Interactions between carbohydrate and lipid metabolism in metabolic disorders

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General discussion

Part of this general discussion has been submitted
The economics of science

The incidence of obesity and type 2 diabetes both in adults and children has grown to pandemic proportions in recent years. More than one billion people is overweight and more than half of black children and one third of white children are overweight by the age of 19 in the United States. Obesity is associated with development of type 2 diabetes and atherosclerosis. Over-consumption of (energy-rich) foods, in addition to lack of physical activity, especially in industrialized societies, has created an ever-growing number of people suffering from diseases such as diabetes and atherosclerosis. There is great irony in the rise in incidence and handling of obesity, diabetes and atherosclerosis on the one hand and the worldwide poverty-related malnutrition on the other hand. Millions of people worldwide still lack adequate nutrition, causing high death rates among infants and high malnutrition-associated morbidity. The World Health Organization recently reported that 27% of all children under the age of 5 are underweight.

One can postulate that market economies stimulate the prevalence of obesity, since most industries benefit from over-consumption of food, from agriculture to drug industries. In the U.S. more than 3800 kilocalories per person per day is produced for national consumption, which is far more than is necessary for most adults. With the obvious relationship between the amount food consumption and the incidence of obesity, health measures to promote lower food intakes are of pivotal importance, next to stimulation of physical exercise. Despite the fact that an increased morbidity of especially the working population has a negative economic impact, a lack of economic incentive still might exist for preventive policies for obesity.

The economic aspects of food consumption and obesity might also influence research in two ways. First of all, available research funds related to obesity are mostly allocated to projects on possible treatments of obesity, type 2 diabetes and atherosclerosis. Although this will benefit scientific knowledge on metabolism greatly and health to some degree, focusing research funding more on prevention might be more sensible with respect to general health benefits. Secondly, since such large amounts of funding are being directed to obesity-related research, this might provide conflicts of interests for research institutes. Promising hypotheses for treatment of obesity or type 2 diabetes are likely to increase levels of funding related to such hypotheses. Research institutes might be tempted to go along with such hypotheses even when they are no longer valid. It might also stimulate researchers to overreport positive data and underreport negative data. One therefore must remain critical to the “why” and “how” of obesity-related research and to available research data and interpretation of this data.

The increasing need for fluxomics in metabolic research

“Metabolism” comprises a complex network of events, with direct and regulatory links between many metabolic routes in protein, carbohydrate and lipid metabolism. Regulation
of these processes has been the point of interest for many researchers in the past and present. Questions such as: “What controls the rate of glucose production?” have been prominent. During the last years, impressive progress in molecular biological techniques and, particularly, in the possibilities for “functional genomics” have provided tools for novel approaches in metabolic research. Functional genomics is currently widely used in biochemical and biomedical literature to describe relationships between genome and phenotype of cells or whole organisms and encompasses the various “omics” approaches, i.e., transcriptomics (mRNA or gene expression profiling), proteomics (describing the protein complement) and metabolomics (describing the organic metabolic complement). The systematic analysis of these classes of molecules in cells or organs (by “bioinformatics”) is often presented as a description of a complex metabolic process or even as an explanation of disturbances herein. As eloquently pointed out by Hellerstein in two excellent reviews, these approaches are based on the assumption that the molecular parameters that are measured by these omics approaches actually represent, and therefore may reveal, “real” sites of metabolic control. However, a wealth of information is available to indicate that this may not be the case: even a complete picture of gene expression and proteins in a living system will not reveal in a predictable or reliable manner the activity of the integrated metabolic pathways that they comprise. One must realize that metabolic regulation does not only take place at a molecular level which means that a priori transcriptomics are limited in the sense that they cannot account for all metabolic changes taking place. Regulation taking place at a time scale of seconds to minutes is performed by reversible binding of metabolites to enzymes and activation and deactivation of enzymes through covalent modification. Protein levels can be regulated, not only by their rate of synthesis, but also by modulation of the rate of degradation. For example, secretion of Apolipoprotein B, a structural component of very-low-density lipoproteins, by liver cells appears to be regulated exclusively by changing the rate of its intracellular degradation. Another limitation of studying transcriptional regulation of one enzyme, is that multiple enzymes are usually involved in a specific metabolic pathway. It has long been thought that there must be rate-controlling enzymes in metabolic pathways, visualized as a chain with the weakest link controlling its strength. However, in recent years, evidence has grown towards the idea that the rate-limiting enzyme theory is not sufficient to explain the regulation of metabolism. Rather, it has been proposed that quantitative scales for the influence of an enzyme exist in metabolic flux regulation. All enzymes have an influence on the rate of a certain metabolic flux and they all might have multiple possibilities for regulation and interaction with feedback and feedforward systems. Regulatory mechanisms for metabolic pathways are therefore often extremely complex with many points of interaction and integration. It is therefore virtually impossible to accurately predict the effect of alteration of one protein in that pathway on whole body kinetics. Studying protein levels or intermediates or endproducts of a metabolic cascade, i.e., proteomics and metabolomics, respectively, have the shortcoming that they do not explain why or how a protein or product levels are decreased or increased and, furthermore, these levels represent static parameters that are dependent not only on production but also on uptake and/or
breakdown of that product or protein. One continuously has to realize that changes in RNA and or proteins will only in a limited way predict the changes for a metabolic pathway as a whole. Therefore, over-interpretation of data acquired through a reductionist approach, i.e., by analyzing separate elements of a metabolic pathway, can often lead to incorrect conclusions about the pathway as a whole.

Therefore, although it is evident that important new information is being generated by the current “omics” approaches, it is important to realize that full understanding of control of metabolic pathways and how they are affected by genetic and acquired diseases or drugs, requires additional experimental strategies. Measurement of molecular fluxes through stringently defined metabolic fluxes in vivo, which can be referred to as “fluxomics”, provides such a strategy. This advantage of fluxomics using either radioactive, but more preferably stable isotopes, is that it is dynamic in nature and, if desired, can encompass the entire metabolic pathway that one is interested in. By labeling a precursor and measuring the incorporation into its endproduct in vivo, one is able to accurately calculate the rate of the flux of that metabolic pathway. Data obtained through fluxomics represents the sum of all transcriptional and posttranscriptional points of regulation in an in vivo situation. During the last decades, various approaches for metabolic flux measurements in humans and in experimental animals have been developed for this purpose. Particularly in combination with techniques and/or novel drugs that specifically alter expression of individual genes or series of genes involved in certain pathways, these approaches have and will contribute to our understanding of metabolic control in health and disease. In the following, some recent examples from our laboratory will be presented to delineate the usefulness of this combined genomics-fluxomics approach in metabolic research.

Gluconeogenesis, i.e, *de novo* synthesis of glucose by liver and kidney, is a tightly controlled process in which insulin plays a pivotal role as a powerful suppressor of this pathway. Recent data suggests that certain transcription factors are also involved in the regulation of the gluconeogenic pathway. Peroxisome proliferator-activated receptor alpha (PPARα, NR1C1) is a nuclear receptor that is activated by fatty acids and fibrates and that promotes expression of various genes involved in fatty acid oxidation among other genes. PPARα has also been suggested to be involved in transcriptional regulation of gluconeogenesis by inducing gene expression of phosphoenolpyruvate carboxykinase (PEPCK), an enzyme in the gluconeogenic pathway, although evidence for direct transcriptional control of the PEPCK gene is lacking. Indeed, PPARα knockout (Pparα−/−) mice were shown to suffer from fasting-induced hypoglycemia supporting this concept. The liver X receptor (LXRα, NR1H3; LXRβ, NR1H2) is another nuclear receptor involved in the regulation of gluconeogenesis and also belongs to the superfamily of nuclear hormone receptors. LXR is activated by physiological concentrations of oxidized derivatives of cholesterol. LXR itself has been shown to suppress PEPCK gene expression supposedly by direct inhibition of transcription. To make matters even more complex, recent data indicates that PPARα inhibits LXR signalling. If one takes all available molecular data together, one would expect decreased gluconeogenesis in Pparα−/− mice. In a recent study we infused stably labeled glucose and a gluconeogenic precursor (glycerol) for
6 hours and determined its incorporation into glucose throughout the infusion period at regular intervals in moderately fasted \( \text{Ppar}^{\text{α}-/-} \) and control mice.\(^{16} \) Through analysis of the incorporation of both labels over time it was possible to calculate rate of gluconeogenesis. This study showed that PPAR\( \alpha \)-deficiency was not associated with decreased but rather with similar rates of gluconeogenesis up to glucose-6-phosphate (G6P).\(^{16} \) Using a similar technique, Xu et al.\(^{17} \) determined endogenous glucose production in \( \text{Ppar}^{\text{α}-/-} \) mice and found this to be even increased compared to control mice. Stimulating LXR by pharmacological means, did also not lead to changes in the gluconeogenic flux in mice although mRNA levels of PEPCK were slightly decreased (unpublished data). Taken together, these data imply that the transcription factors LXR and PPAR\( \alpha \) are not of pivotal importance to maintain a normal gluconeogenic flux or able to solely inhibit this flux, although this was expected based on molecular data alone. Compensatory mechanisms must be present or, alternatively, the known molecular modes of regulation are not quantitatively important for the metabolic flux as a whole.

A second example addresses control of bile salt metabolism, which forms an important component of cholesterol homeostasis in the body. Bile salts are synthesized in the liver from cholesterol, after which they are excreted into bile. Bile salt synthesis and metabolism is controlled by at least two transcription factors, \( i.e. \) the nuclear receptor LXR and the Farnesoid X receptor (FXR, NR1H4).\(^{18,19} \) A primary enzyme in the bile salt synthetic cascade is cholesterol 7alpha-hydroxylase. Molecular research has shown that LXR is involved in stimulation of transcription of cholesterol 7alpha-hydroxylase (Cyp7a1) in rodents, but not in humans.\(^{20,21} \) FXR appears to be essential in downregulating Cyp7a1 transcription that is mediated through a mechanism involving two nuclear hormone receptors, \( i.e. \) liver receptor homolog 1 (LRH-1, NR5A2) and small heterodimer partner (SHP, NROB2).\(^{18,22-24} \) FXR is activated by bile salts that return to the liver during their enterohepatic circulation and acts by activating transcription of SHP. SHP subsequently binds and inactivates LRH-1, thereby repressing transcription of Cyp7a1.\(^{24} \) Furthermore, FXR stimulates transcription of the ileal bile acid-binding protein transporter (IBABP), which is a small soluble protein and is expressed in the terminal ileum.\(^{25,26} \) IBABP is thought to be involved in facilitating uptake of bile salts and their intracellular trafficking in the small intestine\(^{27} \), thereby assisting in preserving the bile acid pool. Based on transcriptional data, one might expect strongly upregulated bile salt synthesis and decreased intestinal bile salt uptake in Fxr\(^{−/-} \) mice. However, a recent study showed that the effects of FXR deficiency on the basal expression of Cyp7a1 (+150%) and on bile salt synthesis (+67%) were relatively modest.\(^{28} \) Furthermore, intestinal bile salt uptake was even increased in Fxr\(^{−/-} \) mice in this study. These results showed that although FXR has important regulatory functions, the effects of activation of this transcription factor on bile salt metabolism cannot be predicted on transcriptional data alone.

Transcriptional research suffers from apparent limitations as the data on transcriptional regulation of gluconeogenesis and bile salt synthesis and uptake showed. Apart from fluxomics all other “omics” research is static and results clearly cannot be directly extrapolated to a dynamic in vivo situation. Numerous fluxomics techniques have been
applied sofar, including labeled water techniques and mass isotopomer distribution analysis or MIDA\textsuperscript{29}, which involves use of probability logic to ultimately calculate synthetic rates. Molecular changes that do not lead to changes in the overall rate of production along a certain metabolic pathway, due to counter regulatory mechanisms or lack of regulatory importance in an in vivo situation, will not be detected using fluxomics. Fluxomics is therefore of pivotal importance for verifying the predictions that are made based on results obtained from transcriptomics, proteomics and metabolomics. A schematic representation of fluxomics and other levels of metabolic research are schematically depicted in Figure 1. The examples of transcriptional regulation of gluconeogenesis and bile salt synthesis underscore the fact that combined approaches are needed to be able to fully comprehend the complex regulatory mechanisms involved in a metabolic pathway. Fluxomics is an indispensable tool for integrating data obtained through other levels of metabolic research and to reach conclusions on metabolic regulation that represent true physiological in vivo systems.

\textbf{Figure 1. Levels of metabolic research}

The effects of glucose on lipid metabolism

This thesis comprises studies on metabolic regulation of carbohydrate and lipid metabolism. In these studies physiological and pathophysiological regulation is examined, when possible, at the transcriptional and post-transcriptional level combined with measuring actual fluxes.
Two central questions guided the research presented in this thesis:

1. How are cholesterogenesis and de novo lipogenesis regulated, and what is the role of carbohydrates herein?
2. How are gluconeogenesis and hepatic glucose production regulated, and what is the role of fatty acids herein?

Better insight in these regulatory processes will be helpful in understanding the metabolic changes occurring in diseases as type 2 diabetes, glycogen storage disease type 1 and other metabolic disorders. Multiple animal models and patient groups were used in trying to answer these questions. For measuring metabolic fluxes novel stable isotope methodologies were developed and used.

A long-standing discussion remains on whether hepatic de novo lipogenesis is of quantitative importance in human life. Hepatic de novo lipogenesis was studied under three conditions in this thesis to add to the discussion on this issue, i.e., prematurity, glycogen storage disease and type 2 diabetes. One unanswered question is whether hepatic de novo lipogenesis is important in late intrauterine and early postnatal life, since it has been shown to be of little quantitative importance in adults on a western diet. Our data showed that hepatic de novo lipogenesis was present in pre-term infants, but at very low levels. This is in a sense remarkable, since at the time of the study, infants received almost solely carbohydrates as nutritional intake, which is supposed to increase lipogenic rates. It is known that fatty acid synthesis rates are regulated by various transcription factors, i.e., SREBP1a, 1c, LXR and ChREBP, with the latter one activated by glucose or one of its metabolic derivatives. Whether developmental aspects of this transcriptional pathways account for the lack of high rates of hepatic de novo lipogenesis remains to be seen. This finding also raises questions about how lipid accumulation in the fetus during the third trimester of pregnancy takes place. Some reservations must be included when extrapolating data from early postnatal, prematurely born, life to late intrauterine life. Taken into account these reservations, our data might suggest that extrahepatic lipogenesis contributes to accumulation of lipids during late fetal life. Lipogenesis has been shown to occur in adipocytes, although probably at very low rates in adult humans, but could theoretically be more active during certain early periods in life. On the molecular regulation of lipogenesis in adipocytes, insight is starting to evolve. Recent studies suggest that PPARγ and LXR, which are both expressed in adipocytes, are involved in the regulation of lipogenesis in these cells. Whether these transcription factors are important in the lipid accumulation during fetal life remains to be elucidated but provides a promising hypothesis. Studies using nuclear magnetic imaging techniques might help to elucidate the quantitative role of adipocyte de novo lipogenesis early in human life.

The metabolic state of patients with glycogen storage disease type 1 (GSD-1) is different from that of pre-term infants in many respects, although both states are associated with a high net hepatic glucose uptake. In GSD-1 patients, glucose entering the liver is in a sense trapped and must be metabolized either to glycogen or to acetyl-CoA. In both the
GSD-1 patients and the rat model of GSD-1, we did find strongly elevated levels of hepatic de novo lipogenesis. The transcription factor ChREBP might play a role in the induction of hepatic de novo lipogenesis in GSD-1, since ChREBP responds to changes in intracellular carbohydrate contents. However, in GSD-1, intrahepatic glucose concentrations are lower, because deficient glucose production is an intrinsic part of the disease. An intriguing hypothesis might therefore be that G6P is involved in transcriptional activation of lipogenesis. During the last years several studies have indicated a strong metabolic regulatory function for glucose-6-phosphate (G6P). However, an alternative hypothesis is that another intermediate in hepatic glucose metabolism, i.e., xylulose-5-phosphate (X5P) plays a role as metabolic regulator. Future research is needed to assess if and how G6P might be to perform this regulatory role. Furthermore, it remains to be determined whether G6P/X5P is a specific ligand for ChREBP and what the role is of ChREBP in the development of increased hepatic de novo lipogenesis in GSD-1.

Strongly induced hepatic de novo lipogenesis was found in a type 2 diabetic model, i.e., the ob/ob mouse, which could be attributed to an induced expression of at least one transcription factor, namely SREBP-1c. The expression of the genes encoding for LXR and ChREBP were not induced, although this does not exclude activation of the proteins. Using specific inhibiting systems it should be possible to distillate the relative importance of the various transcription factors in the regulation of de novo lipogenesis in the physiological and diabetic state. Hepatic de novo lipogenesis might not be an important pathway in healthy adults on a western diet, but can be induced substantially in various disease states. An overview of the molecular mechanisms involved in regulation of hepatic de novo lipogenesis are shown in Figure 2.

Cholesterogenesis was studied in the same metabolic situations as hepatic de novo lipogenesis. Cholesterogenesis is transcriptionally regulated almost entirely by SREBP2. In pre-term infants increased rates of cholesterogenesis were found, when compared to adult subjects. Whether preferential development of transcriptional activation of the cholesterogenic pathway is involved in attaining the high rates of cholesterogenesis is unknown. With respect to the situation of GSD-1, only in the GSD-1a patients and not in the animal model of GSD-1b, evidence for increased cholesterogenesis was found. Why cholesterogenesis was upregulated in the GSD-1a patients and not in the rat GSD-1b model remains unclear. Is it related to differences between the acute model in the rats and the chronic patients? In the GSD-1 patients, a “diabetic” plasma lipid profile is present, with hypertriglyceridemia, low HDL-cholesterol, high LDL-cholesterol and decreased VLDL clearance. In addition, indications exist that these patients are insulin resistant, of course without the hyperglycemia normally present in DM2. Since it is known that in DM2 patients cholesterogenesis is elevated, the high cholesterol synthesis rates in GSD-1a patients might be related to the insulin resistance. However, in the animal model used for DM2, i.e., ob/ob mice, no increased rates of cholesterogenesis were found, although plasma cholesterol concentrations were increased. No clear explanation for the lack of increased cholesterogenesis is present at the moment. Since, other proteins (SCAP, S1P, S2P) are required for SREBP processing, overactivation of SREBP1c or SREBP1a to a higher extent
Much debate remains on whether hepatic cholesterogenesis and de novo lipogenesis have a regulatory role in VLDL production and thereby development of hyperlipidemia. The increases in synthesis rates of cholesterol and fatty acids could only partially explain the hyperlipidemia in GSD-1a patients. We hypothesize that increased lipolysis and increased VLDL production account for the hyperlipidemic phenotype in GSD-1a. G6P might also be play a role in this respect, since Brown et al. found that phosphorylation of glucose is essential for upregulation of VLDL secretion by glucose.48 However, we found no indication of increased hepatic VLDL secretion in an acute animal model of GSD-1b. There are some considerations with respect to this finding. First of all, the studies were performed in an acute animal model, whereas GSD-1 patients of course suffer from the disease chronically. It might be that the experiments were not long enough to observe changes in VLDL secretion. Furthermore, rats used in this study were fasted, causing elevation of FFA flux to the liver and perhaps already a maximal stimulation of VLDL secretion. An alternative hypothesis is that compartmentalization of G6P is responsible for the effects of G6Ptranslocase inhibition as is schematically shown in Figure 3. In GSD-1a,
Figure 3. Schematic overview of the G6Pase enzyme complex in GSD-1a (A) and GSD-1b (B).

G6P is able to enter the luminal side of the endoplasmic reticulum via interaction with G6P translocase. However, the formation of glucose is inhibited due to deficiency of the G6Pase catalytic subunit. Theoretically, this situation will lead to elevated levels of G6P inside the ER. In GSD-1b, G6P is unable to enter the ER, due to deficiency of G6P translocase, causing elevated levels of G6P inside the cytosol, but absence of G6P inside the ER. Since at least the early steps in VLDL formation take place inside the ER, fluctuations in G6P content in this compartment might influence VLDL formation. Determining VLDL secretion under the circumstance of specific inhibition of G6P translocase or G6Pase might
help to unravel the importance of G6P compartmentalization for lipoprotein assembly and/or secretion.

Recently, a mouse model for GSD-1a was created. Homozygous $G6Pase^{-/-}$ mice suffer from severe hypoglycemia and newborn pups die soon after birth without continuous exogenous supply of glucose. The severity of the disease makes in vivo studies in adult $G6Pase^{-/-}$ mice practically impossible. Heterozygous $G6Pase^{+/+}$ mice have no apparent phenotype of hypoglycemia or hyperlipidemia. However, when metabolically challenged, a phenotype might become apparent. It would be of interest to determine insulin sensitivity in these mice during, for example, a high-fat diet, which is known to cause insulin resistance in normal mice. In the same line of reasoning, it would also be of interest to study (heterozygous) parents of GSD-1 patients to determine insulin resistance, either by performing glucose tolerance tests, or better, by performing hyperinsulinedemic euglycemic clamps together with carbohydrate flux measurements. If indeed this group of individuals shows a relative insulin insensitivity, it would indicate that even small increases in intrahepatic G6P content is enough to produce insulin resistance. This would perhaps provide more insight in the subtle regulation of carbohydrate metabolism and the primary features occurring in the development of DM2.

Although de novo lipogenesis was increased, basal VLDL secretion was not increased in ob/ob mice with hepatic insulin resistance. This finding underscores the complexity of the mechanisms behind the regulation of hepatic VLDL secretion. Carbohydrates, cholesterol and free fatty acids, all have been found to regulate VLDL secretion. Also SREBP’s have been shown to negatively regulate transcription of an important protein involved in VLDL formation, i.e., microsomal triglyceride transfer protein. SREBP-1c mRNA levels were higher in the ob/ob mice, although SREBP-2 mRNA levels were lower compared to control mice. It would be worthwhile to separate these individual components in an in vivo system, to be able to understand what components are quantitatively important in regulation of VLDL secretion and how they are interrelated. Considering the type of hyperlipidemia observed in GSD-1 and the possible role of ChREBP herein, it is tempting to speculate on a possible role of ChREBP in the phenotype of DM2. Since the functions and ligand(s) of ChREBP have remained largely unknown up to now, future research should be aimed at trying to gain more knowledge on the basic properties of ChREBP.

The effects of lipids on glucose metabolism

The second question that was addressed in this thesis concerned the regulation of gluconeogenesis and hepatic glucose production (HGP) and, especially, the role of fatty acids in this regulation. DM2 is characterized by insulin resistance, leading to fasting hyperglycemia. In addition, hepatic steatosis is a common feature of this disease. The hyperglycemia is related to elevated HGP in DM2. In theory, elevated HGP can be caused by an increase in GNG and/or glycogenolysis. Furthermore, if one defines HGP as the total flux from G6P to glucose, cycling between glucose and G6P could in theory also increase
HGP. We found evidence for highly induced glucose cycling, defined as the cycling between hepatic G6P and glucose, in ob/ob mice. Furthermore, total GNG, defined as the new synthesis of G6P, was significantly decreased in ob/ob mice in comparison with lean control mice, compared to controls. As a consequence, total hepatic glucose production was similar in both mice groups, despite higher insulin concentrations in the ob/ob mice. Insulin is a well-known inhibitor of GNG and a recent study provided molecular evidence for the inhibitory function of insulin on PEPCK.\textsuperscript{51} Insulin resistance could therefore lead to an upregulation of Pepck gene transcription, although this was not observed in the study in the ob/ob mice. Recently, it was discovered that a co-activator of nuclear receptors, PGC-1, and HNF-4α are both responsible for transcriptional activation of Pepck and glucose-6-phosphatase (G6pase).\textsuperscript{52} Activation of PGC-1 has been shown to be present in ob/ob mice\textsuperscript{52}, although we found similar PGC-1 mRNA levels in this study. Although many possible explanations for relatively increased HGP in ob/ob mice are apparent, no conclusive factors can be appointed as of yet.

PPARα is a transcription factor controlling genes involved in fatty acid oxidation, but it has also been hypothesized that PPARα is involved in regulation of hepatic glucose metabolism.\textsuperscript{11} PPARα might provide a molecular link between fatty acid oxidation and glucose production, which could be important for understanding of the pathophysiology of DM2. HGP was decreased in Pparα−/− mice compared to control mice. One can speculate as what caused the specific effect of PPARα deficiency on HGP. It might be that PPARα transcriptionally regulates the G6Pase enzyme complex, although, again, no direct evidence has been produced so far. An alternative hypothesis is that decreases in HGP are related to alterations in glycogen metabolism. Decreased HGP could also be secondary to decreased peripheral glucose utilization. PPARα might be be involved in thermogenesis, either directly or through interaction with leptin. Future studies to study the specific role of PPARα in hepatic G6P partitioning and peripheral glucose utilization are required.

Interestingly, PPARα-deficiency had no effect on total GNG during fasting as already mentioned earlier. Furthermore, an earlier study where the G6Pase flux was pharmacologically inhibited in rats also showed unaffected GNG.\textsuperscript{53} These data suggest that adequate GNG can be maintained under many circumstances, even in the presence of high intrahepatic G6P contents or decreased β-oxidation and that these processes are not directly linked during fasting. Instead of altering the rate of GNG, the liver rather redirects newly formed G6P towards either glucose or glycogen depending on the hormonal status of the organism, which might be related to the regulatory processes mentioned above. The combination of these experiments indicates that other factors are influencing the gluconeogenic flux toward glucose than the molecular regulatory factors known at present. An overview of the molecular mechanisms involved in regulation of GNG and HGP are shown in Figure 4.
**Closing remarks**

Metabolic disorders such as DM2 and GSD-1 represent, in some ways, opposite extremes of a metabolic state, one associated with overexpression of the G6Pase enzyme system (DM2), one with absence of this system (GSD-1). Obtaining a metabolic state associated with limited pathological consequences has been proven difficult in both diseases. Pharmaceutical research in the area of DM2 during the last years has been focused on partial inhibition of the G6Pase system. However, pharmaceutical solutions might not exist with respect to the treatment of obesity and DM2. A study presented in this thesis provided data on the overall metabolic effects of inhibition of the G6Pase system (chapter 4). A drug inhibiting glucose-6-phosphate translocase was developed for treatment of hyperglycemia in DM2. This study showed that partial inhibition of G6Pase caused severe hepatic steatosis in a 6-hour time frame in rats. In a study by Desai *et al.* 54 adenovirus-mediated glucokinase overexpression was achieved to study its effect in type 2 diabetic mice. Although whole body insulin resistance improved in these mice, hepatic triglyceride content did not decrease and hepatic glycogen content increased strongly in comparison to untreated mice. This new phenotype is expected to cause problems of hepatic steatosis and liver fibrosis. These studies underscore the inherent limitations of potential drugs stimulating or inhibiting specific enzymes in treating DM2. Since the various pathways in carbohydrate metabolism...
and lipid metabolism are linked, it is expected that when one pathway is partially blocked by pharmaceutical inhibition, accumulation of an intermediate metabolic product will take place, especially in the case of continuous elevated caloric intake. Pharmaceutical intervention for diseases associated with a western life style, such as obesity and DM2, are therefore limited and perhaps only palliative in nature and should always be used in combination with dietary measures and exercise. This does not remove the relevance of this kind of research for society as a whole, and diseases such as DM2 and GSD-1 specifically, but scientific modesty in this respect is warranted.
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Leo Vroman