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Effects of Diet and Age on Lipoprotein Lipase and Hepatic Triglyceride Lipase Activities in the Rat[1] (41781)

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Abstract. Plasma clearance of triglyceride-rich lipoproteins appears decreased in aged humans and rats and may be due to lowered activities of the lipases responsible for lipid degradation. This study was designed to examine differential effects of age and diet on lipoprotein lipase (LPL) activity of adipose and heart tissue and hepatic triglyceride lipase (HTGL) activity. LPL and HTGL activities were examined in 3- and 13-month-old Sprague–Dawley rats after they had consumed either a high-carbohydrate or a high-fat diet for 14 days. The data were analyzed for age and diet differences by two-way analysis of variance. Although animals in the two age groups consumed diets of equal caloric content, the older rats gained less weight. Rats on the high-carbohydrate diet consumed less calories and gained less weight than the fat fed rats in both age groups. Neither heart nor adipose tissue LPL activity differed when examined for age or diet. HTGL activity levels, while not affected by age, were higher in the carbohydrate fed rats ($P = 0.014$). Regardless of age group, fasting plasma cholesterol levels were significantly higher in the carbohydrate-fed rats than fat-fed rats ($P = 0.002$). Thus, the diet effect was much stronger than the age effect for HTGL and plasma cholesterol levels.

Two triglyceride lipases (triacylglycerol lipase, EC 3.1.1.3) have been described which are released into plasma by heparin and have maximal activity at an alkaline pH. The more widely known of these is lipoprotein lipase (LPL), which is found in adipose tissue, heart, diaphragm, and skeletal muscle (see (1) for a review). LPL is characterized by its dependence on apolipoprotein C-II for optimal activity and its inhibition by salt and protamine sulfate. The physiologically active fraction, synthesized in adipose or muscle cells, is thought to be anchored by a heparinoid binding site to the luminal surface of adjacent capillary endothelial cells, permitting interaction with circulating triglyceride-rich lipoproteins and thus making released fatty acids from plasma triglyceride available to the adjacent cells for oxidation or resynthesis into triglyceride for storage (2–5). Hepatic triglyceride lipase (HTGL), the less well-characterized enzyme, is generally distinguished from LPL by its primary location in the liver together with its lack of dependence on the apolipoprotein C-II and its resistance to inhibition by salt or protamine sulfate (6). Although the physiological function of HTGL has not been clearly defined, the enzyme also appears to be bound to endothelial cells and may be involved in removal of cholesterol and phospholipids from the high- and low-density lipoproteins as they pass through the liver (7–9).

The level of LPL activity is affected by the diet composition. Rats fed high-fat diets have decreased adipose tissue LPL and enhanced heart and skeletal muscle LPL (10–12). A high-carbohydrate diet, on the other hand, is as-

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sociated with higher adipose tissue LPL levels and decreased heart and muscle LPL activity (12, 13). LPL activity is also affected by nutritional status. In fasted rats the pattern resembles that seen with a high-fat diet in that activity is low in adipose tissue and high in heart and muscle tissue (11, 12, 14). While the changes in adipose LPL with diet composition may probably be attributed to changes in the chylomicron load, the mechanism of the inverse relation between adipose LPL and heart and muscle LPL is poorly understood.

Few studies have examined the manner in which diet may affect HTGL. However, consistent with the proposed role of the enzyme in lipid metabolism, it, too, might be altered by diet composition and nutritional status.

The plasma clearance of triglyceride-rich chylomicron and VLDL lipoproteins appears to be decreased in aged humans (15–17) and rats (18, 19). One might predict, then, that LPL and HTGL activities might decrease with age. The total triglyceride lipase activity which is released into plasma by heparin (postheparin lipolytic activity, PHLA) has been reported to decrease significantly in humans with age (20), but it is not known whether these findings represent decreased LPL, HTGL, or both. The current study was therefore designed to examine the differential effects of age and diet (carbohydrate vs fat) on HTGL and LPL from adipose tissue and heart of the rat.

Materials and Methods. *Animals.* Male Sprague–Dawley rats of 3 and 13 months of age were purchased from Tyler Laboratories (Bellevue, Wash.). Following arrival the animals were housed in temperature- and light-controlled animal quarters (22°C, lights on from 6 AM to 6 PM) and fed Purina Laboratory Chow *ad libitum* with water freely available. The animals were transferred to individual cages for at least 5 days before being placed on either the high-carbohydrate or the high-fat diet.

Diet (Table I). Corn starch and sucrose were present in both experimental diets, but provided the bulk of the energy (75.6%) in the high-carbohydrate diet. For the high-fat diet, the bulk of the energy (73.8%) was derived from hydrogenated vegetable oil (polyunsaturated to saturated fat ratio of 1.08). Corn oil (0.5% by weight in both diets) served as the only source of fat in the high-carbohydrate diet and was included to provide essential fatty acids. Caloric density was 3.60 kcal/g for the high-carbohydrate and 6.07 kcal/g for the high-fat diet. Except for the fat (Crisco), corn oil (Mazola), and sucrose (California and Hawaii), all dietary components were purchased from ICN Nutritional Biochemicals (Cleveland, Ohio).

TABLE I. COMPOSITION OF DIETS

Component	Carbohydrate		Fat	
	Weight (%)	kcal (%)	Weight (%)	kcal (%)
Sucrose	52.7	58.2	14.6	9.6
Corn starch	15.8	17.4	4.4	2.9
Fat	0.0	0.0	49.5	73.1
Corn oil	0.5	1.2	0.5	0.7
Casein	20.0	22.1	20.0	13.1
D,L-Methionine	0.3	0.3	0.3	0.2
Cellulose	5.0	0.0	5.0	0.0
Minerals[a]	3.5	0.5	3.5	0.3
Vitamins[b]	2.0	0.3	2.0	0.1
Choline	0.2	0.0	0.2	0.0

[a] AIN Mineral Mixture 76TM.
[b] AIN Vitamin Mixture 76TM.

Experimental procedure. The 21 rats were separated into groups of 11 and 10 and started on the diets at an interval of 2 days apart with sacrifice occurring after the same period (i.e., 14 days, 2 days apart). The animals consumed the diet *ad libitum* with water freely available. Animal weights and food intakes were measured three times a week. On the evening of the 14th day on the diet food was removed and the animals fasted for 16 to 19 hr. The animals were anesthesized with 0.3 ml/100 g Equithesin (4.25 g chloral hydrate, 16–33 ml pentobarbital (60 mg-ml), 37.8 propylene glycol, 10 ml ethyl alcohol, 2.13 g magnesium sulfate, University of Washington School of Pharmacy, Drug Service Department) prior to sacrifice which occurred between 8 and 11 AM. The order of sacrifice was rotated among the groups to maintain comparability.

Blood and tissue collection. Blood from the postcava was withdrawn, placed in Vacutainer tubes (10 ml, Becton Dickinson, Rutherford, N.J.) containing EDTA crystals (1.4 mg/ml of blood) and put in ice. Epididymal fat, liver, and the heart were removed, minced into 10- to 12-mg pieces, and transferred to ice-cold Krebs–Ringer phosphate buffer, pH 7.4, for

the lipase assay. Blood was centrifuged at 2000 rpm and the separated plasma stored at −15°C for cholesterol analysis.

Triglyceride lipase assay. Activity was determined by the release of labeled oleic acid from [1-^{14}C]triolein with bovine serum albumin as the fatty acid acceptor. The assay was performed similarly to that described by Pykalisto *et al.* (21) except that 20% of the volume was used so that the samples could be easily extracted by the method of Belfrage and Vaughn (22). In addition, Triton X-100 (Calbiochem Behring, San Diego, Calif.) was substituted for the lecithin to aid emulsification. Each assay (pH 8.5) contained 0.9 mg of triolein (Sigma, St. Louis, Mo), 0.02 μCi glycerol tri-[1-^{14}C]oleate (New England Nuclear, Boston, Mass.), 0.025 μl of Triton X-100, 1.6 mg bovine serum albumin (Fraction V, fatty acid free, Miles Biochemicals, Elkhart, Ind.), 32 μmol of Tris–HCl, pH 8.5 (Sigma, St. Louis, Mo.), and 4.0 μl of human serum. Before weighing, the minced tissues were blotted dry on sharkskin filter paper (Schleicher and Schuell, Keene, N.H.). The tissue (65–75 mg) was immediately placed into a test tube with 0.7 ml Krebs–Ringer phosphate buffer containing 2 U/ml heparin (Upjohn, Kalamazoo, Mich.) and incubated for 45 min at 37°C. After this incubation, 0.2 ml of the heparin eluate was transferred to a test tube containing the substrate (0.04 ml) and incubated for 45 min at 37°C. Fatty acids were extracted and the top 1 ml of the upper phase removed to a scintillation vial. Ready-Solv HP (Beckman Instruments, Inc., Palo Alto, Calif.) was used for the scintillant and the samples were counted in a Beckman LS-7000 Scintillation Counter. Enzyme activity was calculated as nanomoles free fatty acid released per minute per gram of tissue.

Analysis of cholesterol. Total plasma cholesterol was measured by the Dow Enzymatic Cholesterol Test Kit (Dow Diagnostics, Indianapolis, Ind.) using the cholesterol standard provided with the kit for the calculations.

Statistics. Data are presented as the mean ± standard error of the mean (SEM). Statistical analysis was performed by two-way analysis of variance with the Statistical Package for the Social Sciences from Vogelpack Computing Center (Northwestern University) at the Academic Computer of the University of Washington. The method was chosen because it enables one to determine differences in one variable while considering its interaction with another variable. The results of the ANOVA are presented at the bottom of each table. Age effect refers to the young vs the mature and diet effect compares carbohydrate vs fat.

Results. All of the young animals gained weight during the diet study. The younger rats fed the high-carbohydrate diet increased from 435 ± 16 ($\bar{x}$ ± SEM), to 470 ± 9 g (N = 5); the fat-fed younger animals increased from 479 ± 14 g to 560 ± 16 g (N = 5). In contrast, some of the older rats actually lost weight. The mature rats fed the high-carbohydrate diet declined from 691 ± 15 to 679 ± 18 g (N = 5); those fed the high-fat diet increased from 657 ± 25 g to 679 ± 37 g (N = 6). The effects of the two diets on mean daily food intake and weight gain are presented in Table II. The food intake did not show an age effect, but did show a significant diet effect. The mass of food intake was greater for rats fed high carbohydrate than those fed high fat; but because of the difference in caloric density, the high-carbohydrate-fed animals actually consumed less kilocalories per day. The weight gain showed both an age and diet effect. In both age groups, weight gain was greater on the high-fat than the high-carbohydrate diet. However, there was significantly less weight gain in the mature animals on both diets, and the mean change in weight with the high carbohydrate diet was actually slightly negative.

LPL activity. Both adipose tissue and heart LPL activity failed to show either an age or a diet effect (Table III).

HTGL activity. Like LPL, there was no indication of an age effect. However, there was a diet effect with slightly lower HTGL levels in the fat-fed animals (Table III).

Plasma cholesterol. There did not appear to be an age effect on plasma cholesterol levels, but there was a strong diet effect. Thus, the rats fed the high-carbohydrate diet had significantly higher plasma cholesterol levels than those on the high-fat diet (Table IV).

Relationship between plasma cholesterol and HTGL. Both plasma cholesterol and HTGL activity were influenced by diet. The relationship was examined further by linear regression. The plasma cholesterol levels were positively related to HTGL levels in the fat-

TABLE II. FOOD INTAKE AND WEIGHT GAIN[a]

Group	N	Intake		Weight gain	
		Mass (g/day)	Calorie (kcal/day)	Total (g)	Daily (g/day)
Young					
Carbohydrate	5	28 ± 2	105 ± 3	35 ± 7	2.5 ± 0.5
Fat	5	23 ± 1	136 ± 8	81 ± 7	5.8 ± 0.5
Mature					
Carbohydrate	5	27 ± 1	96 ± 4	−4 ± 7	−0.3 ± 0.5
Fat	6	21 ± 3	128 ± 16	51 ± 21	2.7 ± 1.4
ANOVA (*P*)					
Age effect		NS	NS	0.020	0.002
Diet effect		0.008	0.006	0.001	0.001

[a] Means ± SEM.

fed animals ($r = 0.64$, $P < 0.05$, $N = 11$), but not in the carbohydrate fed rats. These studies would suggest that 40% of the variance in plasma cholesterol values in the fat fed animals could be accounted for by changes in HTGL activity (or vice versa).

Discussion. The present experiments were designed to study the differential effects of diet and age on the activities of both LPL and the HTGL in the rat. Although the high-carbohydrate-fed rats consumed significantly more food by weight than the high-fat-fed animals, the latter had significantly greater caloric intake due to the greater caloric density of the high-fat diet. This was in turn associated with a greater gain in weight in both young and mature animals. While the gram and calorie intake did not differ between the young and mature rats within each diet, their weight gain was significantly different. This marked age difference occurred despite the fact that even the "older" rats in this study might be more appropriately called "middle-aged" at 13 months.

Since the rats were fasted for 16–19 hr before sacrifice and specifically during those nighttime hours of normal maximal feeding, the LPL activity in the heart was high while that of the adipose tissue was low, as has been previously reported (11, 12, 14). However, the fasting may not have altered LPL activity in these animals since the ability of food to in-

TABLE III. TRIGLYCERIDE LIPASE ACTIVITIES[a]

Group	N	LPL		HTGL
		Adipose tissue (nmole/min/g)	Heart (nmole/min/g)	Liver (nmole/min/g)
Young				
Carbohydrate	5	18.6 ± 7.5	66.9 ± 11.8	61.0 ± 4.9
Fat	5	10.9 ± 3.2	49.2 ± 10.0	45.5 ± 3.8
Mature				
Carbohydrate	5	8.1 ± 2.7	56.7 ± 4.7	55.3 ± 9.8
Fat	6	5.2 ± 0.9	48.3 ± 5.6	41.6 ± 3.4
ANOVA (*P*)				
Age effect		NS	NS	NS
Diet effect		NS	NS	0.014

[a] Means ± SEM.

TABLE IV. PLASMA CHOLESTEROL LEVELS[a]

Group	N	Cholesterol (mg/dl)
Young		
Carbohydrate	5	71.5 ± 1.6
Fat	5	68.4 ± 7.1
Mature		
Carbohydrate	5	99.6 ± 5.0
Fat	6	59.5 ± 7.4
ANOVA (*P*)		
Age effect		NS
Diet effect		0.002

[a] Means ± SEM.

duce an increase in adipose LPL activity has been found to be lost in 100-day- and 1-year-old rats (23). The differential effects of the two diets on adipose and heart LPL (10–12) were not seen in the current study. In contrast, HTGL activity did show a difference between the carbohydrate and fat diets.

The lack of an effect of age on LPL activity found in the current study confirms previous results (23) and may imply that the regulation of LPL is not affected between youth and middle age in rodents. No significant difference in HTGL could be attributable to age in the current study, but the relationship between plasma cholesterol levels and HTGL activity supports the idea that HTGL plays an important role in lipoprotein metabolism. No age effect was seen in plasma cholesterol levels in the current study, although such an effect has been previously reported (16, 17). It is not clear why the effect was not present, but may relate to the animals not having a large age difference, the duration of the diet, the duration of fasting or a masking effect attributable to the non-Purina Chow diet employed.

The differential effect of diet on HTGL but not LPL seen in the current study is consistent with these activities representing different enzymes subject to different regulatory mechanisms and performing different physiological functions. As the older animals in this study cannot really be considered aged, it would be of interest to see if HTGL and LPL activities are decreased in much older rats (approximately 2 years old). Considering the relation of lipids to heart disease, it would be valuable to extend this question to the human level.

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