Novel Role of Enteral Monosaccharides in Intestinal Lipoprotein Production in Healthy Humans

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

Objective—Overproduction of triglyceride-rich lipoproteins (TRLs) by liver and intestine contributes to hypertriglyceridemia and may increase cardiovascular risk. Dietary carbohydrates, especially fructose, have been shown to amplify postprandial lipemia but little is known about its effect on intestinal TRL particle production. Here, we examined intestinal and hepatic TRL particle production in response to enteral glucose or fructose in the presence of enteral lipid.

Approach and Results—In 2 randomized studies, 4 to 6 weeks apart, 7 healthy male subjects received intraduodenal infusion of Intralipid plus saline or glucose. TRL-apolipoprotein (apo) B48 and apoB100 kinetics were assessed under pancreatic clamp conditions. In a separate study of another 7 subjects under similar conditions, glucose was replaced by fructose. When coinfused with Intralipid into the duodenum, glucose markedly stimulated TRL- apoB48 production (P<0.01), with a concomitant moderate increase in fractional clearance (P<0.05), resulting in net elevation of TRL-apoB48 concentration. TRL-apoB100 concentration, fractional clearance, and production were not significantly affected by glucose. When glucose was replaced by fructose, both TRL-apoB100 and apoB48 production (P<0.05), but not fractional clearance, were enhanced compared with Intralipid alone.

Conclusions—These results reveal a novel role of monosaccharides in acutely enhancing intestinal lipoprotein particle production, thereby aggravating hyperlipidemia. (Arterioscler Thromb Vasc Biol. 2013;33:00-00.)

Key Words: apolipoprotein B ■ fructose ■ glucose ■ intestine ■ kinetics

The dyslipidemia that is commonly associated with insulin resistance and type 2 diabetes mellitus includes hypertriglyceridemia, low high-density lipoprotein cholesterol, and increased small, dense low-density lipoprotein particles. Postprandial lipemia, which is highly prevalent in obese and insulin-resistant individuals and in patients with type 2 diabetes mellitus, is associated with increased risk of atherosclerosis and cardiovascular diseases, and nonfasting plasma triglyceride (TG) level is a strong predictor of cardiovascular disease risk. Overproduction of TG-rich lipoprotein (TRL) particles, that is, very low density lipoprotein (VLDL, apolipoprotein [apo] B100-containing) from the liver and chylomicrons (apoB48-containing) from the intestine, is an important contributor to the fasting and postprandial hypertriglyceridemia of these conditions. In previous studies, we and others have demonstrated that TRL production both in the liver and in the intestine are subject to regulation by a variety of hormonal, metabolic, and nutritional factors.

Diets high in carbohydrates are known to increase fasting and postprandial lipids (reviewed in Reference 7). Increased consumption of fructose, in the form of sucrose or high-fructose corn syrup, has been implicated in the obesity epidemic and the pathogenesis of metabolic syndrome. Consumption of purified sugars, for example, sucrose and fructose, chronically or acutely, has been shown to exacerbate postprandial TG. On the contrary, oral glucose does not acutely augment postprandial lipemia. Although sucrose decreased postprandial TG clearance in rodents and humans, previous studies in humans have not examined whether the acute exacerbation of postprandial lipemia by simple sugars results from enhanced production or impaired clearance of TRL particles. It is also not clear whether simple sugars differentially affect the source of TRL, that is, liver, intestine, or both. Furthermore, in acute studies, postprandial hormonal responses to oral ingestion of glucose or fructose (such as stimulated secretion of insulin, which per se acutely inhibits intestinal and hepatic TRL production in healthy individuals) make it difficult to deduce the mechanisms whereby these monosaccharides affect TRL metabolism.

In the current study we aimed to examine, in healthy humans, the following: (1) whether enteral monosaccharides affect lipid-induced TRL production by the liver and intestine; (2) whether enteral monosaccharides, added to enteral lipid, affect the fractional clearance of TRL from the circulation; and (3) whether enteral glucose and fructose differentially affect TRL production by liver and intestine. To neutralize

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From the Departments of Medicine and Physiology, Division of Endocrinology and Metabolism, Banting and Best Diabetes Centre, University of Toronto, Toronto, Ontario, Canada.
*These authors contributed equally to this work.

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Correspondence to Gary F. Lewis, MD, FRCP(C), Toronto General Hospital, 200 Elizabeth St, EN12-218, Toronto, Ontario, M5G 2C4, Canada. E-mail gary.lewis@uhn.ca
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the effect of these monosaccharides on pancreatic hormone secretion, we elected to assess TRL kinetics under conditions of a pancreatic clamp, in which insulin, glucagon, and growth hormone secretion were suppressed by somatostatin, with concurrent replacement of these hormones at rates that mimic their basal rates of secretion. In addition, because intestinal infusion of lipids and monosaccharides affect gastric emptying to variable extents,\textsuperscript{20,21} lipids and glucose or fructose were infused directly into the duodenum via a nasoduodenal tube.

Materials and Methods

Materials and Methods are available in the online-only Supplement.

Results

Lipid+Glucose Versus Lipid+Saline Study (n=7)

**Enteral Glucose Elevated Plasma and TRL-TG**

Plasma glucose, TG, free fatty acid (FFA), and TRL-TG were not different before intraduodenal infusion of either normal saline (NS) or glucose (GLU). As expected, coinfusion of glucose with Intralipid resulted in higher plasma glucose levels in GLU versus NS throughout the study period (Figure 1A in the online-only Data Supplement). TG levels in both plasma (GLU=0.91±0.08 versus NS=0.63±0.13 mg/L, \(P<0.05\)) and TRL (GLU=0.43±0.05 versus NS=0.30±0.07 mg/L, \(P<0.05\)) were higher in GLU versus NS during the 10-hour lipoprotein turnover period (Figure 2A and 2C). Plasma FFA were lower in GLU compared with NS (Figure 2B), possibly attributable to relatively higher insulin levels in response to glucose infusion (Figure 1B in the online-only Data Supplement). Circulating levels of C peptide were higher in GLU versus NS during the kinetic study, whereas glucagon levels were similar in both treatments (Figure 1C and ID in the online-only Data Supplement). The increase in insulin and C peptide suggested breakthrough insulin secretion in response to higher glucose concentrations, despite the inhibitory effect of somatostatin in the pancreatic clamp. The levels of these hormones during the lipoprotein turnover study were all within the baseline ranges.

**Enteral Glucose Did Not Significantly Affect TRL-apoB100 Concentrations, Fractional Clearance or Production Rate**

TRL-apoB100 concentrations during the same period tended to be higher in GLU versus NS but did not reach statistical significance (Figure 3A). Both fractional clearance rate (GLU=4.71±0.72 versus NS=4.64±0.90 pools/d, \(P=NS\) and production rate (GLU=10.08±2.20 versus NS=7.83±2.55 mg/(kg·d), \(P=NS\)) were not statistically significant between GLU and NS (Figure 3D).

Lipid+Fructose Versus Lipid + Saline Study (n=7)

**Enteral Fructose Elevated Plasma Glucose, TG, and TRL-TG**

As compared with NS, coinfusion of fructose into the duodenum resulted in an increase in plasma glucose (Figure 2A in the online-only Data Supplement). Similar to the glucose study, plasma and TRL-TG levels were not significantly different between fructose (FRU) and NS during the infusion before the kinetic study (0 hours). Plasma TG and TRL-TG were not significantly different between treatments during the first 3 hours of the kinetic study. However, starting from 4 hours and persisting until the end of the study, mean plasma TG (FRU=0.76±0.08 versus NS=0.62±0.08 mg/L, \(P<0.01\)) and TRL-TG (FRU=0.47±0.07 versus NS=0.30±0.06 mg/L, \(P<0.01\)) were higher in FRU versus NS during this latter part of the kinetic study (Figure 4A and 4C). Differences in the pattern of TG elevation by glucose and fructose are small, and the study sample size was relatively small. We caution against over interpreting these small differences. Circulating FFA levels were consistently lower in FRU versus NS throughout the study period (Figure 4B), possibly attributable to relatively higher insulin levels with fructose infusion (Figure 1B in the online-only Data Supplement). Levels of C peptide were higher in FRU versus NS during the kinetic study, whereas glucagon levels were similar in both treatments (Figure 1C and IID in the online-only Data Supplement).

Figure 1. A. Experimental protocol. Intralipid+normal saline (NS) or Intralipid+glucose was infused into the duodenum through a nasoduodenal tube placed under fluoroscopic guidance into the first part of the duodenum. Triglyceride-rich lipoprotein (TRL) kinetics was studied with a primed, constant infusion of stable isotope deuterated d3-leucine for 10 hours under pancreatic clamp conditions (infusion of somatostatin, insulin, glucagon, and growth hormone). In a separate study, glucose was replaced with fructose. B. Multicompart-mental model for analysis of TRL-apolipoprotein (apo) B100 and apoB48 metabolism.
ENTRAL FRUCTOSE ELEVATED TRL-APoB48 CONCENTRATIONS THROUGH INCREASED PARTICLE PRODUCTION

TRL-apoB48 concentrations were modestly higher with fructose infusion as compared with NS (FRU=2.14±0.50 versus NS=1.39±0.34 mg/L, P<0.05; Figure 5A). Kinetic analysis revealed that TRL-apoB48 fractional catabolic rate (FRU=0.91±0.09 pools/d, NS=0.83±0.17 pools/d, P=NS) was not significantly affected by fructose infusion; instead, the higher TRL-apoB48 concentrations in FRU were because of an increased production rate (FRU=0.10±0.02 mg/(kg·d), NS=0.06±0.02 mg/(kg·d), P<0.05; Figure 5B).

ENTRAL FRUCTOSE ELEVATED TRL-APoB100 CONCENTRATIONS THROUGH INCREASED PARTICLE PRODUCTION

TRL-apoB100 concentrations were higher in FRU versus NS toward the later part of the study, that is, after 3 hours into the kinetic study (Figure 5C). Fractional clearance was not significantly affected (FRU=4.24±0.55 versus NS=3.46±0.27 pools/d, P=0.11); however, the corresponding production rates were ≈40% higher in FRU as compared with NS (FRU=17.15±3.23 versus NS=12.23±2.35 mg/(kg·d), P<0.05; Figure 5D).

Discussion

Chronic consumption of dietary carbohydrates and refined sugars are known to exacerbate postprandial lipid responses and promote metabolic abnormalities such as those that characterize the metabolic syndrome.7,22,23 The current study is the first to examine the acute metabolic effects of enteral monosaccharides on lipid-stimulated intestinal TRL particle production in healthy nonoverweight or nonobese humans. We demonstrate that both glucose and fructose infused directly into the duodenum enhance lipid-stimulated chylomicron particle production. Considering the metabolic consequences of TRL overproduction, these results point to a previously unappreciated potential role of simple sugars in exacerbating cardiometabolic risk.

Previous studies have demonstrated the importance of dietary carbohydrates on lipid metabolism, thus chronic intake of high carbohydrates induces elevation in fasting and postprandial TG (reviewed in References 7,22). This may be because of increased lipoprotein particle size (TG) and particle number (apoB), along with impaired particle clearance.22 Besides the amount, the property and composition of carbohydrates, for example, various sugar forms, also

Figure 2. Intraduodenal coinfusion of glucose with Intralipid affects concentrations of plasma triglyceride (TG; A), free fatty acid (FFA; B), and triglyceride-rich lipoprotein (TRL)-TG (C). Subjects received a constant infusion of Intralipid (20%, 60 mL/h) with equal volume of normal saline (NS) or glucose (20%, 60 mL/h, GLU) directly into the duodenum via a nasoduodenal tube throughout the study. n=7. *P<0.05 GLU vs NS.

Figure 3. Intraduodenal coinfusion of glucose with Intralipid increases triglyceride-apolipoprotein B48 (TRL-apoB48) concentrations (A), FCR and PR (B). TRL-apoB100 concentrations (C), FCR and PR (D) were not significantly affected. n=7. *P<0.05, †P<0.01 GLU vs NS.
differentially affect lipid metabolism. In acute studies, a mixed meal with high glycemic index resulted in higher chylomicron apoB48 concentrations, as compared with a meal with low glycemic index. With purified sugars, exacerbation of postprandial lipemia was observed with sucrose and fructose, but not glucose. Short-term consumption and acute intake of fructose are associated with accentuation of postprandial lipemia. Although these studies demonstrate that certain forms of carbohydrates modulate TRL metabolism, interpretation of results is confounded by the changes in insulin, glucagon, and GLP-1 levels, each of which per se may affect TRL metabolism. Furthermore, lipoprotein particle turnover was not directly assessed as it was in the present study, with consequent inability to distinguish between changes in particle production versus clearance and apoB48-containing (intestinal origin) versus apoB100-containing (hepatic origin) TRL.

It has been postulated that fructose enhancement of postprandial lipemia occurred because of reduced chylomicron clearance, as a result of competition for removal with elevated VLDL. The current study, however, provides direct evidence that intestinal luminal supply of fructose, along with lipids, promoted apoB48 production without impairing its removal. To minimize the differences in insulin and glucagon concentrations between treatments, the lipoprotein turnover was assessed under pancreatic clamp conditions. In addition, both Intralipid and monosaccharides were infused directly into the duodenum. Although insulin levels were higher with glucose or fructose infusion, the levels were modest compared with oral ingestion of these sugars without a pancreatic clamp. It has been well established that insulin acutely inhibits intestinal and hepatic TRL production. If any effects of insulin were present in the current study, it would be expected to be opposite to the observed stimulation by monosaccharides. Similarly, FFA levels were also lower with either monosaccharide than with lipid alone, possibly because of insulin suppression of adipose tissue lipolysis or increased fatty acid utilization, which would also be expected to result in an effect opposite to the observed stimulation of TRL production. The effects by glucose and fructose on TRL production, therefore, likely involve mechanisms beyond their modulation of insulin or FFA levels.
may have been impaired with even this short-term fructose infusion.

Several differences were present between glucose and fructose in their effects on TRL metabolism. A striking point is that glucose, but not fructose, promoted TRL-apoB48 fractional clearance. This effect is independent of changes in circulating glucose, insulin, and FFA levels because changes in these parameters were similar between glucose and fructose infusion. In addition, TRL-apoB100 fractional clearance was not similarly affected as TRL-apoB48, suggesting a mechanism beyond insulin stimulation of LPL activity. Plasma apoC-III concentrations were not different between treatments during each study (GLU=57.7±9.7 versus NS=61.7±12.7 mg/L, \( P=\text{NS} \); FRU=59.4±9.2 versus NS=63.1±15.5 mg/L, \( P=\text{NS} \)). Therefore, this difference in TRL-apoB48 fractional clearance did not seem to be the result of an effect on LPL via apoC-III. One possibility is that glucose may have altered the physical and chemical properties of intestinal lipoproteins in a fashion that enhanced their clearance from the circulation, but this is purely speculative. A second possibility relates to the fact that chronic sucrose feeding reduced fractional removal of postprandial lipoproteins and TRL particles from fructose-fed rats were less effectively cleared than that from glucose-fed rats. If this were the case, an increase in fractional clearance, similar to glucose, would be lost with fructose infusion. Another difference is that enteral fructose stimulated both hepatic and intestinal TRL production, yet the glucose effects on hepatic TRL production were less clear. There was a nonsignificant trend for glucose to increase TRL-apoB100 production. However, this did not reach statistical significance, possibly because of the small sample size. Previous studies on high-dietary carbohydrate-induced hypertriglyceridemia also showed increased TG production without changes in VLDL-apoB production, suggesting secretion of larger VLDL particles. The differential effects of glucose and fructose on hepatic and intestinal lipoprotein production may reflect the difference in their metabolism. Glucose and fructose utilization differ in various aspects, including the sites and metabolic pathways. In contrast to the universal utilization of glucose, fructose is mainly metabolized in the liver. Fructose is absorbed through the glucose transporter GLUT5 on the apical membrane of the enterocytes, but only a minor amount may be utilized by intestinal mucosa for glyceral synthesis. Unlike phosphofructokinase (the enzyme for glucose metabolism), fructokinase, the enzyme in the committed step of fructose metabolism in the liver, is not subject to regulation by energy status and insulin. This results in the majority of dietary fructose being taken up by the liver, where fructose escapes glycolysis and is preferentially used for de novo lipogenesis. With fructose intake, intrahepatic fatty acid availability may be increased through increased DNL, increased re-esterification of fatty acids, and decreased fatty acid oxidation, leading to increased VLDL production. In addition, fructose may induce hepatic insulin resistance (discussed above), which is expected to promote VLDL production. Nevertheless, the differences in magnitude of modulation on TRL production by glucose and fructose were modest in this acute study, which may further diverge with longer treatment.

### Table. Demographic Characteristics and Fasting Plasma Concentrations of Men who Participated in the Glucose Study and Fructose Study

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Glucose Study (n=7)</th>
<th>Fructose Study (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>49.1±2.1 (38–55)</td>
<td>38.1±3.6 (23–52)</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>75.8±6.0 (61.0–100.0)</td>
<td>72.1±3.0 (64.0–83.5)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>23.5±0.9 (20.0–27.0)</td>
<td>23.7±0.3 (22.8–25.2)</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>5.2±0.1 (4.9–5.7)</td>
<td>4.9±0.2 (4.5–5.7)</td>
</tr>
<tr>
<td>Insulin, pmol/L</td>
<td>53.5±8.0 (26.9–82.2)</td>
<td>35.5±6.2 (18.0–59.0)</td>
</tr>
<tr>
<td>Plasma TG, mmol/L</td>
<td>1.0±0.2 (0.5–1.9)</td>
<td>0.7±0.1 (0.4–1.0)</td>
</tr>
<tr>
<td>Plasma FFA, mmol/L</td>
<td>0.4±0.1 (0.2–0.6)</td>
<td>0.4±0.1 (0.2–0.9)</td>
</tr>
<tr>
<td>Plasma TC, mmol/L</td>
<td>4.1±0.2 (3.6–5.1)</td>
<td>3.6±0.1 (3.2–4.2)</td>
</tr>
<tr>
<td>TRL-apoB100, mg/L</td>
<td>55.4±7.6 (31.5–88.6)</td>
<td>89.7±10.0 (49.8–121.1)</td>
</tr>
<tr>
<td>TRL-apoB48, mg/L</td>
<td>3.0±0.9 (0.4–7.0)</td>
<td>1.6±0.2 (0.7–2.1)</td>
</tr>
</tbody>
</table>

Data are mean±SEM (ranges). apoB indicates apolipoprotein B; BMI, body mass index; FFA, free fatty acid; TC, total cholesterol; TG, triglyceride; and TRL, TG-rich lipoprotein.

The exact mechanisms whereby monosaccharides stimulate chylomicron secretion are unknown. However, based on previous studies, several potential mechanisms may be postulated. First, enteral glucose may promote rapid mobilization and release of lipids that are retained postprandially in intestinal enterocytes. In an elegant study, Robertson et al demonstrated that glucose ingestion 5 hours after a fatty meal mobilized lipid droplets in the enterocytes, eliciting an elevation in plasma TG and apoB48. Although this might have occurred in our study during the initial infusion of glucose, the persistent elevation of TG and apoB48 in plasma and TRL particles throughout the study suggests an additional role of glucose in promoting de novo chylomicron particle assembly and secretion. It is not known whether fructose is capable of similarly mobilizing lipid storage in intestinal enterocytes. Second, glucose may directly stimulate intestinal lipoprotein assembly. In the liver, increased DNL in the presence of excess glucose may be mediated by carbohydrate response element-binding protein. Carbohydrate response element-binding protein is also expressed in the small intestine; therefore, a similar mechanism may operate to stimulate intestinal TRL production. Third, hyperglycemia may have contributed to the increased intestinal TRL production. Under the experimental conditions in the current study, both glucose and fructose infusion elevated blood glucose levels (Figures IA and IIA in the online-only Data Supplement), which may reflect the capability of fructose to stimulate hepatic glycogenolysis. VLDL overproduction has been correlated with hyperglycemia. However, the effect of acute hyperglycemia per se on VLDL production has not been directly studied. An association between intestinal lipoprotein production and acute hyperglycemia has not been demonstrated. Forth, insulin resistance with fructose infusion may have contributed to the increased intestinal TRL production. Human intestinal lipoprotein assembly is subject to control by insulin, thus overproduction of intestinal TRL particles in insulin-resistant states is linked to aberrant insulin signaling. Although chronic fructose consumption induces insulin resistance, insulin sensitivity in the intestine (not directly assessed in the present study)
These findings have potentially important clinical implications. First, these results lend scientific support to recommendations in nutrition guidelines in recent years that discourage the intake of sugars and refined carbohydrates.48 Second, the contribution of dietary carbohydrate quantity and composition (e.g., glucose and fructose units) on lipid metabolism should be taken into account when designing meal tests.51 The use of defined lipid and carbohydrate composition in such tests may potentially reveal underlying metabolic abnormalities. For instance, a dual sugar challenge test that uses ingestion of both glucose and fructose could be used to identify individuals with increased risk of dyslipidemia.52

In conclusion, the results of this study reveal a novel role of enteral monosaccharides in enhancing intestinal TRL particle production in healthy humans. This newly recognized phenomenon expands our understanding of the regulation of TRL particle production and the mechanisms whereby carbohydrate consumption may aggravate dyslipidemia and thereby the risk for atherosclerosis and cardiovascular disease.

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Disclosures
None.

References
Increased production of lipid particles from the gut and the liver contributes to lipid disorders and higher cardiovascular risks. Diets with high carbohydrates are known to increase blood lipid levels and are associated with higher cardiovascular risks, but the exact mechanisms are not fully understood. We measured gut and liver lipid particle production in humans in response to continuous supply of fat plus simple sugar (glucose or fructose) directly into the small intestine. Both glucose and fructose promoted lipid particle production from the gut. Fructose also stimulated lipid particle production from the liver. These results reveal novel roles of simple sugars in rapidly enhancing intestinal lipid particle production, thereby aggravating high blood lipids.
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**Supplement Figure I.** Plasma levels of glucose (A), insulin (B), C-peptide (C) and glucagon (D).

Subjects received constant infusion of Intralipid (20%, 60 ml/h) with equal volume of normal saline (NS) or glucose (20%, 60 ml/h, G) directly into the duodenum via a nasoduodenal tube throughout the study. A pancreatic clamp was performed starting from t=−2 h. Primed constant infusion of duterated leucine was started from t=0. *P<0.05 vs NS
Supplement Figure II. Plasma levels of glucose (A), insulin (B), C-peptide (C) and glucagon (D).

Subjects received constant infusion of Intralipid (20%, 60 ml/h) with equal volume of normal saline (NS) or fructose (20%, 60 ml/h, FRU) directly into the duodenum via a nasoduodenal tube throughout the study. A pancreatic clamp was performed starting from t=−2 h. Primed constant infusion of duterated leucine was started from t=0. * P<0.05 vs NS
MATERIAL AND METHODS

Subjects

Fourteen healthy, normolipidemic, male subjects participated in this study, 7 in the glucose arm of the study and 7 in the fructose arm. Their demographic characteristics and fasting biochemical profiles are shown in Table. The subjects had normal glucose tolerance in response to a 75g, 2-hr oral glucose tolerance test performed immediately prior to their enrollment. None of the participants had any previous history of CVD, 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

Each subject underwent 2 separate lipoprotein kinetics studies, in random order, 4 to 6 weeks apart. In one study normal saline was infused intraduodenally with Intralipid. In the other study, either glucose or fructose was infused together with Intralipid. Seven subjects received saline or glucose and a separate set of 7 subjects received saline or fructose. A nasoduodenal tube (Tyco Healthcare, Toronto, ON, Canada) was inserted into the first part of the duodenum under fluoroscopy guidance one day prior to the kinetic study, as previously described, for nutrient infusion. Lipoprotein kinetics was assessed under pancreatic clamp conditions (described below) (Figure 1A).

Following insertion of the nasoduodenal tube, an iv catheter was inserted into a superficial vein of each forearm, one for infusion and one for sampling. The subject was fasted after 7pm and was not permitted to ingest food for the duration of the study. Starting at 4am the next day, subjects received a constant infusion, through the nasoduodenal tube, of Intralipid (20%, 60 ml/h) plus 60 ml/h normal saline (NS study) or plus glucose (20%, 60 ml/h, GLU study). Infusion continued until the end of the study, i.e. 7 pm. Seven additional subjects received infusion of Intralipid plus normal saline or plus fructose (20%, 60 ml/h, FRU study). We elected to match the duodenal lipid load between all studies, rather than to match caloric load between studies by reducing Intralipid infusion rates with monosaccharide co-infusion. As a result, calorie supply was 34% higher in GLU and FRU (168 kcal/h) than NS (120 kcal/h). This intentional design was felt to be a more appropriate comparison of the effect of glucose or fructose on
intestinal TRL particle kinetics, the major regulator of which is fat ingestion, although we cannot exclude an effect of the added calories in the intralipid+monosaccharide studies, particularly on hepatic TRL particle kinetics.

At 7am, i.e. 3 hours after starting the intraduodenal infusion, a pancreatic clamp was started with the following iv infusions: somatostatin (Sandostatin, Novartis Pharmaceuticals Canada, Dorval, QC, Canada) 30 ug/h, insulin (Humulin R, Eli Lilly Canada, Toronto, ON, Canada) 0.05 mU/kg/min, human recombinant growth hormone 3 ng/kg/min (Humatrope, Eli Lilly) 0.65 ng/kg/min. At 9am, 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, USA) was started for assessment of lipoprotein kinetics. Blood samples were collected at 0, 0.5, 1, 2, 3, 4, 5, 7, 8, 9 and 10 h thereafter for isolation of lipoproteins, stable isotope enrichment and kinetic analysis. Blood samples for glucose, TG, FFA and hormone analysis were collected at regular intervals.

**Laboratory Methods**

TRL were isolated from plasma samples at each time point by ultracentrifugation. Separation and hydrolysis of TRL-apoB100 and apoB48 proteins, derivatization of amino acids and analysis of stable isotope enrichment were as previously described. Derivatized samples were analyzed by an Agilent 5975/6890N GC/MS with electron impact ionization (Agilent Technologies Canada Inc, Mississauga, ON, Canada). Selective ion monitoring at m/z =200 and 203 was performed and tracer-to-tracer ratios (TTR) were calculated from isotopic ratios according to a standard curve of isotopic enrichment.

Commercial kits were used to measure cholesterol (Roche Diagnostics, Mannheim, Germany), TG (Roche Diagnostics), FFA (Wako Industrials, Osaka, Japan), insulin (Millipore, Billerica, MA, USA), C-peptide (Millipore) and glucagon (Millipore). TRL-apoB100 and apoB48 mass were measured with ELISA kits specific for human apoB100 (Mabtech Inc, Mariemont, OH, USA) and apoB48 (Shibayagi Co. Ltd., Shibukawa, Gunma, Japan). The intra- and inter-assay variations were 2% and 10% for apoB100, and 4% and 6% for apoB48, respectively. Plasma apoC-III was measured with ELISA (AssayPro, St. Charles, MO) with the intra- and inter-assay variation of 5% and 7%, respectively.

**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 (Figure 1B). The model consisted of synthesis of TRL-apoB from the precursor pool via a delay compartment. Plasma free leucine TTR, measured for each visit of each subject, was used as a forcing function. Individual TTR time course curves were used to derive kinetic rate constants. Production rates (PR) of each apolipoprotein were calculated as
PR = FCR X 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).

**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, apoB100 and apoB48 concentrations, and FCR and PR between the two treatments. All statistics were performed with SAS (version 9, Cary, NC). A p value < 0.05 was considered significant.

**References:**
