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Changes in lipid composition of plasma lipoproteins after total hepatectomy in the rat

BY

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Lipidic composition of plasma lipoproteins were determined at different times after total hepatectomy in the rat and compared with those in sham-operated animals. After hepatectomy there was a progressive decrease in the plasma triglycerides amounts corresponding to changes in the content of very low density lipoproteins (VLDL) triglycerides. Phospholipid concentrations in both low density lipoproteins (LDL) and VLDL decreased after hepatectomy but these lipids were unchanged in high density lipoproteins (HDL) which are the main contributors to plasma phospholipid content. Contrary to the other lipid components, plasma cholesterol levels increased after hepatectomy, due mainly to the increment in HDL-esterified cholesterol. Modifications in plasma triglyceride levels after hepatectomy are interpreted as the result of total reduced synthesis and the effect of extrahepatic lipoprotein lipase activity on VLDL-triglycerides. Changes in cholesterol are considered due to its enhanced production by extrahepatic tissues.

Introduction

Both chylomicrons and very low density lipoproteins (VLDL) are initially metabolized by interaction with lipoprotein lipase at the endothelial surface of capillaries in extrahepatic tissues (FIELDING & HAVEL, 1977), resulting in partially degraded particles called remnants (REDGRAVE, 1970; MJØS *et al.*, 1975). In the rat, both chylomicron and VLDL remnants are taken up and catabolized by the liver (GOODMAN, 1962; NESTEL *et al.*, 1963; REDGRAVE, 1970; BERGMAN *et al.*, 1971; FAERGEMAN & HAVEL, 1975; WINDLER *et al.*, 1980). Studies *in vivo* in several species have shown that after injection of ^{125}I-labelled low density lipoproteins (LDL) or high density lipoproteins (HDL), most radioactivity is found in the liver (ROHEIM *et al.*, 1971; SNIDERMAN *et al.*, 1975). On the other hand, partial hepatectomy does not decrease the catabolic rate of LDL or HDL (SNIDERMAN *et al.*, 1974; VAN TOL *et al.*, 1978*a*), and discrepancies in the catabolic pathways of rat and human LDL in partially hepatectomized rats have been reported (VAN TOL *et al.*, 1978*b*). To clarify the rôle of the liver in lipoprotein catabolism, the effect of total hepatectomy on the lipid composition in the main plasma lipoproteins was investigated in the rat.

Materials and Methods

Female Wistar rats weighing 160-190 g were anaesthetized with sodium pentobarbital (40 mg/kg, i.p.), blood vessels supplying the liver (the coeliac axis and portal vein just above the first division to the hepatic lobes) were tied (RUSSELL, 1942) and medium and left lateral lobes were ligated (HIGGINS & ANDERSON, 1931) to ensure total hepatectomy. The entire surgical procedure took less than 15 min. Animals were sacrificed at 0, 5, 10, 20 or 30 min after hepatectomy, the 0-min group being sham-operated rats in which laparotomy and handling of liver were performed as in the other groups but without lobe ligation or tying of any vessel. Blood was collected from the cava vein into tubes containing 2 mg sodium EDTA and after centrifugation, fresh plasma aliquots were used for lipoprotein fractionation following a dual precipitation technique (WILSON & SPIEGER, 1973). Lipoprotein fractions were identified by agarose electrophoresis (NOBLE, 1968; DYERBERG & HJØRNE, 1970). It was found that the VLDL fraction contained a small proportion of chylomicrons and no attempt was made to obtain better purification. Neither LDL nor HDL fractions showed any other contaminants. Aliquots of whole plasma and lipoprotein fractions were used for lipid extraction and purification (FOLCH *et al.*, 1957) to measure phospholipid-phosphorus (FISKE & SUBBAROW, 1925) after acid hydrolysis (FREINKEL, 1958) and total and esterified cholesterol (CRAWFORD, 1958). For triglyceride determination, another aliquot of the lipid extracts was blown to dryness under N_2 and treated with activated silicic acid in chloroform media. After saponification in 0.7 M ethanolic KOH, it was treated with 0.18 M $MgSO_4$ to determine glycerol in the supernatant following centrifugation, according to an enzymatic method (GARLAND & RANDLE, 1962). Statistical comparisons between groups were done by the Student "*t*" test.

Results

As shown in Table I, hepatectomy in the rat caused a progressive decrease in the plasma triglyceride concentration, the effect reaching significance 10 min after hepatectomy as compared with values in sham-operated controls (time 0). This reduction corresponded to triglyceride values found in the VLDL fraction which were significantly reduced from the 5th min after hepatectomy. The proportion of triglycerides present in VLDL compared with those in whole plasma decreased after hepatectomy, being 76 % in the sham-operated controls and 48 % in the animals sacrificed at 30 min. Hepatectomy did not modify triglyceride concentration in either LDL or HDL. Plasma concentration of phospholipids decreased only slightly after hepatectomy since its content remained constant in HDL, the lipoprotein fraction containing the highest concentration. In both VLDL and LDL, however, there was a significant reduction in phospholipid concentration at 20 and 30 min after hepatectomy. Contrary to other lipids, total cholesterol concentration increased in plasma after hepatectomy, being significant after 10 min (Table I). This effect was due exclusively to the amount of HDL-cholesterol which rose 10 min after hepatectomy, while cholesterol in LDL and VLDL decreased significantly from the 20th and 10th min respectively. Most of the plasma cholesterol was in esterified form (about 70 %, Table I) and the change in whole plasma HDL and VLDL was almost the same as in total cholesterol. The modification of esterified cholesterol in LDL, however, was different than that of total LDL-cholesterol because at 5 min there was a significant rise with a return to basal values (time 0) at 10 min and further decrease at 20 and 30 min.

TABLE I. *Lipid composition of plasma lipoprotein fractions after hepatectomy in the rat.*

Min after hepatectomy :	0	5	10	20	30
Triglycerides glycerol (μmol/dl) Whole plasma	135.6 ± 6.8	107.8 ± 11.6	95.3 ± 5.4***	91.8 ± 5.2**	74.4 ± 3.6***
VLDL.....	103.3 ± 5.3	72.3 ± 9.9*	58.9 ± 4.6***	55.3 ± 2.5**	35.4 ± 2.9***
LDL	15.2 ± 3.0	21.9 ± 2.1	18.5 ± 0.4	15.1 ± 1.2	23.0 ± 1.1
HDL	17.1 ± 2.0	16.5 ± 3.0	17.9 ± 1.3	21.5 ± 2.1	15.9 ± 2.4
Phospholipids (mg/dl) Whole plasma	149.7 ± 9.1	142.5 ± 4.7	133.2 ± 4.9	134.9 ± 14.9	129.5 ± 4.5
VLDL.....	12.9 ± 2.8	9.6 ± 1.9	12.3 ± 2.0	5.4 ± 0.7*	5.1 ± 0.6*
LDL	21.9 ± 1.2	21.6 ± 1.5	21.4 ± 3.2	12.3 ± 3.2*	10.6 ± 2.1**
HDL	114.9 ± 7.6	111.3 ± 3.2	106.6 ± 6.6	117.1 ± 10.9	113.8 ± 4.5
Total cholesterol (mg/dl) Whole plasma	80.5 ± 2.5	89.6 ± 5.1	94.0 ± 4.2*	92.9 ± 4.1*	85.8 ± 5.2
VLDL.....	7.6 ± 1.6	4.6 ± 0.9	3.2 ± 1.0*	2.6 ± 0.7*	3.6 ± 0.8*
LDL	12.4 ± 1.0	18.5 ± 0.4	10.2 ± 7.0	4.2 ± 0.3**	5.4 ± 0.6
HDL	60.0 ± 4.5	66.4 ± 4.5	80.2 ± 3.6**	86.1 ± 4.1**	76.8 ± 4.1*
Esterified cholesterol (mg/dl) Whole plasma	53.3 ± 2.3	65.3 ± 2.5*	71.6 ± 3.8*	71.8 ± 3.6*	74.2 ± 0.9**
VLDL.....	6.2 ± 1.5	3.3 ± 0.9	2.7 ± 1.0	1.1 ± 0.4*	2.8 ± 0.8
LDL	8.1 ± 0.9	15.8 ± 0.2*	7.8 ± 1.0	2.8 ± 0.8*	4.3 ± 0.7*
HDL	45.4 ± 4.7	45.7 ± 1.2	63.2 ± 2.1**	66.6 ± 3.8***	67.2 ± 0.6*

Hepatectomized animals *vs.* sham-operated controls (time 0) : * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.
Number of animals/group = 4-5.

Discussion

The decrease in all lipid components in plasma VLDL after total hepatectomy in the rat demonstrates that the extrahepatic tissues metabolize these particles, as previously suggested (FIELDING & HAVEL, 1977; SMITH *et al.*, 1978). The most rapid and striking change following hepatectomy was the reduction of VLDL-triglycerides in plasma which was probably due to the immediate decrease of their synthesis, known to take place mainly in the liver (SMITH *et al.*, 1978) and to the maintenance of extrahepatic lipoprotein lipase activity. Through the action of this enzyme, VLDL-triglycerides are hydrolysed to free fatty acids (FFA) and glycerol which are partially utilized by extrahepatic tissues in the hepatectomized rat (CARMANIU & HERRERA, 1979 & 1980), but their rate of production seems to exceed the rate of their utilization, as indicated by the rapid increase in both FFA and glycerol concentrations in plasma after hepatectomy (CARMANIU & HERRERA, 1979 & 1980). An augmented lipolytic activity in adipose tissue (PECTOR *et al.*, 1978) and absence of the liver which is the main receptor site in the body for both FFA and glycerol (MAMPEL *et al.*, 1981) also contribute to the increase of these metabolites after hepatectomy as well as to the catabolism of VLDL-triglycerides. After hepatectomy, lipid components are better preserved in plasma LDL than in VLDL. The relative contributions of the various tissues involved in the degradation *in vivo* of LDL are still controversial. It is known that the liver plays an important rôle in LDL catabolism in the rat (VAN TOL *et al.*, 1978*b*) and consequently total hepatectomy should reduce their disappearance rate. It has also recently been proposed that a certain fraction of rat serum VLDL may be catabolized to LDL without intervention of the liver (SURI *et al.*, 1979), and this effect may also contribute after hepatectomy to the maintenance of lipid components in LDL longer than in VLDL. Interpretation of the increase in LDL cholesterol esters 5 min after hepatectomy and their subsequent reduction requires further experimental study, but these effects are probably due to the active interchange of cholesterol esters known to occur between LDL and other lipoprotein fractions in plasma (ZILVERSMIT *et al.*, 1975; SNIDERMAN *et al.*, 1978; BARTER & JONES, 1980). This process is dependent on the activity of a specific esterified cholesterol transfer protein which has been detected in the plasma of several species (ZILVERSMIT *et al.*, 1975; SNIDERMAN *et al.*, 1978; BARTER & LALLY, 1979) including the rat (RUIZ, 1981). The increased circulating cholesterol after hepatectomy may be caused mainly by the esterified cholesterol found in plasma HDL. This explanation coincides with the negative correlation normally observed between HDL-cholesterol, VLDL-cholesterol, and plasma triglyceride levels (SCHAEFER & LEVY, 1979), with the reported participation of HDL in the removal of cholesterol from peripheral tissues (STEIN *et al.*, 1976), and with the described rôle of these lipoproteins as the main substrate of lecithin cholesterol acyltransferase (SMITH *et al.*, 1978) for the esterification of cholesterol. The increase in circulating cholesterol after hepatectomy contrasts with the usual concept of the liver as the main site for production of body cholesterol, although this traditional theory has recently been challenged with the demonstration of important extrahepatic cholesterol synthesizing tissues (CHEVALLIER & MAGOT, 1975; MAGOT & CHEVALLIER, 1979). The contribution of extrahepatic tissues to the total body cholesterol synthesis has been shown to rise following a decreased level of cholesterol synthesis in the liver (BALASUBRAMANIAM *et al.*, 1976; JESKE & DIETSCHY, 1980). It is proposed that the increment in circulating cholesterol found after hepatectomy in the rat is the result of an enhanced production of cholesterol by extrahepatic tissues, and the mechanism of this compensatory effect remains to be established.

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