induces fasting and postprandial hypertriglyceridemia. Although most carbohydrate feeding studies documented increases in VLDL-triglyceride (determining particle size), the effects on VLDL-apoB (particle number) were equivocal. In some studies, VLDL-apoB100 was found to be increased because of lower conversion of VLDL-apoB to LDL-apoB without significant effect on VLDL-apoB secretion or because of both increased production and decreased removal. Other studies have demonstrated dissociated responses of VLDL-triglyceride and apoB100 to high-carbohydrate diet, thus hypertriglyceridemia was not accompanied by increased VLDL-apoB production. It remains to be seen whether prolonged hyperglycemia per se stimulates TRL particle production from the liver. The increase in plasma triglyceride in response to intravenous glucose during the late part of the study (t = 4–10 hours) was accounted for by increases in triglyceride content in both VLDL and chylomicron fractions. The concordant changes in triglyceride and VLDL-apoB secretion further suggest a differential effect of intravenous glucose on the intestine and liver.

Enteral glucose increased TRL-apoB48 FCR, whereas intravenous glucose infusion in this study did not influence apoB48 clearance. The change in apoB48 clearance in response to enteral glucose may not be a result of hyperglycemia because enteral fructose stimulated apoB48 production without affecting FCR, despite induction of hyperglycemia. It has been shown that glucose stimulates apoC-III expression in vitro, and plasma apoC-III concentrations correlate with fasting glucose and glucose excursion after oral glucose load in overweight patients. In this study in healthy individuals, plasma apoC-III concentrations (saline = 31.7 ± 5.4 versus glucose = 38.3 ± 7.2 mg/L at t = 0 and saline = 33.6 ± 7.6 versus glucose = 40.4 ± 7.2 mg/L at t = 10 hours, P = NS) were not increased with short-term hyperglycemia, which is in agreement with the lack of significant differences in TRL-triglyceride and apoB48 clearance between treatments. In addition, enteral glucose delivery stimulated TRL-apoB48 production more dramatically without affecting apoC-III concentrations. The mechanism whereby intravenous or enteral glucose enhances TRL-apoB48 production remains unknown. It is intuitive to speculate that glucose directly stimulated chylomicron production. One potential mechanism is that higher glucose enhanced de novo lipogenesis in the enterocytes, which provided lipid substrate for chylomicron production. De novo lipogenesis in the small intestine has been shown to occur in animal models. This study did not directly measure intestinal de novo lipogenesis. TRL-triglyceride turnover during the entire study period was not significantly affected by intravenous glucose infusion when compared with saline infusion. In humans, de novo lipogenesis is a quantitatively minor mechanism for development of hypertriglyceridemia with carbohydrate consumption that requires at least several days of overfeeding. Therefore, de novo lipogenesis is unlikely to have played a major role in this short-term study. Crosstalk between carbohydrate and lipid metabolism is known to occur in enterocytes. For example, culture of Caco-2/15 cells with high levels of glucose promoted cholesterol uptake, enhanced the expression of the cholesterol transporter NPC1L1 and CD36, and modulated several transcription factors (eg, increased LXR, LXRβ, PPARα, PPARγ, and ChREBP, and decreased expression of SREBP-2). Many of these molecular factors are also involved in chylomicron synthesis and secretion in the intestine. For example, treatment for 2 weeks with ezetimibe, an NPC1L1 inhibitor, suppressed TRL-triglyceride and apoB48 secretion in hamsters fed a high-fructose and high-fat diet. Whether oral ezetimibe can abrogate the enhancement of chylomicron production by intravenous glucose in the acute settings of this study in humans is unknown. ChREBP is expressed in the small intestine, potentially serving as a link between glucose and lipid metabolism in the enterocytes. LXR also senses glucose and integrates glucose and lipid metabolism in the liver. The role of LXR in TRL synthesis in the intestine in response to glucose has not been examined yet. More importantly, stimulation of cholesterol uptake by high glucose concentration is because of the presence of glucose at the basolateral (corresponding to blood circulation) side of the enterocytes. This study further supports that hyperglycemia (ie, elevated glucose from the basolateral side) may modulate intracellular events involved in chylomicron synthesis and secretion in the enterocytes.

The pathophysiological implications of the findings should be interpreted in the context of several limitations of this study. First, metabolic abnormalities in TRL metabolism are closely related to the development of insulin resistance. The subjects in this study had normal insulin sensitivity, as indicated by homeostatic model assessment–insulin resistance. Second, the use of continuous feeding, intravenous glucose infusion, and a pancreatic clamp represent a nonphysiological, controlled, experimental setting. Hormones used in this protocol, alone or in combination, may have unintended effects on metabolism.

Table. Demographic Characteristics and Fasting Plasma Concentrations of Study Participants

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<tr>
<td>Age, y</td>
<td>48.3±3.1</td>
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<tr>
<td>Weight, kg</td>
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</tr>
<tr>
<td>Height, cm</td>
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</tr>
<tr>
<td>BMI, kg/m²</td>
<td>24.9±0.8</td>
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<tr>
<td>Glucose, mmol/L</td>
<td>5.1±0.2</td>
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<tr>
<td>TG, mmol/L</td>
<td>1.1±0.2</td>
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<tr>
<td>Insulin, pmol/L</td>
<td>54.0±6.9</td>
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<tr>
<td>HOMA-IR</td>
<td>2.0±0.5</td>
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Data are mean±SEM. BMI indicates body mass index; HOMA-IR, homeostatic model assessment–insulin resistance; and TG, triglycerides.
Third, the sample size is small. The increased TRL-apoB48 production in this study was observed with experimentally raised plasma glucose level that meets the diagnostic criteria of diabetes mellitus, which implies that hyperglycemia may contribute to the development of dyslipidemia in patients with diabetes mellitus. However, dyslipidemia may occur in the absence of hyperglycemia, which suggests that hyperglycemia-stimulated intestinal lipoprotein production is not a universal mechanism for the development of dyslipidemia. It remains unknown whether intestinal lipoprotein production is increased in other conditions of hyperglycemia, for example when hyperglycemia is found in combination with an elevation of plasma FFAs, as may be the case in those with T2D. Future studies are needed to examine the effects of hyperglycemia on TRL production under physiological conditions in individuals with insulin resistance or T2D.

In conclusion, these results demonstrate that intravenous glucose administration and consequent hyperglycemia may be pathologically linked to hyperlipidemia through enhancement of intestinal lipoprotein production. This new finding adds to the current body of knowledge that the intestinal lipoprotein production contributes to hypertriglyceridemia by responding to systemic metabolic cues in humans.

Acknowledgments
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Disclosures
None.

References

**Highlights**

- Type 2 diabetes mellitus and insulin resistance are associated with high production of triglyceride-rich lipoprotein (TRL) particles from the liver and intestine.
- This study examined the relationship between TRL production and blood sugar levels in healthy volunteers whose blood sugar levels were maintained at normal or increased by intravenous infusion of glucose.
- TRL production from the intestine, but not the liver, was increased when blood sugar levels were elevated.
- This study supports a pathological link between hyperglycemia and hyperlipidemia in type 2 diabetes mellitus.
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Materials and Methods

Subjects

Ten healthy, normolipidemic and normo glycemic, men participated in this study in the Metabolic Test Center at Toronto General Hospital. Their demographic characteristics and fasting biochemical profiles are shown in Table 1. HOMA-IR was calculated as fasting glucose (mmol/L) x fasting insulin (mU/L)/22.5, where fasting plasma glucose and insulin concentrations were obtained the day prior to each lipoprotein kinetics study. The subjects had normal glucose tolerance in response to a 75-g, 2-hr oral glucose tolerance test performed immediately prior to their enrollment. None of the participants had any previous history of cardiovascular diseases, gastrointestinal or systemic illness, surgical intervention within six months prior to the studies, or was taking any medications. The Research Ethics Board of the University Health Network, University of Toronto, approved the study and all subjects gave written informed consent prior to their participation.

Experimental Protocol

The experimental protocol is outlined in Figure 1A. Each subject was randomized using a randomization table into 2 separate studies, 4 to 6 weeks apart. In each study, the subject was fasted overnight following an American Heart Association phase 1 diet as dinner. An iv catheter was inserted into a superficial vein in each forearm, one for infusion and one for sampling. Starting at 4am the next day, a high-fat liquid formula (Hormel Health Labs, GA; total fat 10% by weight, saturated fat 1.5%, trans fat 0%, monounsaturated fat 2.6%, polyunsaturated fat 5.6%, cholesterol 0%; 49% calories from fat, 38% from carbohydrates, 13% from proteins) was ingested every hour during the course of the study. Each hourly aliquot (41.8±/±1.4 mL/h) was calculated to evenly spread the total daily caloric requirement, estimated with the Harris-Benedict Equation, across the duration of the study. TRL kinetics was studied in a constant, fed state because fasting TRL-apoB48 concentrations are too low to allow accurate quantification of stable isotope enrichment. Mixed nutrients were ingested because ingestion of pure lipid emulsion resulted in decline in plasma and TRL-TG concentrations. Also at 4am, during one randomized study visit, the subjects received an iv infusion of 20% dextrose at the rate of 75 ml/h, which was continued until the completion of the study. In another study visit, normal saline was infused at the same rate. Participants were blinded to treatments.

At 7am (i.e. 3 hours after starting the liquid formula ingestion), a pancreatic clamp was started with the infusion of somatostatin (55 µg/h). Insulin (0.05 mU/kg/min), glucagon (0.325 ng/kg/min) and growth hormone (3.0 ng/kg/min) were infused at basal rates. The pancreatic clamp was performed to limit fluctuations in circulating levels of insulin, glucagon and growth hormone, some of which have been shown to affect TRL metabolism. At 9am (referred to as 0 hour of lipoprotein turnover study), a primed constant infusion (10 µmol/kg bolus followed by 10 µmol/kg/h for 10 h) of L-[5,5,5-2H3]-leucine (d3-leucine; Cambridge Isotope Laboratories, Andover, MA) was started. At 9am, subjects also received a bolus of [1,1,2,3,3-2H5]-glycerol.
(d5-glycerol, 75 µmol/kg; Cambridge Isotope Laboratories) in order to assess TRL-TG kinetics. Blood samples were collected at 0, 0.5, 1, 2, 3, 4, 5, 7, 8, 9 and 10 h after administration of d3-leucine for isolation of lipoproteins. Blood samples for TG, FFA and hormone analysis were collected at regular intervals. To prevent protein degradation and lipid hydrolysis, the following preservatives were added to blood collection tubes prior to sample collection, per ml of blood: sodium azide, 70 mg; aprotinin, 1.94 mg; tetrahydrolipstatin, 0.55 mg.

**Laboratory Methods**

TRL fractions were isolated using ultracentrifugation. After delipidation, samples were loaded to SDS-PAGE for separation of apoB100 and apoB48. Following electrophoresis, apoB100 and apoB48 formed 2 distinct bands on SDS-PAGE, which were excised, hydrolyzed, derivatized and quantified for stable isotope enrichment as previously described. Leucine was quantified with an Agilent 5975/6890N GC/MS with electron impact ionization and selective ion monitoring at m/z = 200 and 203. Tracer-to-tracee ratios were calculated from isotopic ratios according to a standard curve of isotopic enrichment. TRL-TG turnover was assessed as previously described. Briefly, deproteinized TRL fractions were separated on thin layer chromatography plates and scrapings corresponding to triglycerides were collected. Glycerol was derivatized with heptafluorobutyric anhydride in ethyl acetate (1:2, v/v), followed by electron impact ionization and selective ion monitoring at m/z = 467 and 472. Commercial kits were used to measure cholesterol, TG, FFA, insulin, C-peptide and glucagon. Plasma apoC-III was measured with ELISA (Assaypro, St Charles, MO). TRL-apoB100 and apoB48 were measured with human specific ELISA.

**Kinetics analysis**

Stable isotope enrichment curves for TRL-apoB48 and apoB100 were fitted to a multi-compartmental 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 synthesis of TRL-apoB from a precursor pool via a 0.5-h delay compartment. Plasma free leucine tracer-to-tracee ratios, measured for each kinetic study of each subject, was used as a forcing function. Individual tracer-to-tracee ratios time course curves were used to derive kinetic rate constants. In steady state, production rates of each apolipoprotein were calculated as the product of FCR and pool size, where pool size = average plasma concentration (mg/L) during the kinetic study X plasma volume (estimated as 0.045 liter/kg body weight). TRL-TG FCR was derived from the monoexponential slopes of TRL-TG glycerol stable isotope enrichment curves determined from the peak of the isotopic enrichment to the last point that, by visual inspection, fell on the log-linear portion of the curve (usually 6 h to 8 h).

**Statistics**

Results are presented as mean ± SEM. Repeated measures ANOVA was used to compare the time course of parameters during the kinetic experiments. The primary outcome (TRL apoB48 production) and secondary outcome TRL apoB100 production) were compared between the two treatments using paired t-tests. Paired t-tests were also used to compare plasma or TRL concentrations of TG, FFA, apoB100, apoB48 and apoC-III, and FCR and production rates, between the two treatments. All statistics were performed with SAS (version 9, Cary, NC). A p value < 0.05 was considered significant.
References


High blood sugar primes the gut to make more artery clogging fats.