Interactions between carbohydrate and lipid metabolism in metabolic disorders

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Carbohydrates and fat represent the most important sources of energy for the human body. Through a fine intricate system of regulation, the body is able to select to what extent it will use either fatty acids or carbohydrates for generation of energy in the form of adenosine-triphosphate (ATP). Breakdown of glucose through the process of glycolysis produces potential precursors for the synthesis of fat, i.e., \textit{de novo} lipogenesis, and of cholesterol. On the other hand oxidation of fat results nearly exclusively in acetyl-CoA, which is not a precursor for the synthesis of glucose, a process that is called gluconeogenesis. However, more and more data indicates that fat are involved in regulation of glucose metabolism and \textit{vice versa}. This thesis is focused on the interactions between carbohydrate and lipid metabolism and especially on the regulation of \textit{de novo} lipogenesis and cholesterogenesis by carbohydrates and the regulation of gluconeogenesis by fat. A special regulatory role for glucose-6-phosphate, an intermediate in the gluconeogenic and glycolytic pathway, in the regulation of \textit{de novo} lipogenesis is suggested. Novel stable isotope techniques have been developed and used to determine the lipogenic, cholesterogenic and gluconeogenic rates in animal models as well as in humans.

In chapter 2 the importance of the hepatic \textit{de novo} lipogenesis and cholesterogenesis is determined in prematurely born infants. Hepatic \textit{de novo} lipogenesis has been found to be an unimportant pathway in adult humans on a western diet, but might be quantitatively important during the fetal period, since 16\% of a full-term baby consists of fat at the time of birth. During this period the unborn infant receives its nutrition primarily through placental delivery of carbohydrates. Preterm infants were infused with a stable isotopically-labeled precursor of cholesterol and fatty acids ($^{13}$C-acetate) during the first 48 hours after birth when dietary intake consisted almost entirely of glucose. The distribution of label incorporation was measured and analysis of the mass isotopomer distribution (MIDA) was performed to determine hepatic \textit{de novo} lipogenesis and cholesterogenesis. Hepatic \textit{de novo} lipogenesis represented only 5\% of fatty acids in secreted very-low-density lipoprotein particles, indicating that this pathway is not quantitatively important during in preterm infants. Extrahepatic lipogenesis, possibly in adipocytes, might contribute to the fat accumulation during gestation. Hepatic cholesterogenesis, however, was about three times the rate per kilogram bodyweight as found in healthy adult subjects. These results might suggest that cholesterogenesis is a quantitatively important pathway in late fetal life.

Glycogen storage disease type 1 is a disease caused by deficiency of the glucose-6-phosphatase (G6Pase) enzyme complex. G6Pase catalyzes the conversion of glucose-6-phosphate (G6P) into glucose and represents the final step in glucose production from either glycogen breakdown or gluconeogenesis. GSD-1 can be separated into at least two distinct types of diseases, \textit{i.e.}, types 1a and 1b, on the basis of the underlying gene defects. The catalytic subunit of the G6Pase complex is deficient in GSD-1a, whereas the G6P translocase, responsible for transport of G6P from cytosol into the lumen of the endoplasmic reticulum, is deficient in GSD-1b. GSD 1 patients do not only suffer from hypoglycemia, but also develop severe hypercholesterolemia and hypertriglyceridemia. We
chose to investigate the origin of the abnormalities in lipid homeostasis in GSD-1 patients (chapter 3). By using the same methodology as used in chapter 2, it was shown that GSD-1 patients had strongly upregulated rates of hepatic cholesterogenesis and de novo lipogenesis, compared to healthy adult subjects. However, hepatic de novo lipogenesis could not fully account for the observed hypertriglyceridemia in these patients. Furthermore, despite their severe atherogenic lipid profile most studies indicate that GSD-1 patients are not prone to early development of atherosclerosis. An additional finding in our study was that lipoproteins from GSD-1 patients have a lower oxidizability rate than lipoproteins from healthy adult subjects. Oxidation of lipoproteins is a key step in the formation of atherosclerotic lesions and its rate is related to the fatty acid composition. In our study GSD-1 patients were found to have strongly elevated synthesis rates of palmitate, a saturated fatty acid. We found an inverse correlation between the relative amount of saturated fatty acid of lipoproteins and their oxidizability. Therefore this study provides data indicating that GSD-1 patients are protected from early development of atherosclerosis by high rates of hepatic de novo lipogenesis.

In chapter 4, an animal model of GSD-1 was used to further clarify the origin of the hyperlipidemia in this disease. A class of chlorogenic acid derivatives had recently been developed for the treatment of hyperglycemic conditions in DM2. These compounds specifically inhibit G6Pase activity by blocking the translocase of the G6Pase complex and thereby increasing hepatic G6P levels. One of these compounds, S4048, was infused in moderately fasted rats to address the question whether acute increases in hepatic G6P levels were associated with increased rates of de novo lipogenesis and cholesterogenesis and whether this would affect hepatic VLDL secretion. Acute inhibition of G6P translocase indeed led to strongly increased rates of de novo lipogenesis as determined by MIDA, i.e., more than ten times compared to untreated rats. However, cholesterol synthesis rates were not affected by inhibition of G6P translocase. Finally, although studies indicated that high rates of de novo lipogenesis are associated with increased rates of hepatic VLDL secretion, these rates were similar between our two study groups, contributing to the observed hepatic steatosis in the S4048-treated rats. From this study we concluded that intrahepatic G6P elevation can lead to a stimulation of de novo lipogenesis within a short-time frame, but that the increase in de novo lipogenesis is not sufficient to stimulate VLDL secretion.

Diabetes Mellitus type 2 (DM2) is associated with increases in cholesterol, and triglyceride concentrations. A model for DM2 is the ob/ob mouse, which is characterized by leptin-deficiency. These mice suffer from fasting hyperglycemia and hyperinsulinemia as well as from hypercholesterolemia and hypertriglyceridemia. In chapter 5 this model was used to further clarify the interactions between glucose and lipid metabolism. It was found that hepatic de novo lipogenesis was strongly increased in ob/ob mice compared to lean littermates and contributed to the hypertriglyceridemia. However, cholesterogenesis was similar between the two groups despite increases in plasma concentrations and hepatic content. Basal hepatic VLDL secretion was also similar in ob/ob mice and lean littermates. Only under influence of acute hyperinsulinemia VLDL secretion was less suppressed in ob/ob mice compared to lean littermates. As found under circumstances of acute elevation
of hepatic G6P content, increases in hepatic *de novo* lipogenesis are not sufficient to upregulate VLDL secretion. Whether a lack of increase in cholesterogenesis limits the amount of VLDL particles secreted remains speculative.

In chapter 6 different labelling techniques were used to answer the question to what extent hepatic gluconeogenesis, glucose cycling and peripheral clearance of glucose contribute to hyperglycemia in an animal model of DM2, *i.e.* the *ob/ob* mouse. Mice were fasted for 9 hours after which they were infused with a sterile solution, containing [U-13C] glucose, [2-13C]glycerol, [1-2H]galactose and paracetamol. By analysis of glucose and paracetamol-glucuronide from respectively bloodspots and urine samples taken throughout the infusion period metabolic flux rates could be calculated. The rate of *de novo* synthesis of G6P in obese mice was significantly decreased in comparison with lean control mice. Partitioning of newly synthesized G6P towards plasma glucose or glycogen was not affected in obese mice when compared to lean control mice. In contrast, glucose cycling was greatly enhanced in obese mice. As a consequence, total hepatic glucose production, *i.e.*, the sum of endogenous glucose production and glucose cycling, was similar in obese and lean mice. Furthermore, metabolic clearance rate of glucose was strongly decreased in *ob/ob* mice compared to control mice. In conclusion, this study demonstrated that in *ob/ob* mice *de novo* synthesis of glucose-6-phosphate was diminished while glucose cycling was increased, resulting in a "normal" total glucose output by the liver. However, these normal values were observed in face of hyperglycemia and hyperinsulinemia pointing to a co-existence of hepatic and peripheral insulin resistance with peripheral insulin resistance as the major cause of hyperglycemia.

Peroxisome proliferator activated receptors (PPARs) are a group of proteins that regulate expression of genes and are activated by fatty acids and fibrates. PPARα regulates the expression of a variety of proteins involved in β-oxidation and lipoprotein metabolism. Interestingly, mice deficient in PPARα (*Pparα-/-*) suffer from severe hypoglycemia during fasting. In a study in *Pparα-/-* mice it was tried to clarify the exact disturbances in carbohydrate metabolism in order to gain insight in the relation between β-oxidation and hepatic glucose production (chapter 7). The same methodology as in chapter 6 was used for this study. Hepatic glucose production was lower in *Pparα-/-* compared to *Pparα+/+* mice after a moderate fast. However, total *de novo* synthesis of G6P was similar between the two groups. Altered partitioning of G6P, which was preferentially directed towards hepatic glycogen stores, was responsible for the observed decrease in hepatic glucose production. This study indicates that β-oxidation and hepatic *de novo* production of G6P are not directly metabolically linked processes.

In conclusion, the studies described in this thesis have given additional insight in the complex interactions that exist between carbohydrate and lipid metabolism. Hepatic *de novo* lipogenesis is not a major pathway in early and adult human life even under circumstances of high carbohydrate intake and might only be a quantitatively significant in specific metabolic disorders, such as GSD-1 and DM2. G6P, an intermediate in the gluconeogenic pathway, might be an important factor in the regulation of hepatic *de novo* lipogenesis by carbohydrates. *De novo* synthesis of G6P is probably not transcriptionally
regulated by fat through PPARα, but PPARα does play a role in the partitioning of G6P. Since data on transcriptional regulation not necessarily corresponded with kinetic data, these studies understate the need to combine molecular with kinetic research to come to any conclusions regarding metabolic regulation.