HEPATIC CHOLESTEROL METABOLISM

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The liver is the only organ where cholesterol can be removed irreversibly from the blood. The unique anatomical structure enables uptake of cholesterol-carrying particles (lipoproteins) on the sinusoidal site of hepatocytes, intracellular metabolism and secretion of the metabolic products in the bile canaliculi. Besides hepatocytes, the liver also contains Kupffer, endothelial and fat-storing cells. These latter cell types are highly specialized in receptor-mediated endocytosis (including lipoproteins) and cholesterol initially internalized by the sinusoidal liver cells will be subject to a multicompartment processing system. Cholesterol from exogenous origin is partly esterified in the small intestine and transported from the lymphe to the blood circulation in chylomicrons (relatively large particles containing triglycerides and apolipoprotein B-48). In the blood the high density lipoproteins (HDL) provide the chylomicrons with apolipoproteins E and C, which are required for proper delivery of fatty acids in extrahepatic tissues and subsequent delivery of cholesterol (ester) in liver parenchymal cells. Recognition of the chylomicron remnants occurs by a so-called apo E-receptor which is specific for liver hepatocytes. Intracellularly the uptake of cholesterol inhibits the biosynthesis of cholesterol while excretion in the bile canaliculi or secretion with very low density lipoprotein (VLDL) may occur. The circulation of VLDL by the liver leads to the socalled endogenous cholesterol pathway because also newly synthesized liver cholesterol is transported in these particles. VLDL is extrahepatically metabolized in a similar way as chylomicrons leading to the formation of triglyceride-depleted VLDL (VLDL-remnants). The VLDL-remnants are either converted to low density lipoproteins (LDL), or re-internalized by the liver with the aid of the apo B,E-receptor. Receptor-deficiency (type II-a hypercholesterolemia) leads to increased formation of LDL. Because also LDL is recognized by the apo B,E-receptor, its catabolism is greatly blocked by an apo B,E-receptor deficiency, leading to a 6-8 fold increased level of LDL in these patients. In rats 60-70% of the LDL is catabolized by the liver for which mainly the liver Kupffer cells are responsible. Probably in other species (including man), a higher proportion of LDL will be processed by the liver hepatocytes. In addition to the apo B,E-receptor the liver endothelial cells do possess a highly active scavenger-receptor which recognizes both chemically or biologically modified lipoproteins. Uptake of cholesterol(esters) by liver endothelial or Kupffer cells is coupled to an endocytotic pathway which includes participation of the lysosomes. After processing, resecretion to the hepatocytes and finally to the bile can occur, leading to the concept of intercellular communication in the metabolism of cholesterol inside the liver. Recent therapies designed to lower serum cholesterol levels, utilize drugs which act on reabsorption of bile acids in the intestine (cholestyramine), inhibit endogenous cholesterol synthesis (mevinolin) or are designed to utilize highly active carbohydrate-recognizing receptors (ref. 1,2) for lipoprotein catabolism.

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HEPATIC CATABOLISM OF CHOLESTEROL

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The liver plays a central part in the metabolism of cholesterol. Firstly, the liver shows a relatively high rate of cholesterol synthesis, a process controlled by the rate-limiting enzyme 3-hydroxy-3-methylglutaryl co-enzyme A (HMG-CoA) reductase. Secondly, the liver is actively involved in the uptake of various cholesterol-containing lipoproteins from the circulation, which is mediated by specific receptors. Thirdly, the main route of cholesterol elimination from the body is via bile, either as cholesterol as such or after conversion to bile acids.

In the past years we have developed an animal model which allows us to study cholesterol metabolism and biliary bile acid excretion in unanestetized, unrestrained rats with a normal feeding pattern (1). For this purpose, rats are equipped with permanent catheters in bile duct, duodenum and heart. All three catheters are attached on top of the skull. There, bile duct and duodenum-catheter are connected to each other in order to maintain an intact enterohepatic circulation. The heartcatheter allows intracardial administration of compounds and repeated blood sampling. Rats are used for experiments after an 8-days recovery period.

Interruption of the enterohepatic circulation in these rats resulted in a strong decrease in biliary bile acid output, due to exhaustion of the endogenous bile acid pool. After approximately 3 hours bile acid output stabilized at 5% of its initial value, representing hepatic synthesis rate. During long-term bile diversion, bile acid output gradually increased, but never exceeded 13% of its value during intact enterohepatic circulation.

Treatment of rats during long-term bile diversion with mevinolin, a potent blocker op HMG-CoA reductase, caused a rapid, dose-dependent and reversible reduction of hepatic bile acid synthesis, which could be abolished by simultaneous administration of mevalonolactone. Maximal inhibition reached a level of 79% and the formation of chenodeoxycholic acid was affected most. In contrast, the synthesis of β-muricholic acid, a primary bile acid in the rat, was not affected by mevinolin.

Intravenously administered human low density lipoproteins (LDL), radiolabeled with $^3$H-cholesteryloleate ($^3$H-CO), were cleared slowly by the liver, mainly by Kupffer cells. After 3 hours only $0.9 \pm 0.2$% of the injected radioactivity had appeared in bile, after 12 hours this value was $4.5 \pm 0.5$%. Induction of LDL (apo B,E) receptors on hepatocytes by 17α-ethinylestradiol (EE) treatment accelerated hepatic uptake of $^3$H-CO from LDL. Biliary excretion was increased to $3.9 \pm 0.5$% after 3 hours and $12.4 \pm 2.0$% after 12 hours. Both in untreated and EE-treated animals biliary radioactivity derived from $^3$H-CO was

mainly in the form of bile acids. Specific activity of the excreted bile acids was 2.5 times higher in EE-treated rats than in untreated controls.
Similar results were obtained using liposomes as artificial vehicles for $^{3}$H-CO. Thus, large multilamellar vesicles (MLV), composed of cholesterol, sphingomyelin, phosphatidylserine and $^{3}$H-CO in a 49½:40:10:½ molar ratio, were cleared very rapidly by Kupffer cells ($t_{½}$~1 min). After 3 hours only 1.9±0.2% of the injected radioactivity had appeared in bile. Small unilamellar vesicles (SUV; cholesterol: phosphatidylcholine: phosphatidylserine: $^{3}$H-CO, 49½:40:10:½) were directed predominantly to hepatocytes ($t_{½}$~10 min) and the 3 hours biliary recovery was 6.5±1.2%. Again, biliary radioactivity was mainly in the form of bile acids. SUV-derived radioactivity was found markedly enriched in the muricholic acid fraction; MLV-Co was mainly converted to cholic acid.
In conclusion: newly synthetized hepatic cholesterol is quantitatively the main source for bile acid synthesis in the rat, although a specific bile acid, β-muricholic acid, is highly dependent on pre-existing cholesterol. Uptake of CO by hepatocytes is followed by a relatively efficient excretion of its derivatives, i.e. bile acids, into bile. Kupffer cell uptake is less efficiently coupled to biliary excretion, and probably depends on the rate of cholesterol transport from Kupffer cell to hepatocyte.

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KETOGENESIS AND CARBOHYDRATE AVAILABILITY

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It is now well-established that ketone bodies are important alternative substrates to glucose in mammalian tissues, in particular the brain (1). Under normal conditions, as the availability of glucose decreases (e.g. during fasting) the level of ketone bodies in the circulation increases and their utilization , which is concentration-dependent, is enhanced. When carbohydrate is made available again, the concentration of ketone bodies rapidly falls. The key question is how this reciprocal relationship between these respiratory substrates is achieved?
The available evidence indicates that it is brought about by changes in concentration of plasma hormones which in turn alter metabolism in adipose tissue and liver (2). As the blood glucose decreases there is a concomitant decrease in plasma insulin which in turn results in a stimulation of lipolysis in adipose tissue. One of the most potent effects of insulin being its antilipolytic action in this tissue. Increases in plasma glucagon or catecholamines (e.g. as a consequence of hypoglycaemia) relative to the prevailing insulin concentration will also increase lipolysis and flux of fatty acids from adipose tissue.
Long-chain fatty acids derived from adipose tissue are the major precursors of ketone bodies and therefore whenever the flux to the liver increases the rate of ketogenesis might be expected to increase. However, the fate of fatty acids within the liver is finely regulated by the hepatic carbohydrate availability as well as to external signals (insulin versus glucagon) (2). Thus long-chain fatty acids can either be esterified and secreted as VLDL (high carbohydrate state) or enter the mitochondria for oxidation to ketone bodies or $CO_2$ (low carbohydrate state).
The integration of fatty acid and carbohydrate metabolism in the liver is brought about by changes in the concentration of malonyl-CoA, an intermediate in the de novo synthesis of fatty acids from glucose (or lactate) (2). This metabolite inhibits the activity of carnitine acyltransferase I (CAT I) which initiates the entry of long-chain fatty acids into the β-oxidation pathway. Insulin can increase the concentration of malonyl-CoA by activation of the enzyme, acetyl-CoA carboxylase, which controls its synthesis.
Glucagon has the opposite effect. Recent studies indicate that CAT I is less sensitive to malonyl-CoA inhibition in insulin-deficiency or fat feeding, which are associated with increased ketogenesis. Glucagon can also activate CAT I by covalent modification (phosphorylation) (3) and it is possible that this may be responsible for the decrease in sensitivity to malonyl-CoA.
This overview of the integration of ketogenesis and carbohydrate availability will be discussed in relation to two inborn errors of hepatic carbohydrate metabolism, namely glycogen synthetase deficiency (4) and glucose-6-phosphatase deficiency (5) which are associated with hypoglycaemia but widely different concentrations of blood ketone bodies.

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THE GLUCOSE PARADODX: IS GLUCOSE THE PRECURSOR OF LIVER GLYCOGEN?

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The consumption of carbohydrate after a fast is followed by glycogen deposition in the liver, and carbohydrate feeding induces hepatic lipogenesis. Common sense suggests that glucose is the precursor for glycogen and fat; and indeed the direct conversion of glucose into glycogen via hexose phosphate and UDP glucose is depicted in most textbooks. However, a large body of experiments shows conclusively that a major part of glycogen is not directly derived from glucose but from 3 carbon precursors formed by glucose cleavage. Such a pathway appears energetically wasteful, and hence has been designated the "glucose paradox". I present here the experimental evidence for the occurrence of the indirect pathway, and I dwell upon the unsolved problems in the area. Relevant literature references are found in two recent reviews (1,2).
The evidence in vivo and in vitro for the limited capacity of liver to take up glucose even in the presence of a substantial glucose load and to convert it directly to glycogen is as follows: a) Isolated perfused liver and hepatocytes have a high capacity for gluconeogenesis, but show very little net uptake of glucose at physiological concentrations (below 15 mM), and form virtually no glycogen when