Metabolic adaptations in models of fatty liver disease

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Chapter 7

General discussion
The increasing body weight of the population is accompanied by a plethora of medical symptoms together called the metabolic syndrome. Non-alcoholic fatty liver, characterized by persistent storage of lipids in the liver, is regarded as the hepatic component of the metabolic syndrome [2, 3]. Our understanding of how hepatic lipid accumulation or steatosis develops is incomplete. The deposition of hepatic triglycerides (TG) can have different origins that may vary between individuals and different animal models (chapters 1 and 6). Research described in this dissertation examined the origin of hepatic steatosis upon liver X receptor (LXR) activation (chapters 2 and 3) and in case of deficiency of the catalytic subunit of glucose-6-phosphatase (G6PC) (chapters 4 and 5). In chapters 4 and 5 also other metabolic fluxes diverging from G6P in the G6pc-deficient liver were examined, providing new insights into how intra- and extrahepatic metabolism is altered in type I glycogen storage disease (GSD Ia).

**The origin of TG in LXR-induced steatosis**

Due to its potent beneficial effects on plasma cholesterol levels, LXR has been considered an anti-atherosclerotic target [76]. Unfortunately LXR activation induces detrimental side-effects on TG homeostasis. Severe hepatic steatosis and maladaptive changes in plasma very low density lipoprotein (VLDL)-TG concentrations [71] were thought to mainly result from increased *de novo* lipogenesis (DNL) [78, 79]. These assumptions are however based upon gene expression patterns. A dynamic balance between input and output fluxes of the hepatic TG pool is needed to maintain homeostasis of hepatic TG content. Thus, to critically assess which metabolic fluxes determine hepatic TG storage it is important to measure all fluxes contributing to steatosis. Until now, tissue-specific catabolism of TG, which we assume is mostly comprised of β-oxidation of fatty acids, cannot be measured *in vivo*. Therefore, in chapters 2 and 3 we examined the cause of transient effects of LXR activation on VLDL-TG concentrations [71, 78, 93] using a multidisciplinary approach of mathematical modeling combined with *in vivo* animal studies. The mathematical modeling approach ADAPT (Analysis of Aynamic Adaptations in Parameter Trajectories) was especially developed to analyze changes in metabolic pathways over time upon a (pharmacological) intervention.

In chapters 2 and 3 we showed the value of a modeling approach such as ADAPT in unraveling and predicting the sequential origin of accumulating hepatic TG upon LXR activation (Figure 7.1). Upon acute LXR activation, *in vivo* studies showed a remarkable discrepancy: hepatic steatosis was increased together with lipogenic gene expression, but without an enhanced *de*
General discussion

*novo* lipogenic (DNL) flux. ADAPT predicted that in this initial phase of LXR activation hepatic TG accumulation was mainly caused by enhanced hepatic fatty acid influx. Indeed, *in vivo* studies using infusion of stable isotope labeled palmitate showed increased incorporation of labeled palmitate in the livers of mice that were treated with the LXR agonist T0901317. The increased hepatic fatty acid influx is probably caused by increased LXR-induced lipolysis in white adipose tissue [125]. Furthermore, ADAPT indicated that both input and output fluxes of the hepatic TG pool are massively increased upon LXR activation and that only a minor imbalance between the two leads to hepatic steatosis. Also, ADAPT taught us that upon prolonged LXR activation a new balance between input and output fluxes is found, which is corroborated by *in vivo* data showing stabilizing of hepatic TG content and plasma VLDL-TG concentrations in mice after two weeks of T0901317 treatment.

**Increased β-oxidation upon LXR activation?**

As stressed before, to adequately evaluate the basis of steatosis, all metabolic pathways involved in influx to and efflux from the hepatic TG pool should be studied in parallel and *in vivo*. It is not yet possible to study β-oxidation in specific tissues *in vivo*. This is unfortunate, since there are strong indications that hepatic peroxisomal β-oxidation is enhanced upon LXR activation. T0901317 treatment led to increased hepatic expression of genes involved in peroxisomal β-oxidation (Figure 7.2A and [346]) and increased capacity for peroxisomal β-oxidation, as measured *ex vivo* in liver homogenates [346]. Together with an increased supply of fatty acids to the liver ([171], chapter 2), this could likely result in enhanced β-oxidation. Next to this, ADAPT showed that upon LXR activation fluxes that diminish the hepatic TG pool, among which TG catabolism consisting for a large part of hepatic β-oxidation, are strongly induced.

We have other strong experimental indications for increased β-oxidation induced by LXR-activation. After infusing U-[\(^{13}\)C]-palmitate we noticed the appearance of palmitate with two \(^{13}\)C molecules (m+2) in livers of both untreated and T0901317 treated mice (Figure 7.2B), showing that uniformly labeled palmitate undergoes β-oxidation and acetyl-CoA formed in the process can be used for *de novo* lipogenesis. Interestingly, an enhanced hepatic fraction of m+2 palmitate in livers of mice treated for 1 day with T0901317 (Figure 7.2B), suggests a two-fold increase in incorporation of acetyl-CoA derived from β-oxidation into hepatic TG. Simultaneous induction of β-oxidation and DNL has been demonstrated before [62, 347] and may be a protective mechanism
Figure 7.1: The origin of LXR-induced hepatic steatosis. Upon acute LXR activation input and output fluxes of the hepatic TG pool are imbalanced. After prolonged treatment the balance is restored. Acute LXR activation induces a short-lasting increase in hepatic FFA influx, accompanied by increased TG output. A minor imbalance between input and output fluxes leads to hepatic steatosis. Upon prolonged LXR activation DNL is enhanced, with a concomitant further increase in TG output. The balance between input and output fluxes is restored, leading to stabilization of hepatic TG concentrations.

against lipotoxicity [348]. It is however to be determined whether both processes take place in the liver and are enhanced upon LXR activation, or whether the fatty acids end up in hepatic TG after being metabolized in other tissues.

Integrating different levels of regulation in mathematical models
Integration of mathematical models into metabolic research is becoming more common and can be used to tackle the limitations of in vivo animal research. Our studies in chapter 2 and 3 illustrate the value of in silico modeling when combined with in vivo studies. ADAPT was developed to examine dynamic changes in metabolic systems over time. In our case, it balances fluxes con-
Figure 7.2: Indications for increased hepatic $\beta$-oxidation upon LXR activation. A) Hepatic expression of genes involved in peroxisomal $\beta$-oxidation. B) Fragmented enrichment ($m^{+2}$) of hepatic palmitate after infusion of U-$[^{13}C]$-palmitate.

tributing to the hepatic TG pool and fluxes that diminish this pool. A mathematical model contains states which typically represent metabolite concentrations. These states are interconnected via fluxes, representing the transport or conversion of metabolites. The data presented in chapter 3 illustrate that mathematical models such as ADAPT gain predictive capability when transcriptional data are included into the model.

Regulation of metabolic pathways occurs at many different levels that interact and it will be interesting to examine the effect of including more modes of regulation into mathematical models such as ADAPT. In some cases it may even be a necessity. In our mouse model of GSD Ia, for instance, it is probable that hormones and other circulating factors play a large role in altered lipid metabolism. On the other hand, concentrations of metabolites, such as hepatic G6P, and induction of lipogenic gene expression do not show large variability between nutritional states. Therefore the differences in lipid metabolism be-
tween nutritional states may not be captured by modeling of fluxes between metabolite concentrations and inclusion of transcriptional data. In ADAPT fluxes in hepatic metabolism are modeled, not taking into account how other organs are affected by treatment. In GSD Ia the importance of inter-organ communications seems evident from increased circulating apolipoproteins [349] and perhaps other regulatory factors that are secreted by the liver [167]. Also, adipose-tissue derived FFA may drive hepatic fatty acid chain elongation in fasted in L-\textit{G6pc}^−/− mice (chapter 4). Inclusion of inter-organ communication is thus another point on which future studies using ADAPT may focus.

The origin of steatosis in GSD Ia is dependent on nutritional status
In GSD Ia G6PC is inactive, resulting in major changes in metabolic pathways upstream and downstream of G6P. Next to fasting hypoglycemia, this enzymatic defect leads to what are considered as secondary metabolic defects, such as hepatic steatosis, hypertriglyceridemia and hepatic adenomas [82]. In chapter 4 we examined how lipid metabolism differs between feeding and fasting in mice with a hepatic \textit{G6pc} deficiency (L-\textit{G6pc}^−/− mice) and show the importance of the metabolic effects of nutritional status in \textit{G6pc} deficiency. We present large differences in the origin of hepatic TG between fasted and fed L-\textit{G6pc}^−/− mice. While in fed L-\textit{G6pc}^−/− mice hepatic DNL is increased, in fasted L-\textit{G6pc}^−/− mice enhanced elongation of existing fatty acids is found. In fasted L-\textit{G6pc}^−/− mice increased chain elongation was accompanied by enhanced white adipose tissue lipolysis. Increased adipose tissue lipolysis has been shown to result in increased delivery of FFA to the liver [180, 350], suggesting that adipose tissue derived fatty acids contribute to hepatic steatosis in fasted L-\textit{G6pc}^−/− mice. Previous studies have shown increased DNL in 'semi-fed' GSD Ia patients [146, 147] and increased hepatic fatty acid uptake and adipose tissue lipolysis in fasted patients [173], but until now no studies compared nutritional states in \textit{G6pc} deficiency.

Importance of nutritional state in GSD I
To maintain blood glucose levels within a normal range patients are kept on an uncooked cornstarch diet. The slow release of carbohydrates from the gut will mostly suppress circulating glucagon concentrations and keep insulin concentrations normal. Therefore fasting-induced changes in lipid metabolism as seen in our studies in mice (chapter 4) will probably not occur in well controlled patients. It may thus be more physiologically relevant to examine models of GSD Ia in a fed state. Examining metabolic differences between fed and fasted
states can on the other hand be useful to learn more about the mechanisms and consequences of poor metabolic control in GSD I.

Although through dietary treatment hyperglycemic episodes are greatly reduced and many secondary metabolic derangements are corrected by dietary treatment, comorbidities still occur frequently (e.g. [82]). One serious complication affecting GSD I patients is the formation of hepatocellular adenomas (HCA) (e.g. [83]). An interesting feature of these adenomas is that with proper metabolic control their incidence seems to be lower [351] and their size may even be reduced [352, 353]. There are however conflicting reports on this phenomena [83, 354]. Despite these conflicting data, it has been shown that GSD Ia patients more often develop hepatic adenomas than GSD Ib patients, which is found to be strongly associated with hypertriglyceridemia [83], an important marker of metabolic control in GSD I. However, in two distinct liver-specific animal models of GSD Ia hypertriglyceridemia is present until two months of age, but does not persist [86, 87], while in GSD Ia patients hypertriglyceridemia does persist [82]. These mice do however develop HCA at a later age [86, 87]. More research should focus on the conditions in which GSD Ia and Ib patients develop HCA. Animal models of both enzymatic defects may be used to examine the decoupling of derangements in fatty acid metabolism and development of HCA and relationships to metabolic control.

Regulators of lipid metabolism in GSD Ia

In chapter 4 we found indications for regulatory mechanisms behind aberrant lipid metabolism in GSD Ia, which will be briefly discussed in the following section.

Insulin and glucagon

The nutritional differences in lipid metabolism between fasted and fed L-\(G6pc^{-/-}\) mice may largely be explained by differences in glycaemia and its effects on the insulin to glucagon ratio. Regarding adipose tissue, insulin-mediated inhibition of HSL activity will be greatly diminished in fasted L-\(G6pc^{-/-}\) mice, while glucagon will stimulate cAMP-PKA induced lipolysis (reviewed in: [44]). In the livers of fasted L-\(G6pc^{-/-}\) mice \(Apoc3\) and \(Fgf21\) expression were enhanced and plasma FGF21 was greatly elevated. Both circulatory factors are regulated at different levels by insulin and glucagon. The expression of \(Apoc3\) is downregulated by insulin via Forkheadbox O1 (FoxO1) nuclear exclusion [355] and FGF21 release is regulated by glucagon [185, 356].
PPARs
Increased fasting-induced adipose tissue lipolysis in \(L-G\text{6pc}^{-/-}\) mice is likely to lead to an enhanced hepatic influx of fatty acids, creating circumstances for hepatic peroxisome proliferator activated receptor (PPAR)\(\alpha\) and/or \(\delta\) activation [65, 356, 357]. The increase in hepatic expression of \(\text{Angptl4}\) and \(\text{Fgf21}\) is also an indication for PPAR activation [65, 166, 356, 358, 359]. Increments in circulating ANGPTL4 and perhaps FGF21 may further increase lipolysis [65, 67, 187, 360] thereby inducing a self-enforcing PPAR activation during the fasting response in GSD Ia.

Apolipoproteins and ANGPTL4
Though we merely show gene expression increments in \(\text{Apoc1}, \text{Apoc3}\) and \(\text{Angptl4}\) in the livers of \(L-G\text{6pc}^{-/-}\) mice, elevated circulating apolipoproteins have been found in GSD I patients [169, 170, 190, 349]. ApoC1 and apoC3 seem to work in concert with ANGPTL4 to inhibit lipoprotein lipase (LPL) activity. At higher concentrations of apoC1 and apoC3, inhibitory effects of ANGPTL4 on LPL were found to be increased [194]. Next to this, apoC1 and apoC3 displace LPL from lipoprotein particles, reducing its hydrolytic activities. After displacement ANGPTL4 seems to have better access and can irreversibly inactivate LPL by converting active dimers into inactive monomers [194]. This process may contribute to hypertriglyceridemia in GSD Ia. ApoC3 is also likely to be involved in VLDL secretion [196, 197], which may further aggravate hypertriglyceridemia.

ChREBP
In GSD Ia aberrances in glucose metabolism may activate pathways that lead to deranged lipid metabolism. Elevated hepatic glycolytic intermediates, such as G6P concentrations (chapter 5) may activate carbohydrate response element binding protein (ChREBP)-mediated gene expression. \(\text{ApoC3}\) and \(\text{Fgf21}\) expression are under transcriptional control of ChREBP [361, 362]. Next to this, ChREBP is known to activate mRNA expression of microsomal triglyceride transfer protein (MTTP) [30], which was induced in livers of \(L-G\text{6pc}^{-/-}\) mice.

ChREBP was previously implicated in regulation of lipogenic gene expression in GSD I in a mouse model of pharmacological G6PT inhibition [363] and its expression was shown to be upregulated in \(L-G\text{6pc}^{-/-}\) mice in chapter 4 and by others [167]. Our results implicate a broader role for ChREBP in deranged lipid metabolism in GSD I, not only involving accumulation of hepatic TG, but also in the induction of hypertriglyceridemia (by both inducing VLDL-TG
production and inhibiting clearance) and adipocyte lipolysis.

**Glucose production in GSD Ia is possible via glycogen breakdown through debranching enzyme and/or lysosomal amylglucosidase**

It is a long standing enigma how GSD Ia patients retain a limited amount of endogenous glucose production (EGP), despite the absence of glucose-6-phosphatase (G6PC). Three hypotheses have been proposed (Figure 7.3). In chapter 4 we show that EGP can be achieved by G6pc-deficient hepatocytes through glycogen degradation via debranching enzyme and/or lysosomal breakdown.

**Glucose production via debranching enzyme**

Glycogen is broken down by the interplay of several enzymes. Glycogen phosphorylase (GP) catalyzes the sequential removal of glycosyl molecules from a glycogen chain, yielding glucose-1-phosphate. GP breaks down glycogen chains until it reaches a point four residues away from a branching point. To cleave the remaining residues two additional reactions are needed, which are catalyzed by one enzyme with two catalytic sites: amylo-α,1,6-glucosidase/4-α-glucanotransferase (AGL). 4-α-D-glucanotransferase, also called transferase, transfers the outer three glycosyl residues to another glycogen branch, upon which α,1,6-glucosidase (debranching enzyme) can hydrolyze the α,1,6-glycosidic bond, releasing a free glucose molecule. Approximately 10% of glycosyl residues is in this manner released as unphosphorylated, or 'free' glucose [13].

In chapter 5 we show slightly increased GP activity in L-G6pc−/− mice. Next to this, UDP-glucose turnover was strongly increased, suggesting increased glycogen formation. When infusing D-[1-2H]-galactose or [1-13C]-galactose in L-G6pc−/− mice we found isotopic enrichment (m+1) of blood glucose. Galactose is primarily taken up by the liver [364], suggesting that galactose is metabolized to glucose via the Leloir pathway and hepatic glycogen.

The kidney however also contributes to galactose metabolism [364] and is capable of producing glucose from galactose [365]. Therefore, labeled blood glucose may (in part) have been produced by the kidneys of L-G6pc−/− mice. Using 13C-MRS measurements we showed that [1-13C]-galactose is incorporated into hepatic glycogen. Next to this, we showed MIDA indications of increased glycogen cycling together with a greatly reduced hepatic glucokinase (GCK) flux. Finally, in G6pc-deficient primary hepatocytes residual glucose production was abolished by an α-glucosidase inhibitor. Together these data strongly
Figure 7.3: Simplified schematic representation of possible manners in which glucose can be produced when G6PC is not present. 1. via debranching enzyme (α-1,6-glucosidase), 2. via lysosomal degradation (α-1,4-amyloglucosidase), 3. via aspecific phosphatases (e.g. G6Paseβ) [204].

suggest that in L-G6pc−/− mice an increased amount of unphosphorylated glucose may be released from the liver via debranching enzyme and/or lysosomal glycogen degradation.

In GSD I patients, the kidney and intestine are also G6pc-deficient. Interestingly, 13C-enriched blood glucose derived from enteral [1-13C]-galactose infusion has been found in GSD I patients [203]. This indicates the relevance of EGP via glycogen breakdown through debranching enzyme and/or lysosomal degradation.

Lysosomal glucose production from glycogen
The physiological relevance of glycogen autophagy is clearly demonstrated in patients with Pompe Disease, or GSD II [14]. GSD II patients do not have a functional lysosomal α-1,4-glucosidase which leads to accumulation of lysosomes full of undegraded glycogen. Studies in newborn animals have furthermore indicated that glycogen autophagy is a selective process [15]. In hepatocytes and cardiomyocytes of newborn animals many autophagic vacuoles have been found in close proximity to cytosolic glycogen stores, while other autophagic cargo besides glycogen is rarely seen in these vacuoles [16]. Also, in GSD II
hepatic expression of a protein involved in targeting glycogen to the lysosome (starch binding domain 1 (STBD1)) was found to be highly upregulated [366]. From studies in a mouse model of GSD II STBD1 appears to be critical for hepatic glycogen accumulation [367].

It was recently reported that markers of autophagy were reduced in livers of \( G6pc \)-deficient mice and dogs [176] suggesting that lysosomal glycogen breakdown may have a minor contribution to glucose production by \( G6pc \)-deficient hepatocytes. In chapter 5 we however showed that hepatic lysosomal amyloglucosidase activity was maintained in \( L-G6pc^{-/-} \) mice, arguing against an impaired capacity for lysosomal glycogen breakdown. Also, hepatic mRNA expression of \( Stbd1 \) was increased in \( L-G6pc^{-/-} \) mice. Because STBD1 targets glycogen to autophagosomes [17, 18], it is conceivable that despite a general reduction in autophagy in \( L-G6pc^{-/-} \) mice [176] lysosomal breakdown of glycogen may be increased because of massive accumulation of hepatic glycogen in these animals [17].

**Reduced hepatic glucose phosphorylation in GSD Ia**

In chapter 5 we show substantial evidence for a greatly reduced hepatic ability to phosphorylate glucose in \( L-G6pc^{-/-} \) mice. We found reduced hepatic \( Gck \) expression together with a reduction in GCK activity and a severely hampered GCK flux. A likely explanation for this are low blood glucose concentrations with an accompanying high glucagon to insulin ratio. Expression of \( Gck \) is regulated through hormonal control of gene transcription; insulin induces and glucagon suppresses its expression [21]. Next to this glucokinase activity is regulated at the posttranslational level by binding to its inhibitory protein: glucokinase regulatory protein (GCKR). At low glucose concentrations glucokinase is retained in the nucleus, which is dependent on the expression of and binding to GCKR [224, 225]. After a meal glucokinase is released from its binding partner and moves to the cytosol [232]. Recently, glucagon has also been shown to induce translocation of glucokinase from the cytosol to the nucleus, but this process was only stimulated by glucagon at high glucose concentrations [368]. It is therefore likely that low fasting blood glucose and accompanying low plasma insulin concentrations in \( L-G6pc^{-/-} \) mice account for diminished expression of glucokinase and increased sequestering of the enzyme to the nucleus.
Is glycogen cycling increased in GSD Ia?
In chapter 5 we present data that indicate increased glycogen cycling in L-
\( \text{G6pc}^{-/-} \) mice. Simultaneous glycogen synthesis and degradation has been
demonstrated in rat liver (e.g. [369]) and in humans [212]. Increases in both
metabolic pathways may theoretically occur in GSD Ia. In GSD Ia extrahepatic
tissues are craving glucose which is signaled by an increased plasma glucagon to
insulin ratio and low blood glucose concentrations, while inside the hepatocytes
highly elevated G6P concentrations signal energy abundance. The conflicting
extra- and intrahepatic glucose sensing systems may lead to increased glycogen
cycling in the fasting \( \text{G6pc} \) -deficient liver.

The control of glycogen synthesis is largely dependent on the activity of
glycogen synthase (GS) [370], which catalyzes the addition of \( \alpha1,4 \)-linked glu-
cose units from UDP-glucose to a growing glycogen chain. Glucose-6-phosphate
(G6P) is an allosteric activator of GS making it a better substrate for dephos-
phorylation by protein phosphatase 1 (PP1) [27, 371, 372]. Elevated intracel-
lar G6P concentrations, as found in GSD I, are likely to activate GS.

On the other hand, the activity of glycogen phosphorylase (GP) is rate
limiting for glycogenolysis [226, 230, 231]. Hepatic GP activity seems to rely
largely on conversion from GP\( \beta \) to GP\( \alpha \) by phosphorylation [373] and the major
regulators of phosphorylase activity in the liver are glucagon and glucose [374],
which cause activation and inactivation of the enzyme, respectively. G6P is also
involved in GP inhibition [28], but hepatic GP is thought to be more responsive
to extrahepatic signals that are involved in the maintenance of blood glucose
levels [26]. Thus, in GSD I elevated circulating glucagon concentrations would
stimulate glycogenolysis while at the same time inhibition of GP by glucose
[375] is relieved.

Glycogen is structured in chains, which grow in different directions from
a central starting point (the protein glycogenin). Branching of the chains
results in sphere-like molecules [376]. Studies in different tissues show that
synthesis and breakdown of glycogen is ordered in a 'last in-first out' man-
nner [377, 378, 379]. Regarding its degradation, it is conceivable that only the
outer glycogen branches are accessible to GP. In chapter 5 we attempted to
measure glycogen cycling, glycogen turnover, synthesis and degradation using
\(^{13}\text{C}-\text{galactose} \) and subsequent \(^{12}\text{C}-\text{galactose} \) infusion together with magnetic
resonance imaging (MRS), as has been attempted before [380, 381]. Unfor-
nately actual measurement of fluxes in glycogen metabolism is troublesome,
since the molecular structure of glycogen and cycling of glycosyl moieties lead to
ambiguity in the interpretation of \(^{13}\text{C}-\text{MRS} \) data. For instance, synthesis rates
of glycogen can be underestimated from increments in the $^{13}$C-glycogen signal because of simultaneous glycogen synthesis and degradation. On the other hand, when infusing $^{12}$C-galactose in a wash-out period after $^{13}$C-galactose infusion, degradation rates can be underestimated from a decline in $^{13}$C-glycogen signal because of simultaneous cycling of $^{13}$C-glycosyl moieties.

The insulin signaling paradox may be explained by hepatic zonation of insulin signaling

In chapter 1 we discussed how we can distinguish between intra- and extrahepatic glucose sensing systems from which information is integrated by hepatocytes. Inside hepatocytes, biochemical processes are adapted accordingly to meet systemic and intracellular needs. In chapter 6 the concept of metabolic zonation of the liver was discussed. The liver is not homogeneous: across the liver acinus, from periportal to pericentral, extrahepatic signals differ and therefore the biochemical outcome of hepatocytes differs across the acinus. In chapter 6 we propose that this difference in intracellular biochemistry between hepatocytes from different zones can explain the insulin signaling paradox.

The insulin signaling paradox poses the following problem: while a totally insulin-deficient liver results in hyperglycaemia without hypertriglyceridemia, the metabolic syndrome is characterized by both [327]. In chapter 6 we argue that this paradox may be explained by zonation of insulin sensitivity in the liver. Insulin resistant hepatocytes in the periportal zone may produce glucose, while insulin sensitive pericentral hepatocytes produce TG [171].

The mechanisms leading to insulin resistance discussed in chapter 6 are mainly based upon the interference of metabolites of fatty acids in the insulin signaling cascade. According to known insulin signaling pathways to gluconeogenic and lipogenic gene expression, insulin resistance should lead to increased gluconeogenesis and reduced lipogenesis (Figure 7.4). This theory on selective insulin signaling is corroborated by animal research using knockdown of enzymes at different sites in the insulin signaling cascade, leading to hyperglycaemia, but not hypertriglyceridemia. Next to this, hepatic steatosis is often zonated (reviewed in: [171], chapter 6).

Other recent studies suggest that substrate-driven mechanisms that regulate hepatic glucose metabolism explain the insulin signaling paradox [382, 383, 384]. Flux of free fatty acids (FFA) from the adipose tissue would induce substrate-driven hepatic glucose production (HGP) in the absence of insulin signaling, without a requirement for insulin resistance [383, 384]. Indeed, when both Akt1, Akt2 and FoxO1 are deleted, liver HGP is suppressed by insulin [382, 384],
suggesting regulation of glucose metabolism by another mechanism. However, when FoxO1 is present in livers with Akt1 and Akt2 deletion, mice are hyper-glycaemic upon feeding and insulin fails to suppress HGP [382]. This actually indicates the requirement for insulin signaling via Akt and subsequent inhibition of FoxO1 for suppression of HGP. Thus it seems that other mechanisms may take over control of hepatic glucose metabolism in the absence of Akt and FoxO1, but the physiological relevance of these processes remains to be determined.

Still, enzymatic regulatory mechanisms that lead to selective insulin signaling cannot be ruled out. Recently, for instance, research on a genetic mouse model for selective insulin signaling has been published [385]. The authors silenced an inhibitor of insulin receptor catalytic activity: the growth-factor receptor binding protein 14 (GRB14). Hepatic GRB14 knockdown in mice enhanced insulin signaling, but at the same time reduced lipogenesis. Down-regulation of GRB14 in obese and insulin resistant mice led to decreased blood glucose concentrations, but also improved hepatic steatosis. Genomic research in humans already indicated a role for GBR14 in insulin resistance and type II diabetes (e.g. [386]), making this an interesting target for further study on selective insulin signaling.

**Outlook**

The key findings described in this dissertation are: 1) The initial contributor to LXR-induced hepatic steatosis is an enhanced influx of fatty acids from the circulation; 2) Upon LXR activation both input and output fluxes of the hepatic TG pool are greatly upregulated and only a minor imbalance between the two leads to steatosis; 3) The origin of hepatic steatosis in the G6pc-deficient liver differs between fasting and feeding; 4) The G6pc-deficient liver is capable of producing glucose via glycogen debranching enzyme and/or lysosomal glycogen degradation; 5) Glucose phosphorylation is severely impaired in the fasting G6pc-deficient liver, hampering hepatic glucose uptake.

In this dissertation we show the adaptability and flexibility of metabolism. In the healthy individual the liver is constantly changing metabolic fluxes to adapt to the energy need of the body. A prolonged imbalance between energy input and output leads to maladaptive or diseased states, such as non-alcoholic fatty liver. LXR activation greatly affects input and output fluxes of the hepatic TG pool, but finally metabolism adapts to the pharmacological intervention by setting a new balance. The flexibility of metabolism is also illustrated by the
Figure 7.4: Simplified scheme of insulin signaling to lipogenesis and gluconeogenesis. Insulin binding to its receptor leads to activation of a signaling cascade leading to phosphorylation of Akt. Below Akt, insulin signaling splits into two distinct pathways, which respectively stimulate hepatic de novo lipogenesis through activation of SREBP-1c and inhibit hepatic glucose production via inhibition of FoxO1.

substantial differences in lipid metabolism in L-G6pc−/− mice under differing nutritional conditions.

Adaptability of metabolism is nicely illustrated by our studies into de novo lipogenesis and hepatic steatosis. We show that de novo lipogenesis is not always the main contributor to hepatic steatosis. Importantly, the origin of hepatic steatosis varies depending on treatment duration (chapter 2 and 3) and nutritional status (chapter 4) within animal models.

Thus, adaptation and flexibility of the whole metabolic system may lead to
differing metabolic outcomes to an intervention. When the outcomes of replicate studies on metabolism do not match, this may mean that metabolism can find different solutions for challenges, and may be able to compensate with different adaptive outcomes. Therefore to adequately examine the effects of an intervention such as a gene deletion or pharmacological agents on metabolism, all metabolic pathways should ideally be studied in parallel.