LIPOLYTIC ACTIVITIES IN POSTHEPARIN SERUM

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Ter nagedachtenis aan mijn vader
Aan mijn moeder
Aan Loes

Contents

IV On the selective measurement of hepatic and extrahepatic postheparin serum lipase activities and the influence of experimental hypercortisolism and diabetes on these activities.
H. Jansen and W.C. Hülsmann (1975) Biochim. Biophys. Acta, accepted for publication.

V Increase in postheparin lipoprotein lipase activity in rat plasma by dietary erucic acid.
C.B. Struijk, U.M.T. Houtsmuller, H. Jansen and W.C. Hülsmann (1973) Biochim. Biophys. Acta 296, 253-256.

VI Influence of rapeseed oil feeding on the lipase activities of rat heart.
H. Jansen, A. van Zuylen-van Wiggen, W.C. Hülsmann, C.B. Struijk and U.M.T. Houtsmuller (1975) Biochem. Biophys. Res. Commun., accepted for publication.

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INTRODUCTION

Dietary fat is, after hydrolysis and reesterification in the intestine, transported by the blood mainly in the form of triglycerides. To enable the transport of the hydrophobic lipid in the aqueous bloodplasma, a protein envelope is synthesized around the triglycerides in the intestine. The particles formed in this way are called chylomicrons. Endogenous triglycerides synthesized in the liver from free fatty acids and carbohydrates are secreted by the liver in an analogous manner. These liver particles are smaller than the chylomicrons and have a relatively high protein content. They are called very low density lipoproteins (VLDL). In the breakdown of the triglyceride moiety of both chylomicrons and VLDL an enzyme located at the surface of endothelial cells of bloodvessels, is involved. This enzyme, lipoprotein lipase, is released into the blood after injection of heparin. The measurement of the lipoprotein lipase activity in postheparin serum is an important tool in the study of serum triglyceride metabolism.

We found that serum of rats injected with heparin (postheparin serum) also hydrolyzes palmitoyl-CoA and the development of a simple continuous assay of the serum lipase activity seemed to be at hand. In subsequent experiments, it became clear that a mixture of different enzymes is released by heparin into the blood. Therefore we attempted to measure these lipase activities selectively. When this was achieved, the influence of some factors on the different lipases was studied.

Chapter I gives a brief literature survey of the lipase activities of postheparin sera of different species. In this chapter the most important facts known about the function of these different lipolytic activities are described.

In chapter II the role of the liver in serum triglyceride metabolism is discussed by an integration of literature data with our own experiments. Some additional experiments are shown.

CHAPTER I

LIPOLYTIC ACTIVITIES IN POSTHEPARIN SERUM

1.1. Lipoprotein lipase in different organs and in postheparin serum

In 1943 it was demonstrated by Hahn[1] that serum of hyperlipemic dogs could be cleared by heparin injection. This activity was called "Clearing Factor". Later it was shown that in a number of other species, including man, this "Clearing Factor" became active upon in vivo administration of heparin[2,3].

In 1955 Korn[4] found that acetone-powders of rat hearts contain a lipase activity, that is activated by serum proteins and heparin. This lipase activity was called lipoprotein lipase. It was postulated that the "Clearing Factor" discovered by Hahn was identically with the lipoprotein lipase, released from the organs by the interaction with heparin. Also in lung, adipose tissue, diaphragma, lactating mammary gland and other organs a lipase activity with the characteristics of lipoprotein lipase is described (comp. ref. 5). The characteristics of lipoprotein lipase, as defined by Korn[4], are: it is inhibited by preincubation with high salt concentrations or protamine sulphate and stimulated by heparin and serum proteins. The function of the enzyme was considered to be the hydrolysis of serum triglycerides at the endothelial cells of the organs, in order to facilitate the uptake of these triglycerides by the organs. The easy release of the lipase activity from the organs by heparin injection led to the conclusion that at least part of the lipase activity is located at the endothelial cells facing the luminal space[6-8]. It was later shown that at least two forms of lipoprotein lipase activity, differing in molecular weights, exist in defatted preparations of rat heart and adipose tissue[9-11]. The high

molecular form was considered to be releasable by heparin and to function in the hydrolysis of serum triglycerides.

It was also postulated that the lipoprotein lipase activity may be altered under different feeding conditions. Fasting was shown to result in an elevation of the lipoprotein lipase activity in (rat) heart[12], while that activity in adipose tissue was found to be lowered[6,7,13,14] and the lipase activity of lung unaffected[15]. The lipoprotein lipase activity in rat heart is perhaps regulated by glucagon[16], although conflicting reports exist[17]. The lipoprotein lipase activity of another triglyceride utilizing organ, lactating mammary gland, was found to be regulated by prolactin[18]. The level of the lipoprotein lipase of an organ seems to correlate with the demand of serum triglycerides by the organ[15,17,19-21]. Especially the activity of the heparin-releasable part of the lipase activity determines the uptake capacity of the organs of serum triglycerides, as was found for rat heart[19] and rat adipose tissue[11].

The estimation of the lipoprotein lipase activity of different organs is important for the understanding of serum triglyceride metabolism. The measurement of activity in an organ requires large biopsies, which is often impossible (especially in humans). Besides, the activity in the organs is composed of heparin-releasable and non-releasable activities (see e.g. appendix paper VI and refs. 9-11) and are therefore not necessarily related to the serum triglyceride-uptake capacities of the organs. Therefore, the heparin-releasable part of the total lipoprotein lipase activities of the organs should be measured selectively. This may be done in the serum after the intravenous injections of heparin. However, the lipase activity of postheparin serum is rather inhomogenous, as different organs might contribute (comp. Discussion in the next sections of this chapter).

1.2. Hepatic lipase activity in postheparin serum

The lipase activity of postheparin serum was considered to be identical with lipoprotein lipase, derived from extrahepatic organs, for a long time, although reports existed that heparin releases lipase activity from rat liver[22], dog liver[23,24] and human liver[25]. La Rosa et al.[26] found in 1972 that the lipase activity of postheparin rat serum resembled a lipase activity in a liver preparation more than the lipoprotein lipase activity of adipose tissue, with respect to inhibition by preincubation with high salt concentrations and protaminesulphate.Since then more attention has been paid to the contribution of the liver to the total lipolytic activity of postheparin serum of different species[27-30]. Most investigators made use of the reported resistence of the liver enzyme to inactivation by protaminesulphate and/or high salt concentrations. However, these criteria have been criticized[32], while it was found that the results obtained were largely dependent on the substrate used[32,33]. Moreover, the enzyme purified from rat liver was found to be equally sensitive to the inhibitors mentioned, as the extrahepatic lipoprotein lipase[34].

We were the first to describe (appendix papers II and III) that the liver enzyme is immunologically different from the extrahepatic lipoprotein lipase in the rat. In preliminary experiments the same was found in man. Therefore a differentiation between hepatic and extrahepatic lipases in postheparin serum became available (appendix paper III). Differentiation of both activities is also possible by the use of substrates, that are preferentially hydrolyzed by one of the enzymes (appendix papers I and IV). We were able to show that the contribution of the liver to the total lipase activity of the serum is dependent on the amount of heparin injected and the time of blood removal after the injection in rat or man (appendix papers III and IV and ref. 30). It was further shown that in the rat the hepatic lipase acti-

vity was lowered during fasting, experimental diabetes and experimental hypercortisolism (appendix paper IV). Diets containing large amounts of fat (in addition to carbohydrate) or large amounts of carbohydrate cause an increase of the hepatic lipase activity (appendix paper III and chapter II). These dietary adaptations of liver activity are possibly related to or exerted by variations in the glucagon/insulin levels of the blood as will be described in chapter II.

1.3. Other lipolytic activities in postheparin serum

In the previous sections the contributions of different organs to the triglyceride hydrolyzing activity of postheparin serum was discussed. Apart from these activities other lipolytic activities are present in postheparin serum. Vogel and Bierman[35] found that a phospholipase activity is released into the serum after heparin injection. This activity can be purified from rat liver parenchymal cells[36]. 1-Monoglyceride and 2-monoglyceride hydrolyzing activities are both found in postheparin serum[37-44] and in heparin-containing rat liver perfusates[45]. The 1-monoacylglycerol-hydrolase activity may be identical with the heparin-releasable phospholipase activity[46]. The 2-monoacylglycerol lipase activity is perhaps exerted by the same enzyme as the triglyceride lipase activity from the liver (appendix paper IV).

The different lipolytic activities of postheparin serum may influence each other when measured in vitro. The phospholipase may transfer acylgroups from different substrates to compounds, produced by other enzymes in the serum. The liver lipase activity may be operative in the breakdown of partial glycerides produced by the action of extrahepatic lipoprotein lipase upon triglycerides (see e.g. appendix paper IV). These facts may help to explain the variable results, that are obtained when using different substrates,

as was mentioned before.

1.4. Concluding remarks

Heparin releases different lipolytic activities from different organs into the serum. The lipoprotein lipase activities of extrahepatic organs correlate well with the capacity of these organs to take up serum triglycerides.The role of the liverlipase is much less clear (see chapter II). The selective measurement of different lipolytic activities in postheparin serum will contribute to a better understanding of the serum lipid metabolism in health and disease. As described in section 1.2. of this chapter, the lipase activities of hepatic and extrahepatic origin can now be measured separately. A further differentiation should be made between the enzyme activities released from extrahepatic sources, such as adipose tissue and (heart-) muscle. If this could be achieved a further understanding of serum lipid metabolism and the role of different organs in this process may be expected.

CHAPTER II

THE ROLE OF THE LIVER IN THE SERUM TRIGLYCERIDE REMOVAL

There is general agreement that the liver is the main site of endogenous serum triglyceride production[47-50]. The removal of serum triglycerides, however, is a controversial subject[51-55]. In the serum of hepatectomized dogs remnant particles are found[56]. These remnant particles or "remnants" may be produced as intermediates in the breakdown of chylomicrons and very low density lipoproteins (VLDL) to low density lipoproteins (LDL). LDL particles with an increased triglyceride content may be considered as remnants, but also VLDL with an increased cholesterol content are found[61]. The production of such remnants has been demonstrated in different species[56-59]. These particles may, in addition to triglycerides, contain partial glycerides, as can be expected from incomplete breakdown of triglycerides by the extrahepatic lipoprotein lipase(s) (comp. appendix paper IV). Recently a number of papers has appeared in which an enhanced level of remnant particles in man was demonstrated in patients with liver disease[60], hypothyreoidism[61] and diabetes[62]. The increased level of remnants seems to correlate with a lowered hepatic heparin-releasable lipase activity[30,60,61]. This suggests a role of the liver lipase in the removal of remnant (tri)glycerides from the serum. Whether also intact VLDL or chylomicrons are taken up by the liver is not clear. Uptake of injected VLDL by rat liver has been demonstrated by Stein et al.[55].

The possibility to measure the lipases of hepatic and extrahepatic origin in postheparin serum selectively (see chapter I) has become available, which allows us to correlate liver lipase activities with the levels of blood lipids found. Moreover, data in the recent literature also suggest a role of the liver lipase in serum triglyceride metabolism. Some of these arguments in favour of a role of liver lipase in the

catabolism of blood (tri)glycerides can be found in the discussion of appendix paper IV. Additional support will be given now.

Stein et al.[55] found VLDL accumulation at the plasma membranes of rat parenchymal liver cells, prior to the uptake of these particles in the cells. The liver lipase is located on the outersurface of the plasma membranes of liver parenchymal cells[34,63]. Parenchymal cells are much less involved in pinocytosis than Kupffer cells. Therefore hydrolysis of the triglyceride moiety of the VLDL particles, which then decrease in size, might preceed uptake of the particles in the parenchymal cells.

The effect of the nutritional state on liver lipase activity (appendix paper III) is in line with the hypothesis of a role for this enzyme in serum triglyceride breakdown. Under fasting conditions the liver is the site at which the major part of the serum triglycerides is produced. Therefore the lowered hepatic and adipose tissue lipase activities under this condition may result in a channeling of the triglycerides formed to the site where they should be used: the musculature, including heart muscle. In this context it may be of interest to note that both the liver lipase activity and the fat clearance by the heart of rapeseed oil fed rats are elevated (appendix papers III, V). During rapeseed oil feeding of rats, the heart rapidly accumulates triglycerides (for references comp. appendix papers V, VI) to reach a maximum in about 6 days, after which the lipid content decreases, although rapeseed oil feeding is continued. The elevated liver lipase might have resulted in a channeling of more triglycerides to the liver, which is in accordance with the observation of Gumpen and Norum[64] that the liver erucylcarnitine level is increased when the triglyceride content of the fatty heart diminishes. Therefore the elevation of liver lipase activity under feeding conditions may provide a mechanism to avoid high levels of remnants to accumulate in the blood. Such an accumulation

TABLE I

ALTERATION OF HEPARIN-RELEASABLE LIVER LIPASE ACTIVITY UNDER THE INFLUENCE OF DIFFERENT FEEDING CONDITIONS AND INSULIN ADMINISTRATION

Rats with an average weight of 280 g were used. Glucose (3 ml 10% glucose in destilled water) was administered 15 min before the subcutanous injection of insulin by a stomach tube. 50 I.U. of heparin were injected intravenously 1 h after the administration of insulin or 1 h after the start of the infusion of mannoheptulose. 6 min after the heparin injection blood was withdrawn. The lipase activity in the postheparin serum was measured with palmitoyl-CoA as the substrate, as described in appendix paper I. As was shown elsewhere (appendix paper IV) this activity mainly reflects the liver lipase activity. Insulin was from Organon (Oss, the Netherlands), mannoheptulose was a gift from the Pfizer Company.

Dietary conditions	Treatment	Palmitoyl-CoA hydrolase activity (nmoles CoASH/ min/ml) ± S.E.
normally fed (n=6)	none	220 ± 8
normally fed (n=4)	0.3 g mannoheptulose infused i.v. in 1 h	194 ± 13
normally fed (n=3)	0.5 I.U. insulin s.c.	251 ± 12
24 h fasted (n=3)	none	166 ± 7
24 h fasted (n=2)	0.5 I.U. insulin s.c.	184 (161,207)
24 h fasted (n=3)	glucose + 0.5 I.U. insulin s.c.	245 ± 5

would be an undesirable situation, as has been pointed out by Bierman et al.[65]. The penetration of these particles under the endothelial lining of bloodvessels, possibly the start of atherosclerotic plaque formation[65] should be avoided. Therefore the elevation of the liver enzyme activity during fat feeding (appendix paper III) will be advantageous. During fat feeding more chylomicrons will be produced in the intestinal cells and subsequently more remnants by the interaction with extrahepatic lipoprotein lipase. The elevated liver lipase activity may then be an adaptation to the increased synthesis of these remnant particles. In TABLE I it is shown that the liver lipase activity is decreased by fasting or mannoheptulose administration. Under these circumstances the glucagon/insulin ratio will be elevated. One hour after the administration of insulin cum glucose to fasted rats the liver lipase activity reaches a value slightly above the value found in the normal fed state. Insulin alone gives a much lower response. It is of interest to note that the liver enzyme may be regulated in the same way as adipose tissue lipoprotein lipase[8,14,66]. The fast elevation of the lipase activity by insulin treatment suggests that protein synthesis is not involved in the activation of the enzyme, but that the activation is exerted by some other mechanism. In appendix paper III we have shown that the liver lipase may exist in different states of activity. In preliminary experiments we found that the aggregation state of enzyme subunits may be involved in the degree of activation of the enzyme. In these experiments we found that the purified rat liver enzyme (appendix paper II) contained four protein bands in polyacrylamide electrophoresis. Antisera raised against each of these proteins inhibited the liver lipase activity completely. Also the liver lipase activity of human postheparin serum may depend on the state of aggregation of subunits, as was suggested by the experiments of Ehnholm et al.[31].

The contradictory results on the ability of the liver to take up serum triglycerides (comp. refs. 52 and 53) can, at least in part, be explained by the feeding conditions of the animals used. Felts and Mayes[53] found no uptake of chylomicron triglyceride by the liver of fasted rats, while in carbohydrate-fed rats 30-40% of injected chylomicron triglyceride may be taken up[52]. As was discussed above and was shown in appendix paper III, the liver lipase activity is in different states of activation during feeding and fasting. Another explanation of the difference of results of Felts and Mayes[53] and of Olivecrona et al.[52] may be found in the different systems used. Felts and Mayes[53] studied the uptake of chylomicrons by in vitro perfused rat liver. Chylomicrons with a diameter above 0.1 μ cannot pass the fenestrated endothelial lining of the liver[67]. However, the much smaller VLDL and remnants can enter the space of Disse much more easily. In the intact rats used by Olivecrona et al.[52] the uptake of chylomicrons might have been preceded by partial breakdown to smaller particles by extrahepatic lipoprotein lipase. In this regard it is explicable also why whole rat liver cannot take up chylomicrons, although isolated cells, without a size-limiting vascular barrier, can hydrolyze chylomicron triglycerides[54,68].

To what extent the liver hydrolyzes and takes up VLDL triglycerides is not clear yet. In TABLE II it is shown that antiserum raised against the liver lipase inhibits the lipase activity of postheparin serum to a greater extent when higher substrate concentrations are used. This means that in vitro more triglyceride is hydrolyzed by the liver enzyme than by the extrahepatic lipase(s), when the substrate concentration is high. At low concentrations triglyceride is predominantly hydrolyzed by the extrahepatic lipase activity. From these data it seems likely that the liver enzyme has a higher K_m for the triglyceride substrate than the lipoprotein lipase. Because of non-hyperbolic

TABLE II

DEGREE OF INHIBITION OF POSTHEPARIN SERUM LIPASE ACTIVITY BY LIVER LIPASE ANTISERUM AT DIFFERENT SUBSTRATE CONCENTRATIONS.

Serum lipase activity of rats, injected with 50 I.U. heparin, was estimated after preincubation with antiserum against the liver lipase or with control rabbit serum, exactly as described in appendix paper III. The substrate concentration was varied. The percentage inhibition of the total lipase activity by antiserum is presented.

Triglyceride concentration in mM	Percentage inhibition by the antiserum
10	52
5	46
2.5	42
1.25	36
0.63	33

kinetics the K_m values were not calculated. The results obtained in vitro suggest that also in vivo the liver lipase is relatively more active at higher serum triglyceride concentrations. If this enzyme activity is rate-limiting in the uptake of triglycerides by the liver, then the liver plays a more important role in the removal of serum triglycerides when the serum concentration is high. In this way the liver can perhpas exert a kind of overflow function, in order to prevent excessive elevation of serum triglyceride levels, at least when the triglycerides can

pass the endothelial fenestrae, as discussed above. When high doses of triglycerides are administered to humans intravenously, a fast removal of triglycerides from the blood precedes a slower hyperbolic phase of clearing[69]. It is possible that in the fast clearing phase the liver is involved, while in the slower phase the extrahepatic organs play a predominant role.

In order to test this hypothesis, fat clearing experiments were carried out in rats. It can be seen from TABLE III that the bulk of the radioactive label was recovered in the liver while in the hepatectomized rats practically all of the radioactivity (present in the triglyceride fraction; details not shown) remained in the blood. Therefore in this model the liver seems to be the main site of uptake. For the human species it was reported[70], however, that artificial fat emulsions were not taken up by the liver. Whether a species difference, or perhaps a difference in particle size (a smaller particle size brought about by sonification of our emulsions) accounts for the different results, is not clear, but requires further investigation. From the results with hepatectomized rats and heparin-treated rats it seems not very likely that the uptake of the labeled fatty acid by the liver is preceded by extrahepatic hydrolysis of the triglycerides. If this would have been the case, the label might have been expected to be present in the serum free fatty acid fraction. In the heparin-treated rats, when part of the triglyceride fraction is hydrolyzed in the serum, an enhanced uptake of the label in the liver would have been expected. Whether the liver lipase activity is involved in the uptake of the fat emulsion cannot be concluded from this TABLE. In other experiments we found that 5 min after injection of the fat emulsion 50% of the triglycerides taken up by the liver was hydrolyzed.

In conclusion: we suggest that the liver may play an important role in the serum triglyceride metabolism, not

TABLE III

DISTRIBUTION OF AN INTRAVENOUSLY ADMINISTERED [^{14}C]-LABELED FAT EMULSION OVER THE ORGANS OF THE RAT.

Male Wistar rats (about 250 g) were injected with 0.2 ml Intralipid. The Intralipid used was fortified with glycerol tri[^{14}C]-oleate as described in appendix paper I. 20 min after the injection the rats were killed, the organs removed and rinsed with 0.9% NaCl. Samples of about 0.1 g were dissolved in "Soluene-350" in 3-4 h at 60°C. After cooling to roomtemp., 0.2 ml 30% H_2O_2 and 0.2 ml isopropanol were added and the mixture was incubated for 30 min at 40°C. The samples were counted with 5 ml "Dimilume" and 5 ml "Instagel". The d.p.m. obtained were divided by the sample fraction taken from the whole organ. Skeletal muscle samples were obtained from the thigh bone and assumed to be representative for the entire musculature. Functional hepatectomy was achieved as described in appendix paper III. In the experiment with the heparin-treated animals 50 I.U. heparin were injected together with the Intralipid. "Soluene", "Dimilume" and "Instagel" were obtained from Packard. Intralipid (10%) was from Vitrum, Stockholm.

Organ	% of the injected radioactivity recovered		
	normal rats	hepatectomized rats	heparin-treated rats
serum	9	95-100	4
Liver	64	-	50
heart	2	-	1
epidydimal adipose tissue	1	-	7
skeletal muscle	7	-	8

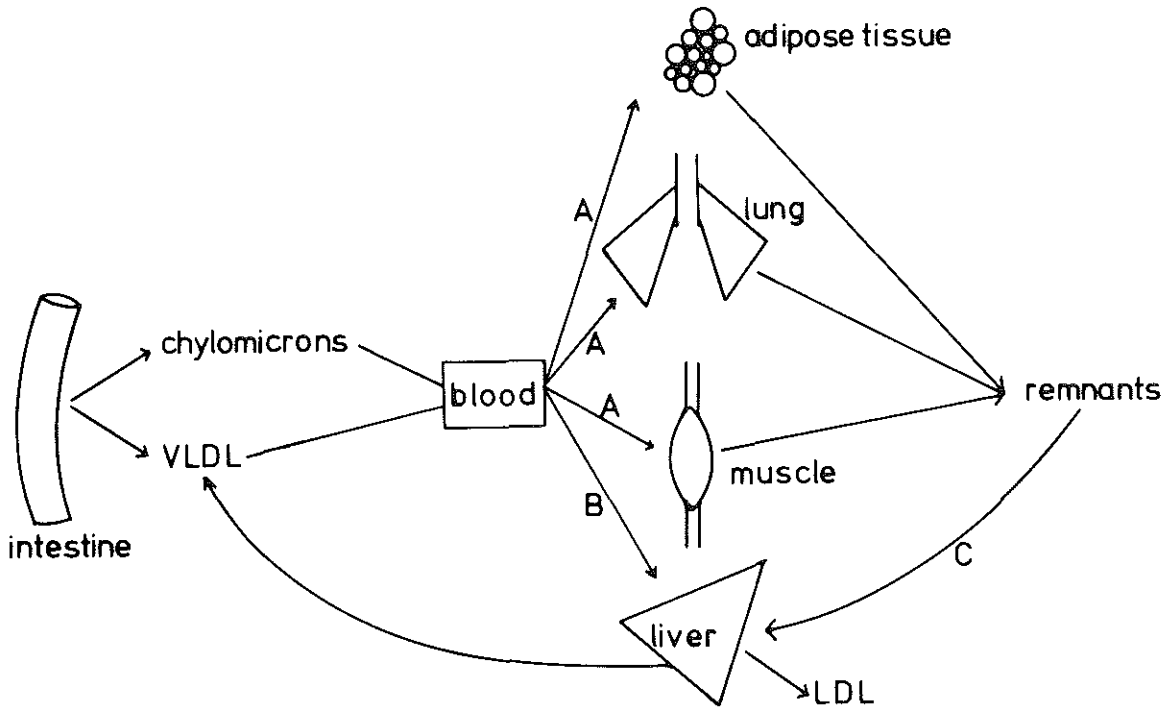

Fig. 1. Schematic representation of serum triglyceride metabolism

only in the production of serum triglyceride, but also in the removal of serum triglycerides. In the latter process the liver lipase activity may be a rate-limiting factor. In accordance with this are the data of other investigators (comp. e.g. refs. 60, 30 and 61). Although under normal conditions only 20% of the daily fat uptake reaches the rat liver directly it seems to be possible that a lowering of the liver lipase activity can lead to hypertriglyceridemia.

The role of the most important organs in serum triglyceride metabolism is summarized in fig. 1. In this figure the reactions designated with (A) are thought to be catalyzed by lipoprotein lipase which hydrolyzes triglycerides to free fatty acids and monoglycerides (comp. also the Introduction and chapter I). In the reactions (B) and (C) liver lipase is thought to be involved. We would suggest that reaction (B) is only of importance at high VLDL levels and perhaps under certain dietary conditions when the liver lipase activity is elevated. Reaction (C) is the major pathway for the breakdown of the triglyceride moiety of remnant particles. This process may take place at the surface of the hepatocytes, where the lipase is located. The fate of the LDL formed is not certain. They may be released into the circulation, taken up by parenchymal or non-parenchymal cell types of the liver. It seems reasonable to state that in the removal of artificial fat emulsions, as shown in TABLE III of chapter II, the liver lipase activity is also involved. In this context it must be emphasized that artificial triglyceride emulsions lack proteins to activate extrahepatic lipoprotein lipase (the same holds for the remnants). However, these activating proteins may be taken up from other serum lipoproteins in the circulation.

For the study of serum triglyceride metabolism in health and disease it will be of considerable importance to be able to measure the liver lipase activity in post-

heparin serum. Of special interest is the possible correlation between the remnant particle level and the development of atherosclerosis. It was postulated by Zilversmit[71] and by us[72] that an increased heart-lipoprotein lipase activity may, under certain conditions, lead to atherosclerosis. Now we have found that the liver lipase activity is low under conditions that the heart lipase activity is high (compare TABLE I and refs. 12 and 16). If this situation is accompanied by a high serum triglyceride level, as in diabetes and under stress conditions, then an elevated level of remnant particles if to be expected. This may lead to hypercholesterolemia together with hypertriglyceridemia. Hypothyroidism[61], type III[73] (possibly type V [74]) hyperlipoproteinemia, diabetes[62] and cholesterol-rich diets[75] are all accompanied by an elevated remnant particle concentration. The atherogeneity of these conditions is well established.

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SUMMARY

The presence of palmitoyl-CoA hydrolase activity in postheparin serum of man and rat has been described. It was found that this activity was released from different organs into the blood after the intravenous injection of heparin. In the rat the liver contributes about 80% of the total activity in postheparin serum.

The immunological difference between lipase species of rat liver and extrahepatic organs was established. By using an antiserum against the liver lipase, a selective measurement of different lipases in postheparin rat serum has become available. It was shown that the contributions of the liver and the extrahepatic organs to the total lipase activity of postheparin serum, were strongly dependent on the amount of heparin injected and on the time of blood removal after the injection. When low heparin amounts are injected, predominantly the extrahepatic organs release their lipase activity. Furtheron it was shown that the liver lipase species was released faster than the extrahepatic lipase activity by heparin in man and rat and that it disappeared from the blood soon after the maximum activity had been reached. Arguments were brought forward that the liver lipase activity was removed from the serum by removal of the enzyme, and not by inactivation in the blood stream. By immunological means it was shown that the liver lipase might exist in more or less activated states. The state of activation was found to be influenced by the blood insulin level and probably related to the glucagon/insulin ratio. A high ratio corresponds with lowered hepatic lipase activity, while the administration of insulin enhances the liver lipase activity. This is an explanation for the decreased hepatic lipase activity during fasting and in diabetes.

The liver and the extrahepatic lipases in postheparin serum have a different substrate specificity and possibly

a different affinity for a triglyceride substrate, the liver enzyme having a relatively low affinity. From in vitro experiments an overflow function of the liver in the control of the blood triglyceride level was postulated. Also a function of the liver in the removal of remnant particles was discussed. In both instances the heparin-releasable lipase activity seems to be a rate-determining factor. Our results were related with those of other investigators.

The effects of experimental hypercortisolism, experimental diabetes and some diets on the liver lipase and extrahepatic lipoprotein lipase activities were studied. The study of the effects of rapeseed oil feeding on hepatic and heart lipase activities revealed that prolonged feeding results in increased activities of the non-heparin releasable (and therefore probably the intracellular-) myocardial enzyme together with the heparin-releasable hepatic enzyme. These adaptations provide an explanation for the gradual removal of myocardial triglyceride after a period of excessive accumulation early during rapeseed oil feeding.

SAMENVATTING

De aanwezigheid van palmitoyl-CoA hydrolase aktiviteit in het bloed van met heparine ingespoten ratten werd vastgesteld. Deze aktiviteit blijkt voor 80% uit de lever afkomstig te zijn. Ook in de mens wordt palmitoyl-CoA hydrolase aktiviteit door heparine in de bloedbaan gebracht.

De lipase welke door heparine uit de lever wordt vrijgemaakt is immunologisch verschillend van de lipase uit andere organen. Met behulp van een specifiek antiserum tegen de rattelever lipase, konden de lipase aktiviteiten van hepatische en extrahepatische oorsprong in postheparine serum afzonderlijk bepaald worden. Het bleek dat de bijdragen van de lever en van de extrahepatische organen, tot de totale postheparine serum lipase aktiviteit, sterk afhankelijk zijn van de toegediende hoeveelheid heparine en de tijd van bloedafname na de injectie.

Na injectie van een lage heparine dosis wordt vooral extrahepatische lipase aktiviteit in het serum gevonden. Bij gebruik van hoge heparine doses, echter, wordt de lever lipase sneller vrijgemaakt dan de extrahepatische lipoproteïne lipase. De verdwijning van lever lipase aktiviteit uit het bloed, nadat een maximale aktiviteit is bereikt, is waarschijnlijk het gevolg van verwijdering van het enzym uit de bloedbaan en geen gevolg van inaktivatie van het enzym in de bloedbaan.

De door heparine vrij te maken lever lipase kan zich in een meer of minder aktieve toestand bevinden. Dit kan worden beïnvloed door de insuline concentratie van het bloed te variëren. In omstandigheden met een hoge glucagon/insuline verhouding, zoals tijdens vasten of diabetes, is de lever lipase aktiviteit laag. Door insuline tezamen met glucose aan gevaste ratten toe te dienen vindt een snelle aktivatie van de lever lipase plaats.

Naast immunologische verschillen tussen de hepatische en extrahepatische lipase aktiviteiten, werd ook een ver-

schil in substraatspecificiteit gevonden. Het lever enzym heeft een lagere affiniteit voor een artificiële triglyceride emulsie, maar katalyseert de hydrolyse van palmitoyl-CoA beter dan extrahepatische lipoproteïne lipase.

Daar in vitro de aktiviteit van het lever enzym bij gebruik van hoge concentraties triglyceride die van het extrahepatische enzym overtreft lijkt het aannemelijk dat ook in vivo de lever zou kunnen bijdragen tot een verlaging van het serum triglyceride gehalte vooral indien deze hoog zou zijn. Met andere woorden, de lever zou een soort "overflow" funktie kunnen vervullen. De rol van de lever in de afbraak van serum lipoproteïne intermediairen (zogenaamde "remnants") werd besproken. De lever lipase lijkt een snelheidsbepalende faktor hierin te kunnen zijn.

Voorbehandeling van ratten met een cortisol preparaat en met bepaalde diëten blijkt van invloed te zijn op de postheparine serum lipase aktiviteiten van hepatische en extrahepatische oorsprong.

Daar raapzaadolie-rijke voeding bij ratten in een vroege fase snel tot hartvervetting leidt, welke op den duur weer vermindert, werd de invloed van deze voeding op de (lipoproteïne-) lipasen van lever en hart nagegaan. Gevonden werd dat deze enzym aktiviteiten toenemen,mogelijk bijdragend tot de verdwijning van excessieve vetstapeling uit de hartspier.

CURRICULUM VITAE

In 1942 werd ik in Deventer geboren. Na het eindexamen HBS-B schreef ik mij in 1960 aan de Universiteit van Amsterdam in voor de studie in de scheikunde. In 1964 werd het kandidaatsexamen f afgelegd. Vervolgens werd als hoofdrichting biochemie gekozen. Keuze-, neven- en bijvakken waren respectievelijk kolloid chemie, fysisch-organische chemie en microbiologie. In 1969 deed ik doctoraal examen. Van 1965 tot aan het doctoraal examen was ik kandidaat-assistent in diverse laboratoria.

In oktober 1970 trad ik in dienst van de Nederlandsche Organisatie voor Zuiver Wetenschappelijk Onderzoek (Z.W.O.). In de afdeling biochemie I van de Erasmus Universiteit te Rotterdam werkte ik eerst aan het enzym carnitine:palmitoyl-CoA transferase, in 1972 begon ik aan het onderwerp waarover dit proefschrift handelt. Sinds oktober 1974 ben ik in dienst van de Erasmus Universiteit.

PAPERS

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LONG-CHAIN ACYL-CoA HYDROLASE ACTIVITY IN SERUM: IDENTITY WITH CLEARING FACTOR LIPASE

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SUMMARY

After intravenous heparin administration, palmitoyl-CoA hydrolase activity strongly increases in the blood. The properties of the enzymatic reaction were studied and compared with published data on lipoprotein lipase (EC 3.1.1.3). In all cases striking similarities were obtained. We therefore conclude that serum postheparin palmitoyl-CoA hydrolase and lipoprotein lipase are identical. Lipoprotein lipase activity in postheparin serum was found to be lowered by fructose feeding.

INTRODUCTION

In our studies on carnitine-palmitoyltransferase (hexadecanoyl-CoA: carnitine *O*-hexadecanoyltransferase) long-chain acyl-CoA hydrolase activity frequently interfered. Therefore, we decided to study the long-chain acyl-CoA hydrolase activity more closely. In accordance with other investigators[1–4], we found the main activity to be present in the microsomal fraction of liver. On the basis of the observations of Barber and Lands[5], who showed that pancreatic lipase is capable of long-chain acyl-CoA hydrolysis, we decided to investigate whether lipoprotein lipase can be determined with long-chain acyl-CoA as the substrate instead of its conventional triglyceride substrate, especially as the conventional specific triglyceride hydrolysis measurements (compare Materials and Methods) are rather laborious.

Hahn[6] showed in 1943 that lipoprotein lipase (or clearing factor lipase) is released into the bloodstream after the intravenous injection of heparin, part of which may be derived from cardiac lipoprotein lipase[7]. We therefore investigated whether postheparin serum contained not only lipoprotein lipase activity, but also long-chain acyl-CoA hydrolase activity. It is the purpose of the present investigation to report on the presence of both activities and on the identity of the activities.

Abbreviation: DTNB, 5,5′-dithiobis-(2-nitrobenzoic-acid).

MATERIALS AND METHODS

The acyl-CoA esters were made by a slight modification of the method of Stoffel *et al.*[8].

Protein was estimated according to Lowry *et al.*[9].

For the lipoprotein lipase assay the method of Kelly[10] was followed. Incubation was carried out in principle according to Boberg and Carlson[11]. The substrate was prepared as follows. About 15 μCi of tri-[^{14}C]oleylglycerol (Radiochemical Centre, Amersham, England), dissolved in benzene, were evaporated to dryness in a plastic tube (when glass was used, emulsification was less reproducible), 10 ml Intralipid (Vitrum, Stockholm, Sweden), containing 1 g triglyceride, 0.12 g lecithin, 0.25 g glycerol and an emulsifier, were added to the residue and sonicated (7 times for 1 min at 20 kHz, with intermittent cooling in ice). 0.35 ml of the radioactive triglyceride emulsion, 2.6 ml of 67 mg/ml fatty acid-poor bovine serum albumin (Pentex, Kankakee, Ill., U.S.A.) in 0.1 M NH_4OH–NH_4Cl buffer of pH 8 and 1.0 ml human serum (citrate plasma clotted by the addition of Ca^{2+} and dialysed) were incubated for 30 min at 37 °C. Then 10 μl heparin (5000 I.U./ml = 50 mg/ml, Organon, Oss, The Netherlands) and 0.5 ml enzyme were added. Samples of 1 ml were removed at 10, 20, 30 and 40 min and extracted with 5 ml isopropanol–H_2SO_4, as described by Dole[12]. 3 ml of the heptane phase were mixed with 0.5 g Amberlite IRA-400 in a scintillation vial. After 1 h of mixing, the fluid was removed and the resin washed 4 times with hexane. Finally 0.5 ml hyamine hydroxide (1 M in methanol) was added, followed by 10 ml toluene with fluors, and the samples were counted. In all experiments male Wistar rats were used with a body weight varying between 240 and 260 g. The activity of *p*-nitrophenylacetate hydrolase (carboxyl esterase; EC 3.1.1.1), expressed in μmoles *p*-nitrophenol formed per min at 30 °C, was determined according to Huggings and Lapides[13] in the presence of 10^{-5} M eserine. In the standard assays, palmitoyl-CoA hydrolase activity was measured at 30 °C in a volume of 1 ml containing 100 mM Tris–HCl, 80 μM palmitoyl-CoA and 100 μM 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) (see Results). The palmitoyl-CoA hydrolase activity was expressed in nmoles of CoASH formed per min. An extinction coefficient of 13600 $M^{-1} \cdot cm^{-1}$ at 412 nm was used for the 5'-thio-2-nitrobenzoic acid liberated from DTNB by CoASH.

Purification of lipoprotein lipase

40 rats were injected intravenously with 1 mg heparin (100 I.U.) per kg body weight. After 15 min the rats were decapitated and the blood collected in 0.05 vol. of 0.25 M sodium citrate. The blood was centrifuged for 15 min at $3000 \times g_{max}$. The clear serum (150 ml) was obtained and mixed with 7.5 ml 10% Intralipid and stirred for 10 min at 37 °C. The mixture was spun in a swing-out rotor in a refrigerated centrifuge for 1 h at $75000 \times g_{max}$. The floating layer was collected and the infranatant again mixed with 7.5 ml 10% Intralipid and stirred at 37 °C for 10 min. After centrifugation, as described above (the infranatant is designated Fraction I in Table I), the floating layer was combined with the first one and suspended in 150 ml ammonia buffer containing 50 mM NH_4OH–NH_4Cl (pH 8.5), 10% sucrose and 10 μg heparin per ml and again centrifuged as described above. The floating cake was now washed 4 times with 20 ml of the ammonia buffer. After washing, the cake was suspended in 9 ml of "Fieldings buffer", containing 50 mM NH_4Cl–NH_4OH, 0.5% deoxycholate (w/v),

TABLE I

SIMULTANEOUS PURIFICATION OF PALMITOYL-CoA AND TRIOLEIN HYDROLYTIC ACTIVITIES; DEPENDENCE OF ACTIVITY ON SERUM PROTEINS

The enzyme fractions are obtained and measured as described under Materials and Methods. The dependence of palmitoyl-CoA hydrolase activity on preincubation with serum was illustrated by mixing 4.5 ml of 0.8 mM palmitoyl-CoA with either 0.5 ml water or preheparin serum. The reactions were carried out under standard conditions. The purified enzyme was diluted 5-fold prior to the assay.

Source of enzyme	*Preincubation of palmitoyl-CoA with serum*	*Palmitoyl-CoA hydrolyse activity (munits/mg protein)*	*Triolein hydrolase activity (munits/mg protein)*
Postheparin serum	−	4.4	
Postheparin serum	+	4.2	10.6
Fraction I	−	1.8	4.3
Purified enzyme	−	12.8	
Purified enzyme	+	636	1900

0.5 mM potassium oleate and 5 μg heparin per ml. The suspension was stirred for 1 h at 0 °C and centrifuged for 1 h at 160000 × g_{max} in a swing-out rotor. The infranatant was designated "purified" enzyme (Table I).

RESULTS

Standardization of the measurement of palmitoyl-CoA hydrolase activity in serum

It can be seen from Fig. 1 that rat serum contains very low palmitoyl-CoA hydrolase activity (4.6 munits/ml), which is strongly increased by the intravenous administration of heparin (1 mg/kg body weight). Serum obtained from blood removed 6 min after heparin administration contained the highest activity (Fig. 1). A similar time dependence for triolein hydrolysis in postheparin plasma was observed by

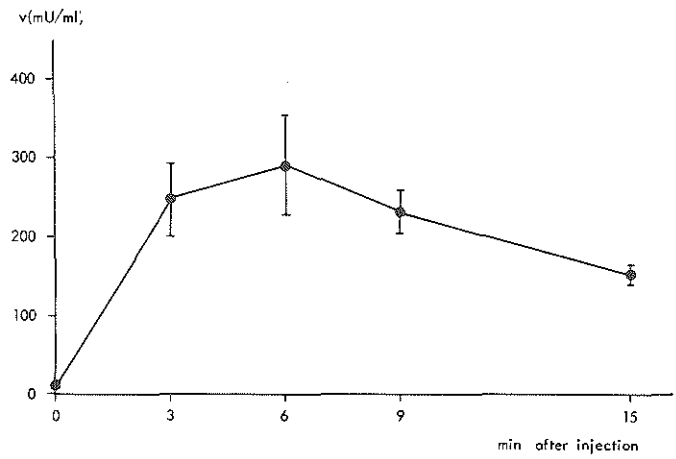

Fig. 1. Release of palmitoyl-CoA hydrolase in blood after heparin injection. At different times, 1 ml of blood was withdrawn from a rat injected with heparin. The zero time sample was obtained by withdrawing blood prior to heparin injection. The hydrolase activity was measured under standard conditions, as described in the text. The data are the mean values of 5 experiments; the standard deviation is shown in the figure.

Fielding[15]. For these reasons serum was generally obtained from blood withdrawn 6 min after heparin injection.

Fig. 2 illustrates that the palmitoyl-CoA hydrolase activity in serum is strongly pH-dependent. The activity at pH 8.5 is almost optimal, which led us to choose this pH for further determinations, since at pH values higher than 9, non-enzymatic palmitoyl-CoA hydrolysis becomes significant.

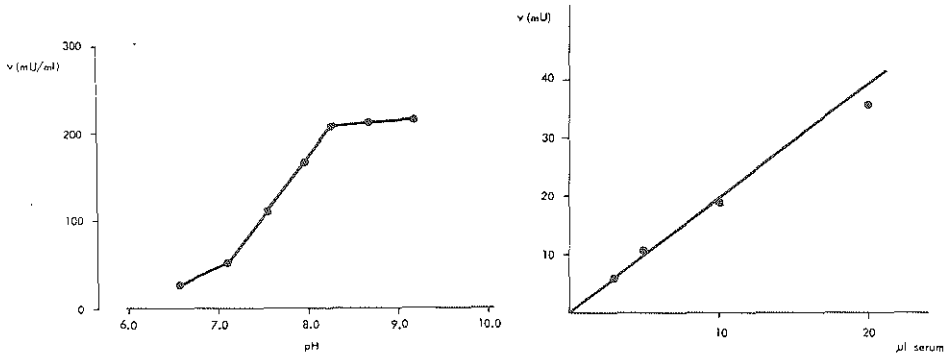

Fig. 2. pH dependence of the palmitoyl-CoA hydrolase activity. In the standard assay the pH of the Tris-HCl buffer was varied between 6.5 and 9.5. The extinction coefficient for DTNB was constant between pH 7 and 9.

Fig. 3. Relation between palmitoyl-CoA hydrolase activity and the concentration of protein. In the assays (under standard conditions) the sample volume was varied between 3 and 20 μl serum.

The enzymatic reaction was found to follow zero order kinetics up to 20 μl serum per ml of incubation medium (Fig. 3). If higher serum concentrations were used, lower specific activities were observed, probably because the concentration palmitoyl-CoA is lowered by adsorption to protein (compare also ref. 2, in which a similar

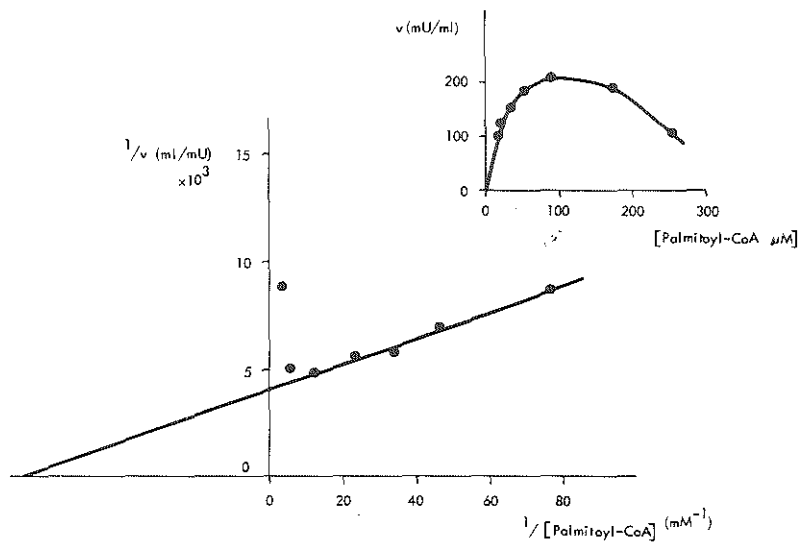

Fig. 4. Lineweaver–Burk plot of palmitoyl-CoA hydrolysis with varying palmitoyl-CoA concentration. At standard conditions (see text) the palmitoyl-CoA concentration was varied between 12.5 and 260 μM.

behaviour was observed with long-chain acyl-CoA hydrolysis catalyzed by rat liver microsomes). In our standard assays we used 5 μl serum per ml incubation medium.

From Lineweaver–Burk plots (Fig. 4) an apparent K_m for palmitoyl-CoA of 15 μM could be calculated. Substrate inhibition occurs at a palmitoyl-CoA concentration higher than 120 μM (see insert of Fig. 4). For these reasons we used in our assays a palmitoyl-CoA concentration of 80 μM. The reaction rate was not influenced when the DTNB concentration was varied between 25 and 400 μM. We have chosen a concentration of DTNB in our standard assays of 100 μM.

Different acyl-CoA esters were tested as substrates under the above conditions (5 μl postheparin serum, pH 8.5, 80 μM acyl-CoA and 100 μM DTNB). No detectable activity was observed with acetyl-CoA, propionyl-CoA, butyryl-CoA, hexanoyl-CoA and octanoyl-CoA. With decanoyl-CoA, lauroyl-CoA, myristoyl-CoA or palmitoyl-CoA, increasing activities, in the order given, were found (data not shown).

Resemblances of the properties of postheparin lipoprotein lipase and postheparin palmitoyl-CoA hydrolase

Korn[7] described in 1955 a number of specific properties of clearing factor (postheparin) lipase (the activity of which was measured with a coconut oil emulsion as the substrate).

(1) Release of lipoprotein lipase activity in the blood after heparin administration. This holds also for palmitoyl-CoA hydrolase activity (Fig. 1). (2) Inhibition of lipoprotein lipase activity by high NaCl concentration. From Table II it can be seen that this is also true for postheparin palmitoyl-CoA hydrolase. (3) Requirement of lipoprotein lipase for serum proteins (compare also ref. 14). The serum dependence is very clear after purification of the enzyme according to Fielding[15] (compare also Yasuoka and Fujii[16]). We did not carry out the last step and obtained significant activity in the palmitoyl-CoA hydrolase assay only after preincubation of the substrate with preheparin serum (Table I). It can also be concluded from this table that,

TABLE II

INHIBITION OF POSTHEPARIN PALMITOYL-CoA HYDROLASE BY NaCl AND HEPARIN

Postheparin serum was tested under standard conditions (see text).

Additions	*Activity (munits/ml)*
None	214
1 M NaCl	17
100 μg heparin	170
250 μg heparin	130

together with palmitoyl-CoA hydrolase, triolein hydrolase activity was purified. The activity in crude postheparin serum was not strongly stimulated by the addition of preheparin serum. (4) Requirement of lipoprotein lipase activity for low concentrations of heparin. We were not able to show, under the standard conditions of the palmitoyl-CoA hydrolase assay, the stimulatory effect of low concentrations of heparin. However, we were able to show that chromatography of postheparin serum on Sephadex G-200, as has been carried out previously by Yasuoka and Fujii[17], yielded no detectable palmitoyl-CoA hydrolase activity in the various eluate fractions (result

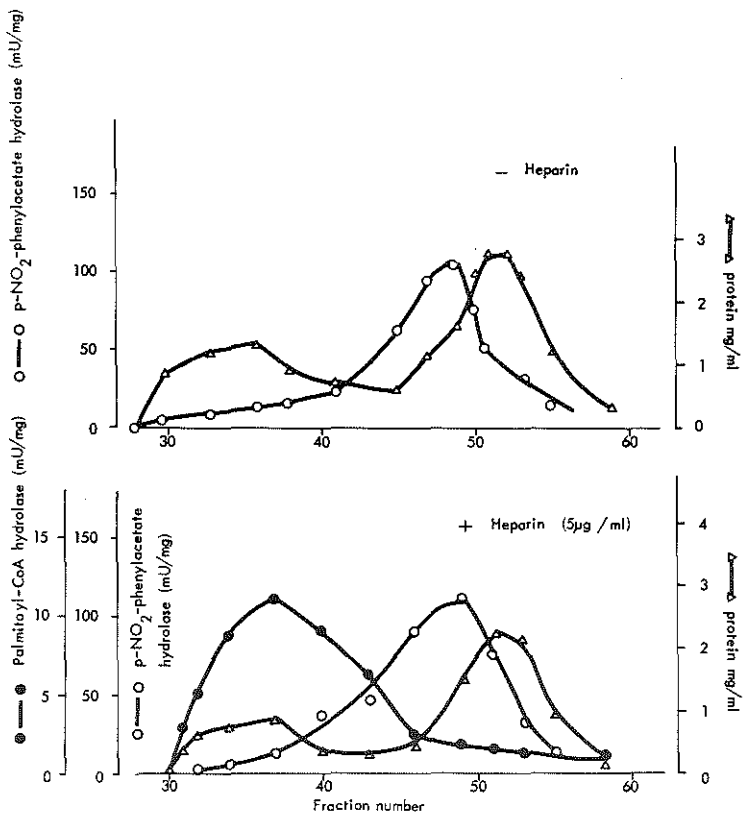

Fig. 5. Gel filtration of postheparin serum. Gel filtration was carried out on a Sephadex G-200 column (5.0 cm × 50 cm). 5 ml postheparin serum were chromatographed at 4 °C. The flow rate was 15 ml/h. Samples of 8 ml were collected. In the samples, protein was estimated according to Lowry *et al.*[9]; *p*-nitrophenylacetate hydrolase (carboxyl esterase) and palmitoyl-CoA hydrolase activities were measured as described in the text. The elution buffer was 50 mM NH_4Cl–NH_4OH at pH 8.5, containing heparin when indicated.

not shown), unless 5 µg heparin per ml elution buffer was present (Fig. 5). The palmitoyl-CoA hydrolase peak corresponds close to the lipoprotein lipase peak obtained by Yasuoka and Fujii[17], and is different from the carboxyl esterase peak (Fig. 5). When the fractions obtained without heparin in the elution medium (top of Fig. 5) were incubated with heparin, the hydrolase activity was not restored. (5) Inhibition of lipoprotein lipase activity by excess heparin. The inhibition by heparin is also observed when palmitoyl-CoA is the substrate (Table II).

Postheparin serum and its hydrolytic activities towards triolein and palmitoyl-CoA

From Table III it can be concluded that the ratio of the hydrolytic activities towards triolein and palmitoyl-CoA does not vary considerably with different enzyme samples, indicating that variation of lipoprotein lipase activity in different sera may be detected with either method.

TABLE III

COMPARISON OF TRIOLEIN AND PALMITOYL-CoA HYDROLYSIS IN VARIOUS POSTHEPARIN SERA

Rats were fed for 40 h with different diets as indicated. Control rats were fed with normal laboratory chow. The casein diet contained 85% casein, 10% crisco, 1%vitamins and 4% salt mixture. The fructose diet contained 17% casein, 68% fructose, 10% crisco, 1% vitamins and 4% salt mixture. After heparin injection the rates of hydrolysis of palmitoyl-CoA or triolein were measured in the obtained sera, as described in the text. The rate of triolein hydrolysis is expressed as nmoles fatty acid formed per min per ml serum. The palmitoyl-CoA hydrolase activity is expressed as munits/ml serum. The average of two experiments is given.

Conditions of the rats	*Blood withdrawn after (min)*	*Substrate*		*Activity ratio*
		Triolein	*Palmitoyl-CoA*	
Control diet	6	720	277	2.6
Control diet	15	573	229	2.5
Casein diet	6	717	268	2.7
Fructose diet	6	448	156	2.9

DISCUSSION

From the results obtained it seems very likely that postheparin lipase and postheparin palmitoyl-CoA hydrolase in serum are identical. The determination with palmitoyl-CoA as the substrate requires only small amounts of enzyme, which may be of advantage in cases where the amounts to be obtained are small. Another advantage is the simple procedure in which continuous recording is possible. Moreover, there is no need for preincubation of the substrate with control (preheparin) serum, which may influence the results. Although the data with both methods, as shown in Tables I and III, correspond fairly well, it is conceivable that in certain situations good correlation is not seen when the activating factors are rate limiting. That in the postheparin palmitoyl-CoA hydrolase reaction activating serum factors are also needed was demonstrated in Table I. This can also be inferred from experiments (not shown) in which less than 5 μl serum were used. In those cases we regularly observed a lag phase in the spectrophotometric assay, probably due to "serum activation". That the hydrolytic activity, presently studied, requires serum proteins for activity, may be of importance in further studies with tissue homogenates in which not only the lipoprotein lipase, but also other lipases, may contribute to palmitoyl-CoA hydrolysis. Pancreas, for instance, contains palmitoyl-CoA hydrolase activity which is not serum dependent[5]. Kurooka *et al.*[18] found in kidney, liver, spleen, testis, heart, brain and erythrocytes considerable long-chain acyl-CoA thioesterase activity in the absence of added serum. Future distribution studies of palmitoyl-CoA hydrolases in various organs will make use of the properties described in the present paper as well of inhibitors, of which paraoxon was found to inhibit lipoprotein lipase (as well as postheparin palmitoyl-CoA hydrolase) at a concentration of 10^{-5} M. It should be mentioned that, after heparin injection, palmitoyl-CoA hydrolase activity appears not only in rat but also in humans (data not given).

In an attempt to obtain different lipoprotein lipase activities in sera, in order to find out whether the activity as measured with palmitoyl-CoA as the substrate correlated with the activity with triglyceride as the substrate, we investigated the influence of fructose in the diet. In an earlier study[19], it was found that fructose stim-

ulated the resorption of fat from the diet. As was shown in Table III fructose feeding led to decreased lipoprotein lipase activity. This phenomenon might contribute to the fructose-induced increase in serum triglyceride levels as observed by McDonald[20] under certain conditions.

ACKNOWLEDGEMENTS

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LIPOPROTEIN LIPASE FROM HEART AND LIVER: AN IMMUNOLOGICAL STUDY

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SUMMARY: Lipoprotein lipase activity was purified from rat postheparin serum. An antibody against this activity was raised. It was found that this antibody inhibits 95% of the postheparin lipoprotein lipase activity. The same inhibition was found in the perfusate of heparin-perfused rat liver. When measuring lipoprotein lipase activity in perfusates of rat hearts, perfused with heparin, no inhibition by this antibody was found. It is concluded that lipoprotein lipase activities from heart and liver are catalysed by different enzymes and that the heart enzyme contributes not more than 5% to the overall lipase activity in postheparin serum.

Korn (1) showed in 1955 that acetone powders of rat heart contained lipoprotein lipase. This enzyme is known also to be localized in lung, adipose tissue, diaphragm and mammary gland. After intravenous injection of heparin, lipoprotein lipase activity appears in the blood (2). This activity is assumed to be released from extrahepatic organs. However a number of authors (3,4,5) have shown recently that lipase activity is also released from the liver. Borensztajn et al. (6) had found that a large part of the original lipoprotein lipase activity was removed from the heart after perfusion of rat heart with a heparin containing medium. Liver shows a very rapid release of lipase activity on perfusion with heparin. Therefore we investigated the contributions of liver and heart to the lipase activity of postheparin plasma.

METHODS

Rats were injected with heparin (100 I.U./kg bodyweight). After 6-15 min they were killed and the blood was collected. Lipoprotein lipase activity was purified from the serum on a sepharose-heparin co-

lumn, as described for lipoprotein lipase from skim milk by Olivecrona and Egelrud (7). We replaced the NaCl gradient by a heparin gradient (0-1.25 mg/ml). The purification was 600-fold. In a subsequent calciumphosphate adsorption step another two-fold purification was obtained. 1 mg of the purified enzyme, mixed with Freunds adjuvant (1:1), was injected in the foot pads of a rabbit. After 4 weeks a booster injection was given intramuscularly and 10 days later 30 ml blood was collected by heart punction. Purification of the γ-globulin fraction was achieved by ammonium sulphate precipitation (50% saturation) and gel filtration on a Sephadex G-200 column, followed by another ammonium sulphate precipitation. Electrophoresis (not shown) indicated that the isolated fraction contained only γ-globulins. Serum of a non-immunised rabbit was handled in the same way. This γ-globulin fraction was used in control experiments.

Rat hearts were perfused, after Nembutal narcose of the animals, according to the Langendorff technique with a modified Tyrode solution for 10 min at 37°C. Then the perfusion medium was replaced by one containing, in addition, 20% (v/v) preheparin rat serum and 4.5 I.U. heparin/ml.

Rat livers (about 8 g) were perfused through the portal vein with a Krebs-Henseleit bicarbonate buffer, saturated with 95% O_2 - 5% CO_2, containing 5 mM glucose at 33 ± 2°C. The inferior caval vein was ligated below the liver and cannulated above the liver. After 10 min perfusion at a rate of 20 ml/min the perfusion medium was replaced by a medium, containing 80% of the medium described above and 20% of rat serum (v/v) as well as heparin (final concentration 4.5 I.U./ml). The top of the latter was briefly sprayed with Antifoam (Dow Corning Corp.).

Lipoprotein lipase activity was estimated with palmitoyl-CoA as the substrate, as described by Jansen and Hülsmann (8). When [^{14}C]trioleate was used as the substrate, the modified method of Kelly, as descri-

Table I

INFLUENCE OF PREHEPARIN SERUM ON PURIFIED AND ANTIBODY TREATED LIPOPROTEIN LIPASE

Enzyme source	Preincubation*	Additions to standard assay	Activity
Purified enzyme	none	none	1200 mU/mg
" "	none	10 µl human serum	1865 mU/mg
" "	none	10 µl rat serum	1880 mU/mg
Postheparin serum	60 min, no additions	none	140 mU/ml
" "	60 min, 15 µl control γ-glob. fraction present	none	136 mU/ml
" "	60 min, 15 µl control γ-glob. fraction present	10 µl human serum	148 mU/ml
" "	60 min, 15 µl control γ-glob. fraction + 10 µl human serum present	none	143 mU/ml
" "	60 min, 15 µl antibody fraction present	none	7 mU/ml
" "	60 min, 15 µl antibody fraction present	10 µl human serum	6 mU/ml
" "	60 min, 15 µl antibody fraction + 10 µl human serum present	none	8 mU/ml

Incubations were carried out as described in Fig. 1. The blood was withdrawn 6 min after heparin injection and centrifuged; the plasma was tested. Palmitoyl-CoA was the substrate.

*Where shown, incubation of 5 µl postheparin serum was for 60 min at 37°C, followed by centrifugation for 2 min at 15 000 x g.

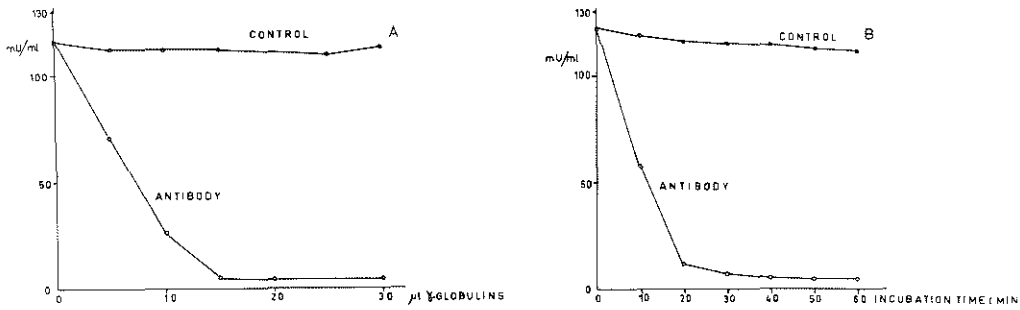

Fig. 1. Influence of different amounts of antibody and different incubation times on postheparin lipoprotein lipase activity.

20 µl postheparin plasma was either incubated for 1 h with different amounts of antibody- or control preparation at 37°C (Fig. 1A) or at different incubation times, as shown in Fig. 1B. The mixtures were centrifuged for 2 min at 15 000 x g. From the supernatants amounts equivalent to 5 µl original postheparin serum were tested. The amounts of control- or antibody preparation used were related to 5 µl postheparin serum.

bed in ref. 8, was used. Activities are expressed as nmoles CoASH released per min in the palmitoyl-CoA assay, and as nmoles FFA released/min, when trioleate was the substrate.

RESULTS

The antibody preparation was diluted with 0.9% NaCl to a protein concentration of 3.35 mg/ml, the same was done with the control serum fraction. By varying the antibody concentration and the incubation time we found that 15 µl antibody inhibited lipoprotein lipase activity of 5 µl whole postheparin serum 95%, when incubated between 30 and 60 min at 37°C, followed by centrifugation for 2 min at 15 000 x g (Fig. 1A and B). The presented values are based on linear reaction rates measured during the first 10 min. In order to exclude that an enzyme labile at 37°C was inactivated during the incubation with control or antibody, we incubated postheparin serum with a non-saturating amount of control and antibody at 0°, 37° and 40°C. It was found that during the incubation at 0°C for 3.5 h no activity was lost and that

with this amount of antibody 70% of the initial activity was inhibited. Incubation at 37°C gave a loss of 19% of the initial activity; of the remaining activity 70% was again inhibited by the antibody. Incubation for 1 h at 40°C with control γ-globulins resulted in 75% inactivation of the enzyme. The remaining activity was again 70% inhibited by the antibody. Therefore the existence of a heat-labile non-inhibited enzyme could be excluded.

In order to exclude that the inhibition resulted from antibody against activating proteins, we incubated antiserum together with preheparin human serum (preheparin human serum showed no precipitation lines in the Ouchterlony immuno diffusion assay with the antiserum) and added human serum to the inhibited enzyme. Table I shows that the addition of human serum before or after incubation with antiserum does not influence the reaction rate. It is also shown that the partially purified enzyme is activated in the same way by human serum as by rat serum. In another experiment (not shown) whole antiserum was mixed with preheparin rat serum and the resulting precipitate removed by centrifugation. From the supernatant, γ-globulins were purified as described before. It was found that with this preparation the same results were obtained as with the original γ-globulin fraction. From this and from Table I it can be concluded that the antibody inhibits the enzyme directly and that the inhibition is not caused by precipitation of activating plasma proteins.

In order to detect the principal source of lipase activity in postheparin serum, liver and heart were perfused *in vitro* with a heparin containing medium (Table II). It was found that lipoprotein lipase activity released from rat heart did not interact with the antibody, but that the liver perfusate is inhibited to the same extent as the postheparin serum lipase activity. After incubation for a longer time (60 min 37°C) the activity of the liver perfusate is completely inhi-

Table II

INHIBITION OF POSTHEPARIN SERUM-, HEART- AND LIVER LIPASE ACTIVITIES BY ANTIBODY AGAINST POSTHEPARIN SERUM LIPASE

Enzyme source	Pretreatment with		Lipoprotein lipase activity substrate used		% Inhibition substrate used	
	control	antibody	palmitoyl-CoA (mU/ml)	trioleate (nmoles/min/ml)	palmitoyl-CoA	trioleate
Postheparin serum	+	-	148	318		
" "	-	+	21	68	86	78
Heart perfusate	+	-	7.4	3.7		
" "	-	+	7.4	3.7	0	0
Liver perfusate	+	-	114	29.0		
" "	-	+	19	4.5	83	84

Liver and hearts were perfused as described. Fractions of 10 ml were collected. Most of the activity in the liver was released in the first 2 min (=40 ml). The heart released its activity more gradually; during 6 min (=60 ml) equal activities were obtained. Pretreatment of the perfusates was as described in Fig. 1. Incubations were done for 30 min at 37°C. 5 µl postheparin serum was incubated with 15 µl antiserum. Heart-and liver perfusates were incubated with equal volumes of anti- or control serum.

bited (not shown), whereas the lipase activity of postheparin serum is inhibited 95% (Fig. 1B).

DISCUSSION

From the data obtained it can be concluded that lipase activities that are released by heparin from heart and liver are catalysed by immunologically different enzymes. The lipase activity in serum, after heparin injection, behaves very similarly to the liver enzyme in response to the inhibitory action of the antibody (Table II). In this table it can be seen that the inhibition of postheparin serum lipase by the antibody, when measured with trioleate as the substrate, is 8% less than when palmitoyl-CoA is the substrate. This can be explained by the higher ratio of palmitoyl-CoA hydrolase activity over trioleate hydrolase activity in liver perfusate, when compared with heart perfusate. The data obtained in Table I allow the conclusion that the heart contributes maximally 5% to the lipase activity in postheparin plasma, when palmitoyl-CoA is the substrate, and not more than 13% when trioleate is the substrate. Kraus *et al.* (5) found, that about 65% of the lipase activity in postheparin serum was derived from the liver. It is tempting to conclude from our results that even up to 95% (87% with trioleate as the substrate) of the lipase activity is released by the liver. It is possible that we find a somewhat higher contribution by the liver because we used a smaller amount of heparin [100 I.U. vs. 250 I.U./kg body weight used by Kraus *et al.* (5)].

Another explanation would be that, in addition to heart and liver, other organs contribute to the serum activity and that this activity would be equally inhibited by the antibody. This is presently under investigation. It has already been found, that the purified postheparin serum enzyme showed only one protein band with hydrolase activity in polyacrylamide gel electrophoresis (pH 8.9) (not shown). Fielding (4) found that the liver enzyme had a different substrate specificity

than the activity released from other organs. The present paper shows, that this difference (at least for heart and liver) is due to different enzymes and not only to the influence of cofactors, as was discussed before by LaRosa et al. (3) and Kraus et al. (5).

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LIVER AND EXTRAHEPATIC CONTRIBUTIONS TO POSTHEPARIN SERUM LIPASE ACTIVITY OF THE RAT

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Summary

The influence of the amount of heparin injected on the contributions of liver and of extrahepatic tissues to the lipase activity of postheparin serum of the rat was studied. It was found that when high doses of heparin (20 I.U./100 g bodyweight) were injected, the liver contributes for 63% to the total triacylglycerol lipase activity of postheparin serum. At low heparin levels (0.4 I.U./100 g bodyweight) the lipase activity in the postheparin serum is almost solely derived from the extrahepatic tissues. The activity of the liver enzyme in the postheparin serum was found to increase very fast after heparin injection. 2 min after heparin injection a maximum activity of the liver enzyme in the serum was found. Subsequently the liver enzyme is removed from the circulation. Not only the activity of the liver enzyme is dependent on the amount of heparin injected, but also the amount of enzyme released from the liver.

Fat feeding increases the hydrolytic activity released from the liver by heparin. Immunological studies suggest that the enzyme may be in a more active state. The role of the heparin-releasable liver enzyme in fat metabolism is discussed.

Introduction

After intravenous injection of heparin, lipase activity is released into the bloodstream [1]. The enzyme activity is released from different organs. Two species of triacylglycerol lipase activity have been characterized in postheparin serum, differing in substrate specificity and in sensitivity to some inhibitors, such as high concentrations of salt and protamine sulfate [2,3]. At least two activities are found to be immunologically different [4] and to be released from liver and extrahepatic organs, respectively [4]. The extrahepatic activity is accepted to be identical with lipoprotein lipase as defined by Korn [5]. The role of this heparin-releasable activity is the hydrolysis of serum triacylglycerols

[6,7], and therefore involved in the utilization of serum triacylglycerols by these extrahepatic organs. The role of the heparin-releasable liver enzyme is much less clear. Maybe that in vivo the lipase activity of the liver is mainly directed towards fatty acid esters other than triacylglycerols, although the postheparin liver enzyme definitely hydrolyzes serum triacylglycerols in vitro [3]. Indeed, liver releases also phospholipase [8] and monoacylglycerol lipase [9] activities after heparin perfusion. That some lipase and phospholipase activities in postheparin serum may be identical has been suggested already in 1965 by Vogel and Bierman [10]. They showed that in postheparin serum a protein is found with lipase and phospholipase activities which are not separable by electrophoresis, ultracentrifugation or gelfiltration on Sephadex G-200. The liver enzyme may contribute considerably to the overall postheparin serum lipase activity [2—4], the magnitude of which may vary to a large extent. Therefore, we studied some factors that could influence the contribution of the liver lipase activity to the serum, such as the amount of heparin injected and the time of blood collection after heparin injection. Another possible influence studied was the effect of fat feeding since previous work [11] indicated that after 6 days rapeseed oil feeding the lipoprotein lipase activity of postheparin serum is elevated when compared with olive oil feeding. Houtsmuller et al. [12] showed that rapeseed oil feeding results in a maximal triacylglycerol accumulation in rat heart after 3 to 4 days and that after 6 days the triacylglycerol content decreases rapidly. We [11] have, therefore, suggested that the increased postheparin lipase activity found after 6 days feeding might be related to the decrease of the triacylglycerol content of the heart. For that reason we raised the questions: is the increased serum activity after 6 days rapeseed oil feeding of extrahepatic or of hepatic origin and, secondly, is the increased activity due to enzyme activation or an increased synthesis of enzyme protein? Both questions can be answered by immunological studies as will be presented.

Methods

The lipase activity in the postheparin serum was measured with palmitoyl-CoA or Intralipid as the substrate as described before [13]. In the feeding experiments the rats had free access to water and food unless stated otherwise. The rapeseed oil diet consisted of 50 cal % rapeseed oil (48% C22 : 1). The olive oil diet was composed of 50 cal % olive oil. In contrast to the earlier studies [11] on the connection between lipoprotein lipase and rapeseed oil feeding, the rats were not fasted overnight before use, unless stated different.

In all experiments male Wistar rats were used. Their weights ranged from 210 ± 30 g. The heparin injections were made intravenously under Nembutal narcosis between 9 and 11 a.m.

Acetonepowders of different organs were prepared as described by Borensztajn et al. [14] and extracted with 25 mM NH_4OH—HCl buffer (pH 8.5). Total hepatectomy was achieved under ethernarcosis by a two-stage procedure. In the first stage 65—75% of the liver was removed according to Higgins and Anderson [15]. Subsequently a porta—cava shunt was made as described by Fisher and Lee [16]. After the operation the rats were allowed to recover for

30 min. Then 50 I.U. heparin (Organon, Oss, The Netherlands) were injected intravenously and blood was withdrawn 2 min later.

Differentiation between liver and extrahepatic lipases in postheparin serum

In the postheparin sera the lipase activity released from the liver was determined with the help of an antibody against this activity. The antibody was raised as described previously [4]. The specificity of the antibody was extended to other organs as is shown under Results (Table I). The incubations of the postheparin sera with the antiserum were carried out in principle as described in ref. 4. The incubation temperature, however, was lowered to 0° C to avoid inactivation of the extrahepatic enzyme. It was found that during 14 h of incubation at 0° C the total lipase activity of postheparin serum in the absence or presence of serum of a non-immunized rabbit (control serum) was stable. The inhibition by the antiserum was already maximal after about 4 h. The postheparin sera were routinely incubated with an excess of antiserum (1 : 2, v/v) for 14 h at 0° C. An increase of the amount of antiserum or incubation time did not result in a further inhibition of the lipase activity in postheparin serum. After the incubation the mixtures were centrifuged for 2 min at 15 000 × *g* and the activities measured in the supernatant. The amount of lipase activity inhibited is taken as the contribution of the liver to the total lipase activity of the postheparin sera. The rabbit sera used, were always preincubated for 1 h at 56° C in order to inactivate lipase activities possibly present in these sera. In the experiments shown in Fig. 1 and Table VII, antiserum, bound to Sepharose as described by Neuwelt et al. [17], was used.

Estimation of the "specific activity" of the inhibited enzyme*

In order to estimate the "specific activity" of the liver enzyme the enzyme activity that could be bound to Sepharose-linked antiserum was measured. In Fig. 1 a typical experiment is shown. A constant amount of Sepha-

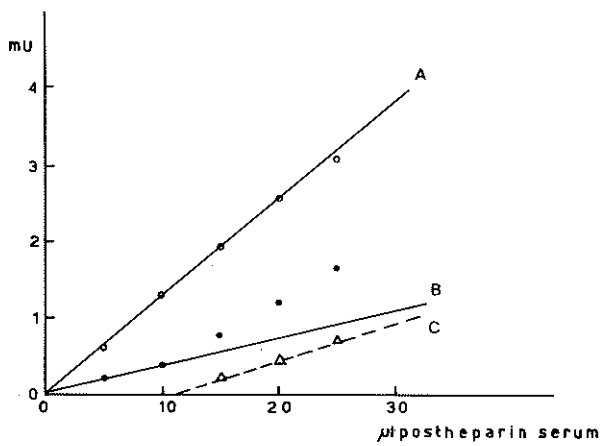

Fig. 1. Relationship between hydrolytic activity and the amount of immunoreactive protein of liver lipase. Each point represents the mean value of duplicate experiments with the serum of a rapeseed oil-fed rat. The experiments were carried out as described under Methods.

* The activity inhibited by a certain amount of antiserum.

rose-linked antiserum was incubated with different amounts of postheparin serum. The activity of the unbound enzyme was measured in the supernatant after centrifugation at low speed. The measured activity was plotted against the amount of serum incubated (closed circles). In control experiments the same incubations were carried out with Sepharose bound to serum of a non-immunized rabbit (Line A, open circles). Fig. 1 shows that at lower serum concentrations in the presence of a fixed amount of antiserum, the activity increases in a linear fashion with the amount of serum added. This activity must be of extrahepatic origin. At higher serum concentrations, however, the antiserum is saturated with the enzyme of hepatic origin and therefore the latter as well as enzyme(s) of extrahepatic origin will contribute to the activity, so that an upward inflection can be observed (Fig. 1, closed circles). By extrapolation of the initial part of the curve (Line B) and subtraction of the calculated extrahepatic activities from the activities measured at higher serum concentrations (closed circles), a curve can be constructed (Line C), which presents the activity of the hepatic contribution at the serum concentration plotted on the abscissa. By extrapolation of Line C an intersection with the abscissa is obtained, the value of which represents the maximal amount of serum of which all (liver) activity is bound by the amount of antibody used. The corresponding (liver) activity of that amount of serum can be calculated.

Results

Specificity of the antiserum

In order to test the specificity of the antisera, lipase activities of acetone-powder extracts of different organs were measured after preincubation with serum of a non-immunized rabbit (control serum) or antiserum. In Table I it can be seen that while the liver activity is completely inhibited, the lipase activities of the other organs are not influenced by the antiserum. Previously [4] we reported that also the lipase activity of in vitro obtained perfusates of rat liver was abolished completely by the antiserum, while heart perfusates were unaffected. In order to study also the in vivo released lipase activities of extrahepatic organs, two rats were hepatectomized as described under Methods.

TABLE I

INHIBITION OF LIPASE ACTIVITIES FROM DIFFERENT SOURCES BY THE ANTISERUM

Acetonepowder extracts of different organs were diluted with buffer so that every extract contained the same order of lipase activity per ml. Then the extracts were incubated with control- or antiserum. Lipase activities were measured with Intralipid as the substrate and expressed in nmoles free fatty acid released/min per ml extract.

Enzyme source	Activity after incubation with	
	Control serum	Antiserum
Liver	14	0
Lung	28	28
Heart	34	33
Adipose tissue	27	28

It was found that the antiserum did not influence the lipase activity of the serum of these rats after injection of 50 I.U. heparin when either palmitoyl-CoA or Intralipid was used as the substrate. From these data it is concluded that the antiserum is inactive against extrahepatic lipases and that the activity released from the liver is completely inhibited.

Influence of the amount of heparin injected and time of bloodremoval on postheparin lipase activity of hepatic origin

The influence of the amount of heparin injected on the contributions of the liver and extrahepatic organs to the total serum triacylglycerol hydrolase activity is shown in Table II. Apparently the activity displayed by the liver enzyme depends on the amount of heparin injected. The extrahepatic contribution seems to be released at lower heparin concentrations and relatively little influenced by the injection of the higher heparin concentrations. In these experiments blood was withdrawn 2 min after heparin injection. Earlier we have found with isolated perfused rat liver that the bulk of the heparin-releasable activity is released within 2 min. From the experiments of Table III it can be concluded that indeed in the intact rat also, the hepatic contribution to the total serum activity is higher at 2 min after the injection of 50 I.U. heparin than at 6 min and that the hepatic contribution beyond this time declines significantly. This contrasts the extrahepatic contribution which remains relatively constant between 2 and 20 min after injection. A similar picture was obtained when the lipase activity in the serum was tested with palmitoyl-CoA as the substrate (ref. 13) instead of trioleylglycerol (cf. also Table IV). With palmitoyl-CoA as the substrate the liver contribution to the hydrolytic activity of postheparin serum was much larger than with trioleylglycerol as the substrate (compare Tables III and IV) In other words, the hepatic contribution to postheparin serum lipase can hydrolyse both palmitoyl-CoA and trioleate efficiently, whereas the extrahepatic contribution preferentially hydrolyses trioleylglycerol and has little activity towards palmitoyl-CoA. In a large series of experiments the latter could be verified (not shown).

TABLE II

INFLUENCE OF THE AMOUNT OF HEPARIN INJECTED ON THE CONTRIBUTIONS OF LIVER AND OTHER ORGANS TO THE LIPASE ACTIVITY IN POSTHEPARIN* SERUM

Four groups of five rats were injected intravenously with 1, 5, 25 or 50 I.U. heparin, respectively. 2 min after the injection the rats were killed and the blood collected. In the serum the contributions of the liver and extrahepatic organs to the total lipase activity was estimated as described under Methods. Lipase activity was measured with "Intralipid" as the substrate. The activities are expressed in nmoles free fatty acid released/min per ml ± S.E.

Amount of heparin injected	Lipase activity		Percentage liver-activities
	Hepatic	Extrahepatic	
1	2 ± 2	65 ± 17	3
5	55 ± 8	80 ± 14	41
25	172 ± 14	120 ± 16	59
50	200 ± 15	115 ± 7	63

* The lipase activity of preheparin rat serum were found to be negligible.

TABLE III

INFLUENCE OF THE TIME OF BLOOD WITHDRAWAL AFTER HEPARIN INJECTION ON THE CONTRIBUTIONS OF THE LIVER AND EXTRAHEPATIC ORGANS TO THE TOTAL LIPASE ACTIVITY IN POSTHEPARIN SERUM

Five rats were injected with 50 I.U. of heparin. 1 ml of blood was withdrawn from each rat at 2, 6 and 20 min after the heparin injection. In the sera the contributions of the liver and the extrahepatic organs to the total lipase activity of the serum was estimated as described under Methods. "Intralipid" was the substrate. The activity is expressed as nmoles free fatty acid released/min per ml ± S.E.

Blood withdrawn after heparin injection (min)	Lipase activity		Contribution of the liver to the total lipase activities (%)
	Hepatic	Extrahepatic	
2	194 ± 16	116 ± 14	63
6	165 ± 7	118 ± 13	60
20	99 ± 14	106 ± 11	48

From Tables II and III it can be concluded that when relatively high concentrations of heparin are used a large part of the postheparin lipolytic activity is of hepatic origin and that the activity rapidly decreases when the removal of blood from the animal is delayed. Subsequently we investigated whether the disappearance of the activity from the bloodstream is due to inactivation of the enzyme, i.e. whether the ratio of active vs. inactive enzyme in the blood would decline, or whether enzyme protein active or inactive would disappear from the bloodstream by e.g. uptake in the liver (compare refs. 21 and 22). Therefore, rats were injected with different amounts of heparin and blood removed at different times. It can be seen from Table IV that delayed removal of blood causes a decrease of the liver contribution to postheparin lipolytic activity and that the amount of antiserum required to inhibit one munit of enzyme activity remains fairly constant. This indicates that enzyme protein completely disappears from the bloodstream and that not inactive,

TABLE IV

PALMITOYL-CoA HYDROLASE ACTIVITY IN POSTHEPARIN RAT SERA; INFLUENCE OF RABBIT ANTI RAT LIVER SERUM

Rats were injected with either 50 I.U. or 20 I.U. heparin as indicated. Blood was withdrawn at various times after the injection. In the sera the hepatic palmitoyl-CoA hydrolase activity was estimated as described under Methods. By varying the antiserum concentration at a constant rat serum concentration the amount of antiserum that inhibited 50% of the liver activity was estimated. From the data obtained the palmitoyl-CoA hydrolase activity that was inhibited by 1 μl antiserum was calculated. Activities are expressed in nmoles CoASH released/min per ml.

Heparin injection (I.U.)	Blood withdrawn after (min)	Hydrolase activity		Activity inhibited by 1 μl antiserum (munits)
		Hepatic	Extrahepatic	
50	2	281	18	0.32
	30	162	28	0.33
20	2	120	21	0.33
	20	52	20	0.30

TABLE V

CONTRIBUTIONS OF LIVER AND EXTRAHEPATIC ORGANS TO THE HYDROLASE ACTIVITY OF POSTHEPARIN SERUM OF RATS FED ON VARIOUS DIETS AFTER A 16-h FASTING PERIOD

Rats were fed during 10 days on various diets (see under Methods). After this period the rats were fasted for 16 h. Then heparin was injected intravenously (10 I.U./100 g body weight). After 6 min blood was withdrawn. Hepatic and extrahepatic contributions were estimated as described under Methods. Activities are expressed in nmoles palmitoyl-CoA hydrolyzed/min per ml serum ± S.E.

Diet	Number of rats	Hydrolase activity in the serum		
		Total	Hepatic	Extrahepatic
Laboratory chow	4	147 ± 7	105 ± 5[a]	42 ± 2[d]
Olive oil	6	139 ± 13	107 ± 11[b]	32 ± 2[e]
Rapeseed oil	7	180 ± 10	140 ± 8[c]	40 ± 2[f]

Statistically significant difference between a and c: $0.02 > P > 0.01$, between b and c: $0.025 > P > 0.02$, between d and e: $0.05 > P > 0.025$, e and f: $0.05 > P > 0.025$.
Not significantly different are a and b nor d and f.

immuno-reactive enzyme protein gradually increases in the blood. In these experiments we determined the amount of antiserum required to inhibit 50% of the liver enzyme activity, as described in Table IV.

Influence of fat feeding on liver lipase activity in postheparin serum

Rats were fed a rapeseed oil-rich or an olive oil-rich diet for 10 days. The rats were fasted for 24 h and subsequently injected intravenously with 10 I.U. heparin per 100 g bodyweight. 6 min later blood was withdrawn to prepare the postheparin serum in which lipolytic activity was assayed. Table V shows that in accordance with earlier observations [11] the total lipase activity in the postheparin serum of the rapeseed oil-fed rats is higher than that of the olive oil-fed rats or of the control rats. From the second and third columns of this table it appears that this elevation is mainly due to increased liver enzyme activity and that the total contribution of the extrahepatic organs to serum palmitoyl-CoA hydrolase activity remains fairly constant. It is known that the state of nutrition may influence the heparin-releasable lipase activity. The same experiment was therefore repeated without fasting the rats before the injection of heparin. The results are shown in Table VI. Clearly the activities of the liver

TABLE VI

CONTRIBUTIONS OF LIVER AND EXTRAHEPATIC ORGANS TO THE HYDROLASE ACTIVITY OF POSTHEPARIN SERUM OF RATS FED ON VARIOUS DIETS

Conditions as in Table IV, but without fasting before the heparin injection.

Diet	Number of rats	Hydrolase activity in the serum		
		Total	Hepatic	Extrahepatic
Laboratory chow	2	224 ± 20	172 ± 10[a]	52 ± 10
Olive oil	4	268 ± 22	229 ± 15[b]	39 ± 7
Rapeseed oil	4	286 ± 12	235 ± 9[c]	51 ± 3

Statistically different is a from b or c ($0.01 > P$ 0.005).

TABLE VII

"SPECIFIC ACTIVITIES" OF THE LIVER HYDROLASE ACTIVITY IN POSTHEPARIN SERUM OF RAPESEED OIL AND OLIVE OIL-FED RATS

The amount of postheparin serum, in which hydrolase activity was inhibited by a fixed amount of antiserum ("specific activity"), was estimated as described under Methods. The same sera were used as in Table V. The activity inhibited by the fixed amount of antiserum was calculated and expressed in munits (nmoles CoASH liberated/min). Palmitoyl-CoA was the substrate.

Diet	Liver hydrolase activity inhibited by a fixed amount of antiserum (munits)	
	Expt I	Expt II
Rapeseed oil	1.97	1.94
Olive oil	1.31	1.32

enzymes from rapeseed oil- or olive oil-fed rats are equal, but significantly higher than from rats fed with a normal laboratory chow diet.

In order to obtain some insight in the mechanism of the activation of the liver enzyme we estimated whether the higher activity of the liver lipase in the rapeseed oil-fed rats (Table V) was due to more enzyme or to a more active enzyme. Table VII demonstrates that the same quantity of antiserum binds more activity in the rapeseed oil-fed rats than in the olive oil-fed rats. From this it could be concluded that the elevation of activity in the rapeseed oil-fed rats is due to a more active enzyme. Both Tables V and VI suggest that feeding rats with an olive oil-rich diet results in low extra hepatic palmitoyl-CoA hydrolase activity. That this could possibly reflect a low (extra-hepatic) lipoprotein lipase activity is suggested in experiments presented elsewhere [18], in which olive oil-rich feeding also results in low cardiac triacylglycerol hydrolysing activity.

Discussion

In this paper it is shown that the relative contribution of liver and extrahepatic organs to the postheparin lipase activity of serum strongly depends on the dosis of heparin injected (Table II) and the time of removal of blood from the animal (Table III). Whereas the extrahepatic organs may remove triacylglycerols from the serum [6,7], for which their lipoprotein lipase activity at the capillary lining may be essential, the liver is thought mainly to contribute to the formation of serum triacylglycerols (as very-low-density lipoproteins) and not to their utilization. Yet the liver, especially when high concentrations of heparin are injected, contributes to a large extent to the serum lipase activity (ref. 4 and Tables II—IV of the present paper). Probably then the high liver activity against triacylglycerols does not represent the capacity of the liver to hydrolyse these substrates in vivo. It should be noted that the high liver activities are measured when high doses of heparin are injected. These high doses are highly unphysiologically. Condon et al. [19] found that after hepatectomy of dogs the release of lipolytic activity by heparin into the serum was somewhat retarded, when compared to control dogs. That the same may hold for rats can be seen from Tables III and IV. We found that the hepatic contribution is removed from the serum more rapidly after heparin injection than the extra-

hepatic contribution. The liver enzyme is completely removed from the serum and not inactivated while still being in the serum (Table IV). What happens to the lipase activity when removed from the circulation is not clear. Whether the activity remains operative or is inactivated has to be investigated. The place where the lipase is removed from the serum is not known although evidence has been brought forward that it may be the liver [21,22].

From the data shown in Table V it can be seen that fat feeding enhances the heparin-releasable lipase activity of hepatic origin in postheparin serum. The activity of extrahepatic origin is slightly lowered by olive oil feeding and significantly after fasting (Tables V and VI). This may be of considerable importance since lipoprotein lipase activity may be involved in atherogenesis (Zilversmit [23], Hülsmann and Jansen [24]) and hence deserves more documentation. In contrast to the lipase activity of the rat heart (ref. 18) the elevation of the liver lipase activity is independent whether erucic acid (C22 : 1; a major component of rapeseed oil) is the major acid or not (Table VI). In the fasted state, however (Table V), the liver lipase of the rapeseed oil-fed rats remains at a higher level than that of the olive oil- or chow-fed rats. Whether the difference in liver lipase activity in the fasted state must be attributed to a direct effect of different fatty acids or to a secondary effect cannot be concluded yet. A possible explanation may be that after rapeseed oil feeding, due to the prolonged alimentary lipemia after rapeseed oil digestion [26], the animals are in a less fasted state than other animals. Of interest is the observation that the higher activity of the liver enzyme is not due to more enzyme but to a more active enzyme (Table VII). The factor that determines the activity of the liver is unknown. One possibility is that the enzyme is normally present in both active and inactive forms and that the equilibrium is shifted to the more active state in the (rapeseed) oil-fed rats. Another simple explanation may be the stabilization of the enzyme by triacylglycerols. The role of the liver lipase activity in the overall triacylglycerol metabolism remains uncertain, but may be involved in the degradation of low- and high-density lipoproteins (compare Introduction).

From the presented data (Tables III and IV) it is concluded that the palmitoyl-CoA hydrolase activity in postheparin serum is mainly of hepatic origin, a conclusion already reached earlier by us [4]. This illustrates the different substrate specificities of the various postheparin serum hydrolases (compare also ref. 25). Whether the lipolytic activities released from the liver by heparin perfusion are due to the release of one enzyme or due to the release of phospholipase [8,10,20], monoacylglycerol lipase [9], palmitoyl-CoA hydrolase and triacylglycerol lipase as separate enzymes, with perhaps overlapping substrate specificity, requires further investigation. The choice of substrate by the in vitro estimation may influence the relative contributions of liver and extrahepatic enzymes considerably. Therefore, no direct conclusions of the in vivo capability of different organs in the hydrolysis of endogenous substrates may be drawn from the activities measured in postheparin serum in vitro. From this paper it becomes clear that the amount of heparin and the time of blood removal may influence the activities in postheparin serum.

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ON THE HEPATIC AND EXTRAHEPATIC POSTHEPARIN SERUM LIPASE ACTIVITIES AND THE INFLUENCE OF EXPERIMENTAL HYPERCORTISOLISM AND DIABETES ON THESE ACTIVITIES

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SUMMARY

The present paper shows that the palmitoyl-CoA hydrolase activity of postheparin serum of the rat is mainly derived from the liver. The identity of this activity with the heparin-releasable hepatic triacylglycerol hydrolase activity is established. The consequence of the different substrate specificities of the hepatic and extrahepatic enzymes for the measurement of the overall postheparin serum lipase activity are discussed.

Treatment of the rats with either a corticosteroid or with streptozotocin was found to lower the lipolytic activity from the liver and to enhance the extrahepatic activity.

Also in human postheparin serum palmitoyl-CoA hydrolase activity is shown to behave identical with hepatic triacylglycerol hydrolase activity.

The possible function of the liver in the serum triglyceride metabolism is discussed in connection with the proposed mechanism for the role of extrahepatic lipoprotein lipase in atherogenesis.

INTRODUCTION

Lipolytic activity of postheparin serum originates from different organs. The enzymes of hepatic and extrahepatic origin differ in substrate specificity[1], sensitivity to

various inhibitors[2] and are immunologically distinguishable[3-5]. The liver may contribute up to 60% of the total triacylglycerol hydrolase activity of postheparin serum of the rat[5]. Also in man a large part of the serum triacylglycerol hydrolase activity is derived from the liver[2,4], but like in the rat the contribution is very dependent on the time of blood removal after the heparin injection[4,6]. The extrahepatic enzyme(s) hydrolyze(s) the triacylglycerol moiety of chylomicrons and very low density lipoproteins . The liver may play a role in the breakdown of remnant particles of very low density lipoproteins and chylomicrons[7]. The role of the heparin-releasable liver lipase activity is not yet established, though recent investigations of Müller et al.[8] point to a key-role for this activity in the breakdown of the lipid moiety of these particles. In a previous paper[9] we showed that postheparin serum also hydrolyzes palmitoyl-CoA. We now present experiments to show that the liver preferentially hydrolyzes palmitoyl-CoA. Since the breakdown of palmitoyl-CoA can be easily followed spectrophotometrically, the estimation of palmitoyl-CoA hydrolase activity offers a simple method for the determination of the heparin-releasable liver enzyme. Furtheron the effects of cortisol and streptozotocin treatment on the lipase activities of rat serum are reported.

MATERIALS AND METHODS

Male Wistar rats of 220 $\pm$ 20 g were used in all experiments, unless stated otherwise. Heparin (100 I.U./mg) was from Organon, Oss, the Netherlands; streptozotocin was a gift from the Upjohn Company, Kalamazoo, U.S.A.; Kenacort (triamcinolonacetonide, 40 mg/ml) was from Squibb and Sons; [2-^{3}H] glyceroltrioleate was from the Radio Chemical Center, Amersham, Great Britain. Palmitoyl-CoA was made as described before[9].

1-Monoacylglycerol hydrolase activity was estimated as follows. The [2-^{3}H] glycerolmonooleate was separated by

thinlayer chromatography on silicagel from the [2-^{3}H] glyceroltrioleate. About 2.5 µCi of the [2-^{3}H] monoacylglycerols were mixed with 68 mg glycerolmonooleate, 8.1 mg lecithin, 1.5 ml 10% fat-free albumin (pH 8.0), 0.8 ml Tris-HCl buffer (2 M, pH 8.2) and water was added to make 3.0 ml. After sonification of this mixture with a Branson sonifier (4 times 1 min with intermittent cooling), the mixture was incubated during 30 min at 37°C with 6 ml preheparin human serum (citrate plasma clotted by addition of Ca^{2+} and dialysed). Then the reaction was started by addition of 25 µl postheparin serum to 0.975 ml of the described substrate. After 30 min the reaction was stopped with 1 ml 10% trichloroacetic acid and glycerol estimated according to Schotz and Garfinkel[10]. In a series of experiments the glycerol liberated was also estimated enzymatically, always with the same results as obtained in the radioactive assay. Triacylglycerol hydrolase activities in postheparin sera were estimated as described before (ref. 9) with "Intralipid" (10%) as the substrate, with one slight modification in that from the reaction mixture no longer samples were taken at 15, 30 and 45 min, but the reaction was now carried out in triplicate and samples were taken after 30 min. Glucose was estimated as described by Werner et al.[11], insulin was determined with a slightly modified method of Yalow and Berson[12].

RESULTS

Stability of palmitoyl-CoA hydrolase activity and triacylglycerol hydrolase activity during incubation at different pH

In earlier experiments[3] we found that palmitoyl-CoA hydrolase activity is rather stable during incubation of postheparin rat serum for 30 min at 37°C. During preincubation with rabbit serum, however, inactivation of the triacylglycerol hydrolase activity occurred. We found that

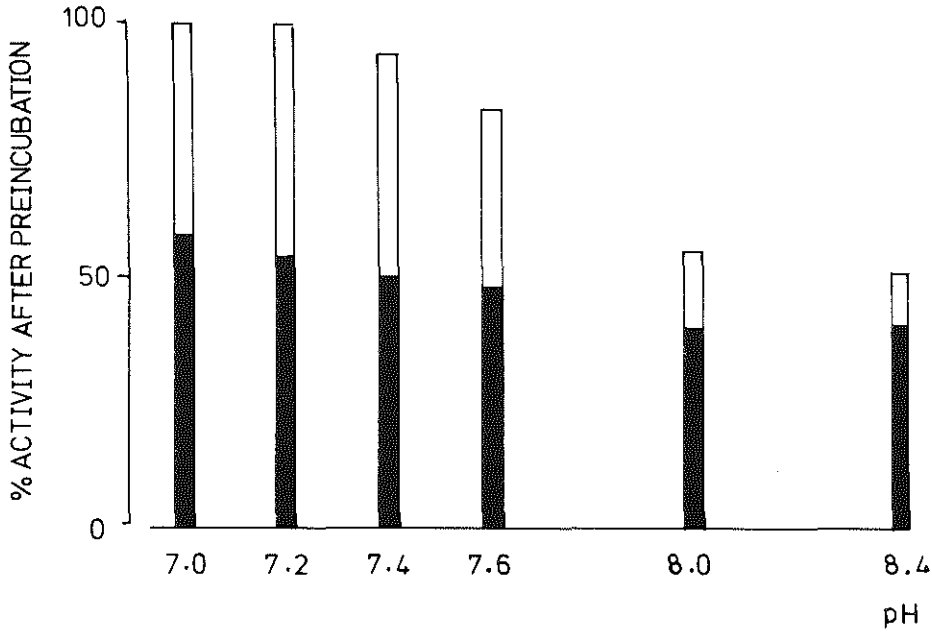

Fig. 1. Inactivation of postheparin serum hydrolase activities during preincubation at different pH. 0.9 ml postheparin serum was incubated with 0.1 ml 1 M Tris-HCl buffer with varying pH. The incubation was for 30 min at 37°C. After the incubation palmitoyl-CoA hydrolase activity and triacylglycerol hydrolase activity (expressed as nmoles F.F.A./min/ml) were estimated (at pH 8.5), as described under METHODS. The bars represent the activities obtained (after the preincubation) expressed as the % of the activities measured without preincubation. The triacylglycerol hydrolase activities and the palmitoyl-CoA hydrolase activities are represented by the black part and the total height of the bars respectively.

the pH could influence the stability of the palmitoyl-CoA hydrolase activity. Therefore, the inactivation of the palmitoyl-CoA hydrolase activity and of the triacylglycerol hydrolase during preincubation at 37°C for 30 min at different pH's was followed (fig. 1). The triacylglycerol hydrolase activity when measured without preincubation was 353 mU/ml. The palmitoyl-CoA hydrolase activity was 251 mU/ml. From this figure it is seen that the palmitoyl-CoA

hydrolase activity is stable at a pH between 7.0 and 7.3, but that the triacylglycerol hydrolase activity is partly inactivated by the preincubation at 37°C. With increasing pH's both activities became progressively inactivated .

Postheparin serum palmitoyl-CoA hydrolase activity: origin and purification by affinity chromatography

In a previous paper[5] we showed with the help of an antibody against liver lipase activity that about 80% of the postheparin palmitoyl-CoA hydrolase activity is derived from the liver. This is now confirmed by experiments with hepatectomized rats (TABLE I). From this TABLE it can be seen that the palmitoyl-CoA hydrolase activity in the serum of hepatectomized rats is only 22% of the activity found in intact rats. This confirms the earlier published observations[5].

TABLE I

RELEASE OF PALMITOYL-CoA HYDROLASE ACTIVITY BY HEPARIN IN INTACT AND HEPATECTOMIZED RATS

4 rats were hepatectomized in a two stage procedure under ether narcosis. In the first stage 65-75% of the liver was removed according to Higgins and Anderson[13]. Subsequently a porta cava shunt was made as described by Fisher and Lee[14]. After the operation the rats were allowed to recover for 30 min. Then 50 I.U. heparin were injected and blood was withdrawn 2 min later. Four intact rats served as controls. In the sera palmitoyl-CoA hydrolase activity was estimated as described before[9].

Rats	Palmitoyl-CoA hydrolase (nmoles CoASH liberated/min/ml serum ± S.D.)
Controls (n=4)	217 ± 15
Hepatectomized (n=4)	49 ± 18

From the liver different lipolytic activities are released by heparin[1,15]. Human, postheparin serum triacylglycerol hydrolase activity of hepatic origin is eluted by about 0.7 NaCl from a Sepharose-heparin column[16]. Lipase activities of rat heart and rat adipose tissue are eluted at a higher salt concentration (1.2 M NaCl)[17]. Ehnholm et al.[18] found two activity peaks after the elution of human postheparin serum lipases from a Sepharose-heparin column with a salt gradient. The activity eluted at 0.7 M NaCl had the characteristics of the liver enzyme and the activity eluted at 1.2 M NaCl the characteristics of the extrahepatic lipoprotein lipase. We now investigated, whether the palmitoyl-CoA hydrolase activity released from the liver (see TABLE I) could be separated from the liver triacylglycerol hydrolase activity by elution from a Sepharose-heparin column (Fig. 2). From this figure it is concluded that about 90% of the palmitoyl-CoA hydrolase activity is eluted in the same activity peak as the liver triacylglycerol hydrolase activity by 0.7 M NaCl (comp. refs. 16 and 18). The recovery of the palmitoyl-CoA hydrolase activity was 81%. Furthermore it can be seen that in the activity peak eluted by 0.7 NaCl the breakdown of triacylglycerol to free fatty acids and glycerol is complete (molar ratio of 3 to 1). In the activity peak eluted by 1.2 M NaCl the release of acylglycerol to free fatty acids greatly exceeds the release of glycerol (molar ratio 8.5 to 1). This is in accordance with the findings of Krauss et al.[20], with enzymes obtained by perfusion of liver and extrahepatic tissues. The recovery of triacylglycerol hydrolase activity when estimated by the fatty acids released from triacylglycerol was 80%. The recovery of triacylglycerol hydrolase activity when estimated as glycerol released from triacylglycerol was only 49%. This can be explained by the incomplete breakdown of the triacylglycerol in the 1.2 M NaCl peak. In the postheparin serum applied to the column the liver enzyme will be able to hydrolyze part of the partial

acylglycerols formed by the extrahepatic enzyme (see also DISCUSSION). The liver enzyme releases from palmitoyl-CoA about 1.7 times more nmoles CoASH per min than nmoles free fatty acids from triacylglycerol. The extrahepatic enzyme releases about 8 times more free fatty acids from trioleate than nmoles CoASH from palmitoyl-CoA. This ratio can be slightly overestimated however, because of the incomplete recovery for the palmitoyl-CoA hydrolase activity. The 80% recovery for the activity measured as free fatty acids released can be ascribed to a low rate of hydrolysis of partial acylglycerols formed by the extrahepatic enzyme as outlined above.

In vitro breakdown of triacylglycerol to free fatty acids and glycerol by postheparin serum, collected after the injection of different amounts of heparin

It is tentatively concluded from the experiments shown above (Fig. 2) that, when measuring glycerol release from triacylglycerol by postheparin serum, the liver enzyme can be the rate-limiting step. TABLE II shows that the ratio of released fatty acids over released glycerol by postheparin serum from a triacylglycerol substrate, depends on the amount of injected heparin. The stimulation of Intralipid hydrolyzing activity by increasing the amount of heparin used, amounts to 255 nmoles fatty acids and 74 nmoles glycerol released per min per ml serum, approximating a fatty acid to glycerol ratio of 3. This suggests that predominantly the liver enzyme is released by the higher heparin dose, in accordance with earlier observations[4,5]. The 1-monoacylglycerol hydrolase activity is less activated by the higher heparin dose than the other measured activities. When the liver enzyme is precipitated with a specific antibody, the molar ratio of released fatty acids to released glycerol in the remaining activity is again 8 to 1 (see data between brackets in TABLE II) as may be expected for the extrahepatic enzyme.

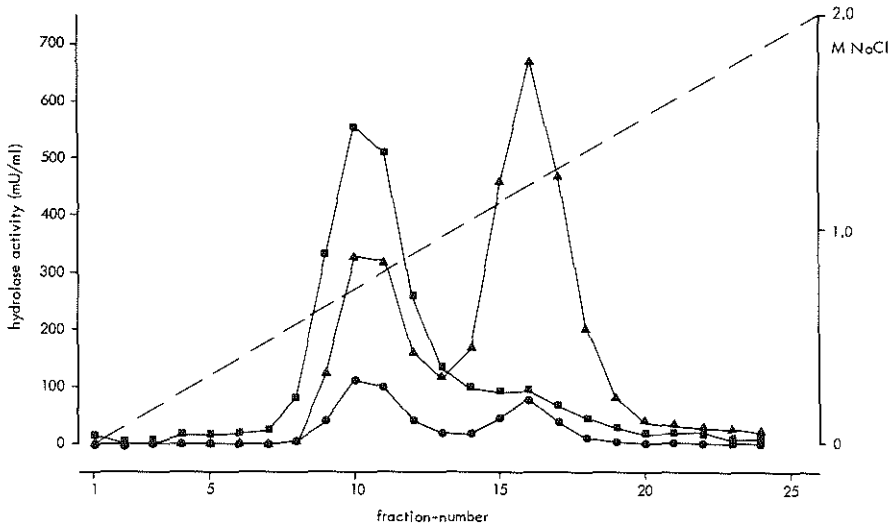

Fig. 2. Elution of hydrolytic activities of postheparin serum from a Sepharose-heparin column by NaCl. 60 ml postheparin serum of rats injected with 50 I.U. heparin was applied to a Sepharose-heparin column (0.9 x 10 cm). After passage of the serum over the column, the column was washed with 50 mM Tris-HCl buffer pH 7.2 until no more protein was eluted. Then a linear NaCl gradient was applied to the column of 0 to 2 M NaCl in the Tris buffer (60 ml). Fractions of 2 ml were collected. Within 2 hrs the fractions were tested for palmitoyl-CoA hydrolase activity and for triacylglycerol lipase activity. In the palmitoyl-CoA assay 10 µl preheparin serum per ml reaction medium was present. When "Intralipid" was the substrate, the reaction was followed in two ways. The substrate was fortified with glycerol tri[1-^{14}C] oleate and [2-^{3}H] glycerol labeled glyceroltrioleate. After preincubation with preheparin serum just as described in ref. 9, 2.70 ml substrate was incubated with 0.15 ml of the column fractions for 30 min at 37°C. After the incubation the released [2-^{3}H] glycerol was estimated as described by Schotz and Garfinkel[10]. The released free fatty acids were estimated in another ml of the reaction mixture as described in ref. 9. (■-■) represents nmoles CoASH released from palmitoyl-CoA/min/ml eluate, (▲-▲) nmoles free fatty acids from trioleate/min/ml and (●-●) nmoles glycerol from trioleate/min/ml.

TABLE II

HYDROLYTIC ACTIVITIES OF POSTHEPARIN SERUM AFTER THE INJECTION OF DIFFERENT AMOUNTS OF HEPARIN

Male Wistar rats (290 ± 10 g) were injected with 2 or 50 I.U. of heparin. After 6 min they were killed and their blood was withdrawn. In the sera the release of free fatty acids and glycerol from "Intralipid" was measured as described in the legends to Fig. 2. Also the 1-monoacylglycerol hydrolase activity and the palmitoyl-CoA hydrolase activity were estimated, as described under METHODS. Between brackets the activities are given, after preincubation of the sera with an antiserum against the liver lipase exactly as described in ref. 5. Activities are given ± standard deviations.

Amount of heparin injected (I.U.)	Hydrolytic activity measured with the substrate			
	"Intralipid"		1-monooleate	Palmitoyl-CoA
	nmoles F.F.A./ min/ml	nmoles glycerol/ min/ml	nmoles glycerol/ min/ml	nmoles CoASH/ min/ml
2 (n=3)	125 ± 15	20 ± 2	180 ± 10	58 ± 4
50 (n=3)	380 ± 40	94 ± 2	304 ± 10	220 ± 9
	(163 ± 15)	(21 ± 3)		

Effect of experimental hypercortisolism and diabetes on the heparin-releasable lipase activities

Earlier we found[5] that fasting lowers the postheparin palmitoyl-CoA hydrolase activity. In order to study whether the insulin of the blood could affect the liver lipase activity and to study whether the hypertriglyceridemia in diabetes (see also ref. 26) could be explained by altered serum lipase activities, we made rats diabetic with "streptozotocin" (TABLE III). From this TABLE it is seen that in the diabetic rats the palmitoyl-CoA hydrolase activity is lowered, but that the total triacylglycerol hydrolase activity in the postheparin serum is significantly elevated. The release of glycerol from triacylglycerol is constant. In experiments with rats made diabetic with alloxan (180 mg/kg body weight) the palmitoyl-CoA hydrolase activity was even lower (30 mU/ml). These experiments were however not further extended because of the high mortality rate of the rats by the treatment.

In TABLE IV it can be seen that the administration of a corticosteroid also influences the liver- and total lipolytic activities of postheparin serum. The possible implications of this finding are pointed out in the DISCUSSION.

Palmitoyl-CoA hydrolase activity in human postheparin serum

Also in humans palmitoyl-CoA hydrolase activity is released by heparin[9]. We found that, as with rat serum (Fig. 2), the palmitoyl-CoA hydrolase activity was eluted by 0.7 M NaCl from a Sepharose-heparin column (not shown). In Fig. 3 it is seen that in man palmitoyl-CoA hydrolase activity reaches a maximum within 5 min after the injection of heparin. The total lipase activity reaches a maximum after about 40 min. From very recent work of Krauss et al.[6] it appears that also the liver triacylglycerol hydrolase activity is maximal shortly after the heparin injection. Therefore in man the palmitoyl-CoA hydrolase activity seems to correlate also with the liver triacylglycerol hydrolase activity.

TABLE III

POSTHEPARIN SERUM LIPASE ACTIVITIES IN EXPERIMENTAL DIABETES

Rats (290 ± 10 g) were injected in the tailvein with 40 mg streptozotocin/kg body weight. After 7 days 50 I.U. heparin were injected and after 6 min their blood was collected. In the serum glucose and insulin were estimated. Rats with a bloodglucose level >300 mg/100 ml were selected as being diabetic. In their sera palmitoyl-CoA hydrolase and triacylglycerol hydrolase activities were estimated. The same rats of TABLE II served as controls. Activities are given ± standard deviations.

Treatment	Triacylglycerol hydrolase		Palmitoyl-CoA hydrolase
	nmoles F.F.A./min/ml	nmoles glycerol/min/ml	nmoles CoASH/min/ml
None (n=3)	380 ± 40[a]	94 ± 2[c]	220 ± 9[e]
Streptozotocin (n=4)	551 ± 21[b]	98 ± 7[d]	131 ± 10[f]

Statistically different are a from b and e from f: $P<0.005$; not significantly different is c from d.

TABLE IV

INFLUENCE OF FEEDING, FASTING AND EXPERIMENTAL HYPERCORTISOLISM ON LIPASE ACTIVITIES OF POSTHEPARIN SERUM OF RATS

One group of rats was normally fed with laboratory chow. From another group the food was withdrawn at 16.oo p.m. when they were used the next day. The third group was injected intramuscularly with 4 mg Kenacort. After 48 h their food was withdrawn and the rats used after 18 h as in the fasted group. 50 I.U. heparin was injected and blood withdrawn 6 min later. Glycerol release and fatty acid release from "Intralipid" was determined as described elsewhere in this paper. Palmitoyl-CoA hydrolase activity was estimated as described in ref. 8. 1-Monoacylglycerol hydrolase was estimated in two rats of the fed and "Kenacort" treated group as described under METHODS. I.R.I. signifies immunoreactive insulin. Activities are given ± standard deviations.

Condition of the rats		Triacylglycerol hydrolase (nmoles fatty acids/min/ml)	Triacylglycerol hydrolase (nmoles glycerol/min/ml)	1-Monoacylglycerol hydrolase (nmoles glycerol/min/ml)	Palmitoyl-CoA hydrolase (nmoles CoASH/min/ml)	I.R.I. µU/ml
Normally fed	(n=3)	324 ± 40[a]	82 ± 3[d]	225 ± 30[f]	190 ± 6[h]	130
Fasted	(n=3)	241 ± 11[b]			133 ± 17[j]	51
"Kenacort" treated	(n=3)	498 ± 92[c]	71 ± 10[e]	450 ± 5[g]	99 ± 17[k]	225

Statistically different are a from b and c: $0.05>P>0.025$; b from c, and f from g, and h from k: $P<0.005$; h from j: $0.01>P>0.005$ and j from k: $0.1>P>0.05$; not significantly different is d from e.

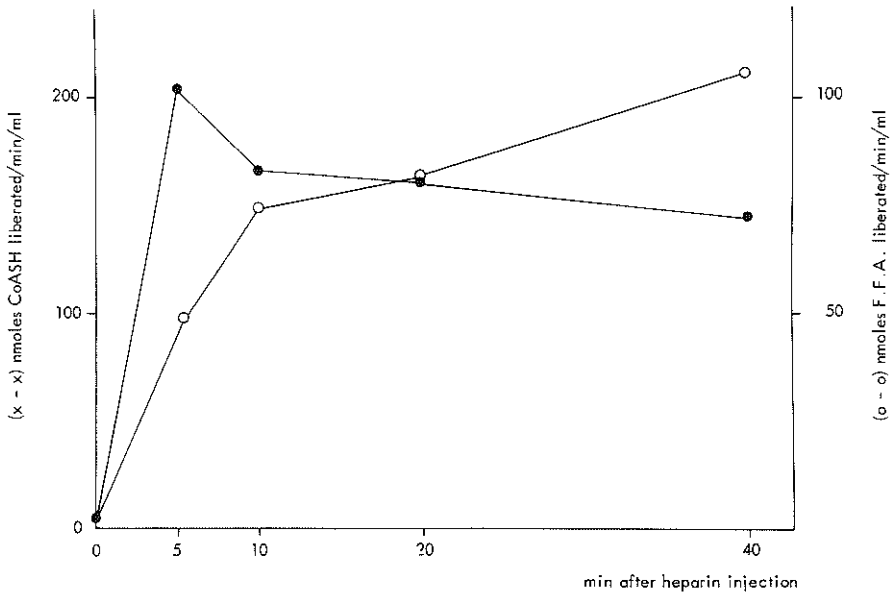

Fig. 3. Palmitoyl-CoA hydrolase and triacylglycerol hydrolase activities after intravenous heparin injection in man. 100 I.U. heparin/kg body weight was injected in a 30 year old volunteer. Blood was withdrawn at different times after the heparin injection. In the sera palmitoyl-CoA hydrolase activity and triacylglycerol hydrolase activity were determined.

DISCUSSION

In accordance with the results of Bensadoun et al.[19], with lipoprotein lipase from pig adipose tissue, we found that the extrahepatic lipoprotein lipase was inactivated faster at a higher pH. The heparin-releasable liver enzyme was found to be stable at a pH between 7.0 and 7.3, but became progressively inactivated with increasing pH (Fig. 1).

Fig. 2 shows that the liver enzyme hydrolyzes triacylglycerol completely to free fatty acids and glycerol. The extrahepatic enzyme(s) release(s) free fatty acids and glycerol in a molar ratio of about 8 to 1, which is in accordance with earlier observations of Krauss et al.

It was shown that the liver releases about 80% of the total palmitoyl-CoA hydrolase activity of postheparin serum (TABLE I). Palmitoyl-CoA hydrolase activity shares many characteristics with heparin-releasable triacylglycerol lipase: a) identical sensitivity towards an antibody preparation[5], b) identical elution patterns of both activities from a Sepharose-heparin column (Fig. 2), c) agreement between our results with palmitoyl-CoA (Fig. 3) and those of Krauss et al.[20] with triacylglycerol as substrate for the liver enzyme. Therefore the conclusion that liver triacylglycerol hydrolase and palmitoyl-CoA hydrolase activities are catalyzed by the same protein seems to be justified. Additional support is given in TABLE II, where it is shown that by the injection of different amounts of heparin a different ratio of *in vitro* released free fatty acids to released glycerol from triacylglycerol by the postheparin sera is found. This ratio is higher at low heparin concentrations (low hepatic enzyme activity) and increases when more heparin is injected. The 1-monoacylglycerol hydrolase activity behaves different. That the 1-monoacylglycerol hydrolase activity is catalyzed by another enzyme was in fact already shown by Nilsson-Ehle[21] who found that postheparin triacylglycerol hydrolase(s) and 1-monoacylglycerol hydrolase activities of human serum could be separated. Recent work of Pykälistö et al.[22] shows a correlation between the phospholipase of liver and 1-monoacylglycerol hydrolase. Waite and Sisson found that 1-monoacylglycerol but not 2-monoacylglycerol or triacylglycerol can act as acyl donor in reactions catalyzed by rat liver phospholipase[23]. Also in TABLE IV it is shown that the 1-monoacylglycerol hydrolase activity is affected differently from the liver palmitoyl-CoA hydrolase activity by cortisol treatment.

During the hydrolysis of triacylglycerol by extrahepatic lipoprotein lipase partial glycerides are formed. These

partial glycerides can be hydrolyzed by the hepatic enzyme. In this way the activity of the extrahepatic lipoprotein lipase can influence the in vitro measured activity of the liver enzyme, particularly when the extrahepatic lipoprotein lipase produces a considerable amount of partial glycerides. Such a mutual interaction of liver and extrahepatic activities does not occur when palmitoyl-CoA is used as the substrate. The liver enzyme hydrolyzes palmitoyl-CoA much faster than the extrahepatic lipase (Fig. 2). Therefore we think that measurement of palmitoyl-CoA hydrolase activity in addition to triacylglycerol lipase activities of postheparin serum may be of use in the study of serum lipases. In TABLES III and IV it can be seen that such a simultaneous estimation of palmitoyl-CoA hydrolase and of triacylglycerol hydrolase activities provides information about the relative contributions of hepatic and extrahepatic lipase activities.

In view of the above mentioned reasons it is worthwhile considering that Orosz et al.[24] and Jaillard et al.[25] measured glycerol release by postheparin serum of normal and atherosclerotic subjects. Since the time course of enzyme activity release after heparin injection as found by Orosz et al.[24] is the same as for the liver lipase (Fig. 3 and ref. 6), it might be suggested that probably the lipolytic activity decrease in atherosclerotic persons stands for a decrease of liver activity. Müller et al.[8] found secondary to liver disease lowered hepatic lipolytic activity and hypertriglyceridemia, due to an elevated remnant particle concentration. Also in diabetes the hypertriglyceridemia may have resulted from an elevated remnant particle concentration. According to Schonfeld et al.[26] accumulation of these particles causes hypertriglyceridemia in diabetes. In the present paper we show that in diabetic rats the liver lipase activity is significantly lowered. The hypertriglyceridemia in these rats cannot be explained by a lowered extrahepatic lipoprotein lipase (TABLE III), nor by

an increased triglyceride production[27]. Boberg[28] found the best inverse correlation of enhanced serum triglyceride content and 5 min postheparin lipase activity in man. From Fig. 3 and ref. 6 it is seen that under these circumstances the liver activity has a maximal contribution, again suggesting the important role that the liver enzyme may play in the removal of serum triglycerides. Putting these facts together, it is clear that the determination of liver and extrahepatic lipolytic activities, which can be done by determination of the ratio of palmitoyl-CoA hydrolase and triacylglycerol hydrolase activities in postheparin serum, may be an important step in the elucidation of serum triglyceride transport. Remnant particles of very low density lipoproteins may be involved in atherogenesis[29]. The possible role of the liver enzyme in the breakdown of these particles suggests the contribution of a lowered liver lipolytic activity in addition to an enhanced (heart-) muscle lipoprotein lipase[30,31] activity in the etiology of atherosclerosis. Results of Orosz et al.[24] and Jaillard et al.[25] seem to be in line with this hypothesis.

Lipoprotein lipase activities of heart and/or adipose tissue are possibly regulated by the serum glucagon[32] and insulin[33,34] levels. For the liver lipase no direct effect of the insulin level of the blood could be demonstrated (TABLES III and IV).

It is known, however, that hypercortisolism leads to an enhanced glucagon production and insulin resistence. Therefore the effective glucagon/insulin ratio in this hormonal state may be enhanced like in diabetic rats (TABLE III).

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INFLUENCE OF RAPESEED OIL FEEDING ON THE LIPASE ACTIVITIES OF RAT HEART

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SUMMARY. Feeding high doses of fat increases the lipase activity of rat heart. The heparin-releasable activity in the hearts of rapeseed oil fed rats remains high after prolonged feeding (10 days), whereas this activity in olive oil fed rats returns to normal values in the same period. After 10 days rapeseed oil feeding the non-releasable lipase activity in the hearts is enhanced significantly, when compared to the activity measured after 3 days. The results suggest an adaptation of heart lipases to dietary fat.

Triglycerides accumulate in the hearts of rats fed a high erucic-acid containing rapeseed oil diet[1]. The fat content of the hearts is highest after 3 to 6 days feeding and is rapidly lowered after 6 days[2]. The fat accumulation in the hearts has been ascribed to the inhibition of the β-oxidation by erucic acid[3] and by a lower rate of the overall metabolism of erucic acid in the heart[4]. Gumpen and Norum[5] suggested that the fat accumulation might arise from an impaired input/output of free fatty acids by the hearts. Rat hearts can take up free fatty acids from the serum directly. Serumtriglycerides can be taken up after previous hydrolysis by heart lipoprotein lipase, in which the heparin-releasable part of this enzyme activity is involved[6]. An impaired input/output of free fatty acids in the hearts could, therefore, be related with changes in the lipoprotein lipase activity of the hearts during rapeseed oil feeding. The heparin-releasable and non-releasable lipase activities of rat hearts were estimated selectively by in vitro heart perfusions. Control hearts were obtained from rats fed a diet containing high levels of olive oil. Olive oil does not introduce gross fat accumulation in heart.

TABLE I

LIPASE ACTIVITIES OF RAT HEART AFTER THE FEEDING OF DIFFERENT DIETS DURING 3 DAYS.

Rats were fed various diets for 3 days as described under METHODS. Then their hearts were perfused in vitro and lipase activities were estimated in the perfusates and in acetone powders of the perfused hearts. The activities are expressed in nmoles of free fatty acid released per min per heart ± standard deviation.

Diet		Lipase activity in perfusates	Lipase activity in the hearts after perfusion	Total lipase activity per heart
Rapeseed oil	(n=5)	253 ± 101	374 ± 150	627
Olive oil	(n=5)	294 ± 67	343 ± 124	637
Laboratory chow	(n=3)	83 ± 32	202 ± 30	285

TABLE II

LIPASE ACTIVITIES OF RAT HEART AFTER FEEDING DIFFERENT DIETS DURING 10 DAYS.

Two groups of 6 rats were fed a rapeseed oil-rich or an olive oil-rich diet for 10 days. Then their hearts were perfused. The perfusates of 3 rats of each group were pooled and lipase activities were estimated. The same was done with acetone-powders of the hearts. The activities are expressed in nmoles of free fatty acid released per min per heart ± standard deviation.

Diet	Lipase activity in perfusates	Lipase activity in the hearts after perfusion	Total lipase activity per heart
Rapeseed oil	296 ± 28	504 ± 64	800
Olive oil	120 ± 7	324 ± 16	444

METHODS. Groups of male Wistar rats, weighing 250 to 300 g, were given for 3 or 10 days fat-rich diets consisting of 50 cal% rapeseed oil (48% C 22:1 n-9) or 50 cal% olive oil (78% C 18:1 n-9) and further 27 cal% carbohydrate (maize starch), 23 cal% casein, different salts and vitamins. The rats had constant access to water and food.

Lipase activity was measured by the method of Kelley[7], which was modified as described elsewhere[8]. Rat hearts were perfused according to the Langendorff technique with a modified Tyrode solution at a pressure of 100 cm H_2O and at 33 $\pm$ 3°C. After 5 min the perfusion medium was replaced by one containing, in addition, 20% (v/v) rat serum and 4.5 I.U. heparin/ml (Organon, Oss, The Netherlands). The hearts were perfused with 50 ml of the latter medium. The lipase activities of the perfusates, which were cooled to 0°C immediately after collection, were estimated within 4 h. The lipoprotein lipase activity of the heart tissues was determined in total acetone powder homogenates[9].

RESULTS

The average weights of the hearts of the rats were 1.04 $\pm$ 0.10 g for the rapeseed oil fed rats, 0.98 $\pm$ 0.08 g for the olive oil fed rats and 0.90 $\pm$ 0.04 g for the rats fed laboratory chow.

TABLE I shows the heparin-releasable lipase activities as well as the residual activities in the hearts of rats fed various diets. It can be seen from this TABLE that both activities are elevated in the rats fed a diet with a high fat content when compared to rats fed the normal laboratory chow. The variation in the measured activities is large as can be seen from the standard deviations. The total lipase activity in the hearts of the groups fed olive oil or rapeseed oil is not significantly different. After 10 days feeding, however, the pattern has changed considerably (TABLE II). The lipase activity of the olive oil fed group is lowered, due mainly to a lowering in the heparin-releasable lipase activity. The lipase of the rapeseed oil fed rats, however, is further elevated, as a result of an increase of the non-releasable or residual activity.

DISCUSSION

From the data shown it can be seen that the lipase activities of rat heart are greatly influenced by fat feeding. Both the heparin-releasable and the non-releasable lipase activities are significantly

enhanced, irrespective of the nature of the fat consumed. The enhanced activity of the heparin-releasable part of the lipase will lead to an increased uptake of serumtriglycerides in the rat hearts[6]. Gumpen and Norum[5] calculated that an increased flow of only 3% of the daily fat intake to the hearts can account for the triglyceride accumulation in the rapeseed oil fed rats. Our results show that this increased flow may be caused by the elevated lipoprotein lipase activity of the rat hearts. In the olive oil fed rats the lipoprotein lipase activity of the hearts is equally enhanced by the diet. Oleic acid (the major component of olive oil) is however rapidly metabolized, in contrast to erucic acid (component of rapeseed oil).

When the rats are maintained on the olive oil diet for a longer period (10 days), the heparin-releasable lipase activity is lowered to an almost normal level (TABLE II). However, in the rapeseed oil fed animals this activity remains elevated, allowing a continuous flow of triglycerides to the hearts.

From TABLE II it can be seen that, contrary to the olive oil feeding experiments, the non-releasable lipase activity in the rapeseed oil fed rats is further elevated after 10 days feeding. The role of this lipase activity is not very well understood. It is not clear whether it is operative <u>in vivo</u> inside the heart cells or not. That an active lipase in the cardiocytes is present was shown by Olson and Hoeschen[10], since triglycerides were shown to serve as an energy source during substrate-free perfusion. It was found also (E.A.M. de Deckere, Unilever Research, unpublished) that hearts of rapeseed oil fed rats continue to beat much longer during substrate-free perfusion than hearts of control rats. The removal of the triglycerides from the hearts could be explained by increased lipolysis of the endogenous triglycerides, which is in accordance with the observation that the FFA content of hearts from rats fed rapeseed oil, remains elevated[2]. Perhaps then a flux of FFA from the hearts to the blood would exist, aiding the removal of fat. Part of the FFA might then be directed towards the liver, in which Gumpen and Norum[5] were able to demonstrate increased levels of erucyl-carnitine. That liver metabolism is indeed affected by prolonged rapeseed oil

feeding is also suggested by our finding[11] of increased liver lipase activity.

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Increase in postheparin lipoprotein lipase activity in rat plasma by dietary erucic acid

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SUMMARY

A diet of 50 cal% rapeseed oil with a high content of erucic acid (C 22:1 *n*-9) given to rats increases the postheparin lipoprotein lipase activity of the plasma by about 45% after a feeding period of three to six days.

Dietary erucic acid (C 22:1 *n*-9) causes fat infiltration and accumulation in the rat heart, especially in short-term feeding experiments[1,2], the amount of accumulated fat being highest after 3 to 4 days[3]. The increase of the total fat content is mainly due to an increase of triglycerides containing large amounts of C 22:1. After the sixth day the triglyceride content decreases rapidly.

Some authors showed that different tissues, such as heart[4], liver, kidney and adipose tissue[5,6], contain an enzyme which appears to be identical with the lipoprotein lipase in plasma. This enzyme is released into the blood after intravenous heparin injection, thus increasing the postheparin lipoprotein lipase activity. If this lipoprotein lipase is not only localized in the capillary endothelium but also in the tissue cells, this enzyme could play a role not only in the removal of intravascular triglycerides but also in the disappearance of tissue triglycerides. Therefore we started to study the postheparin lipoprotein lipase activity in the plasma of rats fed either rapeseed oil with a high C 22:1 content, sunflower seed oil or olive oil.

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In our first series of experiments (Table I) groups of two male Wistar rats, 8 weeks old, were given 50 cal% rapeseed oil (48% C 22:1), 50 cal% sunflower seed oil or 50 cal% olive oil for 6 or 11 days. In a second type of experiment (Fig.1), groups of two male Wistar rats, also 8 weeks old, were given 50 cal% rapeseed oil (48% C 22:1) for 1, 2, 3, 6, 10 and 14 days.

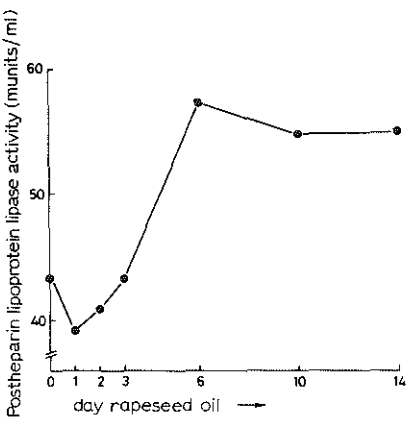

Fig.1. Postheparin lipoprotein lipase activity of plasma from rats fed 50 cal% rapeseed oil for 1, 2, 3, 6, 10 and 14 days. Heparinization time 15 min. Postheparin lipoprotein lipase activity was measured at pH 7.4, temperature 25°C.

After overnight fasting, the rats, weighing 250–300 g, were anaesthetized with Nembutal. Heparin (0.1 mg or 10 I.U. per 100 g body weight) was administered intravenously. The rats were killed by exsanguination from the aorta. Blood samples were collected at 6 or 15 min after the heparin injection. Postheparin lipoprotein lipase activity of the plasma was determined, as described by Jansen and Hülsmann[7].

It is shown in Table I that a rapeseed oil diet with a high C 22:1 (*n*-9) content increases the postheparin lipoprotein lipase activity in rat plasma by about 45%. This seems to be independent of the various pH values and temperatures during the incubations in the various experiments.

The time dependence of the postheparin lipoprotein lipase activity increase on rapeseed oil feeding is presented in Fig.1. The enzyme acitivities are lower than those in the previous experiments due to lower pH and temperature. The postheparin lipoprotein lipase activity in rat plasma increases obviously between 3 and 6 days of rapeseed oil feeding. This rise in postheparin lipoprotein lipase activity coincides with the rapid decrease in triglyceride content, which suggests a causative correlation with the removal of accumulated triglycerides from the heart.

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TABLE I

POSTHEPARIN LIPOPROTEIN LIPASE ACTIVITY IN RAT PLASMA, AFTER FEEDING 50 CAL% RAPESEED OIL, SUNFLOWER SEED OIL OR OLIVE OIL FOR 6 AND 11 DAYS

Postheparin lipoprotein lipase activity (munits/ml) was determined as the rate of hydrolysis of palmitoyl-CoA as described by Jansen and Hülsmann[7]. Differences in pH and temperature of the incubations affect the activity of the enzyme, whereas the heparinization time affects the amount of enzyme.

Heparinization time (min)	*pH*	*Temperature (° C)*	*Feeding period (days)*	*Diet*			*Ratio Rapeseed oil / Sunflower seed oil or Olive oil*
				Rapeseed oil	*Sunflower seed oil*	*Olive oil*	
		25	11	111.6 (±3.6)	–	71.0 (±4.8)	1.57
6	8.5						
		29	6	179.3 (±10.1)	136.7 (±10.2)	–	1.31
	8.5	29	6	153.0 (±21.50)	102.3 (±9.9)	–	1.50
15							
	7.5	32	11	100.5 (±4.2)	70.6 (±4.7)	–	1.42

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