Control of metabolic flux by nutrient sensors
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Introduction
METABOLIC FLUX CONTROL

Complex organisms possess multiple ‘nutrient sensing’ systems that allow adequate biochemical adaptations to ensure sufficient energy supply to organs and tissues and to facilitate the storage of superfluous nutrients. The efficacy of these systems is illustrated by the body’s ability to cope with the diurnal alternation between feeding and fasting, and with excessive energy demands during exercise. Oversupply of energy and nutritional dysbalance trigger adaptive physiology and interfere with regulatory pathways. As a consequence, these conditions predispose to development of metabolic disturbances such as type 2 diabetes and cardiovascular diseases. Insight into the pathophysiological mechanisms is required to define strategies for prevention and treatment of these diseases.

The breakdown, rearrangement and storage of nutrients is accounted for by metabolic fluxes, which are defined as the flow rates of molecules through biochemical pathways. These flow rates are determined by substrate availability and enzyme activities. Cellular nutrient status per se determines substrate availability. Some nutrients furthermore control enzyme activities via feedforward control upon substrate binding or via feedback control by downstream metabolites. Changes in nutrient and/or energy status may also induce sensing systems that exert post-translational modifications of regulatory proteins. These modifications shift the equilibrium between the active and inactive state of an enzyme and/or affect its stability. Thus, nutritional status determines metabolic flux by both direct and indirect mechanisms.

For decades, hormonal networks have been considered as the major sensing pathways responsible for indirect flux control by nutrients. The recent identification of transcription factors that control the gene expression of enzymes has added a new level of complexity to the regulation of metabolic fluxes. Many of these transcriptional regulators are activated by nutrients or their metabolites. Therefore, these small-molecule sensors are interesting targets to modulate metabolic fluxes.

The studies described in this dissertation consider the adaptive mechanisms by which the body responds to changes in nutrient availability. Glucose, fatty acids and amino acids are the major dietary energy suppliers. The work focuses on glucose and fat metabolism, and particularly addresses the role of transcriptional regulators.

GLUCOSE HOMEOSTASIS

Glucose is the primary metabolic fuel for complex organisms. Although most cells are able to exhibit substrate switching when glucose supply is limited, some almost exclusively depend on glucose. This is particularly true for brain cells and erythrocytes: their functioning is severely impaired if glucose concentrations are persistently low. High glucose concentrations, on the other hand, are cytotoxic and cause tissue damage. Tight control of glycemia is thus required to guarantee optimal functioning.

Most dietary glucose is ingested as a multimer complex (i.e., carbohydrates). After digestion of these complexes, glucose molecules are taken up into intestinal cells from where they are transported into the bloodstream. Intestinal uptake represents the major route for glucose input into the circulation in the absorptive or postprandial phase (i.e., following the intake of a meal). Glucose transport across plasma membranes is facilitated by glucose transporters (GLUTs). Once inside the cell, glucose is phosphorylated to glucose-6-phosphate (G6P) by glucokinase (GK) in hepatocytes.
and pancreatic β-cells and by hexokinases (HK) in all other cell types. G6P has different intracellular fates. A limited amount of G6P is stored as glycogen in liver and skeletal muscles. Furthermore, G6P is used for energy supply by its conversion into pyruvate via the glycolytic pathway. Pyruvate kinase (PK) catalyzes the final step of glycolysis. Pyruvate enters the mitochondria and is used as a substrate in the tricarboxylic acid (TCA) cycle to generate energy in the form of adenosine triphosphate (ATP). If ATP supply is sufficient, pyruvate is used for de novo fatty acid synthesis. The pentose phosphate pathway (PPP) represents another route for intracellular G6P. Although the PPP involves glucose oxidation, its primary role is anabolic rather than catabolic because it generates reducing equivalents required for biosynthetic routes.

Blood glucose concentrations decrease as soon as intestinal absorption is completed (postabsorptive phase). In this phase, glucose consumption by most tissues is reduced while glucose production represents the major route of glucose input into the circulation and secures substrate supply for the brain. Glucose-6-phosphatase (G6Pase) systems enable glucose production from G6P in liver, kidney and intestine. In the postabsorptive state, the relative contributions of kidney and intestine are limited [1]. Under these conditions, hepatic G6P is derived from glycogen breakdown via glycogen phosphorylase (GP). Upon prolonged fasting, de novo synthesis of G6P from 3-carbon precursors, or gluconeogenesis, is induced. Lactate and alanine are converted into pyruvate, while glycerol can be used as a gluconeogenic substrate via triose phosphate. G6Pase activity mediates G6P transport from the cytosol into the endoplasmic reticulum (ER) by glucose-6-phosphate translocase (G6Pt) and its subsequent hydrolysis to glucose by glucose-6-phosphate hydrolase (G6Ph). Glucose is finally transported from the ER into the circulation.

**FATTY ACID HOMEOSTASIS**

Fatty acids represent the second major metabolic substrate. Non-esterified fatty acids (NEFA) are extremely cytotoxic [2]. Circulating NEFA concentrations show relatively little variation and cellular fatty acid uptake, transport and storage are heavily regulated [3].

In the postprandial phase, dietary fatty acids mainly enter the body in the form of triglycerides (TGs), consisting of fatty acids complexed to glycerol. After TG digestion, fatty acids are transported into intestinal cells where they are re-esterified to form TG or cholesterol-esters (CEs) which are assembled into chylomicrons. These relatively large particles enter the circulation via the lymphatic system. Chylomicron-associated TGs are hydrolyzed by the action of lipoprotein lipase (LPL). Uptake of NEFA by tissues and their intracellular trafficking is mediated by fatty acid transporters and fatty acid binding proteins [4,5]. Catabolism of dietary long-chain fatty acids by β-oxidation in mitochondria and peroxisomes is mediated by multienzyme complexes [6]. Fatty acids enter mitochondria and peroxisomes as carnitine complexes, which are transported across the membranes by carnitine transferases. Surplus fatty acids are re-esterified with glycerol and cholesterol and stored as TGs and CEs. These processes are mediated by glycerol-3-phosphate acyltransferase (GPAT), diacylglycerol acyltransferases (DGATs) and acyl-CoA:cholesterol acyltransferases (ACAT), respectively. Fatty acids can also be synthesized from excess glucose in liver and adipose tissue in the postprandial phase. This process, which is called de novo lipogenesis, is initiated by transport of citrate across the mitochondrial membrane into the cytosol. Here, citrate is converted into acetyl-CoA by ATP citrate lyase (ACL). Acetyl-CoA is subsequently converted into malonyl-CoA by acetyl-CoA carboxylase (ACC).
Seven malonyl-CoA molecules are condensed to a singly acetyl-CoA molecule, thereby forming palmitic acid. This process is mediated by the fatty acid synthase (FAS) complex. Specific types of fatty acids are stored as TG and CE while others are required for particular physiological functions, such as eicosanoid production and phospholipid synthesis. In the ER, different fatty acid elongation and desaturation enzymes facilitate the synthesis of these fatty acids from newly synthesized palmitic acid or from diet-derived fatty acids [7,8]. Part of the TGs and CEs synthesized in the liver are packaged into very low density lipoprotein (VLDL) particles, which are released into the circulation. These particles provide fatty acids to peripheral tissues via LPL-mediated lipolysis.

In the postabsorptive phase, TGs stored in adipose tissue are hydrolyzed into glycerol and NEFA, which are released into the circulation. Under these conditions, most tissues switch to fatty acid oxidation and circulating NEFAs (complexed to albumin) serve as the major energy substrates. Part of the acetyl-CoA generated from hepatic β-oxidation is converted into ketone bodies, which provide an alternative fuel for the brain during prolonged fasting. The liver also plays a role in fatty acid supply to peripheral tissues via the secretion of VLDL. Glycerol released by adipocytes is used as a 3-carbon precursor for hepatic gluconeogenesis. Not all NEFA that are taken up by the liver are immediately oxidized. This results in hepatic TG accumulation upon fasting [9,10]. An overview of cellular glucose and fatty acid metabolism in the postprandial and postabsorptive phase is given in Figure 1.
Figure 1. Schematic overview of glucose and fatty acid metabolism in the postprandial and postabsorptive phases.
REGULATORY PATHWAYS OF GLUCOSE AND LIPID HOMEOSTASIS

Direct regulation by energy and nutrient availability

Cells adjust ATP production to their needs. Regulatory systems that control energy homeostasis therefore not only sense nutrient availability but also shift the balance between nutrient utilization and storage. Many of the metabolic adaptations in the postprandial and postabsorptive phase coincide with the reciprocal actions of the nutrient sensors insulin and glucagon. Cellular energy and nutrient status, however, also directly affect metabolic fluxes. Low energy availability activated adenosine monophosphate kinase (AMPK), which modulates the activity of metabolic enzymes by phosphorylation. For example, if ATP supply is sufficient, citrate consumption in the TCA cycle is inhibited. Citrate is then converted into acetyl-CoA, which is used for fat storage via de novo lipogenesis. Furthermore, glucose and fatty acids exert substrate competition. This phenomenon was first described by Randle et al. as the glucose-fatty acid cycle [11]. Malonyl-CoA produced from excess glucose inhibits carnitine palmitoyltransferase-1 (CPT1) activity. This enzyme catalyzes acylcarnitine transport across the outer mitochondrial membrane and hence inhibits fatty acid oxidation when the glycolytic flux is high. On the other hand, the inhibition of CPT1 activity will be released if glycolysis is low and fatty acid oxidation will consequently increase. β-Oxidation products inhibit pyruvate dehydrogenase (PDH) action via an increased pyruvate dehydrogenase kinase (PDK) activity. As a consequence, pyruvate conversion into acetyl-CoA is blocked.

Indirect regulation by hormones

Insulin and glucagon are the major hormones that exert indirect flux control in response to changes in glucose availability. These hormones not only affect post-translational modification systems (i.e., protein kinase and phosphatase activities) but also regulate enzyme expression at the transcriptional level. In the postprandial phase, increasing blood glucose concentrations trigger insulin release by pancreatic β-cells. Circulating insulin binds to its receptor (insulin receptor, IR), which initiates several complex signalling cascades. The insulin receptor substrate (IRS)/phosphatidylinositol 3-kinase (PI3K) pathway activates protein kinases, which mediate most of insulin’s metabolic actions relevant for glucose homeostasis. These include:

- Translocation and fusion of intracellular GLUT4 vesicles to the plasma membrane, thereby promoting glucose uptake into skeletal muscle, adipose tissue and heart.
- Induction of glycogen synthase (GS) activity, which facilitates glycogen storage in liver and skeletal muscles.
- Suppression of phosphoenolpyruvate carboxykinase (Pepck) expression, which encodes a key gluconeogenic enzyme.
- Induction of sterol regulatory element binding protein 1c (Srebp-1c) expression, which encodes a regulator of fatty acid synthesis in liver and adipose tissue.
- Inhibition of lipolytic enzyme activity in adipose tissue, thereby suppressing TG hydrolysis.
A decrease in the blood glucose concentration arrests insulin release and its actions. Low blood glucose concentrations furthermore trigger glucagon secretion by pancreatic α-cells. Glucagon interaction with its receptor ultimately results in:

- Suppression of GS and induction of GP activity, thereby promoting glycogen breakdown.
- Induction of G6ph and Pepck expression, which encode gluconeogenic enzymes.
- Suppression of PK activity, thereby inhibiting glycolysis.
- Induction of lipolytic enzyme activity in adipose tissue, thereby promoting TG hydrolysis.

NUCLEAR RECEPTORS AND TRANSCRIPTION FACTORS: THEIR ROLE IN NUTRITIONAL CONTROL OF METABOLIC FLUX

Transcriptional regulators are proteins that control gene expression by binding to specific response elements (REs) located in the promoter sequences of genes. The activity of nuclear receptors and transcription factors depends on their cellular location and structural conformation. Nuclear receptors represent a superfamily of transcription factors that are mostly ligand-activated. These receptors share a common structural and functional organization. This consists of a NH2-terminal domain for ligand-independent transactivation, a DNA-binding domain required for proper targeting to the REs, a connecting hinge region that allows protein flexibility and a ligand-binding domain that exerts ligand-dependent transactivation. Upon binding, some nuclear receptors are first translocated to the nucleus upon ligand binding. Nuclear receptors bind to their REs as monomers, but more often as homodimers or heterodimers. In addition, dephosphorylation and ligand activation of (RE-bound) receptors induce conformational changes, thereby modulating the affinity for certain co-repressor and co-activator proteins. These in turn determine whether a target gene is induced or suppressed. Nuclear receptor action is depicted in Figure 2.

![Diagram of transcriptional regulation upon ligand-activation of nuclear receptors.](image-url)
Based on their ligand-binding properties, nuclear receptors can be divided into three classes. Nuclear hormone receptors represent those that bind hormones with a high affinity. Well-known examples are the glucocorticoid receptor and the estrogen receptor. Those for which the ligand still needs to be identified are called orphan receptors. Several of the recently adopted receptors bind metabolic substrates and intermediates, but also xenobiotics and drug metabolites. Nutrients and their metabolites exert indirect flux control via these regulators. They serve as ligands for certain adopted receptors and hence modulate their transcriptional activity. Table 1 provides an overview of the different transcriptional regulators, their nutrient sensitivity and metabolic regulation.

Table 1. Overview of nutrient-sensing transcription factors and their metabolic actions.

<table>
<thead>
<tr>
<th>Transcription factor</th>
<th>Sensitive to</th>
<th>Metabolic regulation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ligand-activated nuclear receptors</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PPARα</td>
<td>fatty acids</td>
<td>fatty acid oxidation</td>
</tr>
<tr>
<td>PPARβ</td>
<td>fatty acids</td>
<td>fatty acid oxidation, cholesterol transport, glucose transport</td>
</tr>
<tr>
<td>PPARγ</td>
<td>fatty acids</td>
<td>lipid storage, glucose transport</td>
</tr>
<tr>
<td>LXRα/β</td>
<td>oxysterols</td>
<td>cholesterol transport, bile acid synthesis, fatty acid synthesis, glucose transport</td>
</tr>
<tr>
<td>FXRα/β</td>
<td>bile acids</td>
<td>bile acid synthesis, cholesterol transport, glucose transport, glucose oxidation, fatty acid synthesis</td>
</tr>
<tr>
<td><strong>Other transcription factors</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SREBP-1c</td>
<td>cholesterol</td>
<td>fatty acid synthesis</td>
</tr>
<tr>
<td>ChREBP</td>
<td>glucose</td>
<td>glucose oxidation, fatty acid synthesis</td>
</tr>
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Peroxisome Proliferator Activated Receptors (PPARs)

PPARs are key regulators of lipid homeostasis. PPARs are ligand-activated by fatty acids, in particular by polyunsaturated fatty acids (PUFA) and eicosanoids [12]. PPARs form heterodimers with the Retinoid X Receptor (RXR), which is ligand-activated by retinoic acid. There are three PPAR isotypes, encoded by separate genes.
PPARα (NR1C1) is highly expressed in liver, brown adipose tissue, heart and skeletal muscle. Upon activation, PPARα induces the expression of enzymes involved in fatty acid mobilization, uptake, transport and catabolism. In the fasted state, PPARα is activated by NEFA released from adipose tissue. This facilitates energy supply and enables ketogenesis. Because of this, PPARα is an important mediator of the adaptive response to fasting [13,14].

Two PPARγ (NR1C3) isoforms exist. PPARγ1 is mainly expressed in adipose tissues, but also in the colon, spleen, retina, hematopoietic cells and skeletal muscles. PPARγ2, on the other hand, is predominantly expressed in white and brown adipose tissue. PPARγ coordinates adipocyte differentiation and proliferation [15].

PPARβ/δ (NR1C2) is ubiquitously expressed. PPARβ/δ promotes fatty acid oxidation in skeletal muscle and adipose tissue [16]. In addition, PPARβ/δ is involved in cholesterol export in intestine and macrophages [17]. All three PPAR isoforms furthermore mediate inflammatory responses [18].

Liver X Receptors (LXRs)

LXRs are major players in control of cholesterol and fatty acid metabolism [19] and inflammatory responses [18]. The two LXR isotypes are ligand-activated by mono-oxidized derivatives of cholesterol. LXRs also heterodimerize with RXR. LXR binding to its response elements is inhibited by PUFA [20]. LXRα (NR1H3) is highly expressed in liver, and to a lower extent in kidney, intestine, adipose tissue and macrophages, while LXRβ (NR1H2) is ubiquitously expressed. LXR target genes encode enzymes involved in cholesterol efflux and disposal, i.e., bile acid synthesis, hepatobiliary transport and fecal excretion. In addition, LXRs increase fatty acid synthesis, both directly and indirectly via the induction of Srebp-1c [21].

Farnesoid X Receptors (FXRs)

FXRs, which control bile acid and cholesterol metabolism are ligand-activated by bile acids. FXRs can either act as monomer, or form heterodimers with RXR. There are two FXR isotypes. FXRα is mainly expressed in liver and adrenals. FXRβ expression is higher compared to that of FXRα and most dominant in intestine and kidney. FXR activation serves to protect from toxic accumulation of bile acids, by inhibition of bile acid uptake and synthesis genes while inducing bile acid export systems. FXRs have also been implicated in the regulation of glucose and fatty acid homeostasis [22].

Sterol Regulatory Element Binding Proteins (SREBPs)

SREBPs are transcription factors that regulate cholesterol and fatty acid metabolism [23]. There are two SREBP isotypes (SREBP-1/2), which are predominantly present in liver and adipose tissue. SREBPs are synthesized as 125 kDa precursor proteins anchored in the ER membrane. Maturation of SREBPs requires the activation of the SREBP cleavage activating protein (SCAP). SCAP is a sensor of the cholesterol content in the ER membrane, where it is retained in the presence of high cholesterol levels due to its interaction with the INSIG proteins [24]. When the cholesterol content drops, SCAP escorts SREBPs from the ER to the Golgi apparatus. Here, SREBPs are cleaved by two different proteases. The mature 68 kDa SREBP proteins are translocated to the nucleus where they bind to the DNA as monomers. This maturation process of SREBPs is depicted in Figure 3.

SREBP-2 is mainly involved in control of cholesterol biosynthesis [25]. There are two SREBP-1 isoforms. SREBP-1a expression is relatively low compared to that of SREBP-1c. SREBP-1c regulates the expression of fatty acid biosynthesis and esterification genes. Furthermore, Srebp-1c expression is
controlled by LXR and insulin [26, 27]. The subsequent increase in fatty acid synthesis is thought to support cholesterol esterification and thereby to facilitate cholesterol storage upon LXR activation. Insulin’s induction of Srebp-1c expression on the other hand, enables storage of excess glucose as fat. Both SREBP-1 and -2 also induce systems that generate reducing equivalents required for cholesterol and fatty acid synthesis [28]. PUFA arrest SREBP-1 but not SREBP-2 action, by enhancing its decay and/or inhibition of its maturation process [29, 30].

**Figure 3.** Schematic overview of transcriptional regulation upon sterol-induced activation of SREBPs.

*Carbohydrate Responsive Element Binding Protein (ChREBP)*

ChREBP promotes storage of glucose as fatty acids. This transcription factor that is mainly expressed in liver, adipose tissue and kidney, is activated in response to increased glucose availability. Inactive ChREBP is phosphorylated by protein kinase A and localized in the cytosol. Activation of ChREBP occurs by a two-step dephosphorylation: the first triggers its nuclear translocation while the second allows its binding to DNA. The transcriptional activity of ChREBP requires its heterodimerization with the Max-like protein X (Mlx). ChREBP regulates the expression of glycolytic and lipogenic genes. The PPP intermediate xylulose-5-phosphate is thought to promote protein phosphatase 2A (PP2A) activity, which in turn dephosphorylates ChREBP, thereby increasing its activity [31]. On the other hand, AMPK [32] and PUFA suppress ChREBP activity by inhibition of its nuclear translocation [33]. This 2-step activation of ChREBP is depicted in Figure 4.
DETERMINATION OF METABOLIC FLUXES UPON INTRODUCTION OF STABLE ISOTOPES IN VIVO

As stated earlier, metabolic flux is determined by substrate availability and enzyme activities. Substrate concentrations can be assessed by biochemical analysis (metabolomics). This only provides a static measure of metabolite status: insight into the origin of a substrate pool (i.e., the contribution of input versus output) is lacking. Information on the actions of a specific enzyme can be derived from analysis of gene expression level (genomics), its cellular abundance (proteomics), and by determination of its (maximal) activity ex vivo. However, these analyses do not necessarily reflect the true activity under physiological conditions.

Fluxomics allows realtime assessment of substrate flow in vivo [34,35]. Such measurements can be performed in isolated cells, perfused organs or intact organisms, thereby providing detailed information of metabolic processes from a single cell to complex whole-body organ interplay. Fluxomics therefore enables the identification and evaluation of (supposed) critical or rate-limiting steps in a physiological relevant manner. Most commonly used fluxomics procedures are based on isotopic labeling. Labeled molecules are introduced into the system and assumed to be metabolized in a similar manner as those endogenously present. Fluxes are consequently quantified by assessment of the degree of labeling in the metabolite of interest within a certain timeframe. Secreted or circulating metabolites can be studied in a dynamic manner by taking serial samples over time. In the past decades, stably labeled compounds have been proven an excellent alternative for the traditional
radioisotopes. The advantage of stable isotopes is their safe application in human studies. The mass isotopomer abundances are determined by gas chromatography and mass spectrometry (GC-MS). The mass isotopomer distribution in turn allows realtime assessment of biosynthetic fluxes in vivo. Mass isotopomer distribution analysis (MIDA) represents one of the mathematical approaches to quantify these fluxes. This method will be discussed in detail below.

The turnover or rate of appearance (Ra) of a metabolite is derived from the dilution of a labeled form of this metabolite that is introduced. If physiology and label enrichment are constant (i.e., in steady-state), the turnover represents in- and output of the metabolite, thus Ra equals the rate of disappearance (or disposal, Rd). Such measurements are for instance applied to determine whole-body glucose production and disposal in vivo following introduction of $^{13}$C-labeled glucose. The contribution of different anabolic routes and label recycling are however not accounted for by this methodology. Accurate assessment of the flux through biosynthetic pathways therefore requires a more sophisticated approach. This is provided by MIDA, introduced by Hellerstein and Neese [36,37]. MIDA enables the analysis of biopolymer synthesis from repetitive addition of monomeric precursors. The precursor pool is isotopically enriched by the introduction of labeled precursor. The isotope distribution of the synthesized polymer is conform to binominal expansion and depends on the enrichment of the precursor pool (p) and the number of monomers in the polymer (n). The relationship between the different isotopomers of the polymer is uniquely determined by p, and therefore insensitive to dilution by unlabeled polymers [36,38]. Stable isotopes are naturally abundant (~1%) and the measured isotope abundance must therefore be corrected to obtain the excess isotope enrichment due to label incorporation [39]. The theoretical undiluted isotopomer abundance is subsequently calculated at the specific p and n. Its dilution (i.e., the relative excess isotopomer abundance) finally represents the fraction of the polymer pool that is newly synthesized (f). The introduction of $^{13}$C-acetate allows the assessment of fractional fatty acid and cholesterol synthesis. In addition, we have developed methods to determine individual fluxes of hepatic glucose metabolism upon introduction of $^{13}$C-glucose, $^{13}$C-glycerol and $^2$H-galactose [40,41].
SCOPE AND OUTLINE

Obesity, insulin resistance and hepatic steatosis represent three components of the metabolic syndrome that are typically associated with energy oversupply and nutritional dysbalance. Obesity and insulin resistance are furthermore characterized by an impaired capability to balance nutrient availability and substrate utilization [42]. Transcription factors sense nutrient availability and control the expression of genes encoding metabolic enzymes. Impaired action and overactivity of transcription factors are associated with metabolic disturbances [21,41,43–48]. Therefore, metabolic abnormalities can be corrected by modulation of transcriptional activity. There are several examples of drugs that are used to treat disturbances in lipid and glucose metabolism via the action of nuclear receptors. Fibrates are pharmacological PPARα agonists that are widely used to treat dyslipidemia in humans [49]. Pharmacological PPARγ activation by thiazolidinedione (TZD) treatment lowers blood glucose concentrations in insulin-resistant subjects [50]. Most transcription factors are however expressed in multiple tissues and global targeting may therefore result in undesirable side-effects. For example, pharmacological LXR agonists are potential anti-atherosclerotic drugs because they reduce cholesterol accumulation in macrophages. These compounds however also induce hepatic steatosis and the secretion of large VLDL particles [51]. Furthermore, gene expression manipulations per se will not always result in altered metabolic flux, and biochemical changes reflect a shift in the balance between anabolic and catabolic processes. Finally, physiological systems are interrelated. The induction or suppression of a certain a metabolic pathway will therefore affect the flux through another route [52].

Altogether, insight into tissue-specific actions of transcriptional regulators as well as the whole-body consequences for intermediary metabolism are required to define optimal strategies to treat and prevent metabolic diseases. By modulating the activity of several nuclear receptors, we studied their role in control of metabolic fluxes relevant for glucose and lipid homeostasis. In Chapter 2, we investigated the role of LXRα in the control of hepatic carbohydrate metabolism during the feeding-to-fasting transition. We furthermore assessed the physiological relevance of the postulated hepatic glucose-sensing function of LXR [53]. Chapter 3 focuses on the regulatory action of FXR in glucose transport across enterocytes. The consequences of pharmacological PPARα activation for hepatic carbohydrate and lipid metabolism were determined in Chapter 4. We also evaluated the metabolic adaptations in response to chronic dietary fat oversupply in mice. Therefore, we used two different high-fat diets. The first was based on beef fat, while in the other diet this fat was partially replaced by fish oil. The consequences for whole-body glucose metabolism and hepatic fatty acid synthesis are described in Chapter 5 and 6, respectively. We also assessed the effects of these dietary interventions on substrate utilization and energy expenditure (Chapter 5).