

A Sucrose-Rich Diet Affects Triglyceride Metabolism Differently in Pregnant and Nonpregnant Rats and Has Negative Effects on Fetal Growth^{1,2}

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ABSTRACT A sucrose-rich diet (SRD) causes hypertriglyceridemia in nonpregnant rats. To determine whether a SRD further enhances gestational hypertriglyceridemia, female rats were divided into the following two groups: 1) rats fed a SRD (63 g sucrose/100 g), and 2) rats that received the same diet except that the sucrose was replaced by an equal amount of cornstarch (CD). Half of the rats were mated and studied at d 20 of gestation. Body weight increase did not differ between virgin rats fed either diet, but the final body weight of pregnant rats fed SRD was lower than that of rats fed CD due to fewer fetuses per litter and lower fetal and placental weights. The SRD enhanced plasma glucose and insulin concentrations in virgin but not in pregnant rats; plasma triglycerides and FFA concentrations and the rate of triglyceride secretion into the plasma were higher in pregnant than in virgin rats fed SRD, but the increase in liver triglycerides due to SRD was higher in virgin rats. Both removal rate of a fat emulsion and adipose tissue lipoprotein lipase activity (LPL) were lower in virgin rats fed SRD than in those fed CD. They were lower in pregnant than in virgin rats fed CD. Placental and fetal liver triglyceride concentration and placental LPL were higher in rats fed SRD than in those fed CD. Both the increased triglyceride secretion by the liver and the decreased triglyceride removal from blood resulting in maternal hypertriglyceridemia may contribute to the negative effect of SRD on the developing fetus. *J. Nutr.* 126: 2481–2486, 1996.

INDEXING KEY WORDS:

- sucrose • pregnancy • triglycerides • rats
- lipoprotein lipase

A carbohydrate-rich diet causes hypertriglyceridemia in both humans (Mancini et al. 1973) and rats

(Reaven et al. 1979). Several investigators, including ourselves, have shown that normal rats fed a sucrose- or fructose-rich diet for 3 to 4 wk develop hypertriglyceridemia, insulin resistance and impaired glucose tolerance (Bernal et al. 1989, Gutman et al. 1987, Lombardo et al. 1983, Zavaroni et al. 1982). The hypertriglyceridemia produced by high sucrose feeding appears to be caused by the combined effects of both enhanced liver VLDL-triglyceride synthesis and secretion and decreased extrahepatic clearance (Bernal et al. 1989 and 1995, Lombardo et al. 1983, Zavaroni et al. 1982). Moreover, the insulin resistant condition caused by a sucrose- or fructose-rich diet has been associated with an oversupply of lipids produced through either an increase in circulating levels of free fatty acids (FFA)⁴ and triglycerides or in the increased triglyceride (TG) accumulation in insulin responsive tissues (Thorburn et al. 1989). Both hypertriglyceridemia and insulin resistance are occurrences in pregnancy in humans (Freinkel 1980, Montelongo et al. 1992) and rats (Knopp et al. 1970b, Martín-Hidalgo et al. 1994). Gestational hypertriglyceridemia seems to be the result of the com-

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⁴ CD, control diet; FFA, free fatty acids; LPL, lipoprotein lipase; SRD, sucrose-rich diet; TG, triglyceride; TGSR, triglyceride secretion rate.

bined effects of the enhanced adipose tissue lipolytic activity (Knopp et al. 1970a), which allows a greater production of triglycerides by the liver (Wasfi et al. 1980), and the decreased clearance of circulating triglycerides, which is a consequence of reduced lipoprotein lipase (LPL) activity in adipose tissue (Herrera et al. 1988, Martin-Hidalgo et al. 1994). Decreased insulin responsiveness in pregnancy is the result of counterregulatory factors, including gestational hormones and hyperlipidemia (Freinkel 1980, Zorzano et al. 1992), rather than a decreased number of insulin receptors.

A sucrose-rich diet during pregnancy in rats has teratogenic effects (Ornoy and Cohen 1980) and/or reduces newborn weights (Jen et al. 1991), but to our knowledge, no study has previously compared the effects of a sucrose-rich diet in pregnant and nonpregnant rats. Because exaggerated hypertriglyceridemia in pregnant rats fed a sucrose-rich diet could contribute to its negative effects in the progeny, the present work was designed to determine the effect of such a diet on triglyceride metabolism in pregnant and nonpregnant rats.

MATERIAL AND METHODS

Animals and diets. Female Wistar rats initially weighing between 170 and 185 g and purchased from the National Institute of Pharmacology, Buenos Aires (Argentina) were used in the present study. Rats were kept in our animal quarters under controlled temperature ($22 \pm 1^\circ\text{C}$), humidity, air flow and a 12-h light:dark cycle (light 0700–1900 h). After 1 wk of acclimation during which the rats were given free access to a standard laboratory nonpurified diet (cornstarch pelleted, type AIN-76 #111751 from Ralston, Purina, St. Louis, MO), they were randomly divided into two groups: 1) experimental, in which rats were fed a purified sucrose-rich (63 g/100g) diet (SRD); and 2) control, in which rats were fed the same purified diet except that the sucrose was replaced with cornstarch (63 g/100g) (CD). The composition of the diets is given in Table 1. Both diets had a paste structure and were prepared twice a week and kept refrigerated at 4°C ; they were isoenergetic (15.28 kJ/g), and the rats had free access to food and tap water. The experimental protocol was approved by the Human and Animal Research Committee of the School of Biochemistry, University of Litoral, Argentina.

After 4 d of feeding the diets, half of the rats from each group were mated with males of the same strain. The day spermatozooids appeared in vaginal smears was considered d 0 of gestation. Pregnant and virgin rats were housed in collective polycarbonate cages (4 rats/cage) with wood chips as bedding, and body weight and food intake were measured three times per week as previously described (Lombardo et al. 1983). On d 20 of

TABLE 1
Composition of experimental diets¹

Ingredient	Control diet (CD)	Sucrose-rich diet (SRD)
	g/kg diet	
Casein	170	170
Salt mix ²	35	35
Vitamin mix ³	10	10
Choline chloride	2	2
DL-Methionine	3	3
Cellulose	100	100
Cornstarch	630	—
Sucrose	—	630
Corn oil	50	50

¹ Based on the AIN-76A diet.

² Salt mix (g/kg diet): dibasic calcium phosphate, 17.5; magnesium oxide, 0.84; potassium citrate monohydrate, 7.7; potassium sulfate, 1.82; sodium chloride, 25.9; chromium potassium sulfate, 0.019; cupric carbonate, 0.05; potassium iodate, 0.0003; ferric citrate, 2.1; manganese carbonate, 0.1225; sodium selenite, 0.0003; zinc carbonate, 0.056.

³ Vitamin mix (mg/kg diet): retinyl palmitate, 2.4; cholecalciferol, 0.025; DL- α -tocopheryl acetate, 50.0; menadione sodium bisulfite, 0.8; biotin 0.22; cyanocobalamin, 0.01; riboflavin, 6.6; thiamin hydrochloride, 6.6.

gestation, food was removed at 0800 h unless otherwise indicated and rats were decapitated between 0900 and 1000 h. Trunk blood was collected in ice-chilled heparinized tubes and after centrifugation at 4°C , plasma aliquots were stored at -20°C until assay. Liver and lumbar fat pads were dissected and placed in liquid nitrogen and kept at -80°C until processing. The two uterine horns of the pregnant rats were immediately dissected and weighed with their contents to determine the whole conceptus weight. A few fetuses were rapidly decapitated to collect their blood as above and to dissect the liver, which was then placed into liquid nitrogen; others (3–5 fetuses/litter, chosen at random) were used for weighing. Placentas were also dissected and some placed immediately in liquid nitrogen while the rest were weighed.

Analytical methods. Triglycerides (Laurell 1966), free fatty acids (Dumcombe 1963) and glucose (Bergmeyer et al. 1974) were measured in plasma, and plasma immunoreactive insulin was measured by the method of Herbert et al. (1965) by using rat insulin (Novo Nordik, Copenhagen, Denmark) as standard. Triglycerides were also measured in homogenates of frozen livers and placentas (Laurell 1966). LPL activity was measured in certain tissues as previously described (Llobera et al. 1979) and was expressed as pkat (pmoles of substrate transformed per second) per amount of protein that was determined in the enzyme extracts (Lowry et al. 1951) with the exception of activities in adipose tissue which were expressed as pkat/g of fresh tissue because homogenates from this tissue had to be

supplemented with preheated (60°C) plasma to ensure quantitative extraction.

Triglyceride secretion rate (TGSR). Rats deprived of food for 16–18 h were anesthetized intraperitoneally (0.3 mL/200 g) with a ketamine cocktail (ketamine 50 g/L, diazepam 5 g/L and atropine 1 g/L, 5:4:1 v/v/v) and kept in a 28°C environment by means of a heat lamp throughout the experiment. The triglyceride secretion rate was evaluated by blocking the removal of plasma triglycerides with Triton WR-1339 (Sigma Chemical, St. Louis, MO) and calculated from the slope of the linear increase of triglycerides with time, as previously described (Bernal et al. 1989). Briefly, Triton WR-1339 (600 mg/kg, dissolved in 9 g/L NaCl) was injected intravenously and blood samples were drawn before and at 60 and 120 min thereafter for triglyceride measurements (Laurell 1966). In a separate experiment, plasma volume was determined in pregnant and virgin rats fed the two diets by calculating the isotopic dilution of ¹³¹I-labeled albumin (Radiochemical Center, Amersham, UK), as previously described (Chaves and Herrera 1980).

Fractional removal rate of fat emulsion (intravenous fat tolerance test). The method followed was based on that previously described (Rössner 1974) with some modifications. Rats deprived of food for 16–18 h were intravenously injected with 0.1 mL/100 g body weight of 10% Intralipid® (Kabivitrum, Alameda, CA) through a vein in the tail and serial blood samples (0.2–0.3 mL) drawn immediately before and over the next 30 min thereafter. The hematocrit changed from 0.47 ± 0.007 at 0 min to 0.46 ± 0.06 at 30 min after the injection (*n* = 8). Blood was centrifuged at 60 × *g* for 10 min at 4°C; the plasma was collected with care to avoid red cells, and then recentrifuged under the same conditions to remove any remaining red cells and debris. Plasma (0.2 mL) was diluted to 10 mL with 9 g/L NaCl and mixed gently to avoid bubble formation; light-scattering was measured by nephelometry. A calibration curve was constructed using serial dilutions of an initial 1:2000 dilution in saline of the same Intralipid® batch. The nephelometry values for the samples obtained from each rat gave a straight line when plotted on a semilogarithmic scale against time. The first-order constant (*K*₂) for the disappearance of the fat emulsion from the blood stream (fractional removal rate) was calculated by the least-squares method.

Statistical methods. Results are expressed as means ± SE. Statistical significance between two groups was determined by the Student's *t* test or, when appropriate, data were subjected to two-way ANOVA (BMDP, University of California, Los Angeles, CA), with diet and pregnancy as the main effects. When treatment effects were significantly different (*P* < 0.05), means were tested by the Scheffé test and linear regressions were calculated by the least-squares method (Steel and Torrie 1960).

TABLE 2

Maternal, fetal and placental weights in pregnant rats fed sucrose-rich (SRD) or control (CD) diets¹

	Control diet (CD)	Sucrose-rich diet (SRD)
Initial body weight, g	198.5 ± 2.2	195.2 ± 2.1
Final body weight (d 20), g	340.7 ± 9.0	299.8 ± 5.1***
Conceptus weight, g	74.0 ± 2.3	57.1 ± 2.6***
Increment in maternal conceptus-free body weight, g/20 d	57.9 ± 2.9	50.0 ± 3.2
Fetuses/litter, <i>n</i>	12.1 ± 0.3	10.8 ± 0.4*
Average fetus body weight, g	3.88 ± 0.07	3.52 ± 0.20*
Average placenta weight, g	0.557 ± 0.009	0.471 ± 0.022**

¹ Values are means ± SE, *n* ≥ 8.

* *P* ≤ 0.05; ** *P* ≤ 0.01; *** *P* ≤ 0.001.

RESULTS

At the beginning of the experiments there were no differences in the body weights of the rats; however, at d 20 of gestation rats fed the sucrose rich diet (SRD) weighed less than those fed the control diet (CD) (Table 2). This difference corresponded in part to the conceptus weight which was significantly lower in the pregnant rats fed SRD than in those fed CD, whereas the increment in the net maternal body weight (free of conceptus) during gestation did not significantly differ between the two groups (Table 2). The lower conceptus weights in the rats fed SRD corresponded to the smaller number of fetuses per dam as well as the lower fetal and placental weights compared with those in dams consuming the CD diet (Table 2). Average daily energy intake was similar in the two groups of pregnant rats (227.7 ± 7.5 kJ/d in SRD and 237.0 ± 8.3 kJ/d in CD, *n* = 7).

Virgin rats had a significantly lower energy intake than pregnant rats, and once again, average energy intake did not differ between rats fed the SRD and those fed CD (170.8 ± 4.6 kJ/d vs. 173.2 ± 5.4 kJ/d, respectively). The average weight gain was also similar in the two groups (1.58 ± 0.8 g/d in SRD vs. 1.57 ± 0.04 g/d in CD, *n* = 7).

Plasma metabolite levels and liver triglyceride concentrations. Plasma glucose, insulin, triglycerides and free fatty acid (FFA) concentrations as well as liver triglyceride concentration were significantly higher in virgin rats fed SRD than in those fed CD (Table 3). Pregnant rats fed CD had significantly lower plasma glucose and higher plasma insulin levels than virgin rats fed the same diet. However, unlike the effect found in virgin rats, the SRD did not modify either glycemia or insulinemia in pregnant rats. Pregnant rats fed either CD or SRD had significantly higher plasma triglyceride and FFA concentrations than the corresponding values in virgin rats. The SRD further enhanced the basal hyperlipidemic condition of the pregnant rats, and both plasma triglyceride and FFA attained the highest levels

TABLE 3

Plasma glucose, insulin, triglycerides and free fatty acids (FFA) concentrations and liver triglyceride concentration in pregnant and virgin rats fed sucrose-rich (SRD) and control (CD) diets¹

Diet	Pregnancy	Plasma				Liver
		Glucose	Insulin	Triglycerides	FFA	Triglycerides
		<i>mmol/L</i>	<i>pmol/L</i>	<i>mmol/L</i>	<i>μmol/L</i>	<i>μmol/g wet tissue</i>
CD	-	7.07 ± 0.18 ^b	273.0 ± 15.8 ^c	0.43 ± 0.07 ^c	248.0 ± 16.7 ^d	9.30 ± 0.49 ^c
SRD	-	7.64 ± 0.14 ^a	446.2 ± 15.8 ^b	1.42 ± 0.14 ^b	400.0 ± 41.9 ^c	27.30 ± 0.55 ^a
CD	+	5.46 ± 0.10 ^c	768.7 ± 83.3 ^a	1.45 ± 0.07 ^b	656.0 ± 44.8 ^b	8.10 ± 0.24 ^c
SRD	+	5.53 ± 0.20 ^c	622.9 ± 46.7 ^a	4.23 ± 0.47 ^a	1100.2 ± 88.2 ^a	11.30 ± 0.52 ^b
2 × 2 ANOVA ²						
Diet		NS	NS	S	S	S
Pregnancy		S	S	S	S	S
Diet × Pregnancy		NS	S	S	S	S
Residual mean square		0.194	1969.2	0.4486	17019.7	1.43

¹ Values are expressed as means ± SE, $n \geq 8$. Values in each column that do not share the same superscript are significantly different ($P \leq 0.05$).

² ANOVA: effect significant, S ($P \leq 0.05$) or not significant, NS ($P > 0.05$).

in pregnant rats fed SRD (Table 3). Liver triglyceride concentration was similar in pregnant and virgin rats fed CD; however, SRD enhanced liver triglyceride concentration much more in virgin than in pregnant rats, and the difference between the two groups was statistically significant (Table 3).

Triglyceride secretion rate, removal rate of fat emulsion and adipose tissue lipoprotein lipase activity. At d 20 of gestation, pregnant rats fed CD had a higher triglyceride secretion rate (TGSR) and a lower removal rate of a fat emulsion (Intralipid®) as well as lower adipose tissue LPL activity than virgin rats fed the same diet (Table 4). Virgin rats fed the SRD had higher TGSR and lower fat emulsion removal rate and adipose tissue LPL activity than those fed CD. Pregnant rats fed SRD

had higher TGSR and lower adipose tissue LPL activity than those fed CD, whereas the SRD did not modify the already very low fractional fat emulsion removal rate in pregnant rats (Table 4).

Placentas and fetuses. Placentas from pregnant rats fed SRD had a significantly higher triglyceride concentration and LPL activity than those from rats fed CD (Table 5). Moreover, a linear and significant correlation could be established when maternal plasma triglycerides were plotted against the placental triglyceride concentrations from all of the individual animals studied [(plasma triglycerides) = $-5.6506 + 2.2632$ (placental triglycerides)]; $r = 0.8816$, $n = 13$, $P < 0.001$].

Liver triglyceride concentration in fetuses from dams fed SRD was significantly higher than in those

TABLE 4

Rate of triglyceride secretion into the plasma (TGSR) and removal rate of a fat emulsion (K_2) and adipose tissue lipoprotein lipase activity (LPL) in pregnant and virgin rats on sucrose-rich (SRD) and control (CD) diets¹

Diet	Pregnancy	TGSR	K_2	LPL
		<i>nmol/(min · 100 g body weight)</i>	<i>%/min</i>	<i>pkat/g wet tissue</i>
CD	-	291 ± 11 ^d	11.91 ± 0.37 ^a	2971 ± 249 ^a
SRD	-	419 ± 23 ^b	7.00 ± 0.60 ^b	880 ± 123 ^c
CD	+	385 ± 15 ^c	1.61 ± 0.36 ^c	1016 ± 101 ^b
SRD	+	527 ± 21 ^a	2.19 ± 0.71 ^c	644 ± 66 ^c
2 × 2 ANOVA ²				
Diet		S	S	S
Pregnancy		S	S	S
Diet × Pregnancy		NS	S	S
Residual mean square		1829	1.736	19383.4

¹ Values are expressed as means ± SE, $n \geq 6$. Values in each column that do not share the same superscript letter are significantly different ($P < 0.05$).

² ANOVA: effect significant, S ($P \leq 0.05$) or not significant, NS ($P > 0.05$).

TABLE 5

Placenta and fetal liver triglyceride concentration and placenta lipoprotein lipase activity (LPL) of rats fed sucrose-rich (SRD) or control (CD) diets¹

Diet	Placenta		Fetus
	Triglyceride <i>μmol/g wet tissue</i>	LPL <i>pkat/g wet tissue</i>	Liver triglyceride <i>μmol/g wet tissue</i>
CD	3.15 ± 0.10	104 ± 12	2.94 ± 0.03
SRD	3.90 ± 0.13**	148 ± 14*	4.97 ± 0.02**

¹ Results are given as means ± SE of samples from 6–13 separate dams; * $P \leq 0.05$; ** $P \leq 0.001$.

from dams fed CD (Table 5). A significant and linear correlation was again found when maternal plasma triglycerides were plotted against the corresponding fetal liver triglyceride concentration [(maternal plasma triglycerides) = $-0.1739 + 0.6543$ (fetal liver triglycerides); $r = 0.6392$, $n = 11$, $P < 0.05$]. However, no significant differences between fetuses from the SRD-fed dams and those from the CD-fed dams were detected in either plasma triglyceride or FFA concentrations (data not shown).

DISCUSSION

The present results show that a sucrose-rich diet exaggerates the hypertriglyceridemic effect of pregnancy by enhancing the rate of liver VLDL-triglyceride secretion, and these effects are proportional to those seen in virgin rats. Although the maternal conceptus-free body weight was unaffected by the SRD, this diet decreased conceptus weight, which corresponded to a smaller number of fetuses per litter and a decreased mass of both the fetus and the placenta.

In virgin rats, the increase in circulating triglycerides caused by the SRD paralleled the increase in both circulating FFA levels and the liver triglyceride concentration, suggesting that an enhanced release of adipose tissue lipolytic products allows an increase in liver triglyceride synthesis and their release into circulation in the form of VLDL-TG. This interpretation fits well with the enhanced rate of triglyceride secretion seen in these rats. A decreased clearance of triglyceride-rich lipoproteins could also contribute to the hypertriglyceridemia seen in virgin rats fed the SRD, as suggested by both the decreased removal rate of Intralipid® and the lower LPL activity seen in the adipose tissue of these rats. All of these changes are exaggerated in pregnant rats, where most of these pathways are already modified in rats receiving a control diet in the same direction as seen in the virgin animals fed a sucrose-supplemented diet. Thus, the hypertriglyceridemia present in control pregnant rats may be associated with

their increased plasma FFA consequent to their known enhanced adipose tissue lipolytic activity (Knopp et al. 1970a, Martin-Hidalgo et al. 1994). This would increase the liver synthesis rate and content of triglycerides, as occurred in virgin rats receiving the SRD. However, the enhanced arrival to the liver of lipolytic products in the pregnant rats fed CD compared with virgin rats fed the same diet is not reflected by an accumulation of triglycerides in the liver, probably as a consequence of the enhanced capacity of the liver of the pregnant rats fed CD to release the newly formed VLDL-TG into circulation. This is suggested by the augmented rate of triglyceride secretion seen in these rats. A decreased clearance of circulating triglyceride-rich lipoproteins also seems to contribute to maternal hypertriglyceridemia in pregnant rats fed CD, as suggested by the decreases in both the Intralipid® removal rate and the adipose tissue LPL seen in these animals. These findings also agree with previous reports (Herrera et al. 1988, Martin-Hidalgo et al. 1994).

The SRD further enhanced the hypertriglyceridemia in pregnant rats, and present results indicated that such a response is the result of an additional increase in adipose tissue lipolytic activity together with the enhanced capacity of the liver of these rats to release the newly formed triglycerides into circulation. This interpretation is supported by the exaggerated increase in plasma FFA levels, the greater increase in the rate of triglyceride secretion into plasma and the moderate increase in liver triglyceride concentration seen here in these rats compared with both the pregnant rats receiving CD and the virgin rats fed either the CD or the SRD. An additional decrease in circulating triglycerides clearance cannot be claimed in the pregnant rats receiving the sucrose-rich diet because the removal of administered Intralipid® did not change when compared with rats in the same animals receiving the control diet.

Although the present findings do not explain the mechanism through which the SRD impairs fetal growth, the increase in both the placental and fetal liver triglyceride concentrations as well as the significant correlation of maternal plasma triglycerides with both of these variables would indicate a relationship between them. Maternal circulating triglycerides are unable to directly cross the placental barrier (Herrera et al. 1992), but the presence of lipolytic activity in the placenta allows fatty acids present in maternal plasma triglycerides to reach the fetus for use in triglyceride synthesis in the fetal liver (Herrera et al. 1992). Enhanced LPL activity in the placenta of the rats fed the SRD in the presence of maternal exaggerated hypertriglyceridemia could facilitate the placental transfer of fatty acids from the maternal triglycerides. Such a mechanism could be responsible for the great increase in triglyceride concentration in the fetal liver.

Finally, the present results show that the SRD enhances the plasma concentration of both insulin and glucose in virgin rats, suggesting an insulin-resistant

condition in agreement with previous reports (Reaven et al. 1979). However, in pregnant rats, this diet modified neither the plasma insulin nor the plasma glucose concentrations. Although more direct experiments are required, the results indicate that in contrast to what occurs in virgin rats, the SRD does not modify insulin sensitivity in rats at late gestation. This different response insofar as insulin sensitivity is concerned between virgin and pregnant rats fed SRD is not surprising, because there are conditions in which the same stimulus provokes an insulin response which is different in pregnant than in virgin animals (Ramos and Herrera, 1995). A condition of exaggerated hyperinsulinemia without hypoglycemia, such as that caused by prolonged intravenous glucose infusion, reduces insulin sensitivity in virgin rats; however, the hyperinsulinemia reverts the insulin-resistant condition of the animal in late pregnancy (Ramos and Herrera 1995). Although the mechanism acting in the pregnant rats fed the SRD may be similar, more direct experiments are required to test this hypothesis.

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