The body possesses sensor systems that respond to changes in nutrient supply, thereby enabling adequate adaptation of metabolic processes to nutrient availability. Superfluous nutrients are stored if energy supply exceeds energy consumption. These stores are in turn used when energy supply is limited. Such metabolic flexibility ensures proper functioning of living organisms under changing conditions.

Adaptive metabolic responses occur via modifications of metabolic fluxes. A flux represents the flow of molecules through a series of chemical reactions that constitute a biochemical pathway. The flow rate is determined by nutrient availability and enzyme activities. Enzyme activity per se may also be altered by nutrient availability. Thus, nutrient status determines metabolic flux by both direct and indirect mechanisms.

The presence and activity of metabolic enzymes are tightly controlled. Enzyme synthesis is initiated by the transcription of a specific genetic code from DNA. The enzyme is synthesized upon translation of this code. The transcription process is partly dependent on DNA-binding of specific transcription factors. Some transcription factors (referred to as nuclear receptors) are activated upon binding of ligands, and subsequently promote or inhibit transcription of their target genes. In recent years, nutrients have emerged as ligands for a select group of nuclear receptors. These represent nutrient sensors that are able to adapt metabolic enzyme transcription in response to changes in nutrient status. Thus, metabolic fluxes can be attenuated by alterations in transcription factor activity.

As stated earlier, metabolic flexibility enables short-term adaptation to acute changes in nutrient availability. However, metabolic fluxes will be persistently modified upon chronic energy oversupply and nutritional dysbalance. These adaptive responses may in the long term predispose to the development of metabolic abnormalities such as obesity, hepatic steatosis and type 2 diabetes. Therefore, it is important to gain insight into the adaptive modulations of metabolic fluxes. Furthermore, the possibility to attenuate metabolic disturbances via enzyme transcription has sparked the interest to design drugs that modulate transcription factor action. Current knowledge on the regulation of metabolic fluxes is limited. Deeper insights into these regulatory pathways will contribute to the development of new drugs.

The studies described in this thesis consider physiological adaptations that occur in response to changes in nutrient availability. In particular, the role of specific transcription factors was addressed. In Chapter 2, we determined the role of the ‘Liver X Receptor’ (LXR) in the liver during the feeding-to-fasting transition. The two LXR isotypes α and β are both involved in the regulation of cholesterol and fatty acid metabolism. LXR activity is determined by cellular cholesterol content. Furthermore, glucose has been postulated to serve as a LXR ligand. We therefore challenged mice with a glucose-rich diet. Surprisingly, we did not observe an induction of direct LXR target genes upon glucose exposure. Furthermore, no differences in gene expression were observed between normal (‘wild-type’) mice and mice in which LXRα action was abolished (‘LXRα knockouts’). However, when these animals were fasted, hepatic glycogen depletion was found to be delayed in LXRα knockout mice. Furthermore, major fluxes involved in hepatic glucose metabolism were found to be reduced in these animals. We also observed that fasting-induced hepatic steatosis was diminished in LXRα
knockouts. Therefore, LXRα appears to be required for an adequate attenuation of metabolic fluxes under conditions of low nutrient supply, when the body starts using its reserves.

The 'Farnesoid X Receptor' (FXR) is a transcription factor that is activated by bile acids. Upon fasting, FXR knockouts exhibit an impaired ability to maintain hepatic glucose metabolism. The results of the studies described in Chapter 3 indicate that FXR exerts a regulatory role in intestinal glucose metabolism. The increase in blood glucose concentrations upon an oral glucose challenge was found to be diminished in FXR knockouts. Additional studies revealed that intestinal glucose uptake was reduced in these mice. This is explained by an increased glucose flux through an alternative route. From these studies we conclude that FXR inactivation induces changes in intestinal glucose metabolism.

'Peroxisome Proliferator Activated Receptors' (PPARs) represent a group of transcription factors that is activated by fatty acids. PPARα is an important regulator of hepatic lipid metabolism, and its action has been shown to be crucial for the adaptations that occur in response to fasting. These include the induction of fatty acid oxidation while limiting glucose consumption. PPARα furthermore induces a number of systems that protect against damage by fatty acid oxidation products. The studies in Chapter 4 add to the current understanding of the adaptations that occur in response to an increased PPARα activity in the liver. Mice were treated with a pharmacological PPARα agonist. The hepatic expression of fatty acid oxidation genes was consequently increased. To our surprise, we also observed an induction of genes involved in hepatic fatty acid synthesis (‘lipogenesis’). This induction was found to depend on the presence of the transcription factor ‘Sterol Regulatory Element Binding Protein 1c’ (SREBP-1c). These transcriptional changes were translated into an increase in the lipogenic flux, and were furthermore paralleled by an increased hepatic lipid content. We also found that glucose fluxes were altered. Altogether, our data support the co-existence of hepatic fatty acid oxidation and lipogenesis. The induction of hepatic fatty acid synthesis and the accumulation of lipid in the liver may represent a physiological mechanism by which the liver is protected against damage by fatty acids and their oxidation products.

Chapter 5 and 6 address the metabolic consequences of an increased dietary fat supply. We studied the effects of two different high-fat diets. The first diet was based on beef tallow and therefore rich in saturated fatty acids. In the second diet, the beef tallow was partially replaced by fish oil, rich in n-3 polyunsaturated fatty acids (PUFA). Intake of PUFA reduces atherosclerotic and cardiovascular risk in humans. Mice were fed either of the two high-fat diets during six weeks. The increased dietary fat intake resulted in an increase in whole-body fat oxidation as compared to mice receiving a regular (low-fat) diet. This effect was most pronounced in mice fed the fish oil-enriched diet (Chapter 5). Both high-fat diets induced adiposity, because energy consumption was increased, while energy expenditure remained unaltered (Chapter 5). Mice fed the tallow-rich diet exhibited an increased lipogenic flux in their livers. This was paralleled by an increased expression of lipogenic genes (Chapter 6). The amount of lipid secreted by the liver was however comparable to mice fed the low-fat diet. As a consequence, the net storage of hepatic lipid was increased in tallow-fed mice. Partial substitution of the saturated fat by fish oil resulted in a suppression of both lipogenic gene expression and the flux through this pathway, thereby protecting against hepatic lipid accumulation. Therefore, fish oil exerts beneficial effect on lipid metabolism, which contribute to the prevention of hepatic steatosis. We finally evaluated the consequences for glucose fluxes. Intake of the tallow-rich
diet induced insulin resistance of glucose metabolism. This predisposes to development of type 2 diabetes in the long term. Chronic oversupply of dietary fat resulted in a persistent reliance on fat oxidation, which resulted in a reduction of glucose uptake and oxidation. In contrast to the reported improvements in lipid metabolism, fish oil substitution did not rescue glycemia: it even potentiated the development of insulin resistance in these high fat-fed mice (Chapter 5). A protective effect of fish oil on the development of insulin resistance has been observed in previous studies. Therefore, additional studies are required to evaluate the effects of fish oil under different conditions.

These studies add to the current understanding on the action of transcription factors that control metabolic fluxes. An increased activity of specific transcription factors appears to be required to ensure proper handling of fatty acids, in order to limit hepatic damage. However, in most cases, this is accompanied by hepatic lipid accumulation. Although hepatic steatosis per se is relatively harmless, lipid accumulation in the liver may increase the risk to develop liver disease, in particular if it is accompanied by an inflammatory event. The studies furthermore show that inhibition of transcription factor action can result in the re-arrangement of metabolic fluxes. Such adaptations may be different from what is predicted from target gene expression patterns. In addition, the interactions of enzymes and the net effect on global fluxes may vary under different metabolic conditions. The metabolic consequences of transcription factor action should therefore always be evaluated under relevant conditions. Finally, a change in the flux through a particular pathway may affect the flux through another route. Thus, the application of fluxomics in vivo is required to obtain a complete picture on the effects of drugs designed to modulate transcription factor activity. Careful evaluation of the global metabolic consequences will contribute to the development of future drugs.