The Effect of Portacaval Shunt on Hepatic Lipoprotein Metabolism in Familial Hypercholesterolemia

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The hyperlipidemia observed in familial hypercholesterolemia can be reduced by portacaval anastomosis. We report the effects of a portacaval shunt on hepatic morphology and biosynthetic pathways crucial to hepatic cholesterol homeostasis in homozygous receptor-negative familial hypercholesterolemia. Portacaval anastomosis was associated with a dramatic change in hepatocyte morphology, 28% reduction in plasma low-density lipoprotein concentration, and a decrease in hepatic total and free cholesterol content by 27 and 75%, respectively. Furthermore, the rate-limiting enzyme in cholesterol biosynthesis, 3-hydroxy-3-methylglutaryl coenzyme A reductase was decreased by 56%. Finally, the reduced binding of low-density lipoproteins to hepatic membranes preoperatively was increased following the portacaval shunt. These combined results indicate that the changes in circulating lipoprotein concentrations observed after portacaval shunt are due to alterations in the metabolic consequences of the defective recognition of low-density lipoproteins by the liver of familial hypercholesterolemic subjects.

INTRODUCTION

The mammalian liver plays a central role in lipid and lipoprotein metabolism. It is the primary site for endogenous cholesterol and lipoprotein biosynthesis as well as the principal organ for cholesterol excretion through bile and bile acid formation [11, 18, 48]. Studies in rats [9, 19, 54], swine [33], and rabbits [27, 34] indicate that a significant portion of an injected dose of radiolabeled low-density lipoproteins is removed by the mammalian liver. The initial step in the cellular uptake of circulating LDL is the binding of the lipoprotein to a receptor in the plasma membrane [8, 15]. Isolated hepatic membranes from a number of animal species have been shown to contain a receptor which [1, 23, 26, 28, 55] specifically binds LDL. Recently, hepatic membranes from normal adult humans have also been demonstrated to bind LDL [16, 22]. Thus, coordinate control of lipoprotein uptake, catabolism, and synthesis may occur in normolipidemic man.

Familial hypercholesterolemia is an autosomal dominant disorder characterized clinically by hypercholesterolemia, xanthomas, and premature atherosclerosis [24]. By analysis of skin fibroblasts from patients homozygous for FH, Brown and Goldstein determined that FH was due to one of several mutations in the gene coding for the cellular receptor for LDL [7, 51]. The most frequent allelic mutation resulting in FH is the loss of the functional high-affinity receptor for LDL and is referred to as receptor-negative FH [51]. We have recently demonstrated that the loss of the fibroblast
LDL receptor in FH homozygotes is paralleled by a defect in the hepatic membrane recognition of LDL [22]. Therefore, the profound hypercholesterolemia and accelerated atherosclerosis observed in FH may occur as a result of the loss of the hepatic LDL receptor and the resulting changes in hepatic lipid and lipoprotein metabolism.

Patients with FH frequently are refractory to all lipid lowering drug regimens. However, the use of a portacaval anastomosis to shunt blood flow from the liver has been shown to be effective in lowering the plasma lipids in these patients [42]. The present studies were performed on hepatic tissue in a receptor-negative FH subject to evaluate the effect of the portacaval shunt on both the binding of LDL to hepatic membranes as well as to assess the impact of the portacaval shunt on hepatic cholesterol metabolism.

METHODS

Patient history. The patient studied was first noted to be hypercholesterolemic at the age of 13. Despite therapeutic trials of cholestryamine, niacin, hydroxymethylglutamic acid, and neomycin, the plasma cholesterol ranged from 915 to 1210 mg/dl and she developed symptomatic coronary artery disease. The patient's mother (age 40) had a plasma cholesterol of 462 mg/dl and an LDL cholesterol of 359 mg/dl. The patient's father was noted to be hypercholesterolemic at the age of 40.

At age 21, after suffering two myocardial infarctions and receiving a double coronary artery bypass graft, the patient received a therapeutic portacaval anastomosis at the University of Pittsburgh in an effort to lower the plasma lipid concentrations. A liver biopsy taken at the time of the anastomosis provided the basis for the biomedical and histopathologic studies referred to as “preshunt” values. Three months after the initial surgery, hepatic tissue was obtained; informed consent had been given. Biochemical and morphologic studies performed on this tissue are referred to as “postshunt” values.

The hepatic tissues from normolipidemic control subjects were obtained at the time of laparotomy for kidney donation for renal transplantation. All hepatic biopsies were performed after informed consent had been given and the protocol used for hepatic biopsy and portacaval anastomosis was approved by the human experimentations committee at the University of Pittsburgh School of Medicine.

Lipoprotein preparation. Preparation of human LDL (d 1.030–1.050) was from 500 ml plasma collected in 0.01% EDTA by plasmapheresis from fasting, healthy volunteers. Lipoproteins were separated by preparative ultracentrifugation at 4°C for 16–24 hr [17] using KBr for density gradient adjustment [36]. These subfractions were then dialyzed 34 hr at 4°C against 150 vol of phosphate-buffered saline (pH 7.0) (GIBCO, Grand Island, N.Y.). Each isolated lipoprotein fraction was sterilized by 0.45-µm Millipore filtration (Millipore Corp., Bedford, Mass.) and used within 1 month of preparation. ¹²⁵I-LDL was prepared by the iodine monochloride method [31] as modified for lipoproteins [4]. A 25–30% efficiency of iodination was obtained and less than 6% of the radioactivity was soluble in the organic phase following a chloroform-methanol extraction. After dialysis over 24 hr at 4°C against 500–600 vol PBS, specific activities ranged from 2.7 to 4.6 × 10⁶ Bq/ml LDL protein. The LDL protein concentration was determined by the method of Lowry et al. [30] using bovine serum albumin standard. After Millipore filtration, ¹²⁵I-LDL was stored at 4°C and used within 2–3 weeks of preparation.

Liver membrane preparation. Biopsy specimens were immediately placed in a beaker and all processing occurred at 4°C similar to that described by others [1, 2]. After mincing the tissue with a razor blade, it was washed with an ice-cold buffer containing 0.9% (w/v) NaCl, 1 mM EDTA, and 10 mM TrisCl (pH 8.0). Homogenization was performed by six
strokes of a motor-driven Teflon pestle in a buffer containing 0.25 M sucrose, 1 mM EDTA, and 10 mM TrisCl (pH 8.0). The homogenized preparations (10 mg/ml) were centrifuged for 10 min at 1000g. The supernatant solution was recentrifuged for 25 min at 10,000g, followed by ultracentrifugation at 100,000g for 60 min. The pellet from this ultracentrifugation was resuspended in a buffer containing 150 mM NaCl, 10 mM TrisCl (pH 8.0), and flushed through a 22-gauge needle 10 times. These membranes were recentrifuged for 15 min at 100,000g and the membrane pellets were then frozen in dry ice and stored in liquid nitrogen until used for binding assays and quantitation of HMG-CoA reductase activity. The 10,000g supernatant was used for cholesteryl esterase assays.

Lipoprotein quantitation. Blood was obtained in 0.01% EDTA from patient TH after a 12- to 14-hr overnight fast, and the plasma was separated at 4°C in a refrigerated centrifuge. Plasma cholesterol and triglycerides were quantitated on a Gilford spectrophotometer (Beckman, Fullerton, Calif.) and the VLDL was separated from the other plasma lipoproteins by tube slicing [17]. The cholesterol concentration in the 1.006 g/ml infranate was measured, and the VLDL and LDL cholesterol were calculated.

Hepatic cholesterol and cholesteryl ester determination. Liver biopsy samples from normal and familial hypercholesterolemic subjects were weighed, extracted three times with chloroform–methanol (2/1 v/v) at 41°C. The samples were then extracted overnight with chloroform–methanol (2/1 v/v) at 30°C. The chloroform–methanol extracts were blown to dryness and the liquid was resuspended in 2-Propanol (J. T. Baker Chemical Co., Phillipsburg, N. J.). Free and total cholesterol were then measured by the enzymatic, fluorimetric method of Heider and Boyette [20]. Esterified cholesterol was determined as the difference between total and free cholesterol. The nanomoles of cholesterol extracted from the biopsy samples were normalized to initial sample weight.

Hepatic enzyme activities. The activity of HMG-CoA Reductase (EC 1.1.1.34) was quantitated in the 100,000g pellet, as previously described [3]. The cholesteryl ester hydrolase activity (EC 3.1.1.13) was determined in the cytosolic fraction at both pH 4.0 and pH 7.0 [21].

Binding of 125I-LDL to liver membranes. Hepatic membrane binding of 125I-LDL was assessed using previously described methods [1, 22, 28]. Briefly, frozen liver membrane preparation was thawed and resuspended in 50 mM NaCl, 30 mM Tris–HCl (pH 7.5) buffer (10–12 mg/ml) and passed through a 22-gauge needle. Membranes were then sonicated by five 4-sec pulses at the 55-W setting using an ultrasonics microtip (Heat Systems Ultrasonics, Inc., Plainview, N. Y.). From 100 to 200 µg membrane protein was then added to a 50 mM NaCl, 20 mM Tris–HCl (pH 7.5) buffer containing 1 mM CaCl2. 125I-LDL was added at the indicated concentrations with or without unlabeled LDL with a total assay mixture volume of 0.1 ml. Incubations were carried out at 37°C for 30 min at which time preliminary studies had demonstrated that equilibrium had been reached. Bound 125I-LDL was separated from free ligand by a 3 min, 100,000g centrifugation of 50 µl of the assay mixture through 125 µl PBS in a 50°C angle rotor in an air-driven ultracentrifuge (Beckman, Palo Alto, Calif.). The supernatant was removed from the pellet by vacuum aspiration, and the pellet was washed once with 125 µl of PBS. The cellulose nitrate tube tips containing the membrane pellet were sliced and the radioactivity in the pellet quantitated in a Biogamma II scintillation counter (Beckman). Specifically bound 125I-LDL was defined as the difference in 125I-LDL quantitated in samples which were incubated with and without 390 µg of unlabeled LDL.

Histopathologic studies. One sample of each liver specimen was fixed in 10% neutral formalin and was then processed for examination...
by light microscopy. A second piece of the biopsy tissue was fixed in buffered glutaraldehyde, postfixed in osmium tetroxide, and then embedded in epoxy resin (Epon 812). Some of the liver postshunt tissue was also postfixed in osmium and processed for electron microscopy. Ultrathin sections were stained with lead citrate and examined in a Phillips 300 electron microscope. The sizes of the midzonal hepatocytes before and after portal diversion were determined on hematoxylin and eosin-stained sections by a method previously described [43]. Midzonal hepatocytes identified in 1.0-μm-thick epoxy resin sections were also used for measuring the length of rough endoplasmic reticulum per area of cytoplasm by a morphometric method [29].

Statistical methods. Statistical comparisons of paired and unpaired lipoprotein determinations and biochemical assays were made using two-tailed t tests assuming the samples were independent [39].

RESULTS

The effects of portacaval shunt on the lipid and lipoprotein concentrations in the plasma in FH are summarized in Table 1. All of the lipid and lipoprotein concentrations decreased following the portacaval shunt. The decline of 291 mg/dl in total cholesterol and 266 mg/dl in the LDL cholesterol represented a 28% change (P < 0.01). Although the concentrations of VLDL, HDL, and total triglycerides also declined, these changes were not statistically significant.

These changes in lipoprotein concentration were paralleled by striking alterations in hepatocyte morphology (Fig. 1). Before portacaval diversion the hepatocytes were enlarged and their cytoplasm was vacuolated. Lipid deposits were demonstrated by staining frozen sections with Sudan IV. Ultrastructurally, the cytoplasmic droplets possessed a double membrane. The amounts of rough and smooth endoplasmic reticulum were normal. Free polyribosomes were also present in the cytoplasm. Glycogen was abundant.

After portacaval shunt, the size of the hepatocytes was nearly halved and the amount of fat in the cytoplasm of the liver cells decreased. Ultrastructurally, the lipid droplets remained enclosed in a double membrane. Morphologic analysis showed that the area of rough endoplasmic reticulum was reduced to 47% of the quantity found in the preoperative biopsy. The amount of smooth endoplasmic reticulum was reduced to 55% compared to the first biopsy. Free ribosomes were abundant. Glycogen particles were rare. Therefore, marked alterations in hepatocyte morphology, independent of any autolytic artifact, were observed both before and after surgery.

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>Cholesterol (mg/dl)</th>
<th>Triglycerides (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>VLDL</td>
</tr>
<tr>
<td>Normal* range</td>
<td>126-190</td>
<td>5-25</td>
</tr>
<tr>
<td>Preshunt</td>
<td>1034 ± 156</td>
<td>47 ± 17</td>
</tr>
<tr>
<td>Postshunt</td>
<td>743 ± 37*</td>
<td>24 ± 6</td>
</tr>
<tr>
<td>% Change</td>
<td>28*</td>
<td>49</td>
</tr>
</tbody>
</table>

*Note. Plasma lipid and lipoproteins were measured five times preshunt and four times postshunt. The values represent the mean ± SE.

* Normal values are those reported for the 10-90 percentile for females ages 14-19 from The Lipid Research Clinics Prevalence Study [49].

* These values represent a significant difference from preshunt values (P < 0.05).
The portacaval shunt was also associated with major changes in hepatic cholesterol content (Table 2). Before the portacaval shunt, the total cholesterol content in the FH liver was 1.7 times that of normal liver. Although the portacaval shunt reduced total hepatic cholesterol by 27%, the postshunt cholesterol content was still 21% higher than normal. The most striking change included the 75% decline in free cholesterol that was observed after the operation. As the free cholesterol declined, the amount of cholesterol in the esterified form increased by 79%. The normal livers had 24% of the cholesterol esterified while the FH liver preshunt had 32% of the cholesterol esterified. By performing the portacaval anastomosis, the fraction of hepatic esterified cholesterol in FH increased to 77%. Thus, the portacaval shunt had a profound effect not only on the absolute cholesterol content in the liver of TH, but also on the cholesterol distribution between free and esterified forms.

**TABLE 2**

<table>
<thead>
<tr>
<th>Hepatic cholesterol (nmol/mg wet tissue)</th>
<th>Total</th>
<th>Free</th>
<th>Esterified</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>5.80 ± 0.46</td>
<td>4.99 ± 0.24</td>
<td>0.81 ± 0.52</td>
</tr>
<tr>
<td>Familial hypercholesterolemia</td>
<td></td>
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</tr>
<tr>
<td>Preshunt</td>
<td>9.61 ± 0.21</td>
<td>6.58 ± 0.24</td>
<td>3.03 ± 0.32</td>
</tr>
<tr>
<td>Postshunt</td>
<td>7.04 ± 0.19</td>
<td>1.62 ± 0.34</td>
<td>5.42 ± 0.29</td>
</tr>
</tbody>
</table>

*Note.* Three replicate samples of hepatic biopsies were measured and the values represent the mean ± SE.
Enzymes central to hepatic cholesterol metabolism were quantitated in liver biopsy specimens taken before and after surgery (Table 3). The activities of the enzymes HMG-CoA reductase, acid cholesteryl ester hydrolase, and neutral cholesteryl ester hydrolase in the preshunt liver biopsy were comparable to the activities observed in normal liver. Although the portacaval shunt had no apparent effect on neutral esterase activity, changes in both HMG-CoA reductase and acid esterase activities were observed (Table 3). A 56% decline in HMG-CoA reductase activity was paralleled by a 220% increase in acid esterase activity. Thus, portacaval shunt of blood flow from the liver appreciably altered the enzyme activities relevant to hepatic cholesterol homoeostasis.

The ability of hepatic membranes to specifically bind LDL was directly determined in membranes isolated from the FH liver before and following portacaval anastomosis (Fig. 2). There was a significant reduction in LDL binding to the FH hepatic membranes compared to binding to hepatic membranes from normolipidemic subjects. After the portacaval shunt, however, the specific binding of 125I-LDL to the hepatic membranes was significantly increased compared to preshunt values. Thus, portacaval shunt enhanced the hepatic membrane binding of 125I-LDL.

TABLE 3

The Effect of Portacaval Shunt on Hepatic Enzymatic Activities in Familial Hypercholesterolemia

<table>
<thead>
<tr>
<th>Enzymatic activity (% control)</th>
<th>HMG-CoA</th>
<th>Acid esterase</th>
<th>Neutral esterase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preshunt</td>
<td>129 ± 26</td>
<td>110 ± 3</td>
<td>92 ± 22</td>
</tr>
<tr>
<td>Postshunt</td>
<td>57 ± 4</td>
<td>249 ± 19</td>
<td>102 ± 5</td>
</tr>
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</table>

Note: The enzymatic activity in the FH hepatic tissue was compared to that from liver taken from three normolipidemic subjects. The control specific activities expressed as pmole/min/mg protein were HMG-CoA reductase 3.24 ± 0.75, acid esterase 71.5 ± 3.34, and neutral esterase 11.4 ± 2.1. Values represent the mean ± SE of three or four replicate samples.

FIG. 2. Specifically bound 125I-LDL to hepatic membranes from normal subjects and a familial hypercholesterolemia before and after portacaval shunt. Membranes were prepared from hepatic biopsy specimens from three normal subjects (C, N = 3), and familial hypercholesterolemic hepatic tissue preshunt (∆), and postshunt (●). From 100 to 200 μg of membrane protein was added to a 75 mM NaCl, 150 mM TrisCl, 1 mM CaCl2 (pH 7.5) buffer with the indicated concentrations of 125I-LDL in the presence and absence of excess unlabeled LDL. Specifically bound 125I-LDL, defined as the difference in 125I-LDL bound in the presence and absence of excess unlabelled LDL, was normalized to the amount of membrane protein in the sample. Values represent the mean ± SE of triplicate determinations.

DISCUSSION

The extreme hypercholesterolemia and rapidly progressive premature cardiovascular disease observed in familial hypercholesterolemia have prompted the search for an effective, definitive treatment. After observing a marked decline in serum lipid concentrations with a portacaval shunt for Type I glycogen storage disease [47], Starzl and co-workers successfully employed this procedure to lower the plasma cholesterol levels in a patient with familial hypercholesterolemia [42]. Subsequent observations of lowered LDL cholesterol levels, xanthoma regression, and safety of the procedure have been reported by Starzl as well as several other investigators [6, 10, 12, 13, 25, 40, 41, 44, 54]. Thus, the use of portacaval anastomosis appeared to be one of the few successful hypocholesterolemic maneuvers in patients homozygous for FH.

The 28% reduction in total cholesterol and LDL observed in TH after portal diversion was typical of the 20–55% decline in these values.
previously reported [6, 10, 12, 13, 25, 40-42, 44, 54]. This decline could occur because of a fall in the synthesis of cholesterol containing lipoproteins, an enhanced clearance of the lipoproteins from the plasma, or a combination of these two possibilities. Metabolic turnover studies of radiolabeled lipoproteins indicated that the fractional catabolic rate of FH homozygotes was significantly less than in normal individuals [5, 37, 38, 50] and that the synthesis rate of apoB into LDL was two- to fourfold normal [5, 37, 38]. Bilheimer et al. reported that portacaval shunt in a patient homozygous for FH enhanced the clearance of LDL from the circulation by 17% and a 48% decline was measured in the rate of LDL synthesis in a patient homozygous for FH [5]. A similar response has been reported in a patient heterozygous for FH [14]. Thus, the fall in LDL observed after portacaval shunt appeared to result from changes in both LDL synthesis and removal.

Since the mammalian liver may be important for both cholesterol and lipoprotein synthesis as well as degradation, a disruption in the blood supply rich in hepatotrophic factors would be anticipated to alter these biochemical pathways. As in the present case, the cellular structures involved with lipid and lipoprotein metabolism have consistently been shown to undergo profound morphologic changes [35, 43, 45, 46]. The reduction in hepatocyte size and the development of cytoplasmic lipid droplets in the FH liver after portacaval shunt were similar to the changes induced by portacaval shunt in the dog [45]. These anatomic alterations are paralleled by biochemical changes observed in the liver of FH after portacaval shunt. First, quantitative as well as qualitative changes in hepatic cholesterol content were observed. Total hepatic cholesterol content decreased; however, the fraction of cholesterol in the free form decreased from 68 to 23%. Second, the activity of the rate-limiting enzyme for cholesterol synthesis, HMG-CoA reductase, was reduced by more than half following the portacaval shunt. Thus, the biosynthetic pathway of cholesterol synthesis in the FH liver was shown for the first time to decline in parallel with the fall in hepatic content of total and free cholesterol following portacaval shunt.

The distribution and hydrolysis of cholesterol ester within the FH hepatocyte may reflect aberrant metabolism of cholesterol ester-containing lipoproteins. The LDL receptor-mediated uptake mechanism leads to lysosomal localization and degradation of LDL [8, 15]. Since TH had no high-affinity LDL receptor, delivery of LDL to cells could only occur through a pinocytotic or an alternate pathway for LDL uptake. Such an alternate pathway may lead to ineffective delivery of cholesterol ester-rich lipoproteins to appropriate subcellular compartments. The existence of such an alternate pathway has recently been shown to be the major source of LDL delivery to the liver of the Watanabe heritable hyperlipidemic (WHHL) rabbit, the only existing animal model for FH [34]. In TH, the portacaval shunt was associated with an increase in the "receptor-independent" binding of LDL to the hepatic membranes (Fig. 2). An enhancement of LDL transport, though a less efficient alternate pathway, could account for the striking modifications observed in hepatic cholesterol metabolism.

In summary, portacaval shunt in FH has been shown to increase hepatic LDL recognition, markedly alter the intracellular enzymes central to cholesterol metabolism, and modify hepatic cholesterol concentration and distribution. These changes parallel the increased clearance of plasma LDL and reduced LDL synthesis observed in FH patients after portacaval shunt. By manipulating the metabolic consequences of the hepatic LDL receptor loss in FH, more effective therapeutic approaches to the hypercholesterolemia and accelerated atherosclerosis present in FH can be developed.

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REFERENCES


