Effect of ingestion of unsaturated fat on lipolytic activity of rat tissues

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ABSTRACT Homogenates of some rat tissues, incubated in Tris–maleate buffer containing bovine serum albumin, olive oil emulsion, heparin, and serum, liberated free fatty acids. The total lipolytic activity in tissues of rats fed a low fat, 20% lard, or 20% corn oil diet for 6 wk was measured.

Similar activities were found in all the livers, but there was a significant increase in the total lipolytic activity of the mucosa, epididymal fat, and mesenteric tissues after ingestion of an unsaturated fat diet as compared with that containing a more saturated fat. From measurements of the lipolytic activity in the presence of 1 M NaCl or 0.2 M NaF and in the absence and presence of heparin and serum, the conclusion is drawn that more lipoprotein lipase was present in adipose tissue of rats on unsaturated fat diets.

An increase in available lipoprotein lipase after unsaturated fat diets may aid in clearing lipids from the blood of these rats and thus in producing the lower blood lipid levels obtained.

KEY WORDS polyunsaturated fat · lipoprotein lipase · rat liver · epididymal tissue · mucosa · mesenteric tissue · lipolytic activity

TISSUE LIPASE APPEARS TO PLAY a major role in the metabolic exchange of lipids between the blood and the body tissues. Robinson (1) has described in a review article the role of lipases in the transport of fatty acids between blood and tissues. A major part of this activity has been ascribed to lipoprotein lipase, although some other lipases seem to be involved. The nutritional state has been shown to affect both the lipolytic activity (2) and the rate of release of lipoprotein lipase from rat epididymal fat bodies after incubation with heparin (3).

If enzymatic lipolysis is an important physiologic mechanism for removal of triglycerides from the blood, the greater availability of tissue lipase might increase the rate of removal of such lipids from the blood. Since the lipase content of some tissues appears to be influenced by changes in the nutritional state of the animal, it might also be affected by the composition of the diet. We therefore studied the effect on lipolytic acitivity of some rat tissues after ingestion of diets containing predominantly saturated or polyunsaturated fats.

MATERIALS AND METHODS

30 male albino rats from the Holtzman Company, Madison, Wis., weighing about 80 g initially, were maintained on commercial rat pellets up to the start of this experiment. The rats were divided equally into three groups and fed ad libitum diets composed either of rat pellets[1] (low fat diet), or of ground pellets enriched with either lard or corn oil to give a final concentration of 20% of the added fat. After 6 wk, the rats on each diet were used to determine the total lipolytic activity of some tissues in the presence of heparin and serum. The rats were anesthetized with ether and as much blood as possible was drawn from the heart for subsequent determination (4) of esterified fatty acids in the plasma. The liver, distal mesenteric tissue, epididymal fat pads, and intestine were immediately removed and chilled. The lower half of the intestine was flushed with cold 0.9% saline and slit longitudinally. After the mucosal surface has been blotted with filter paper, the mucosa was scraped off. The other tissues were well washed with cold saline and blotted. About 1.0 g of each tissue was weighed and homogenized in 15 ml of Tris–maleate buffer (pH 8.5 and 0.05 M).

Total Lipolytic Activity

A substrate was prepared by mixing purified olive oil, emulsified in arabic gum (5), with a buffered bovine al-

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[1] Uncle Johnny's BSD laboratory animal diet contains not less than 26.0% protein, 5% fat (prepared by Uncle Johnny Mills, Houston, Tex.).

bumin solution so as to have a final concentration of 5% fat, 5% albumin (Armour Fraction V) in 0.05 M Tris–maleate buffer; 40 μg (100 U/mg) of heparin per ml was added and the pH was adjusted to 8.5 with NH_4OH. Flasks containing 12 ml of this substrate and 1.2 ml of fresh pooled serum of normally fed rats were incubated for 30 min at 37°C in a Dubnoff incubator. 4 ml of each tissue homogenate was then added to each flask and mixed thoroughly; an aliquot, immediately pipetted into the extraction mixture of Dole (6), served as a control. The remainder was incubated for 2 hr. At the end of the incubation period duplicate aliquots from each flask were pipetted into a modification of Dole's mixture in which the more readily available hexane was substituted for heptane (7). After extraction, the free fatty acids were titrated electrometrically to pH 10.0 with 0.05 M NaOH (CO_2-free) while the solution was constantly stirred with a stream of nitrogen. The amount of fatty acid liberated per hr per g of wet tissue was calculated.

Lipoprotein Lipase Activity

In order to determine the proportion of lipoprotein lipase in total tissue lipases, we measured the lipolytic activity of tissues under special conditions. The effects of serum, heparin, and some characterizing inhibitors were examined. The substrate described above was employed except that heparin was omitted in half the tests (Tables 3 and 4, below). After 30 min of incubation, both with and without serum and heparin, 4 ml of medium was mixed with 1 ml of tissue homogenate and incubated again for 1 hr at 37°C in the shaking incubator. Although it has been previously reported (3) that under slightly different conditions free fatty acid evolution proceeds at a constant rate for 2 hr and is a linear function of the enzyme concentration, we found this to be true for only a little more than 1 hr, and used incubation periods of 1 hr in these experiments. Other tests were made similarly in the presence of 1 M NaCl and 0.2 M NaF as potential inhibitors of lipolytic activity.

RESULTS AND DISCUSSION

The total lipolytic activity was significantly higher in the mucosa, epididymal fat bodies, and mesenteric tissue of rats on the polyunsaturated fat diet (Table 1), while in liver no difference between rats on different diets was seen. The increase (51%) in total lipolytic activity in the epididymal tissue of rats on the corn oil diet was almost twice that in two other tissues (28%).

Since rats on the fat diets ingested the same amount of food and gained weight to the same extent (Table 2) during the experimental periods, the results obtained cannot be a result of differences in dietary intake; the only variable appears to be the composition of the fat ingested.

TABLE 1 DIETARY EFFECT ON TOTAL LIPOLYTIC ACTIVITY

Tissue	Lard Diet*	Corn Oil Diet*	Diff.
	μeq/g wet wt/hr		
Liver	36.2 ± 2.75	32.4 ± 3.14	0
Mucosa	28.2 ± 2.06	35.8 ± 1.30	+28
Adipose	14.1 ± 0.83	21.2 ± 1.23	+51
Mesenteric	11.3 ± 0.80	14.5 ± 1.10	+28
	μeq esterified fatty acids/ml		
Plasma lipid levels	11.0 ± 0.54	8.4 ± 0.10	−24

* Mean values from six rats ± SEM.

TABLE 2 WEIGHT GAINS OF RATS ON THREE DIETS*

Diet	Initial Weights	Final Weights	Gain in Weight
		g	
Low fat	79.8 ± 1.87	413.0 ± 7.31	333.2 ± 8.73
Lard (20%)	81.2 ± 1.85	421.2 ± 13.48	340.0 ± 12.78
Corn oil (20%)	81.8 ± 2.15	422.8 ± 12.51	341.0 ± 13.22

* Mean values from 10 rats in each group ± SEM.

The small amount of cholesterol in the lard has been shown to not affect cholesterol synthesis (8).

Some tissues contain a variety of lipases, but according to current concepts only lipoprotein lipase is involved in the assimilation of plasma triglycerides by the tissues. It has been shown that lipoprotein lipase activity of rat adipose tissue may be altered by changes in carbohydrate intake (2); Table 3 shows that dietary fat promotes still greater alterations in the enzyme content of the tissue. The percentage increase in lipase activities induced by heparin was greatest for epididymal fat pads from corn oil-fed rats; there was also an increase for mesenteric tissue, but the corresponding increase for animals fed lard was not statistically significant. The absolute amounts of lipase activity in liver and mucosal tissue were small by comparison (note units in Table 3).

Employing the only two tissues (epididymal and mesenteric tissues) that appeared to have any possibility of an increased lipolytic action with heparin, we added sufficient NaCl and NaF to the media to give 1 M and 0.2 M concentrations, respectively, both in the presence and absence of heparin and serum. In epididymal fat, the increased lipase activity evinced in the presence of heparin and serum was inhibited 71% after a corn oil diet (Table 4) but only 28% after the other two diets. In mesenteric tissue, the inhibition was increased from 45% in the lard-fed rats to 68% in corn oil-fed rats. The less specific inhibitor NaF did not give significant differences between tissues from rats that had ingested different fats. While it is true that similar differences in percentage inhibition of lipase activity by NaCl occurred in the absence of heparin and serum (Table 4), the total activity under these circumstances was lower (Table 3).

TABLE 3 HEPARIN EFFECT ON LIPOLYTIC ACTIVITY OF TISSUES AFTER VARIOUS DIETS*

Tissue	Low Fat Diet			Lard Diet			Corn Oil Diet		
	Heparin		Increase With Heparin	Heparin		Increase With Heparin	Heparin		Increase With Heparin
	Without	With		Without	With		Without	With	
	μeq/mg protein/hr†		%	*μeq/mg protein/hr*		%	*μeq/mg protein/hr*		%
Epididymal fat	2.78 ± 0.10	3.16 ± 1.13	12.0	2.72 ± 0.48	4.98 ± 0.97	45.4	2.24 ± 1.12	8.20 ± 1.01	72.7
Mesenteric tissue	1.00 ± 0.15	1.48 ± 0.12	32.4	3.30 ± 1.15	4.92 ± 2.18	32.9	3.16 ± 0.53	5.41 ± 0.74	41.6
	μeq/100 mg protein/hr			*μeq/100 mg protein/hr*			*μeq/100 mg protein/hr*		
Liver	5.02 ± 0.60	5.36 ± 0.57	6.3	6.70 ± 0.51	9.35 ± 0.82	28.3	7.96 ± 0.63	9.67 ± 0.19	17.6
Mucosa	3.40 ± 0.76	3.60 ± 0.46	5.5	4.06 ± 0.31	4.32 ± 0.21	6.0	4.78 ± 0.79	5.30 ± 0.06	9.6

* Mean values from 10 observations on rats on each diet ± SEM.
† Protein determination, ref. 9.

TABLE 4 DIETARY EFFECT ON INHIBITION OF LIPOLYSIS BY CHLORIDE AND FLUORIDE WITH AND WITHOUT SERUM AND HEPARIN

Tissue	Diet	Without Heparin and Serum		With Heparin and Serum	
		NaCl	NaF	NaCl	NaF
		% inhibition			
Epididymal fat	Low fat	37.1	73.3	27.8	49.5
	Lard	31.5	84.9	28.0	26.1
	Corn oil	83.3	50.0	71.2	20.0
Mesenteric tissue	Low fat	18.9	100.0	12.2	18.7
	Lard	34.0	67.8	45.3	39.9
	Corn oil	44.2	81.7	67.6	32.0

An increase in the lipoprotein lipase content of a tissue would be expected to increase its availability for lipolytic activity, especially since the level of lipolytic activity in the blood has been reported to be dependent upon prior dietary fat intake (10). Also, Persson, Björntorp, and Hood (11) have reported that a highly significant negative correlation exists between the triglyceride level in serum and the lipoprotein lipase activity that can be released from adipose tissue of man. Thus if more lipase is present and hence available for enzymatic activity in the rats fed the polyunsaturated fatty acids, then such increased activity may aid in clearing lipids from the blood, producing the lower plasma lipid levels obtained (about 24% lower, Table 1) after the ingestion of corn oil instead of lard.

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