Characterization of a model of dietary-induced hypertriglyceridemia in young, nonobese rats

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Abstract Healthy, nonobese, young rats developed hypertriglyceridemia (mean triglyceride levels of 250 mg/dl) following consumption of a sucrose–lard diet. The hypertriglyceridemia was apparent three days after start of the diet and persisted throughout the 4-week experimental period. Body weight, liver weight, and serum glucose levels were similar in animals eating either the sucrose–lard diet or standard rat chow. On the other hand, serum free fatty acid levels were slightly increased and serum insulin levels were substantially increased in animals eating the sucrose–lard diet. Determination of very low density lipoprotein turnover revealed that total triglyceride secretion in rats eating the sucrose–lard diet was significantly ($P < 0.01$) increased over that of rats eating standard chow. Direct measurement of hepatic and intestinal very low density lipoprotein secretion indicated that the observed rise in total triglyceride secretion was secondary to increased secretion of very low density lipoproteins by the liver. Finally, lipoprotein lipase activity of adipose tissue from rats eating the sucrose–lard diet was equal to, or greater than (depending upon sampling time), the activity of the enzyme from adipose tissue of rats eating the control diet. These data indicate that young, nonobese, rats develop hypertriglyceridemia when they ingest a sucrose–lard diet, and that the rise in plasma triglyceride levels results from an increase in hepatic very low density lipoprotein secretion. The dietary-induced hypertriglyceridemia is associated with elevated serum insulin levels, and, as such, may provide a useful animal model to use in studies aimed at defining the pathogenesis of endogenous hypertriglyceridemia in man.—**Reaven, G. M., T. R. Risser, Y-D. I. Chen, and E. P. Reaven.** Characterization of a model of dietary-induced hypertriglyceridemia in young, nonobese rats. *J. Lipid Res.* 1979. **20:** 371–378.

Supplementary key words endogenous hypertriglyceridemia · sucrose–lard diet · hyperinsulinemia · hepatic VLDL-TG secretion · intestinal VLDL-TG secretion · lipoprotein lipase

We have previously suggested that circulating insulin levels play a central role in the development of endogenous hypertriglyceridemia in man (1). This hypothesis evolved from our observation that the fasting plasma triglyceride (TG) concentration attained on a given diet was highly correlated with the plasma insulin response elicited by that diet (1–3). Furthermore, we indicated that the increase in fasting plasma TG level in the majority of patients was secondary to increased very low density lipoprotein (VLDL) secretion (1–3). During the succeeding years, considerable evidence has been published with is consistent with this formulation. Thus, many investigators have subsequently noted that there is a significant correlation between plasma insulin and TG levels (4–11). Although the relationship between plasma TG concentration and VLDL secretion rate has been more controversial (1, 11–19), results of recent studies, using a variety of techniques (20–24), seem to indicate that endogenous hypertriglyceridemia is associated with increased VLDL secretion in most individuals. In addition, considerable support for the overall hypothesis has been derived from studies of animal models with hypertriglyceridemia. In particular, the elegant studies of the *ob/ob* mouse by Assimacopoulous-Jeannet et al. (25) and Loten, Rabinovitch, and Jeanrenaud (26) have emphasized the crucial role that hyperinsulinemia plays in the hypertriglyceridemia seen in this genetic variant.

Although there is a good deal of agreement concerning the fact that there is a relationship between plasma TG levels, insulin response, and VLDL secretion rate in subjects with endogenous hypertriglyceridemia, questions remain as to the meaning of the observed relationships. In this regard, the absence of an entirely satisfactory animal model for the study of endogenous hypertriglyceridemia has inhibited investigation of the biochemical and physiological mechanisms responsible for the rise in plasma TG levels. Thus, endogenous hypertriglyceridemia develops spontaneously in obese, genetic variants such as the *ob/ob* mouse (25, 26) or the Zucker rat (27). It can also be seen when the Egyptian sand rat is allowed to eat ad libitum in captivity (28), as well as when normal rodents are subjected to hypothalamic lesions (29). However,

Abbreviations: TG, triglyceride; FFA, free fatty acid; VLDL, very low density lipoprotein.

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even under these conditions, the existence of morbid obesity is essential for the development of hypertriglyceridemia. Although valuable information can be gained from study of such models, the relevance of these data to endogenous hypertriglyceridemia in man is open to question. In an effort to avoid this potential dilemma, we have tried to induce hypertriglyceridemia in otherwise normal rats by increasing their glucose or their fructose intake as previously described (30, 31). We could not predictably induce a sustained increase of plasma TG levels in young, nonobese rats with these methods, and this variability in TG response to different dietary carbohydrates is apparent from the work of others (30–35). However, we have more recently adopted the procedure described by Bar-On, Roheim, and Eder (36) and, with their prescribed diet, can consistently induce sustained hypertriglyceridemia in rats without the development of concomitant obesity. In this communication we describe the results of our use of this approach to define the characteristics of dietary-induced hypertriglyceridemia in nonobese and otherwise normal rats.

METHODS

Experimental protocol

Male Sprague–Dawley rats, weighing 180–200 g, were used for all experiments. Prior to experimentation, the rats were fed standard rat chow and maintained on a 12-hr light/dark (6 AM/6 PM) cycle. At the start of each study, the rats were weighed and some rats were placed on the experimental diet (which will be subsequently referred to as the sucrose–lard diet) and other rats continued to eat regular laboratory rat chow (standard diet) for periods up to 4 weeks. The sucrose–lard diet (Teklad Labs, Madison, WI) contains 350 calories/100 g chow; diet constituents (as percent calories) are 66% sucrose, 12% lard, and 22% casein.[2] Standard rat chow (Wayne Lab Blox, Allied Mills, Inc., Chicago, IL) also contains 350 calories/100 g, but its composition (as percent calories) is 60% vegetable starch, 11% unsaturated oils, and 29% animal proteins. Unless otherwise indicated, food was removed from the animals at 8 AM on the final day of a study, the animals were weighed, then blood was taken and/or experimental procedures were begun 5 hr later.

[2] In order to keep the sucrose–lard diet palatable for the rats, it was necessary to use diet that had been stored under refrigeration for less than 3 months.

Measurements

Serum glucose, insulin, triglyceride, and free fatty acid (FFA) levels. Blood samples for glucose, insulin, triglyceride, and FFA levels were obtained from the tail or after decapitation of unanesthetized animals. The resulting serum was separated by centrifugation, aliquoted, frozen, and later assayed for glucose (37), insulin (38), TG (39), and FFA (40) levels.

Total body TG (liver and intestine) turnover. VLDL turnover rates were determined by following the rate of removal from the plasma of prelabeled VLDL-TG (41, 42). This was done in two ways. Female donor rats, weighing 200–250 g were maintained on a fat-free diet for 4–7 days. On the day of the experiment their food was removed at 8–9 AM, and 4 hr later they were injected via the tail vein under light ether anesthesia with 400 μCi of [2-^{3}H]glycerol. The donor rats were exsanguinated under sodium thiamylal anesthesia 20 min later. (Previous experiments had shown maximal incorporation of [^{3}H]glycerol into TG 20 min after injection, at which time 95% of the lipid-extractable radioactivity was incorporated into VLDL-TG as determined by ultracentrifugation and thin-layer chromatography.) In some experiments plasma was separated by centrifugation, and 0.8-ml aliquots were injected without anesthesia into the tail veins of rats eating either the control or sucrose–lard diet. In other experiments the plasma obtained was used to isolate VLDL by ultracentrifugation, and aliquots of isolated VLDL, rather than plasma, were injected into the experimental rats. After the administration of either labeled VLDL or plasma, the tail was amputated proximal to the site of injection and 0.4 ml of blood was collected into capillary tubes rinsed with a 5% EDTA solution 5, 10, 15, and 20 min after the injection. (These samples took 1–2 min to collect and, in all subsequent calculations, the time used for a collection period was the mean of the beginning and ending times of the blood collection.) The plasma was separated by centrifugation and stored frozen until analyzed. Samples were extracted with chloroform–methanol, polar lipid was removed with silicic acid, and each sample was evaporated to dryness. Radioactivity was measured by liquid scintillation counting (Beckman LS-235), using a standard toluene scintillation mixture. The half-time ($t_{1/2}$) of VLDL-TG removal was directly determined from these measurements by a least-squares linear regression analysis, and was found to be comparable regardless of the source of the prelabeled TG (i.e., plasma or VLDL). Therefore, in order to avoid the possible alteration of the VLDL that might occur as the result of the isolation procedure, we carried out the studies of VLDL-TG

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removal rate with prelabeled unextracted plasma. VLDL-TG turnover rate was calculated from the following formula: VLDL-TG turnover rate = ($ln2 \div t_{1/2}$) $\times$ (plasma TG concentration) $\times$ (plasma volume). Since these studies were carried out under steady state conditions, VLDL-TG turnover rate = VLDL-TG removal rate = VLDL-TG secretion rate. For the purposes of this communication we shall subsequently refer to this measurement as VLDL-TG secretion rate.

Plasma volume of animals from each diet group was estimated by a standard dye dilution technique after the intravenous injection of Evans blue; the average plasma volume obtained by this method for both categories of animals was found to be 4.0 ml/100 g body weight and this figure was used in the above formula to calculate VLDL-TG secretion rate.

Hepatic TG secretion. Hepatic TG secretion by livers isolated from rats eating the standard or sucrose–lard diet was determined by in situ cyclic perfusion techniques previously described (43, 44). The basal perfusing medium consisted of a filtered mixture of fresh 90% defibrinated rat blood and Krebs–Ringer bicarbonate buffer (containing 3 g of bovine serum albumin/100 ml) which was infused with an oleic acid–albumin mixture, bringing mean FFA levels to 0.42 μEq FFA/ml during the course of the perfusion. The perfusate was recycled through the livers at flow rates of 1.0 ml/min per g of liver. Samples of 1.0 ml were removed from the recycling perfusate of all rats at 0, 30, 60, 90, and 120 min and were stored at −20°C for subsequent FFA and TG determinations. Mean FFA levels were determined by averaging the 30–90 min values for each liver/perfusion. Net VLDL-TG secretion was calculated from TG changes in perfusate concentration, corrected for sampling losses and infusate additions at each time point between 30 and 90 min; these values are expressed as mg TG secreted/hr per g liver weight.

Intestinal TG secretion rates. Intestinal TG secretion was determined from measurements of TG entry rate into the mesenteric lymph. To obtain these values, mesenteric lymph duct cannulations (45) were performed on animals eating either the standard or sucrose–lard diet. In addition, each animal received a duodenal fistula for infusion of saline. Following surgery, the animals were maintained in restraining cages similar to those described by Bollman (46), and were permitted to stabilize overnight while receiving a continuous duodenal infusion of saline (3 ml/hr). The morning after surgery, three 2-hr collections of lymph were obtained in test tubes containing 100 μl of EDTA solution. Lymph volume was measured for each collection period to determine the flow rate per hr. Only those animals that had established a stable lymph flow were included in the study. The TG entry rate into intestinal lymph (mg/hr) was calculated by multiplying the mean hourly lymph TG concentration by the mean hourly lymph flow.

Lipoprotein lipase activity. For examination of adipose tissue lipoprotein activity, samples were obtained from postabsorptive rats (killed 1–2 PM) or from fed rats (killed 9 AM) who previously had eaten the standard or sucrose–lard diet. Samples of perirenal adipose tissue were weighed, immediately frozen with dry ice, and stored at −70°C. Acetone–ether powders, as described by Nilsson-Ehle, Tornquist, and Belfrage (47) were subsequently prepared, homogenized in NH_4Cl–NH_4OH buffer (pH 8.0) and lipoprotein lipase activity was assayed according to Schotz et al. (48). Different concentrations of homogenate were reacted with a mixture containing 10 mM radioactive and unlabeled triolein (sp act 0.1 μCi/μmol), 4% BSA, and 8% horse serum in 0.1 M Tricine-HCl buffer, for 30 min at 37°C. Nonspecific hydrolytic activity was obtained for each sample by using sodium chloride (1 M) to inhibit specific lipoprotein lipase activity in duplicate samples. The results are expressed as μmol FFA released/hr per g wet weight tissue.

RESULTS

Effect of diet on weight and TG, glucose, FFA, and insulin levels

Rats given the sucrose–lard diet ate identical amounts of food (22 g/rat per day) during the 4-week experimental period, and, as indicated in **Fig. 1,** they gained the same amount of weight (4.6 g/rat per day) as animals eating the standard diet. Furthermore, there were no differences in liver weights of rats eating either diet. Despite these similarities in food consumption and weight gain, serum TG levels were substantially ($P < 0.01$) higher in the group eating the sucrose–lard diet. Fig. 1 indicates that the incremental rise in plasma TG concentration averaged 150 mg/dl at 1 week and 190 mg/dl after 4 weeks of the sucrose–lard diet.

The effect of the sucrose–lard diet on serum glucose, insulin, and FFA concentrations is seen in **Fig. 2.** These data indicate that serum glucose did not change, but that both insulin and FFA levels were higher after both 1 and 4 weeks of eating the experimental diet. Although the incremental increase in insulin level was relatively greater, the rise in both insulin and FFA was statistically significant ($P < 0.01$) at both time intervals.

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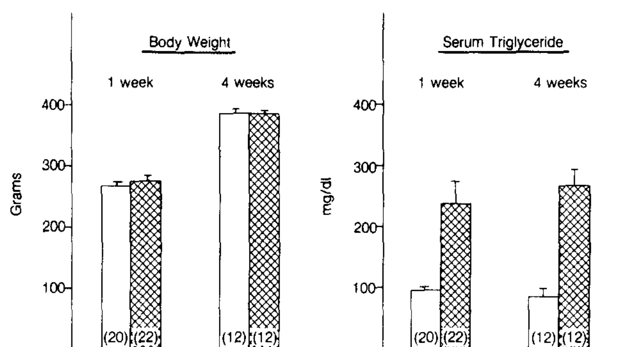

Fig. 1. Body weight and serum TG concentrations in rats eating the standard (clear bars) or sucrose–lard diet (hatched bars) for 1 or 4 weeks. Measurements are from postabsorptive rats (1–2 PM). Results are expressed as mean ± SE; the number of rats in each category is indicated in each bar.

TABLE 1. Effect of diet on serum TG levels and total TG secretion rates

Diet[a]	Number of Rats	Body Weight	Serum TG	TG Secretion Rate
		g	*mg/dl*	*mg/min/100 g*
Standard (1 week)	13	268 ± 6	94 ± 8	0.56 ± 0.06
Sucrose–lard (1 week)	14	277 ± 7	241 ± 29[b]	0.90 ± 0.09[b]
Standard (4 weeks)	11	389 ± 7	84 ± 13	0.53 ± 0.07
Sucrose–lard (4 weeks)	12	384 ± 6	270 ± 22[b]	1.00 ± 0.13[b]

[a] Food was removed from all rats at 8 AM and experimental procedures began 5 hr later. Results are expressed as mean ± SE.
[b] $P < 0.01$ as compared to results from animals eating standard diet.

Effect of diet on VLDL-TG secretion rates

Mean TG concentrations and secretion rates of the four experimental groups are seen in **Table 1.** These results show that the hypertriglyceridemia that results from eating the sucrose–lard diet is associated with significant ($P < 0.01$) increases in VLDL-TG secretion rates. The individual data are seen in **Fig. 3,** in which VLDL-TG secretion rates obtained from animals fed both diets (for 1 and 4 weeks) are plotted against the TG concentration that obtained during the determination of the VLDL-TG turnover in these animals. The plot suggests that the rats in this study comprise a single population, in which higher serum TG levels are associated with higher VLDL-TG secretion rates. No distinction can be made between groups of animals eating their respective diets for 1 week versus 4 weeks. However, the rats consuming the sucrose–lard diet in general had higher values of both VLDL-TG secretion and TG concentration. Thus, it seems clear that their hypertriglyceridemia is secondary to dietary-induced increases in VLDL-TG secretion.

Effect of diet on hepatic and intestinal TG secretion

Although the measurement of VLDL-TG secretion rates indicated that animals eating the sucrose–lard diet had higher overall secretion rates, it was not clear what proportion of this increased VLDL-TG secretion was contributed by the liver and what proportion was contributed by the intestine. To answer these questions, direct measurements of hepatic and intestinal TG secretion were made. **Fig. 4** indicates that hepatic TG secretion was almost doubled in livers from animals that had eaten the sucrose–lard diet for 1 week. In contrast, only a slight increase in intestinal TG secretion rate was observed in animals eating the sucrose–lard diet, and this difference was not statistically significant.

Effect of diet on adipose tissue lipoprotein lipase

As seen in Fig. 4, adipose tissue lipoprotein lipase activity was not different in animals eating the standard or sucrose–lard diet when samples were taken at 2 PM (or 5–6 hr after food was removed from the rat cages). However, when tissue was removed from

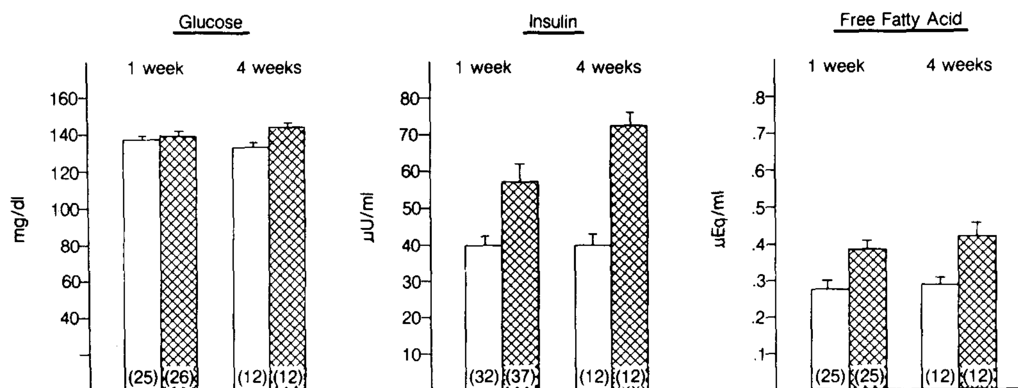

Fig. 2. Serum glucose, insulin, and FFA concentrations in rats eating the standard (clear bars) or sucrose–lard (hatched bars) diet for 1 or 4 weeks. Measurements are from postabsorptive rats (1–2 PM). Results expressed as in Fig. 1.

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animals at 9 AM, adipose tissue lipoprotein lipase activity of animals eating the sucrose–lard diet was significantly ($P < 0.01$) elevated as compared to samples from animals on the standard diet: mean (±SE) lipoprotein lipase activity at 9 AM was found to be 73 ± 8 and 44 ± 4 μmol FFA/hr per g wet weight tissue, respectively, for animals eating the sucrose–lard or standard diet.

DISCUSSION

The results we have presented confirm the earlier observations of Bar-on, Roheim, and Eder (36) that feeding young, nonobese rats a sucrose–lard diet can lead to hypertriglyceridemia. The hypertriglyceridemic effect of the diet was expressed in the vast majority of the rats studied, and the elevation of plasma TG concentration persisted throughout the duration of our experimental observations. Our results provide no insight as to which component (or components) of the diet was most important in inducing the rise in TG concentration. However, there are some observations that suggest that sucrose may be the key element. For example, Cohen, Briller, and Shafrir (49) fed diets containing butter and either sucrose or cornstarch to rats for one year, and found that the TG levels of sucrose-fed rats were twice as high as their starch-fed controls. Furthermore, Shiff, Roheim, and Eder (50) described a 2.5-fold increase in plasma TG levels when rats were fed a sucrose and corn oil diet, which is comparable to the degree of elevation seen with the sucrose–lard diet used in this study. Finally, several investigators have found that increasing dietary fructose intake will also lead to hypertriglyceridemia under certain conditions (31–36). Thus, the available evidence suggests that it is the increase in dietary carbohydrate which is most likely to have been responsible for the development of the hypertriglyceridemia. On the other hand, this question was outside the scope of the current investigation, and it is certainly possible that the effects of the lard and sucrose were synergistic. In either event, these results do point out that dietary-induced hypertriglyceridemia can develop in normal rodents who are neither genetic variants, obese, nor subjected to hypothalamic lesions. As such, this model may prove quite useful in efforts to define the mechanisms of endogenous hypertriglyceridemia in man.

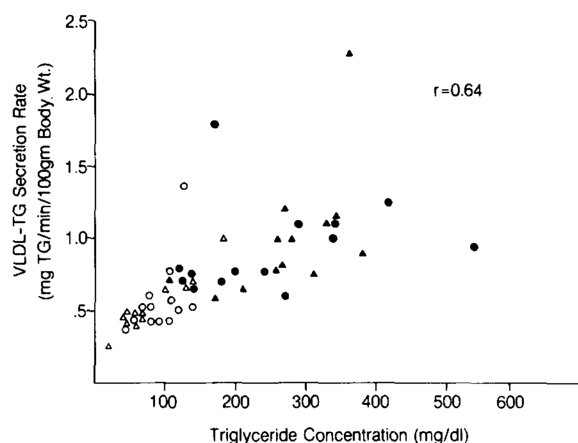

Fig. 3. Correlation between serum triglyceride levels and VLDL-TG secretion rates in animals fed the standard diet for 1 week (open circles) or 4 weeks (open triangles) or the sucrose–lard diet for 1 week (closed circles) or 4 weeks (closed triangles). The plot shows that rats in this study comprise a single population in which the higher TG secretion rates are associated with higher serum TG levels; in general, rats consuming the sucrose–lard diet had higher values for both TG secretion and TG concentration. Correlation coefficient, $r = 0.64$.

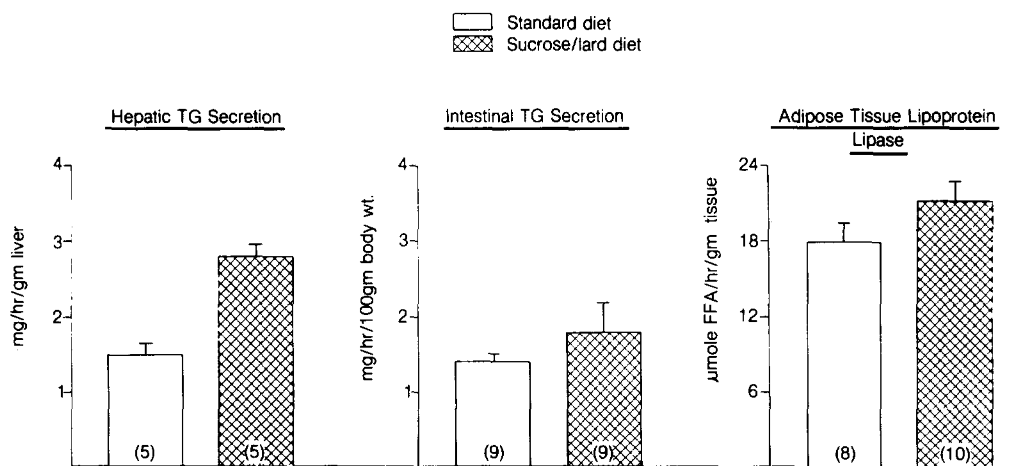

Fig. 4. Values for hepatic TG secretion, intestinal TG secretion, and adipose tissue lipoprotein lipase activity in rats eating the standard (clear bars) or sucrose–lard diet (hatched bars) diet for 1 week. Measurements are from postabsorptive rats (1–2 PM). Results expressed as in Fig. 1.

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Obviously, the utility of any animal model for the study of human disease depends upon the degree to which the animal model simulates the situation in man. On the basis of this criterion, the current model seems particularly propitious. Indeed, all of the well-recognized features of endogenous hypertriglyceridemia as it occurs in man seem to be replicated in this example of dietary-induced hypertriglyceridemia in normal rats. Thus, the increase in plasma TG level resulting from this diet is highly correlated with an apparent dietary-induced rise in plasma insulin level. Furthermore, the hypertriglyceridemia seems to be secondary to increases in VLDL-TG secretion rate (12). This conclusion is supported by the demonstration that elevations in plasma TG levels were correlated with direct measurements of VLDL-TG secretion rate, and by the observation that adipose tissue lipoprotein lipase activity was, if anything, somewhat higher in the hypertriglyceridemic rats. Finally, direct estimates of both hepatic and intestinal VLDL-TG secretion suggest that the rise in plasma TG concentration was secondary to increased secretion of VLDL-TG by the liver—a finding yet to be confirmed in man.

We have suggested that the increase in VLDL-TG secretion that occurs in man is secondary to the observed hyperinsulinemia, and there is evidence that insulin can augment hepatic lipoprotein synthesis and secretion (1–2). In man the hyperinsulinemia seems to be associated with a loss of normal insulin sensitivity (3), and the same phenomenon may have occurred in the sucrose–lard-fed rats. Thus, Vrána and Kazdová (51) have documented the presence of insulin resistance in adipose tissue from sucrose-fed rats, and the combination of hyperinsulinemia and slightly elevated plasma glucose levels suggests that insulin sensitivity may also be decreased secondary to the sucrose–lard diet. The relationship between hyperinsulinemia and insulin resistance is a complicated one. For example, insulin resistance could have developed in these animals as a direct consequence of the diet, and the hyperinsulinemia could be viewed as a compensatory effort aimed at maintaining normal glucose homeostasis. Alternatively, sucrose-rich diets could lead to increased insulin secretion, hyperinsulinemia, "down-regulation" of the insulin receptors (52, 53), and insulin resistance. Obviously, the effect of the sucrose–lard diet on these relationships deserved further study. Finally, it must be pointed out that an increase in FFA delivery to the liver could also lead to an increase in VLDL-TG synthesis and secretion. Although disagreement exists as to whether this phenomenon does (54) or does not occur (1) in patients with endogenous hypertriglyceridemia, it is obvious that it might play an important role in the development of this syndrome. In this regard, the slight increase in FFA levels produced by the sucrose–lard diet may add another dimension to the similarity between this animal model and endogenous hypertriglyceridemia in man.

In conclusion, we have described an animal model of dietary-induced hypertriglyceridemia with metabolic characteristics that strongly resemble those seen in patients with endogenous hypertriglyceridemia. The observation that changes in carbohydrate intake can modify plasma TG levels of rats was not unexpected, and several earlier publications (30–36, 49, 50) have documented hypertriglyceridemia as the result of a variety of protocols aimed at increasing the intake of glucose, fructose, or sucrose. Although previously published results differ somewhat in such details as which carbohydrate was most potent, the age at which rats will or will not respond, and the time it takes to induce hypertriglyceridemia, the overall unanimity of the results serves to highlight the central role that carbohydrate intake plays in control of plasma TG levels. Furthermore, several of these earlier studies have contributed significant insights as to the mechanism of carbohydrate-induced lipemia in the rat. However, in none of these models has the pathogenesis of the hyperlipidemia been so thoroughly characterized as has now been done with the sucrose–lard diet. As such, we suggest that this example of dietary-induced hypertriglyceridemia provides a useful experimental model for studies aimed at increasing our understanding of the pathogenesis of endogenous hypertriglyceridemia in man.

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