EFFECT OF HYPOTHYROIDISM, DIABETES AND POLYUNSATURATED FATTY ACIDS ON HEPARIN-RELEASABLE RAT LIVER LIPASE

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SUMMARY. In the rat both hypothyroidism and diabetes decrease heparin-releasable liver lipase activity. This defect may be reversed by feeding a diet rich in polyunsaturated fatty acids. It is suggested that a diet-induced increase of membrane fluidity restores liver lipase activity, which contributes to the hypolipidemic effect of polyunsaturated fatty acids.

A decrease in heparin-releasable liver lipase activity may be expected[1,2] to occur in a number of atherogenic conditions, in which the plasma concentrations of intermediate density lipoprotein particles (IDL) have been shown to be increased. Indeed, in diabetes and in hypothyroidism not only the IDL levels are increased[3-5], but liver lipase activities have been shown to be low. We have described the latter for streptozotocin diabetic rats[1], which was recently confirmed by Elkeles and Hambley[6], while Krauss et al.[7] described a 40% decrease in 4 hypothyroid women. The reason for the decreased activity is not known, although a decreased membrane fluidity[8] may be involved to some extent since diets rich in polyunsaturated fatty acids have repeatedly been shown to have a hypolipidemic effect. In the present paper it will be shown that also in the hypothyroid rat heparin-releasable liver lipase activity is low and that both in diabetes and hypothyroidism the normal levels of activity may be restored by a diet rich in polyunsaturated fatty acids.

METHODS AND MATERIALS. Fed, male Wistar rats of 220-270 g were used. Hypothyroid rats were obtained by including methimazole (1-methyl-2-mercaptoimidazole of Nogepha, Alkmaar, The Netherlands) in the drinking water (0.5 mg/ml). After 2 weeks the thyroxin concentration in the plasma had dropped from 36 ± 1.6 to 4.7 ± 0.7 nmol/l (n=8), while the total plasma cholesterol level had increased from 55 + 6 to 74 ± 7 mg/100 ml. Diabetes was induced by injecting 50 mg per kg body weight of streptozo-

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tocin (Calbiochem, dissolved in 10 mM citrate buffered saline of pH 4.5) into a tailvein. These rats were sacrificed 6-10 days later, when the blood glucose level had increased from 6.5 ± 0.5 (n=14) to 32.8 ± 1.2 mM (n=8). Glucose was determined as described by Werner et al.[11], thyroxin as described by Visser et al.[12] and cholesterol plus cholesterolester as described by Röschlau et al.[13]. Postheparin serum was obtained by injecting 200 I.U. heparin (Organon)/kg body weight intravenously. After 6 min, blood was withdrawn and liver lipase measured in the plasma with either tri-1-[^{14}C]-oleoylglycerol, in the presence of 1 M NaCl, or with 2-[^{3}H]-glycerolmonooleate as the substrate, as described previously[14]. Since the monoglyceridase assay measures both "preheparin" and extrahepatic postheparin plasma activities, the liver contribution could only be determined by comparing activities in plasma, preincubated with control rabbit serum, with plasma preincubated with an antiserum raised in rabbits against purified heparin-released liver lipase[1] as described previously[14]. The amount of antiserum used was sufficient to remove the liver activity completely[14] during a 3 h preincubation at 0°C (followed by centrifugation). The antiserum was kindly provided by Dr. H. Jansen. The figures given are mean values ± S.E.M.

RESULTS

Control sera contain a "NaCl-resistent" triglyceridase activity, which reflects the heparin-releasable liver lipase activity[7] (TABLE I). The enzyme may also be measured with a monoglyceride substrate, provided the preheparin and extrehepatic contributions are excluded by preincubation with antibody (see METHODS). Both activities are decreased significantly in hypothyroidism and in diabetes. The latter has been reported earlier[1]. When the standard chow (containing 18 cal% fat, including 6.4 cal% linoleic acid) was replaced by a diet rich in sunflowerseed oil (containing 40 cal% fat, including 66 cal% linoleic acid and 4 cal% arachidonic acid), liver lipase activities in both hypothyroid and diabetic rats increased to the normal range (TABLE I).

DISCUSSION

The reversal of the lowered liver lipase activity by a diet rich in polyunsaturated fatty acids, as observed in diabetes and hypothyroidism, suggests that in both diseases membrane fluidity may be decreased. Mercuri et al.[15] and Lerner et al.[16] observed a fatty acid pattern in the diabetic liver in which the arachidonic acid is low in comparison to the linoleic acid level. This suggested a desaturase deficiency, which indeed has been demonstrated[15]. A similar defect may be expected in hypothyroid

TABLE I

LIVER LIPASE ACTIVITY IN POSTHEPARIN RAT SERA UNDER DIFFERENT HORMONAL AND DIETARY CONDITIONS

$C_{18:2}$ rich diet was given for one week; for composition see text. The significance (Student t-test) of the differences in the triglyceridase activity on the one hand and the monoglyceridase activity on the other hand between the normal and hypothyroid states are $P<0.005$ and $P<0.001$ respectively; between the normal and diabetic states $P<0.05$ and $P<0.001$ respectively. The significance of the increase of activity by the $C_{18:2}$ rich diet in hypothyroidism was for the triglyceridase and monoglyceridase activities $P<0.005$ and $P<0.05$ respectively; for the increase of activities by diet in diabetes were the P values <0.025 and <0.001 respectively. The alterations of activities by the $C_{18:2}$ rich diet in control rats were not significant.

Condition	Hydrolysis of	
	Trioleoylglycerol	Monooleoylglycerol
	(nmoles fatty acid/min/ml serum	
Control rats	517 ± 20 (n=7)	2937 ± 93 (n=6)
Control rats; $C_{18:2}$ rich diet	625 ± 33 (n=3)	2719 ± 219 (n=3)
Hypothyroid rats; control diet	411 ± 9 (n=5)	2056 ± 135 (n=6)
Hypothyroid rats; $C_{18:2}$ rich diet	564 ± 32 (n=4)	2500 ± 114 (n=4)
Diabetic rats; control diet	445 ± 21 (n=5)	1405 ± 41 (n=5)
Diabetic rats; $C_{18:2}$ rich diet	558 ± 34 (n=6)	2402 ± 173 (n=6)

liver membranes, where Chen and Hoch[17] also found decreased arachidonic acid and increased linoleic acid levels, resulting in an overall lowered desaturation index. Thyroxin-stimulated microsomal fatty acid desaturation has been reported recently[18].

Decreased membrane fluidity could influence membrane-bound enzymes[8] and binding proteins, which are laterally mobile[19]. Defective binding may cause cholesterol accumulation in the blood, a finding well known to occur in both diabetes and hypothyroidism. This in turn may further decrease membrane fluidity since cholesterol has been shown to increase the phospholipid packing [20-22], especially of the less unsaturated phospholipid species. According to Cooper[8], an increase in the cholesterol/phospholipid

ratio may be expected to lead to instable particles from which cholesterol may escape into cell membranes.

Liver lipase probably does not extend through the liver plasma membrane, because it is easily removed by heparin perfusion. Therefore it is possible that the lowered activity observed in diabetes and hypothyroidism results from defective binding. Normal binding requires intact surface glycosylation. In diabetes sialic acid synthesis is low[23]. The linoleic acid rich diet, however, did not increase the sialic acid content of liver microsomes and plasma proteins (not shown) in the diabetic rat. It is most likely therefore that the diet increased binding and possibly insertion of newly synthesized protein molecules by increasing the membrane fluidity.

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