Dietary Rat Models in Which the Development of Hypertriglyceridemia and That of Insulin Resistance Are Dissociated

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The consequences of chronic ingestion of a high-carbohydrate (starch + glucose [HCHO]) and high-fat (lard + corn oil [HFAT]) diet on triglyceride metabolism and insulin sensitivity were evaluated in fasted and fed rats. Compared with their HFAT counterparts, animals fed the HCHO diet displayed fasting and postprandial hypertriglyceridemia that was apparent after 3 weeks of feeding and persisted after 6 weeks. It was determined that hypertriglyceridemia was due to oversecretion of triglycerides into the circulation. During fasting, triglyceride accumulation in plasma after administration of Triton WR1339 was indeed twofold higher in HCHO than in HFAT rats, whereas the global capacity for intravascular triglyceride hydrolysis, as assessed by an intravenous fat tolerance test and measurement of postheparin plasma lipoprotein and hepatic lipase activities, was comparable in both dietary cohorts. The postprandial increase in triglycerides after a high-carbohydrate meal was larger in HCHO than in HFAT rats. A fasting intravenous glucose tolerance test (IVGTT) showed that HFAT animals displayed insulin resistance after 3 weeks of feeding, which worsened after 6 weeks of treatment. Thus, the HCHO diet elicited fasting and postprandial hypertriglyceridemia without impairment of insulin sensitivity as compared with the HFAT diet, whereas the latter brought about deterioration of the sensitivity of glucose metabolism to insulin without affecting triglyceridemia. From these studies and other animal models, it is suggested that rapid delivery of fatty acids to tissues from chylomicron-derived triglycerides leads to insulin insensitivity, while fatty acids may not be available to increase endogenous production of triglycerides because they are mainly oxidized. In contrast, dietary starch/glucose increases hepatic synthesis and secretion of triglycerides that result in hypertriglyceridemia, but the deleterious effects of glucose–fatty acid competition on insulin sensitivity are prevented because endogenously derived triglycerides are catabolized more slowly and glucose is available for oxidation. The present results support the concept that coexistence of hypertriglyceridemia and resistance of glucose metabolism to insulin may be frequent but not obligatory.

HYPERTRIGLYCERIDEMIA and resistance to insulin-mediated glucose uptake frequently occur in tandem. For instance, the two metabolic abnormalities are part of the multiple metabolic syndrome, or syndrome X, and sometimes coexist in obesity and non–insulin-dependent diabetes mellitus.[1-4] Their common occurrence has also been reported in type IIB, IV, and V hyperlipidemias, familial combined hyperlipidemia, and familial dyslipidemic hypertension.[5-8]

Prospective studies have suggested that insulin resistance can precede, as well as follow, hypertriglyceridemia.[9] Several links between hypertriglyceridemia and insulin insensitivity may be invoked as possible causes for their common occurrence. Insulin influences both the rate of hepatic triglyceride secretion into the circulation and the rate of disappearance from the bloodstream through its modulatory action on lipoprotein lipase (LPL) activity.[10,11] Thus, loss of insulin action and hyperinsulinemia consequent to insulin resistance are likely to alter triglyceride metabolism. For instance, hyperinsulinemia and increased fatty acid mobilization triggers the synthesis of very-low-density lipoprotein (VLDL) triglycerides in the insulin-resistant liver.[12] On the other hand, that insulin resistance per se leads to decreased catabolism of triglyceride-rich lipoproteins is still uncertain, but triglyceride hydrolysis may become saturated, especially postprandially, because of elevated hepatic secretion of VLDL.[13] Thus, impairment of both the production and catabolism of triglycerides could lead to hypertriglyceridemia in insulin resistance. Conversely, it has been proposed that circulating levels of triglycerides per se may be able to influence insulin homeostasis and sensitivity of glucose metabolism to the hormone.[14] Fat intake or infusion blunts the insulin-induced reduction of hepatic glucose output and decreases glucose transport, oxidation, and storage through the glucose–fatty acid cycle.[15-18] Since increased oxidation of fatty acids can inhibit glucose oxidation and storage, the presence of high levels of triglycerides could constitute a source of increased fatty acid availability and oxidation, thereby reducing sensitivity of glucose metabolism to insulin.

Despite the relatively frequent coexistence of hypertriglyceridemia and resistance to insulin-mediated glucose uptake, situations in which one occurs in the absence of the other have been reported. For instance, normalization of triglyceridemia with gemfibrozil did not improve insulin resistance in type II diabetic subjects.[19] Recently, the presence of frank hypertriglyceridemia without impairment of insulin-mediated glucose metabolism has been reported in transgenic mice expressing the human apolipoprotein C-III gene.[20] These findings emphasize the fact that a link between impairments in triglyceride and glucose metabolism may be frequent but not obligatory. This concept is further supported by the present studies, which describe dietary rat models in which the development of hypertriglyceridemia and that of insulin resistance are dissociated from each other.

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Table 1. Diet Composition and Percentage of Energy Derived From Nutrients in the HCHO and HFAT Diets

Nutrient	HCHO		HFAT	
	% Weight	% Energy	% Weight	% Energy
Corn starch	46.8	47.4	13.1	9.8
D-Glucose	15.6	15.8	4.4	3.2
Corn oil	3.2	7.2	19.8	32.5
Lard	3.2	7.2	19.8	32.5
Casein	20.0	20.3	27.6	20.2
DL-Methionine	0.3	0.3	0.4	0.3
Vitamins*	1.0	1.0†	1.4	1.0
Minerals‡	4.9	0.6§	6.7	0.6
Alphacel	5.0	0.0	6.8	0.0

*Teklad vitamin mix no. 40060 (Teklad, Madison, WI).

†Energy from corn starch diluent.

‡AIN-76 mineral mix (ICN Biochemicals, Montreal, Quebec, Canada).

§Energy from sucrose diluent.

MATERIALS AND METHODS

Animals

Male Wistar rats (Charles River, St. Constant, Canada) initially weighing 150 to 175 g (study 1) or 100 to 125 g (study 2) were housed individually in stainless steel cages. They were placed in a room at 22 ± 1°C lighted between 8 PM and 8 AM, and had free access to tap water. The animals were divided into two dietary groups. One group had access to a purified high-starch, high-dextrose diet (HCHO), and the other was fed a purified high-fat diet (HFAT) with a polyunsaturated to monounsaturated to saturated fat ratio of approximately 1:1:1. Except for the carbohydrate-fat proportion, all nutrients had a similar caloric density in both diets (Table 1). The care and killing of animals were approved by our institutional review board.

Protocols

Two studies were performed in which animals were fed the experimental diets for a total of 3 weeks (study 1) or 6 weeks (study 2). The general outline of the procedures and measurements is presented in Table 2. In study 1, fasting and postprandial plasma concentrations of triglycerides, nonesterified fatty acids (NEFA), glucose, insulin, glucagon, and corticosterone were determined. An intravenous glucose tolerance test (IVGTT) was performed, and rates of triglyceride secretion into and clearance from the circulation were evaluated in separate groups of animals. Finally, activities of LPL and hepatic triglyceride lipase (HTGL) were assessed in postheparin plasma. Details of the above procedures are given later. In study 2, measurements of the same fasting and postprandial plasma variables, as well as the IVGTT, were performed in animals fed the diets for 6 weeks.

Meal Feeding

To measure postprandial plasma variables at a well-defined moment after food intake, animals were subjected to a meal-feeding protocol. The two dietary cohorts were allowed to acclimate to their environmental conditions and diets for 2 weeks (study 1) or 5 weeks (study 2) before procedures were initiated. During this time, the animals had free access to the diet. Body weight and food intake were recorded every other day. Thereafter, ad libitum food intake was restricted to the dark period, and the animals were adapted during 5 days to eat a meal 30 minutes after the beginning of the dark period, according to a protocol previously reported,[21] with slight modifications. Meal composition was similar to the chronic diet. Food was not restricted during meal intake, and the animals spontaneously ingested approximately 100 kJ (~25% of their average daily food intake). Any uningested food was removed 30 minutes later, followed by restoration of ad libitum access to food after an additional 90 minutes until the end of the dark period. This dietary protocol did not alter total 24-hour food intake. On the day of sampling, all acutely fed rats received the HCHO meal to allow between-cohort comparisons of the response of plasma variables to the same nutritional stimulus. On the day of killing, 10 rats from each of the two dietary cohorts were kept in the fasted state. An additional 10 animals in each cohort were given a meal composed of the HCHO diet. Two to three hours after the beginning of meal intake, the animals were decapitated. Trunk blood was collected and kept on ice until centrifuged (1,500 × *g*, 4°C, 15 minutes). The separated serum was stored at −70°C until later biochemical measurements. Inguinal and retroperitoneal adipose depots, vastus lateralis and soleus muscles, and the heart were removed and weighed.

Intravenous Glucose Tolerance, Triglyceride Secretion and Clearance Rates, and Postheparin Plasma Lipases

Rats were cannulated via the jugular vein under isoflurane anesthesia and were allowed to recover for 3 days before being used for the following procedures. After a 12-hour fast, an IVGTT was performed in 10 rats from each of the two dietary cohorts. The animals were injected through the venous catheter with 1.5 mL · kg body weight^{-1} of a 35% glucose solution dissolved in saline. The catheter was then flushed with saline. Blood samples (250 μL) were taken through the catheter with EDTA-containing syringes (1.5 g · L^{-1} blood) before (0 minutes) and 5, 10, 15, and 30 minutes after glucose administration. Glucose and insulin were determined in plasma obtained from these samples as described later. Ten additional animals in each of the two dietary cohorts were used for determination of lipid emulsion clearance rate. After a 12-hour fast, 0.75 mL · kg body weight^{-1} of a 20% Intralipid emulsion (Vitrum, Stockholm, Sweden) was administered within 1 minute through the venous catheter. Samples of 0.15 mL blood were taken 1, 4, 8, and 12 minutes after injection of lipid emulsion. Plasma triglyceride concentration was determined as described later. The

Table 2. Flow Diagram of Experimental Procedures

16 or 37 Days Ad Libitum	16 or 37 Days Ad Libitum	18 or 39 Days Ad Libitum	18 Days Ad Libitum	18 Days Ad Libitum
5-day meal-feeding*	5-day meal-feeding	Cannulation†	Cannulation	Cannulation
12-hour fast	HCHO meal	12-hour fast	12-hour fast	12-hour fast
Fasting blood	Postmeal blood‡	IVGTT	Intralipid§	TGSR
			12-hour fast	
			LPL, HTGL	

NOTE. Ten animals fed the HCHO diet and 10 fed the HFAT diet were used for each of 5 procedures described in columns. Procedures related to fasting blood, postmeal blood, and IVGTT were performed in two studies that lasted for a total of 3 weeks (study 1) or 6 weeks (study 2). The other procedures were performed only in study 1.

Abbreviation: TGSR, triglyceride secretion rate.

*Meal composition during training period was similar to that of chronic diet.

†Rats were allowed to recover from surgery for 3 days.

‡Blood was collected 2 to 3 hours after meal intake.

§Rats were returned to ad libitum feeding for 2 days after the Intralipid procedure and before sampling for postheparin plasma LPL and HTGL activities.

rate of elimination of triglycerides from the circulation was calculated as the slope of the semilogarithmic plot of plasma triglycerides versus time, and was defined as k_2 (percent per minute).[22] Two days after measurement of Intralipid clearance rates, the same animals were used for determination of postheparin plasma LPL and HTGL activities. After a 12-hour fast, the 10 rats in each dietary group were administered 200 IU · kg body weight^{-1} of heparin through the jugular catheter. Ten minutes later, a blood sample (0.15 mL) was withdrawn and centrifuged, and plasma was stored at −70°C until later determination of LPL activity. Rats were then killed by decapitation. Samples of 2 μL postheparin plasma diluted to 100 μL with saline were used for determination of postheparin plasma LPL and HTGL activities as described later. A final group of 10 animals in each dietary group was used for determination of triglyceride secretion rate. After a 12-hour fast, rats were injected through the jugular catheter with 600 mg · kg body weight^{-1} of Triton WR1339 (Sigma, St Louis, MO), a detergent that prevents intravascular triglyceride catabolism. Blood samples (0.15 mL) were taken before (0 minutes) and 20, 40, and 60 minutes after Triton injection. Rates of VLDL-triglyceride secretion were determined from regression analysis of triglyceride accumulation in plasma versus time, and adjusted for plasma volume.[23]

Serum/Plasma Measurements

Serum/plasma glucose was determined using the Beckman glucose analyzer (Beckman Instruments, Palo Alto, CA). Insulin and glucagon levels were measured by radioimmunoassay using reagent kits from Incstar (Stillwater, MN) and ICN Biomedicals (Carson, CA), respectively. Incremental areas under the glucose and insulin curves obtained during IVGTT were calculated with a computer-graphics program, using values at 0 minutes as the baseline. Corticosterone level was also measured by radioimmunoassay.[24,25] Triglycerides were assayed by an enzymatic method using a reagent kit from Boehringer Mannheim (Montreal, Canada), which allowed correction for free glycerol. NEFA levels were measured enzymatically using reagents from Wako Chemicals (Richmond, VA). Postheparin plasma LPL and HTGL activities were assessed using a method described in detail elsewhere,[26] in which enzyme activities are evaluated by measuring the amount of in vitro hydrolysis by postheparin plasma samples of a labeled triolein emulsion in the presence of 0.1 or 1 mol/L NaCl.

Statistical Analysis

The data are expressed as the mean ± SEM. Data obtained in fasting and postprandial states were compared using a 2 × 2 factorial ANOVA to determine main treatment effects (diet, with two levels, HCHO and HFAT, and meal, with two levels, fasting and postprandial) and treatment interactions. Individual between-group comparisons were made using the post hoc Fisher's protected least-squares difference test. Data obtained from serial blood samplings were analyzed using ANOVA with repeated measures, with time as the variable with repeated measures. Postheparin plasma LPL activities were compared using an unpaired Student's *t* test.

RESULTS

After 3 weeks of treatment, rats displayed comparable body and organ weights regardless of whether they had been fed the HCHO or the HFAT diet (Table 3). Rats fed the HFAT diet for 6 weeks were slightly heavier than their HCHO counterparts, a difference reflected in the mass of adipose depots, which were 25% larger. Muscle tissues had comparable mass in both dietary cohorts after 3 or 6 weeks of treatment. Table 3 also shows that in each of the two studies, animals fed either diet spontaneously ingested comparable amounts of energy. The average daily ad libitum intake was less in animals fed for 6 weeks than in those fed for 3 weeks because the former were younger at the onset of the study. The size of the last high carbohydrate meal was slightly smaller in rats chronically fed the HFAT diet for 3 weeks compared with that ingested by HCHO rats. This difference in meal size, which was not significant in the cohort fed for 6 weeks, was not of sufficient magnitude to affect plasma variables, since glucose, lipid, and hormone levels did not correlate with meal size (data not shown).

Table 3. Final Body and Tissue Weights (g), Average Daily Food Intake (kJ), and Size of Last Meal (kJ) of Rats Fed a HCHO or a HFAT Diet for 3 or 6 Weeks

	3 Weeks		6 Weeks	
Parameter	HCHO	HFAT	HCHO	HFAT
Body weight (g)†	329 ± 5	333 ± 4	400 ± 7	421 ± 7*
Inguinal WAT (g)	1.6 ± 0.1	1.7 ± 0.1	2.8 ± 0.1	3.5 ± 0.2*
Retro WAT (g)	2.0 ± 0.1	2.3 ± 0.1*	3.5 ±0.1	4.4 ± 0.2*
Vastus lateralis (g)	0.96 ± 0.02	0.96 ± 0.02	1.20 ± 0.02	1.20 ± 0.02
Soleus (g)	0.133 ± 0.004	0.143 ± 0.003	0.166 ± 0.005	0.167 ± 0.004
Heart (g)	1.00 ± 0.01	0.98 ± 0.02	1.07 ± 0.02	1.12 ± 0.02
Food intake (kJ/d)	409 ± 6	408 ± 6	346 ± 6	351 ± 5
Last meal (kJ)	119 ± 7	97 ± 7*	106 ± 5	92 ± 8

NOTE. Values represent the pooled mean ± SEM of groups in the fasting and postprandial states (18 to 20 animals), except for size of last meal, for which values represent the mean ± SEM of groups in the postprandial state (8 to 10 animals).

Abbreviations: Retro, retroperitoneal; WAT, white adipose tissue.

*Different from HCHO group of similar treatment duration, $P < .05$.

†Initial weight of rats treated for 3 weeks was 150 to 175 g, and that of rats treated for 6 weeks was 100 to 125 g.

Plasma glucose and insulin concentrations during an IVGTT performed in rats fed a HCHO or HFAT diet for 3 or 6 weeks are shown in Fig 1. Peripheral insulin resistance in HFAT animals was suggested at both treatment periods. Indeed, intravenous glucose elicited higher plasma insulin concentrations in HFAT than in HCHO rats, as reflected by larger incremental areas under the insulin curve (3 weeks, 52% > HCHO, $P < .03$; 6 weeks, 68%, $P < .04$). Despite this, plasma glucose was higher in HFAT animals (incremental areas at 3 weeks, 51% > HCHO, $P < .01$; 6 weeks, 97%, $P < .02$), suggesting that insulin was less efficient in

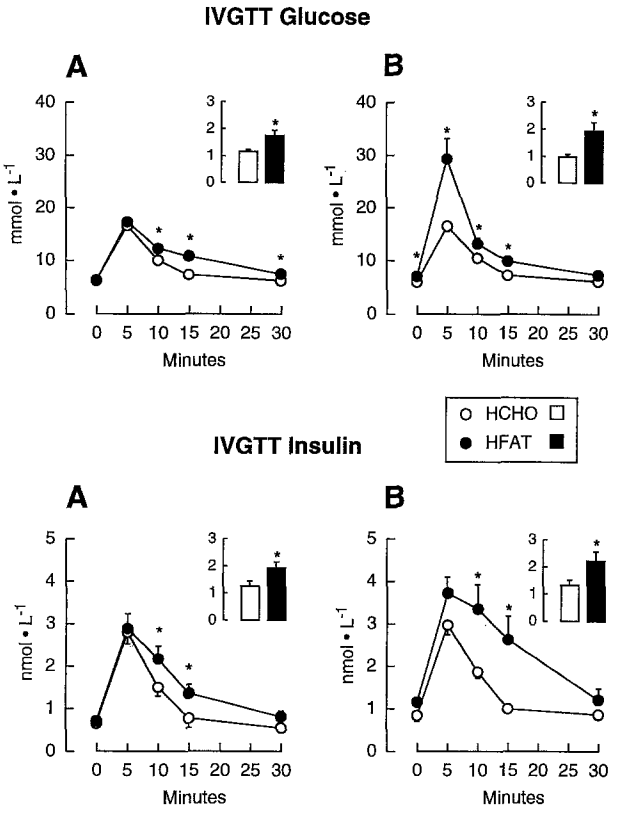

Fig 1. Plasma glucose and insulin concentrations during an IVGTT in fasted rats chronically fed either a HCHO or a HFAT diet for 3 weeks (A) or 6 weeks (B). Results are the mean ± SEM of 8 to 10 animals. * Different from the HCHO group, $P < .05$. Inserts represent the means of incremental areas (above 0 minutes under the curves in arbitrary units.

stimulating glucose disposal in HFAT rats. Keeping in mind that the two treatment periods were evaluated in separate experiments, the sensitivity of glucose metabolism to insulin action nevertheless appeared to deteriorate gradually with treatment duration in the HFAT cohort.

Figure 2 shows triglyceride and NEFA levels in serum collected in the fasted state or 2 to 3 hours after a high-carbohydrate meal in animals fed the diets for 3 or 6 weeks. For both treatment periods, animals fed the HCHO diet displayed fasting hypertriglyceridemia as compared with HFAT animals, on the order of twofold and threefold after 3 and 6 weeks of treatment, respectively. The high-carbohydrate meal increased serum triglycerides in both cohorts, but more so (fourfold and twofold at 3 and 6 weeks, respectively) in rats chronically fed the HCHO diet than in the HFAT group, as confirmed by a significant diet-meal interaction ($P < .0002$ at 3 weeks; $P < .05$ at 6 weeks) revealed by factorial ANOVA. Fasting serum NEFA were also higher in the HCHO than in the HFAT cohort, a difference that persisted up to 6 weeks of treatment. The absolute postprandial decrease in NEFA was also larger in the HCHO than in the HFAT cohort for both treatment periods (diet-meal interaction, $P < .007$ at 3 weeks and $P < .002$ at 6 weeks).

In the fasted state, both at baseline of the IVGTT (Fig 1) and before meal intake (Table 4), blood glucose levels were comparable in the two dietary groups treated for 3 weeks, but HFAT rats displayed hyperglycemia as compared with the HCHO group after 6 weeks of treatment. Fasting insulin concentrations were comparable in both dietary groups regardless of treatment duration (Fig 1 and Table 4). Intake of the high-carbohydrate meal resulted in a significant increase in serum glucose, which was larger in HFAT than in HCHO rats at both treatment periods (Table 4). Serum insulin was elevated twofold to threefold over fasting values by meal intake. Insulin tended to reach higher levels in response to the high-carbohydrate meal in HFAT than in HCHO animals, and did so significantly after 3 weeks of treatment. (Table 4).

It was determined in pilot studies that the postprandial response of triglycerides, glucose, and insulin to the high-carbohydrate meal over a 6-hour period was affected by the long-term diets only in their magnitude, not in their time course (data not shown). Therefore, diet-induced differences reported here at a single time point after the meal reflect alterations in the absolute magnitude of the postprandial increase in the variables.

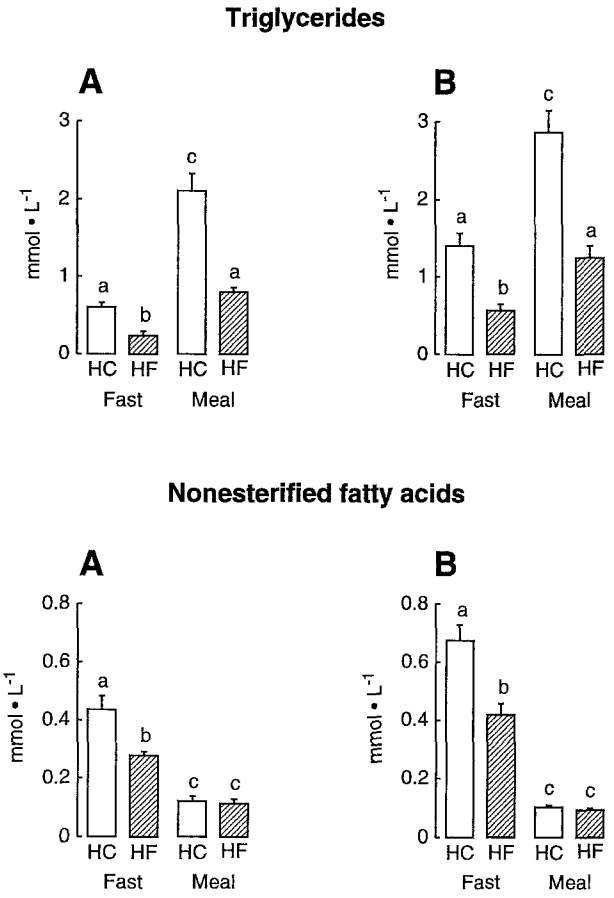

Fig 2. Serum triglyceride and NEFA concentrations in the fasted state and 2 to 3 hours after intake of a high-carbohydrate meal in rats chronically fed either a HCHO diet (□) or a HFAT diet (▨) for 3 weeks (A) or 6 weeks (B). Bars represent the mean ± SEM of 8 to 10 animals. Bars not sharing a common superscript are different from each other, $P < .05$.

Table 4. Serum Glucose, Insulin, Glucagon, and Corticosterone Concentrations in the Fasted State and After Intake of a High-Carbohydrate Meal in Rats Chronically Fed Either a HCHO or a HFAT Diet for 3 or 6 Weeks

	Fasting		Postprandial*	
Parameter	HCHO	HFAT	HCHO	HFAT
Glucose (mmol · L^{-1})				
3 weeks	7.0 ± 0.1[a]	7.5 ± 0.2[a]	8.5 ± 0.2[b]	9.3 ± 0.3[c]
6 weeks	7.1 ± 0.1[a]	7.8 ± 0.1[b]	8.1 ± 0.1[b]	9.0 ± 0.2[c]
Insulin (nmol · L^{-1})				
3 weeks	0.86 ± 0.08[a]	0.95 ± 0.06[a]	1.84 ± 0.17[b]	2.43 ± 0.26[c]
6 weeks	0.84 ± 0.07[a]	0.83 ± 0.08[a]	2.18 ± 0.23[b]	2.67 ± 0.33[b]
Glucagon (mmol · L^{-1})				
3 weeks	62 ± 4[a]	73 ± 4[a]	109 ± 4[b]	126 ± 9[c]
6 weeks	62 ± 3[a]	66 ± 3[a]	105 ± 4[b]	100 ± 6[b]
Corticosterone (mmol · L^{-1})				
3 weeks	0.76 ± 0.12[a]	0.77 ± 0.05[a]	0.47 ± 0.08[b]	0.56 ± 0.06[ab]
6 weeks	0.82 ± 0.06[a]	0.53 ± 0.07[bc]	0.58 ± 0.06[b]	0.36 ± 0.06[c]

NOTE. Values represent the mean ± SEM of 8 to 10 animals. Means on the same line not sharing a common superscript are different from each other, $P < .05$.

*Postprandial values were measured in serum obtained 2 to 3 hours after meal intake.

Table 4 shows the effects of the long-term diets and of meal intake on serum levels of the counterregulatory hormones glucagon and corticosterone. After 3 weeks of treatment, fasting and postprandial glucagon concentrations were approximately 15% higher in the HFAT than in the HCHO cohort (main effect of diet, $P < .02$), a difference that was not observed after 6 weeks of treatment. Intake of the high-carbohydrate meal almost doubled circulating glucagon levels, to a comparable extent in both dietary cohorts and at both treatment periods. Corticosterone concentrations were comparable in HCHO and HFAT cohorts after 3 weeks of treatment in both the fasted and postprandial states. Meal intake was followed by a decrease in circulating corticosterone in both dietary cohorts. A comparable meal-induced decline was observed after 6 weeks of treatment, although this longer period of ingestion of the HFAT diet decreased circulating levels of corticosterone as compared with levels in animals fed the HCHO diet ($P < .002$).

To gain insight into the mechanisms whereby triglyceride metabolism was affected by diet, measurements of the rates of appearance into and disappearance from the circulation of triglycerides and the activity of LPL and hepatic lipases in postheparin plasma were performed. Figure 3 shows that fasting hypertriglyceridemia in the HCHO cohort was due to oversecretion of triglycerides into the circulation (triglyceride secretion rate: HCHO, 3.0 ± 0.3 μmol · min^{-1}; HFAT, 1.8 ± 0.2 μmol · min^{-1}; $P < .0001$), which was not compensated for by a change in the capacity to hydrolyze triglycerides, as evaluated by the rate of clearance of a triglyceride emulsion from the circulation (k_2: HCHO, 5.2 ± 0.8 % · min^{-1}; HFAT, 5.2 ± 0.8 % · min^{-1}; NS). Measurement of lipase activities confirmed the latter observations by showing that the global availability of LPL and HTGL activities in the fasted state was not altered by the nature of the long-term diets (Fig 3).

DISCUSSION

In the present studies, two diets differing from each other in their content of carbohydrate (starch and glucose) and lipid (lard and corn oil) were chronically fed to rats. Indices of insulin sensitivity and variables of triglyceride metabolism were evaluated in the fasted state and following a high-carbohydrate insulinogenic meal, both before (3 weeks) and after (6 weeks) differences in body composition have been established. During this period, the HCHO diet brought about hypertriglyceridemia without insulin resistance relative to the HFAT diet, whereas chronic consumption of the latter was followed by a deterioration of indices of insulin sensitivity relative to the HCHO diet, without development of hypertriglyceridemia. Inasmuch as the two separate studies can be compared with one another, the development of one dysfunction seemed to be amplified with duration of treatment without the concomitant appearance of the other dysfunction. In fact, even after 6 weeks of treatment, glucose tolerance in HCHO animals was within a range comparable to that of rats fed nonpurified chow,[27] whereas fasting triglyceride levels in HFAT animals were comparable to those measured in chow-fed rats (Y. Deshaies and A. Boivin, unpublished observations, January 1994).

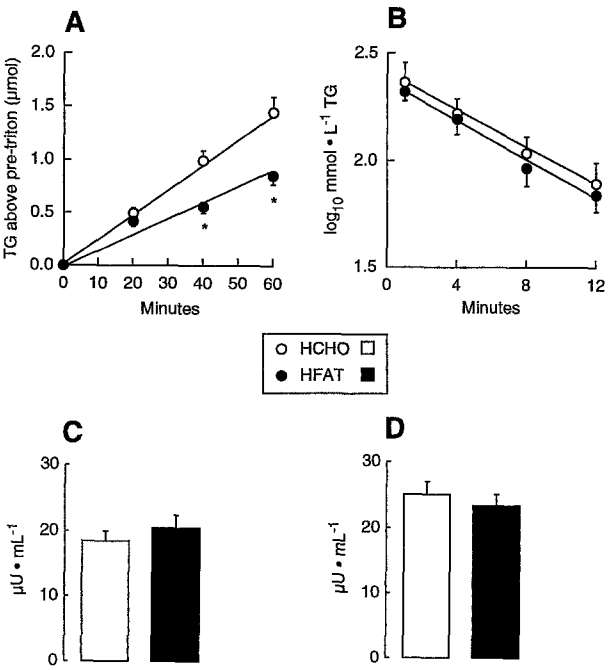

Fig 3. Fasting rate of triglyceride (TG) secretion (A), rate of Intralipid clearance (B), and fasting postheparin plasma LPL (C) and HTGL (D) activities in rats chronically fed either a HCHO diet or a HFAT diet for 3 weeks. For B, values are log_{10} TG over fasting values. Results are the mean ± SEM of 8 to 10 animals. *Different from the HCHO group, $P < .05$.

The higher body and adipose tissue weights observed in HFAT rats, despite an energy intake similar to that of HCHO animals, is consistent with the lower metabolic cost of converting dietary fat into body fat as compared with dietary carbohydrate.[28] However, it is worth noting that hypertriglyceridemia in HCHO rats and deterioration of indices of insulin resistance in HFAT animals were already established before fat mass accretion had become noticeable.

Increases in glucose and insulin during the fasting IVGTT and after the high-carbohydrate meal were higher in HFAT animals than in their HCHO counterparts. Although indirect, this evidence of insulin resistance induced by a high-fat diet agrees with direct measurements by the euglycemic-hyperinsulinemic clamp method in adipose tissue, muscle, and liver.[17,29,30] Several mechanisms have been proposed to account for the development of fat-induced insulin resistance, including both receptor and postreceptor defects. The nature of dietary fatty acids may alter membrane composition and thus impede the binding of insulin to its receptor.[31] A decrease in GLUT4 glucose transporters has also been invoked as a possible causative defect in glucose metabolism.[32] An increased oxidation of fatty acids derived from a high-fat diet is associated with blunting of the insulin-induced reduction of hepatic glucose output, and it decreases glucose utilization through the glucose—fatty acid cycle.[16-18] Acute fat infusion reproduces these phenomena in a matter of hours in vivo.[15,33] Finally, it should be noted that although a causal relationship between dietary fat and insulin resistance is less clear in humans than in animal models fed very high levels of fat, cross-sectional studies support the notion that dietary fat may induce insulin resistance also in humans.[34]

In contrast to dietary lipids, consumption of diets high in carbohydrate is associated with a long-term increase in plasma triglycerides. This is achieved mainly through stimulation of hepatic triglyceride synthesis and subsequent elevation in VLDL-triglyceride secretion,[35-37] perhaps combined in the postprandial state with saturation of an unaltered global hydrolytic capacity.[38,39] The present results corroborate these notions, since the fasting rate of triglyceride appearance in plasma was twice as high in HCHO than in HFAT rats, whereas the global capacity for intravascular triglyceride hydrolysis, assessed by a lipid tolerance test and measurement of postheparin plasma lipoprotein and hepatic lipase activities, was comparable in both dietary cohorts. Finally, the higher level of plasma NEFA during fasting in HCHO rats may have contributed to their hypertriglyceridemia. Indeed, fasting levels of NEFA have been correlated with triglyceridemia, and circulating NEFA are an important substrate for hepatic VLDL production. In the case of high-fat diets, NEFA that reach the liver appear to be mainly oxidized.[40] These findings are in keeping with recent human studies, in which acute and chronic consumption of high-carbohydrate diets was paradoxically associated with greater postprandial lipemia than that of high-fat diets.[41,42]

The counterregulatory hormones glucagon and corticosterone are unlikely to have been involved in the diet-induced differences in insulin sensitivity and triglyceridemia. Indeed, fasting levels of glucagon and the postprandial increase following the high-carbohydrate meal were comparable in the two dietary cohorts. Corticosterone levels during fasting were also similar at 3 weeks. The postprandial decreases in corticosterone levels are in accordance with the diurnal pattern of plasma corticosterone in the rat, with a peak just before the onset of feeding and a nadir some hours after morning food intake.[43] After 6 weeks of treatment, HFAT rats had lower fasting plasma corticosterone, and at both treatment periods, corticosterone tended to decrease less than in HFAT rats postprandially, in accordance with the previously reported decrease in responsiveness of the adrenals to corticotropin with high-fat feeding.[44] Although long-term hypercorticosteronemia stimulates hepatic lipid synthesis and secretion,[45] the fact that treatment-related differences in corticosterone were modest and present only after 6 weeks of treatment makes it unlikely that this hormone was of importance in the development of HCHO-induced hypertriglyceridemia. Moreover, corticosterone, high levels of which are associated with reduced insulin sensitivity,[46] was decreased in HFAT rats in parallel with worsening insulin sensitivity.

In the present models, the origin and delivery of triglycerides were important determinants of whether exposure to large amounts of triglycerides became associated with insulin resistance. Hence, large amounts of dietary triglycerides, absorbed mainly as chylomicrons, are rapidly delivered to tissues through the action of LPL without accumulating in plasma in the long term. This is associated with a rapid deterioration of insulin action on glucose metabolism. This situation is akin to in vivo human studies cited earlier in which glucose metabolism was diminished shortly after an intravenous Intralipid/heparin infusion.[33] In contrast, following consumption of a diet high in starch/glucose, triglycerides that are produced endogenously and transported mainly into VLDL particles accumulate in the plasma compartment, and this hypertriglyceridemia is not associated with a deterioration of the efficiency of insulin-mediated glucose metabolism. It may be that triglycerides carried mainly by VLDL in starch-glucose-fed rats are taken up more slowly by muscle tissue than those carried by chylomicrons in rats fed high-fat diets. With large amounts of dietary glucose being available and incorporated into muscle postprandially, glucose oxidation may not be impaired as with a sudden arrival of fatty acids derived from chylomicron-triglycerides. Thus, the rate at which triglyceride-derived fatty acids are delivered to muscle for oxidation, coupled with the availability of intestinal and hepatic glucose, may be factors that differentiate the impact on insulin sensitivity of dietary triglycerides versus those of endogenous origin. However, it must be emphasized that consumption of high amounts of certain carbohydrates, such as sucrose, does lead to insulin resistance.[47-50] It has been suggested that sucrose-induced insulin resistance is secondary to increased fatty acid oxidation,[50] as is the case with high-fat diets. Perhaps the degree of induction of hepatic lipogenesis achieved through metabolism of the fructose moiety of sucrose combined with a lesser availabil-

ity of dietary glucose as compared with starch/glucose diets are of sufficient magnitude to alter the ratio of fat to glucose oxidation in peripheral tissues, which would be liable to lead to insulin resistance.

The present results are not contradictory to a causal relationship between diet-induced hypertriglyceridemia and insulin resistance. For instance, the human diet is considered high in fat when the contribution of lipid to energy intake is in the range of 40% to 55%, and therefore contains a sizable amount of various carbohydrates. This may be a situation in which insulin resistance and hypertriglyceridemia can occur simultaneously. On the other hand, the insulin-resistant state would be liable to contribute to hypertriglyceridemia when hyperinsulinemia, which was not severe in the present study, is established and when frank abdominal obesity results in a large elevation of circulating NEFA. These conditions, in the presence of liver insulin resistance, would stimulate production of VLDL. However, the opposite scenario in which hypertriglyceridemia per se would result in the development of resistance of glucose metabolism to the action of insulin has been suggested[14] but not unambiguously established, and some animal models of primary hypertriglyceridemia,[20] as well as the present results, do not support such a possibility.

In conclusion, the present studies showed that hypertriglyceridemia and insulin resistance produced by chronic ingestion of diets high in starch/glucose or in lard/corn oil, respectively, occur independently of each other. The diets were spontaneously consumed in eucaloric amounts, and the abnormalities in triglycerides, glucose, and insulin developed before the appearance of overt obesity in animals fed the HFAT diet. Although these diets obviously do not reflect the habitual food consumption of humans, they constitute models for the specific study of each of these two frequently occurring abnormalities without the confounding presence of the other.

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