Chronic exogenous insulin and chronic carbohydrate supplementation increase de novo VLDL triglyceride fatty acid production in rats

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Abstract We have investigated hepatic de novo lipogenesis and the ratio of apoB-48/apoB-100 during chronic carbohydrate supplementation with or without administration of exogenous insulin in rats. Two groups received chronic (2 weeks) carbohydrate supplementation either as 10% glucose or 10% fructose (wt/v) in their drinking water. Two other groups received exogenous insulin chronically, in addition to the monosaccharides above. The insulin was given for 2 weeks as daily human ultralente insulin injections in increasing doses up to 6 units per day. A fifth group of rats (normal control) received only chow and water. The fractional synthetic rate (FSR), the fraction of very low density lipoprotein triglyceride (VLDL-TG) palmitate that was newly made during an 8-h infusion with sodium [1-^{13}C]acetate, was evaluated. The glucose and fructose groups had a 4-fold (0.60%/h) and 7.5-fold (1.13%/h) increase in FSR from baseline, respectively, compared to chow-fed controls (0.15%/h). Chronic exogenous insulin administration resulted in a 11.5 (1.73%/h) and 11.0 (1.65%/h)-fold increase over baseline in the synthesis of newly made VLDL-TG palmitate in the glucose and fructose groups, respectively. The ratio of apoB-48/apoB-100, i.e. apoB-48 enrichment, in VLDL was positively correlated with insulin levels ($r = 0.41$, $P < 0.01$) and with FSR ($r = 0.39$, $P < 0.01$). The present study shows that carbohydrate supplementation significantly increases the FSR of newly made VLDL-TG palmitate and that this increase is further augmented by chronic hyperinsulinemia.—**Park, J., S. Lemieux, G. F. Lewis, A. Kuksis, and G. Steiner.** Chronic exogenous insulin and chronic carbohydrate supplementation increase de novo VLDL triglyceride fatty acid production in rats. *J. Lipid Res.* 1997. **38:** 2529–2536.

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The rate of hepatic production of very low density lipoprotein (VLDL) in vivo is largely regulated by the availability of lipid substrate to the liver and by the hormonal state of the organism. How these factors affect the regulation of the biosynthesis of the lipid and apolipoprotein to form the nascent VLDL particle is not clearly understood. We previously showed in vivo that VLDL-triglyceride (TG) production in rats is elevated by both chronic fructose supplementation and chronic insulin treatment (1, 2). This increase in VLDL-TG production occurred despite a significant reduction in plasma free fatty acid (FFA) concentration. It is known that chronic carbohydrate feeding increases the activity and mRNA level of lipogenic enzymes in the liver (3) and that hyperinsulinemia increases transcription of the genes for fatty acid (FA) synthase and acetyl coenzyme A (CoA) carboxylase (4, 5). In addition, chronic carbohydrate supplementation could increase the supply of subtrate available to the liver for the production of new fatty acids that are subsequently incorporated into TG. Therefore, we previously postulated that during chronic carbohydrate supplementation and chronic hyperinsulinemia de novo lipogenesis may play a greater role in supplying the fatty acids in VLDL-triglyceride (VLDL-TGFA). To date this postulate has not been tested.

Methods to measure de novo lipogenesis in humans both in the fasting state (6) and after a high carbohydrate diet (7, 8) have recently been described. Although de novo lipogenesis was found to contribute only a small fraction of the total VLDL-TGFA (9), this contribution increased significantly when high carbo-

Abbreviations: FSR, fractional synthetic rate; VLDL, very low density lipoprotein; TG, triglyceride; FFA, free fatty acid; MIDA, mass isotopomer analysis.

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hydrate diets were given (7). It has been suggested that newly synthesized TG serves an important role in the regulation of apolipoprotein B (apoB) secretion in HepG2 cells (10). In addition, as rat liver secretes both apoB-48 and apoB-100 it is possible that changes in de novo lipogenesis may alter the proportion of these two forms of apoB in VLDL.

These methods, utilizing stable isotopes and analysis by gas chromatography–mass spectrometry (GC/MS), permit us to examine our earlier postulate not just by measuring the production of VLDL-triglyceride, but by specifically measuring the de novo production of the fatty acids in VLDL triglyceride. Furthermore, they enable us to do this in vivo, thereby allowing examination of the overall nutritional and hormonal interaction that exists in animals that are chronically supplemented with monosaccharides and made hyperinsulinemic. This paper reports on the effects of 2 weeks of carbohydrate supplementation on the proportion of VLDL-TGFA derived from de novo lipogenesis, on the added impact of chronic insulin administration on this, and on the relation between de novo lipogenesis and the relative amounts of apoB-48 and apoB-100 in plasma VLDL.

METHODS

Experimental animals and procedure

Five groups of male Sprague-Dawley rats with an initial weight of 150–200 g were studied: a normal group (n = 11), two carbohydrate-supplemented groups [glucose (n = 11) and fructose (n = 10)], and two groups supplemented with the same carbohydrates and also treated chronically with exogenous insulin [insulin+glucose (n = 13) and insulin+fructose (n = 15)]. The carbohydrate was given either as 10% glucose or 10% fructose in the solution that the rats drank ad libitum for 2 weeks. Two other groups of rats, one given glucose and the other given fructose, also received subcutaneous injections of human ultralente insulin (Novo Nordisk, Toronto, Canada) in gradually increasing doses for 15 days. The injection regimen consisted of one-third of the daily dose at 9:00 AM and the remaining two-thirds at 4:30 PM. The dose was gradually increased from day 1 (0.5 units), the maximum daily dose of 6 units per rat being reached by day 11. Previous studies showed that such rats drank the same amounts of sugar solutions and were normoglycemic whether they were given fructose or glucose and whether they received insulin or not (1, 2). All the rats were fed regular chow ad libitum.

Rat weights were recorded at the start and at the end of 2 weeks. To assay initial levels of insulin, glucose, FFA, and TG, a blood sample was taken from the tail vein at day zero from all rats. On the morning of day 15, all rats were anesthetized with Somnotol (sodium pentobarbital, 50 mg/kg) intraperitoneally and PE_{50} cannulae were inserted into the carotid artery (for blood sampling) and into the jugular vein (for infusion). The cannulae were filled with saline to prevent clotting. The insulin-treated rats were given only the afternoon insulin injection on day 15, after full recovery from anesthesia. Starting at 9:00 AM on day 16, all rats were infused with sodium [1-^{13}C]acetate, 20 μmol/kg · min, (Isotec Inc. Matheson, Miamisburg, OH; >99% isotopically enriched) for 8 h to measure de novo lipogenesis. The insulin-treated rats were given only the morning insulin injection on day 10 before the infusion. During the infusion, the rats were gently restrained in a small cage with sufficient room to permit free movement. They continued to have ad libitum access to their usual drinking solutions.

Blood samples were collected into 5% EDTA before and after the acetate infusion and were immediately spun to obtain the plasma fraction. The plasma was frozen in −20°C until the assays were performed. For apoB measurements, plasma VLDL was isolated from the fresh post-infusion plasma by ultracentrifugation at d 1.006 g/ml using a TLX 100.3 rotor (Optima TLX, Beckman, Palo Alto, CA) at 100,000 rpm and 16°C for 2.5 h.

Lipid assays

TG concentration was measured enzymatically with a commercially available kit (Boehringer Mannheim #450032, Dorval, Quebec, Canada) both in serum and VLDL. Plasma glucose levels were determined using a Beckman glucose analyzer (Beckman Instruments, Fullerton, CA) by the glucose oxidase method. FFA levels were measured by the copper colorimetric procedure (WAKO NEFA-C, Wako Chemical, Neuss, Germany). Plasma insulin concentrations were assayed by radioimmunoassay using an insulin kit (Pharmacia and Upjohn; Don Mills. Ontario, Canada).

ApoB assays

ApoB-48 and apoB-100 in rat VLDL were assayed by the slab gel technique (11). The bands were read against an apoB-100 standard curve obtained from human LDL (1.035 < d < 1.050 g/ml). This standard was used as it has been demonstrated that human apoB-100 and rat apoB-48 and apoB-100 had the same chromogenecity (12, 13). The total protein content of this standard was determined by the method of Lowry et al.

(14). An LDL preparation was used as standard only if more than 97.5% of its protein was found on analytical SDS-PAGE to be a single band with an apparent molecular weight of about 500 kD (the molecular weight of apoB-100).

Four to 20% linear polyacrylamide slab gels (8 cm × 8 cm; 1.5 mm thick; 15 wells) were used. The electrophoresis was performed using a vertical Xcell II electrophoresis apparatus connected to a power supply (EPS 400/500, Pharmacia) and was run at 130V for approximately 2 h. Gels were then stained in Colloidal Coomassie stain kit (Novex, San Diego, CA) overnight and destained in distilled water for 5 days. Staining and destaining were performed at room temperature under constant agitation. The gels were scanned with a laser densitometer (ImageMaster DTS, Pharmacia-LKB) connected to a personal computer equipped with software that allowed integration of areas under the scanned curves. The serum concentrations of VLDL-apoB-48 and VLDL-apoB-100 were then obtained by relating the samples to the standard curve.

Sample preparation and GC/MS

A total lipid extract was obtained from the plasma samples using chloroform–methanol 2:1 according to Folch, Lees, and Sloane Stanley (15). The lipid components (cholesteryl esters, TGs, cholesterol, free diacylglycerol (DG), free fatty acids, and phospholipids) were resolved as bands by thin-layer chromatography on 20 × 20 cm silica gel H plates using heptane–isopropyl ether–acetic acid 60:40:4 (16). The silica gel containing the TG band was scraped off and transesterified in the presence of the gel using 6% H_2SO_4 in methanol at 80°C for 2 h. The methyl esters were extracted with hexane, dried over anhydrous sodium sulfate, and reduced to a small volume (17). The fatty acid methyl esters (FAME) were analyzed by GC/MS with electron impact ionization (16). The GA (Model 5890 Series II, Hewlett-Packard, Palo Alto, CA) was equipped with an HP non-polar (5% phenyl methyl silicone) column (Ultra 2, 25 m × 0.2 mm ID, 0.33 μm film thickness) which was interfaced with a Model 5989A Quadrupole mass spectrometer (Hewlett-Packard, Palo Alto, CA).

Fractional synthetic rate (FSR) calculation

The FSR represents the fractional rate of incorporation of the precursor, acetyl CoA, into the product, VLDL-palmitate. The FSR calculations were based on the approach described by Chinkes et al. (18) for mass isotopomer distribution analysis (MIDA). We analyzed the methyl ester of palmitate (16:0 FA; *m/z* 270) and its isotopomers M+1 and M+2 (*m/z* 271, 272). The precursor enrichment (p) was calculated from the tracer-tracee ratios (TTR) of the isotopomers M+1 and M+2 using the following formula:

$$p = [2 \times TTR\ (M+2)/TTR\ (M+1)]/[(n-1) + 2 \times TTR\ (M+2)/TTR\ (M+1)]$$

where

$$TTR\ (M+1) = [(M+1)/(M+0)]_{post} - [(M+1)/(M+0)]_{pre}$$

$$TTR\ (M+2) = [(M+2)/(M+0)]_{post} - [(M+2)/(M+0)]_{pre} - dT1 \times TTR\ (M+1)$$

For palmitate, n is 8, the number of acetate molecules in one palmitate molecule. M+0 represents the parent ion. 'Pre' and 'post' refer to the parent and isotopomer values before and after the 8-h infusion with sodium [1-^{13}C]acetate. The 'pre' values are subtracted as they represent the natural abundance of ^{13}C in palmitate. dT1 is a theoretical value of 18.05% for palmitate, which, as Chinkes et al. (18) indicate, takes into account the contribution of singly labeled palmitate to the M+2 peak. The FSR (%/h) was then calculated based on the p value using the following formula:

$$FSR = [EB(t_2) - EB(t_1)]/[8 \times p \times (t_2 - t_1) \times (1 - p)^7]$$

where t_1 and t_2 represent the time at which the samples were taken (i.e., before and after 8 h) and EB(t) is the singly labeled product enrichment at time t ($TTR_{(M+1)}/(100+TTR_{(M+1)})$. The factor of $(1-p)^7$ accounts for the probability the seven non-labeled acetate molecules are incorporated into a palmitate molecule.

Statistical analyses

The values are expressed as means ± SE. The two-way ANOVA on repeated measurements was used to detect the effects of group, of time, and of the interaction (group × time) for variables measured both at baseline and after the 2-week treatment. A one-way ANOVA was used to compare variables measures only after the 2-week treatment (FSR, VLDL-TG, VLDL-apoB-48, apoB-100 and the ratios of VLDL-apoB-48/apoB-100 and VLDL-TG/VLDL-apo-B). Student Newman-Keuls multiple comparisons were performed after the analysis of variance. Pearson correlation coefficients were used to examine associations among variables (19).

RESULTS

Changes in weight, plasma triglyceride, free fatty acids, glucose, and insulin levels before (Pre) and after (Post) treatment are shown in **Fig. 1**. No differences in weight and plasma FFA levels were observed after 2

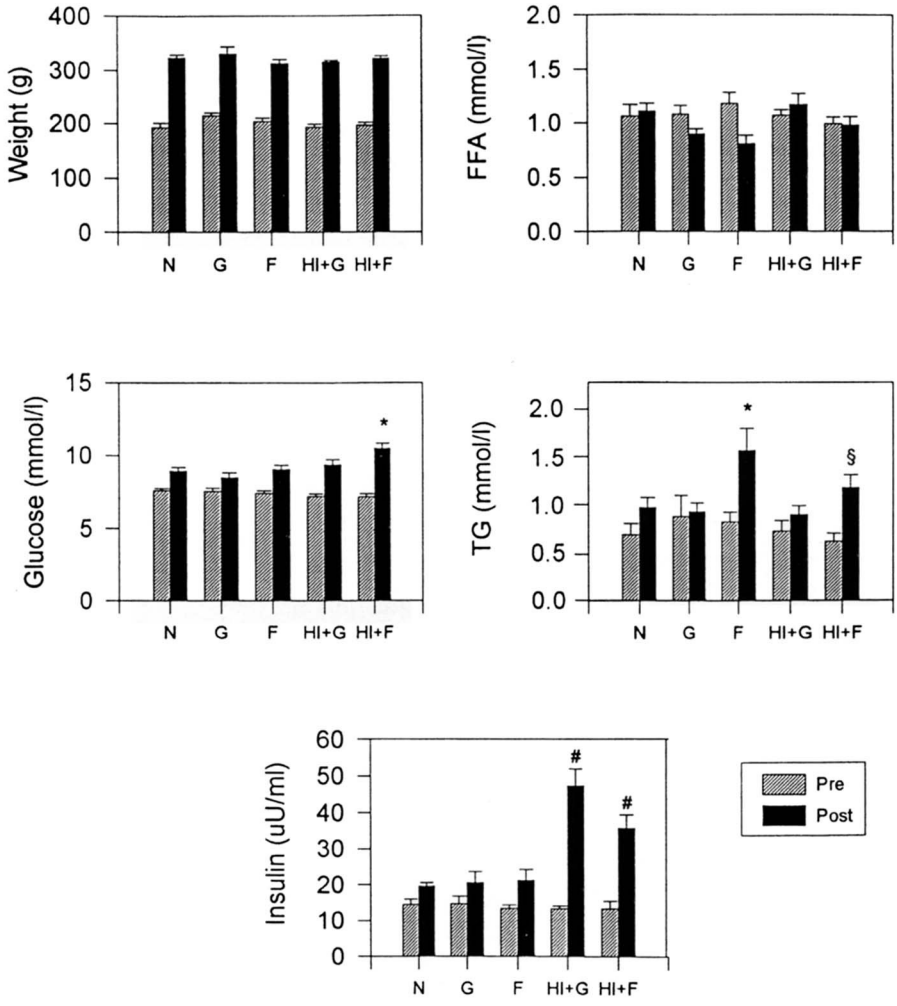

Fig. 1. Measurement of weight, plasma levels of free fatty acids, glucose, triacylglycerol, and insulin before (pre) and after (post) 2 weeks of treatment in normal rats (N, n = 11), glucose-supplemented (G, n = 11), fructose-supplemented (F, n = 10), chronically insulin-treated given glucose (HI+G, n = 13), and chronically insulin-treated given fructose (HI+F, n = 15). Data are expressed as means ± SE. All the 'pre' means are not significantly different in any of the five groups. *Significantly higher than the pre and post means of N, G, F, and HI+G groups ($P < 0.05$). §Significantly higher than the pre and post means of N, G, and HI+G groups ($P < 0.05$). #Significantly higher than the pre and post means of N, G, and F groups ($P < 0.05$).

weeks of treatment among any of the five groups. Plasma glucose levels also were normal in all groups except for the insulin+fructose group which was marginally, but significantly, higher than the rest ($P < 0.05$). Differences due to treatment were observed in plasma TG and insulin levels. Plasma TG levels were significantly higher ($P < 0.05$) in both the fructose and insulin+fructose groups than in the normal group. Also, the fructose group had higher TG levels ($P < 0.05$) than the insulin+fructose group. The TG levels of the glucose and insulin+glucose groups after 2 weeks of treatment did not differ from either those of normal rats or from the pre-treatment values of those groups. Plasma insulin levels were not different from controls in either the glucose- or fructose-fed groups, but were significantly increased in the groups given exogenous insulin.

Table 1 shows the FSR for TG palmitate in each rat group. Both glucose- and fructose-supplemented rats showed a significant increase in FSR compared to normal rats ($P < 0.05$). FSR was elevated 4-fold in the glucose group and 7-5 fold in the fructose group compared to the normal group. Both insulin+glucose and insulin+fructose goups had significantly elevated FSR

TABLE 1. Fractional synthetic rate of VLDL-TG palmitate in rats

Treatment	n	Fractional Synthetic Rate
		%/h
Normal	10	0.15 ± 0.03
Glucose	10	0.60 ± 0.11[a]
Fructose	9	1.13 ± 0.30[ab]
Insulin + glucose	13	1.73 ± 0.48[ab]
Insulin + fructose	15	1.65 ± 0.20[ab]

Data are expressed as mean ± SE.
[a]Significantly higher than normal group ($P < 0.05$).
[b]Significantly higher than glucose group ($P < 0.05$).
[c]Significantly higher than fructose group ($P < 0.05$).

values compared to the normal group ($P < 0.01$), but were not significantly different from each other. The HI+Glu group also had an increased FSR compared to the glucose group ($P < 0.05$). Although not statistically significant, the FSR in the HI+Fru group was 1.5-fold higher than that in the fructose group.

Mean values in each group for VLDL-TG, VLDL-apoB levels (total, B-48, and B-100) as well as the ratio of VLDL-apoB-48/apoB-100 are presented in **Table 2**. Fructose-supplemented rats showed significantly higher VLDL-TG levels than either normal rats or hyperinsulinemic fructose-supplemented rats ($P < 0.05$). Total VLDL-apoB was higher in fructose-supplemented rats compared to the normal and to the two insulin-treated rats. VLDL-apoB-48 concentrations were significantly higher in fructose-supplemented groups compared to all the other groups studied. VLDL-apoB-100 in the fructose group was higher than in either insulin-treated group. The ratio of VLDL-apoB-48/apoB-100 was highest in the insulin-treated groups. However, despite a significant group effect according to the analysis of variance ($F = 2.8$, $P = 0.04$), none of the multiple group comparisons were found to be significant.

TG, insulin, FFA (measurements obtained after treatment), VLDL-apoB levels (total, B-48, B-100) as well as the ratio of VLDL-apoB-48/apoB-100 were tested as potential correlates of FSR. It was found that FSR was significantly positively associated with the ratio of VLDL-apoB-48/apoB-100 ($r = 0.39$, $P < 0.01$) and negatively with the levels of VLDL-apoB-100 ($r = -0.37$, $P < 0.01$). No other significant associations were found with FSR. In addition insulin levels, after 2 weeks of treatment, were similarly associated with both VLDL-apoB-100 ($r = -0.30$, $P < 0.05$) and with the ratio of VLDL-apoB-48/apoB-100 ($r = 0.41$, $P < 0.01$).

DISCUSSION

In this study we have shown that chronic supplementation with either glucose or fructose increases the contribution of de novo lipogenesis, as indicated by the increase in the fractional synthetic rate of palmitate, to VLDL-TGFA. The FSR of VLDL-TG palmitate was significantly higher in the fructose group than in the glucose group. Treatment with insulin for 2 weeks further increased the FSR of VLDL-TG palmitate.

The difference between fructose- and glucose-supplemented rats may be explained by the fact that the in vivo metabolism of glucose and fructose differs substantially. After an oral load, glucose is primarily taken up by extrahepatic tissues, whereas fructose is removed mainly by the liver (20). The fractional hepatic uptake of an oral load of glucose has been shown to be 5% (20) and of fructose about 50% (21). This increased uptake by the liver may reflect the ability of fructose, unlike glucose, to bypass the regulatory step of phosphofructose kinase and enter glycolysis as fructose-1-phosphate which is then converted to dihydroxyacetone phosphate (21). An increase in the latter will favor the reduction of dihydroxyacetone phosphate into glycerol-3-phosphate, which is the main precursor of phosphatidic acid. This would increase the activity of the well-known phosphatidic acid pathway for de novo synthesis of TGs and glycerophospholipids. These differ-

TABLE 2. VLDL-TG, apoB, apoB-48, and apoB-100 in rats

Variable	Normal n = 9	Glucose n = 8	Fructose n = 9	Insulin + Glucose n = 12	Insulin + Fructose n = 10
VLDL-TG, mg/dl	24.1 ± 3.1	46.2 ± 8.8	55.7 ± 10.5[a]	36.3 ± 6.5	27.3 ± 3.5[c]
VLDL-apoB, μg/ml	13.4 ± 1.7	19.1 ± 3.6	24.5 ± 3.9[a]	13.3 ± 1.2[c]	11.3 ± 1.5[c]
VLDL-apoB-100, μg/ml	6.8 ± 1.1	9.2 ± 1.7	10.1 ± 1.8	5.2 ± 0.7[c]	4.8 ± 0.9[c]
VLDL-apoB-48, μg/ml	6.7 ± 0.7	9.9 ± 2.1	14.4 ± 2.2[ab]	8.1 ± 0.9[c]	6.5 ± 0.8[c]
ApoB-48/apoB-100	1.1 ± 0.1	1.1 ± 0.1	1.6 ± 0.2	1.9 ± 0.3	1.7 ± 0.2

Data are expressed as mean ± SE.
[a]Significantly higher than normal group ($P < 0.05$).
[b]Significantly higher than glucose group ($P < 0.05$).
[c]Significantly higher than fructose group ($P < 0.05$).

ences may explain the observed increase in secretion of VLDL-TG in fructose- but not in glucose-supplemented rats seen in our previous study (22). In addition to these metabolic differences, chronic carbohydrate supplementation leads to adaptive changes in liver enzyme activities (23). Park et al. (24) have shown that both chronic fructose and glucose feeding stimulates hepatic pyruvate dehydrogenase, increasing the flux of acetyl CoA towards de novo FA synthesis. Others have also shown an increase in acetyl-CoA carboxylase (21, 25) and fatty acid synthase (3) in chronic fructose feeding. Bruckdorfer, Khan, and Yudkin (3) reported increased activity of fatty acid synthase in rats fed 68% glucose or fructose for 30 days. Glycerol-3-phosphate dehydrogenase, the enzyme that reduces dihydroxyacetone phosphate to glycerol-3-phosphate, was also found to be greatly increased in chronically carbohydrate-fed rats, providing the glycerol moiety for TG synthesis (26). These differences in hepatic sugar uptake and in lipogenic enzyme activities have previously been postulated (22) to increase de novo lipogenesis and to be responsible for the observed increases in VLDL-TG production. However, previous studies have not specifically quantified the de novo production of the fatty acids in VLDL-TG in vivo under these conditions.

The effect of insulin on hepatic VLDL production is controversial. This is partly due to the differences between in vivo and in vitro models studied and to the duration of exposure of hepatocytes in culture versus animals to hyperinsulinemia. Studies of insulin's role on VLDL-TG secretion in isolated hepatocytes (27–29) and perfused livers (30, 31) came to the general agreement that long-term exposure to insulin increases secretion of VLDL-TG. Our in vivo studies (1, 2) have also shown that VLDL-TG production is increased in the chronically hyperinsulinemic state, despite a reduction in the plasma levels of free fatty acids. This suggests a greater role for newly made FAs, in contrast to plasma FFA, as a source for VLDL-TGFA (1, 2). The present study provides support for this suggestion and demonstrates that exogenous insulin has an additional stimulatory effect on de novo lipogenesis. The insulin treatment, both of rats given glucose and of rats given fructose ad libitum, increased the FSR compared to those rats given either monosaccharide alone. The FSR in fructose-supplemented insulin-treated rats was similar to that in glucose-supplemented insulin-treated rats. This may be because fructose supplementation itself elevated the FSR to near maximal levels, allowing only a limited further increase with the addition of insulin treatment. Declercq, Debber, and Mannaerts (32) have shown in cultured hepatocytes that as the glycerol-3-phosphate content is increased, the rate of newly secreted TG, as measured by the incorporation of [1-^{14}C] palmitate, becomes saturated. This relationship was shown to be hyperbolic, reaching plateau levels. This is consistent with our FSR results in the insulin-treated fructose-supplemented rats and it suggests that there is an upper limit to the stimulatory effects of carbohydrate and chronic hyperinsulinemia.

We also observed that, after 2 weeks of treatment, insulin levels were significantly and positively associated with the ratio of VLDL-apoB-48/apoB-100. This is concordant with studies in primary rat hepatocytes where long exposure to insulin (>72 h) increased apoB-48 secretion selectively by altering mRNA editing (33). Also, Phung et al. (34) reported that in Zucker rats, which are genetically obese, hyperinsulinemic, and hypertriglyceridemic, both the ratio of VLDL-apoB-48/apoB-100 and apoB-48 production, were significantly higher than in lean rats. Although we did not measure apoB turnover, our experiments permitted us to examine whether or not insulin altered the proportion of apoB-48/B-100 in the circulating VLDL. Further studies will be needed to determine the effect of hyperinsulinemia on apoB production and catabolic rate.

The physiological importance of de novo lipogenesis to total VLDL production is still unclear (9). Complete blockage of fatty acid synthesis with the agent TOFA (5-tetradecyloxy-2-furoic acid), a potent inhibitor of acetyl CoA carboxylase, results in almost complete abolition of VLDL production in perfused rat livers (35) and in hamsters (36). The studies raise the possibility that de novo lipogenesis may regulate VLDL secretion. Wang, McLeod, and Yao (37) have suggested that the production of apoB-48- and apoB-100-containing VLDL may involve two distinct pathways and that the contribution of pre-existing TG to each of these may not be the same. If this is so, it also raises the possibility that the contribution of de novo made FAs to apoB-100 and apoB-48 VLDL production may differ. Our studies demonstrating a positive association of de novo lipogenesis (FSR) to the ratio of VLDL-apoB-48/B-100 supports this suggestion.

The present studies have measured not just the production of the TG of VLDL, but the de novo production of the fatty acids, as reflected by palmitate, in this TG. They have allowed us to provide experimental data in support of earlier postulates from in vivo studies of carbohydrate supplementation and chronic hyperinsulinemia. Furthermore, they indicate that in vitro studies of the effects of monosaccharides on lipogenesis can be extended to the in vivo state. In summary, we have shown that carbohydrate feeding in rats significantly increases de novo lipogenesis, with fructose having a greater effect than glucose. Exogenously administered insulin has an additive effect in increasing de novo lipogenesis, suggesting that the latter is

one potential mechanism explaining the increased production rate of VLDL in the chronically hyperinsulinemic state. Further studies will be needed to quantify the actual contribution of de novo synthesized FAs to VLDL-TG, and to simultaneously measure the production rates of VLDL-TG, apoB, and de novo lipogenesis.

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