Intestinal nuclear receptors in control of energy metabolism
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**General Introduction**

**Metabolic imbalance caused by our modern lifestyle**

Changes in our lifestyle have resulted in a worldwide increase in the prevalence of chronic metabolic diseases such as type 2 diabetes mellitus (T2DM), non-alcoholic fatty liver disease (NAFLD), cardiovascular disease (CVD) and certain types of cancer. These obesity-related lifestyle diseases are currently the largest health threat in our society and are mainly caused by excessive energy intake and a sedentary lifestyle. Fructose may be an important contributor to this pandemic as its consumption in the past 50 years has dramatically increased to more than 10% of our daily calorie intake, mainly due to the use of inexpensive corn-based sweeteners such as high-fructose corn syrup (HFCS) that was introduced in the early 1970’s[1,2]. Studies aimed to elucidate the mechanisms that contribute to the worldwide increase in metabolic health problems have traditionally focused on the development of insulin resistance in metabolically active organs such as adipose, muscle and liver. Only recently, however, the importance of the gut as an endocrine organ with a major role in whole body energy homeostasis has been underscored.

**Nutrient digestion and absorption in the gut**

The primary role of the gastrointestinal tract is to ensure nutrient absorption which in most cases entails digestion of dietary components before they can be transported into enterocytes (Table 1). Active transport over the plasma membrane takes place via membrane transporters at the cost of energy in the form of ATP. Alternatively, passive transport is mediated by facilitative transporters, channels and, in case of small hydrophobic molecules, direct diffusion. Dietary carbohydrates, fats and proteins are macronutrients that provide the building blocks for cellular structures as well as energy after being absorbed by the small intestine. Simple sugars are readily transported into and through enterocytes and, therefore, cause an acute raise in blood glucose levels. In contrast, complex carbohydrates, like starch, must first be digested before they can be absorbed. This results in a more gradual release of glucose molecules into the bloodstream and more stable plasma glucose levels. Complex carbohydrates are hydrolyzed into smaller polysaccharides by α-amylase in the oral cavity and the proximal part of the small intestine. Polysaccharides and disaccharides are further digested in the small intestine by enzymes in the brush border membrane (BBM) of enterocytes, like sucrase-isomaltase (SI), maltase-glucoamylase (MGAM) and lactase-phlorizin hydrolase (LPH)[3]. The resulting monosaccharides can subsequently be transported into enterocytes by various membrane transporters. Glucose molecules are absorbed from the intestinal lumen by the high affinity sodium-dependent glucose cotransporter 1 (SGLT1) and, with a lower affinity, by the facilitative glucose transporter 2 (GLUT2, SLC2A1). Transport of glucose from the enterocyte into the bloodstream is primarily mediated by GLUT2, as it localizes both to the apical and basolateral membrane[4].

Dietary proteins, composed of chains of amino acids, are digested in the stomach into polypeptides by the enzyme pepsin. Large polypeptides enter the duodenum where they are further digested by the pancreatic enzymes trypsin and carboxypeptidase and by brush border enzymes. Di- and tripeptides are taken up in the proximal part of the
small intestine by hydrogen-peptide transporter (PepT1), a process that is dependent on a pH-gradient which is maintained by transepithelial sodium/hydrogen exchange[5–7]. Free amino acids are absorbed into enterocytes via various sodium-dependent (proton-coupled transporter PAT1) and sodium-independent transporters[8]. Amino acids can be transported over the baso-lateral membrane via the portal vein into the systemic circulation and subsequently to all tissues. Within cells, amino acids can be used as building blocks to synthesize needed proteins.

Dietary fats are predominantly ingested in the form of triglycerides (TGs). Since fat is highly hydrophobic it forms lipid droplets, which are emulsified and, upon lipolysis, solubilized in the intestinal lumen by bile. Pancreatic lipase in turn can break the TGs down into monoacylglycerol and free fatty acids (FAs) that can diffuse through the cell membrane into enterocytes[9]. Within the enterocytes, absorbed FAs and monoacylglycerols are reassembled into triglycerides and as such transported towards peripheral tissues via lipoproteins called chylomicrons (reviewed in[10]).

Lastly, undigestible food components will reach the colon and are either digested by gut bacteria or excreted. Resistant starch for example is digested by gut microflora which produce short chain fatty acids (SCFAs). SCFA can be absorbed by the host and have beneficial effects on human metabolism[11].

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Table 1 Key hormones, secretory stimuli, and physiological processes occurring along the gut axis[12]. The details are based on mouse data. 5-HT, serotonin; CCK, cholecystokinin; EECs, enteroendocrine cells; GIP, glucose-dependent insulintropic polypeptide; GLP-1 and GLP-2, glucagon-like peptides 1 and 2; InsL5, insulin-like peptide 5; Nts, neurotensin; PYY, peptide YY; SST, somatostatin.
The role of the gut in whole-body metabolism

The contribution of the gastrointestinal tract to energy homeostasis has long been considered to be limited to the digestion and absorption of nutrients. Nowadays, however, it is generally accepted that the intestine is not only involved in nutrient absorption, but is also a metabolically active organ with endocrine functions governing systemic metabolism[13]. The major contribution of the gastrointestinal tract in the regulation of systemic energy homeostasis became apparent when gastrointestinal (bariatric) surgery was shown to be a very effective treatment for obese patients suffering from T2DM[14,15]. Although the mechanisms underlying the success of bariatric surgery are still being investigated, it is clear that the beneficial effects are not simply due to decreased food intake or reduced intestinal absorption of nutrients. Hypertension and blood glucose levels for example already improve after bariatric surgery before the weight loss occurs, indicating that bariatric surgery is beneficial for metabolic outcomes independent of weight loss[16].

The gut controls energy balance via various mechanisms but the intestine by itself also directly impacts total energy expenditure, due to its large size and high turnover rate of epithelial cells of 3-5 days. In addition, it has been shown that the intestine is an insulin-sensitive organ and during intestinal insulin resistance, which can develop independently of systemic insulin resistance, the intestine increases lipogenesis and lipoprotein synthesis resulting in dyslipidemia in obese patients[17,18].

Nutrient sensing in the gut is essential to regulate energy homeostasis and can be mediated via at least three different mechanisms. 1) Nutrient status can be communicated by the gut-brain axis via the vagus nerve to match food intake to energy demands; 2) The secretion of gut hormones, which regulate energy homeostasis by acting as satiety and hunger signals that control food intake and are needed to effectively regulate catabolic and anabolic reactions in other organs; 3) The gut microbiota can significantly affect the inflammatory and metabolic status of the host. These three nutrient sensing mechanisms are explained in more detail below.

Gut-brain axis

The systemic regulation of energy homeostasis is governed by the hypothalamus, an area within the central nervous system (CNS) that regulates feeding behavior[19]. The brain receives information about the energy status from peripheral organs via the vagus nerve and via signaling molecules like gut hormones and leptin, an adipose tissue derived satiety molecule[20,21]. This information from the periphery is used to match feeding behavior to energy demand.

The nervous interaction between the gut and brain is mainly taken care of by the enteric nervous system (ENS), a large network of neurons and glial cells in the gut which plays a key role in the regulation of energy homeostasis[12]. This so-called “second brain” consist of approximately 70% vagal afferent nerves that send information from gut hormones and gastric mechanoreceptors to the CNS. Interestingly, it has been shown that both caloric restriction and a high-fat diet (HFD) disturb the gut-brain axis in
mice. Indeed, in both situations, the effects of satiety signal peptides on the vagal afferent nerves of the ENS are blunted, while the ghrelin signaling is enhanced, ultimately promoting obesity[22,23].

Gut hormones

The gut produces many peptides that regulate satiety, including cholecystokinin (CCK), peptide YY (PYY), oxyntomodulin (OXM), glucose-dependent insulinotropic peptide (GIP), glucagon-like peptide 1 (GLP-1) and the appetite stimulant ghrelin. Incretins are gut hormones which are secreted upon food intake and have insulinotropic properties. The main incretins are GIP and GLP-1 which promote insulin secretion in response to glucose. Since GIP loses its insulinotropic effect during insulin resistance, most research focuses on GLP-1[24]. GLP-1 improves beta-cell function through enhanced sensitivity to glucose, higher beta-cell survival and increased proinsulin gene and protein expression, ultimately resulting in enhanced glycemic control[25–27]. Since GLP-1 is rapidly degraded by dipeptidyl peptidase-4 (DPP-4) upon secretion, DPP-4 inhibitors as well as GLP-1 receptor agonists have been studied as drug candidates to treat obesity and T2DM. Several GLP-1 based drugs have reached the clinic and they promote glycemic control without causing hypoglycemic side effects in T2DM patients[28]. The effect on weight control, however, differs between specific GLP-1 receptor agonists and DPP-4 inhibitors, where several GLP-1 based treatments result in weight loss while others are weight-neutral[29].

Surprisingly, it has recently been shown that Metformin, the most commonly prescribed drug for T2DM, maintains its glucose-lowering property if it can selectively and exclusively exert its effects via the gut. The improved glycemic control after intestinal Metformin treatment was ascribed to changes in the gut microbiome, increased GLP-1 levels and influencing the gut-brain axis[30,31]. Before this discovery, the beneficial actions of Metformin were primarily attributed to the liver where it causes a reduction in hepatic glucose production. Low doses of Metformin, however, improve glycemic control due to its effects in the gut[32]. These findings potentially can help to avoid adverse effects that are associated with higher doses of Metformin treatment.

Microbiota

Gut bacteria influence energy homeostasis mainly via retrieving energy from otherwise indigestible nutrients, producing SCFAs, causing low-grade systemic inflammation and regulating bile acid homeostasis[33–35]. Importantly, the gut microbiota composition is crucial for the metabolic outcome since substrate cross-feeding between bacterial stains determine the fermentation end-products. The gut microbiota composition is unique for each individual and can be modulated by the diet, especially by the presence of indigestible dietary components. In addition, dietary prebiotics, (indigestible dietary fibers) and probiotics (live bacteria) are used to influence the intestinal microbiota composition in order to reach an improved metabolic status[36].

SCFAs are volatile fatty acids, produced by microbiota. The total SCFA pool and the relative contribution of a specific SCFA is determined by diet and gut microbiota
composition (reviewed in[37]). The three most studied SCFAs, butyrate, propionate and acetate, are suggested to promote metabolic health. Interestingly, supplementation of any of these SCFAs has beneficial effects on obesity and insulin resistance in mice fed a HFD, although the extent of these effects differ between the specific SCFAs[38]. The mechanisms to explain these effects on metabolism are plural, including, the reduction of food intake, the stimulation of gut hormones and the downregulation of PPARγ signaling in adipose tissue[38,39].

Gut microbiota derived lipopolysaccharide (LPS) causes a pro-inflammatory status that is associated with metabolic perturbations such as leptin resistance and inadequate satiety signal transmission through the vagus nerve[23,40,41]. Interestingly, an altered gut microbiota was found after bariatric surgery and this was associated with reduced levels of low-grade systemic inflammation, explaining at least in part the metabolic beneficial effects independently of weight loss[42,43].

**Nutrient sensors in the gut**

Several nutrient sensing mechanisms are present in the intestinal tract to match food intake with energy homeostasis. Here, we review the most important intestinal nutrient sensors that are activated by dietary cues and that govern whole-body metabolism. The superfamily of G-protein coupled receptors (GPCRs) are membrane bound receptors that are activated by nutrients and other cues present in the intestinal lumen. In addition, intracellular transcription factors such as the nuclear hormone receptor (NR) family of ligand-activated transcription factors, and members of the basic-helix-loop-helix family of transcription factors such as carbohydrate response element-binding protein (ChREBP) and sterol regulatory element binding protein (SREBP) are activated by ligands or metabolites within the cell.

**G protein-coupled receptors**

The largest family of plasma membrane bound receptors are G protein-coupled receptors (GPCRs), characterized by 7 transmembrane loops and intracellular G protein signaling (reviewed in[44,45]). The GPCR-superfamily is involved in the detection of a wide range of signals including nutrients, bile acids, SCFAs, (gut) hormones and neurotransmitters. With about one third of FDA approved therapeutics on the market today targeting this family, GPCRs are the largest group of drug targets for a wide variety of diseases[46–49]. Since these receptors can be activated by agonists present in the intestinal lumen, many GPCR targeted drugs can be taken orally. For example, TGR5, also known as G protein-coupled bile acid receptor 1 (GPBAR1), is activated by bile acids in enteroendocrine L-cells and increases the proglucagon expression. Systemic activation of TGR5 protects against diet-induced obesity via increased energy expenditure and regulates glucose homeostasis via induced GLP-1 production in animal models[50–52]. However, this was also associated with gall bladder and heart problems[53]. Intestine-specific TGR5 ligands have therefore been developed to enhance GLP-1 release while avoiding systemic adverse effects[54,55]. This demonstrates the potential of intestinal GPCRs as putative drug targets for anti-diabetic therapy and indeed more studies are now conducted in this research field[48,56,57].
ChREBP and SREBP

An adequate postprandial response to carbohydrate intake on de novo lipogenesis is regulated via the activation of ChREBP and SREBP1c by glucose and insulin, respectively. Insulin regulates SREBP1c, the hepatic activator of de novo lipogenesis and thereby mediates the induction of lipogenic gene expression in response to glucose intake[58,59]. In response to intracellular sugar levels, the glucose-activated transcription factor ChREBP activates target genes involved in glycolytic and lipogenic pathways and is also required to mediate a postprandial response (reviewed in[60]). Synergistic actions of these transcription factors have been discovered, which were at least partly mediated by the sterol sensor LXR (Figure 1)[59].

Figure 1. Regulation of hepatic lipogenesis requires the concerted actions of ChREBP, LXR, and SREBP-1c. This schematic depicts the synergistic action of insulin and fructose/glucose in promoting hepatic lipogenesis. Insulin increases SREBP-1c mRNA as well as the proteolytic processing of SREBP-1c protein, whereas fructose/glucose activates ChREBP. SREBP-2, which regulates genes of cholesterol biosynthesis, controls the production of endogenous sterol ligands to activate LXR. LXR regulates the transcription of SREBP-1c, ChREBP and other lipogenic genes. ChREBP also directly regulates SREBP-1c expression and, at least under certain conditions, SREBP-1c can also regulate ChREBP. Together, SREBP-1c, LXR, and ChREBP are required for maximal induction of postprandial hepatic lipogenesis[59].

After activation by glucose or glucose metabolites such as glucose-6-phosphate, ChREBP migrates to the nucleus. Together with its dimerization partner Max-like protein X (Mlx), ChREBP binds to the promoter of target genes to control transcription[60]. In 2012, the isoform ChREBPβ was discovered as the most potent activator of ChREBP target genes[61]. ChREBPα is the more glucose-responsive isoform and is a positive regulator of ChREBPβ, completing a positive feedforward pathway. ChREBPs are ubiquitously expressed and recently the importance of intestine-specific ChREBP in fructose absorption and metabolism was highlighted. Similar to the whole-body ChREBP knockout mice, intestinal-specific ChREBP deficient mice displayed fructose malabsorption and intolerance[62,63]. In contrast, hepatic ChREBP was not essential for fructose metabolism, at least partly because hepatic ChREBP does not control first-pass fructose absorption whereas intestinal ChREBP governs fructose transporter Glut5 levels in the small intestine.
The SREBP-family consists of 3 members: SREBP1a, SREBP1c and SREBP2, which are key regulators of lipid- and cholesterol metabolism and predominantly expressed in metabolically active tissues[64]. SREBP2 regulates cholesterol metabolism, and induces the synthesis of endogenous oxysteroid ligands to activate LXR, which subsequently leads to lipogenic gene activation via SREBP1c, a major target gene of LXR[65]. SREBP1c predominantly regulates hepatic triglyceride and fatty acid synthesis. In addition, SREBP1c is under the transcriptional control of insulin, resulting in enhanced lipogenic activity after glucose intake. SREBP precursors are inactive and located in the endoplasmic reticulum (ER). These precursors are transported towards the Golgi when intracellular sterol levels are low. Inside the Golgi, SREBPs are processed by proteases to reach their mature, active forms. Mature SREBPs can enter the nucleus where they exert their transcriptional regulation to restore sterol homeostasis (reviewed in[64,65]).

**Nuclear Receptors**

The nuclear receptor (NR) family consist of 48 members in humans (49 members in mice) and are ligand-activated transcription factors. NRs govern the transcriptional regulation of genes involved in a multitude of biological processes including inflammation, differentiation, reproduction and metabolism[66]. The best known NR ligands are the steroid hormones but also a wide variety of other small lipophilic molecules like bile acids, fatty acids, oxysterols and xenobiotics can serve as natural ligands for NRs (reviewed in[67]). For many NRs, the so-called orphan receptors, a physiological ligand has not been identified. After ligand binding, activated NRs bind directly to a hormone response element (HRE) sequence in the promoter region of target genes to regulate their transcription. NRs can also directly bind to other transcription factors thereby obstructing their actions. This indirect mechanism is called transrepression and is predominantly seen in the regulation of inflammation[68]. The work in this dissertation focuses on the role of intestinal NRs in controlling systemic metabolism and this will be discussed more extensively in the next sections.

**Pharmaceutical potential of nuclear receptors**

The NRs are one of the largest groups of drug targets, with about 16% of FDA approved therapeutics on the market today targeting this family, including drugs for the treatment of insulin resistance (glitazones, TZDs), hyperlipidemia (fibrates), inflammation (dexamethasone) and cancer (tamoxifen)[49]. Development of synthetic ligands with agonistic (full, partial, dual, inverse) or antagonistic effects have provided novel therapeutics and research tools to study NR function, which resulted in a major leap forward in NR research[66].

Drugs that exert their effects via NR activation also include glucose lowering therapies. For example, thiazolidinediones (TZDs) bind and activate the peroxisome proliferator-activated receptor gamma (PPARγ) causing an insulin sensitizing effect[69]. Despite their efficacy in glycemic control, TZDs are associated with various serious adverse side effects, including weight gain, fluid retention, osteoporosis and cardiovascular toxicity, which has strongly limited their clinical use. Current research on PPARγ and other NRs therefore focuses on the development of partial agonists, or so-
called selective pharmacologic modulators, which either act only on a subset of genes or have tissue specific activity in order to circumvent systemic off-target effects (Figure 2,[70,71]). Another approach to minimize adverse effects of drugs targeting NRs is via directly targeting of downstream targets. An example of a downstream target of PPARy with anti-diabetic activity is fibroblast growth factor 1 (FGF1)[72].

**Figure 2. Nuclear receptor mechanisms of action. Schematic illustrating the principle of selective receptor modulation.** NRs are regulated by small molecule ligands, which generally stabilize the receptor into a conformation suitable to bind coregulator proteins (coactivators or corepressors). Ligands can also modulate posttranslational modification of the receptor. Ultimately, these events have an impact on the expression of receptor-specific target genes by modulating coregulator recruitment at specific DNA-response element sites in the target gene promoter (adapted from[70]).

**Nuclear hormone receptors in the intestine**

Considering the emerging acknowledgement of gastrointestinal control in systemic energy homeostasis, intestine-specific NR activation has recently gained popularity as a strategy to regulate systemic energy balance. Many NRs are expressed in the gastrointestinal tract but their specific roles in this tissue remain largely unexplored[73–75] So far, NRs in the intestine have been demonstrated to be involved in a wide variety of (patho-) physiologies. NRs in the gut regulate metabolism by controlling metabolic genes involved in intestinal inflammation, gut hormone secretion, nutrient absorption and bile acids metabolism[76,77].
Regulation of the enterohepatic circulation

As mentioned previously, the gut microbiota governs whole-body metabolism via various pathways, including influencing the bile acid pool size and composition. For instance, gut microbiota can induce GATA4 and, thereby, inhibit the intestinal bile acid transporter ABST blocking the recycling of bile acids[35]. The intestinal NR Farnesoid X receptor (FXR) is a bile acid sensor and influences energy metabolism in response to changes in the bile acid pool, while FXR in turn also controls the bile acid homeostasis[78,79] (Figure 3).

Figure 3. Model of negative feedback regulation of hepatic bile-acid synthesis via FXR in a tissue-specific manner[80]. Bile acids are synthesized by hepatocytes, secreted into the bile and released from the gallbladder into the small intestine upon feeding to assist in lipid absorption. Approximately 95% of the bile acids are reabsorbed in the ileum and transported back to the liver. Intestinal FXR is activated by bile acids in the gut and induces the production and secretion of hormone fibroblast growth factor 19 (FGF19) (FGF15 in mice) which in turn suppresses bile acid synthesis via CYP7A1 in hepatocytes thereby completing the negative feedback cycle[81].

Besides FXR, the NRs Vitamin D receptor (VDR) and Pregnane X receptor (PXR) are also activated by bile acids in the intestine. Similar to FXR, VDR induces FGF15 to reduce hepatic bile acid synthesis in mice and thereby controls bile acid homeostasis[82]. Intestinal VDR has also been demonstrated to protect against colon cancer[83]. PXR is a xenobiotic sensor that is activated by many ligands including bile acids, steroid hormones and xenobiotic compounds. Intestinal PXR activation leads to increased cholesterol uptake from the intestinal lumen via Niemann-Pick C1-Like 1 (NPC1L1), CD63 and other lipogenic target genes, thereby linking a high xenobiotic exposure to the increased prevalence of chronic metabolic diseases[84,85][86].
Systemic FXR activation is associated with reduced cholesterol and triglyceride levels in liver and plasma in mice[87] and improved glycemic control in wild-type mice and db/db mice[88]. Interestingly, Fang et al showed that intestinally-restricted FXR activation by a non-absorbable FXR ligand named Fexaramine[89] protected against weight gain and improved the glucose tolerance and insulin sensitivity in mice during a HFD challenge, highlighting the role of intestine-specific FXR in maintaining metabolic homeostasis. However, increased energy expenditure by enhanced browning of adipose tissue in mice treated with Fexaramine was at least partly mediated by TGR5 signaling, since TGR5 deficient mice treated with Fexaramine were blunted in these metabolic improvements[74].

Intestine-specific inhibition of FXR signaling in mice has also been investigated using glycine-β-muricholic acid (Gly-MCA) treatment demonstrating that intestinal FXR deficiency protected against diet-induced obesity and obesity-related metabolic derangements[75]. These seemingly contradictory results of intestinal FXR actions can be explained by the differential activation of the TGR5 pathway. Whereas, TGR5 was activated by fexaramine, this was not case upon Gly-MCA treatment[75]. These studies indicate the complexity of the molecular pathways by which NRs govern metabolism.

Glycemic regulation

Several NR members including hepatocyte nuclear factor 4 gamma (HNF-4γ), FXR and PPARδ have been suggested to regulate intestinal expression of the proglucagon gene which encodes incretins such as GLP-1 and GIP[90][91][92]. As mentioned above, incretin-related drugs have insulinotropic properties and are therefore promising treatments to improve the metabolic status. Consequently, targeting NRs that regulate incretin production may also have potential as a new therapeutic strategy[93]. HNF-4γ deficient mice displayed increased proglucagon gene expression and GLP-1 secretion[90]. FXR deficiency improved glycemic control in mice fed a HFD, which is mediated by GLP-1 levels. In addition, FXR activation directly decreased proglucagon mRNA levels in human and mice[91]. Together, these data suggest that intestinal antagonist treatment of HNF-4γ and FXR can be beneficial to ameliorate glucose control via incretin actions. On the other hand, PPARδ activation by the selective agonist GW501516 increased proglucagon gene expression and GLP-1 secretion in mice resulting in improved glucose tolerance[92]. This suggests that intestinal activation of PPARδ could be a putative treatment strategy to treat metabolic derangements in glucose homeostasis.
General Introduction

Regulation of cholesterol metabolism

High density lipoprotein cholesterol (HDL-c) exerts a transport function in the reverse cholesterol transport (RCT) pathway, which reduces the lipid deposition in the peripheral tissues, a process associated with the protection against cardiovascular diseases and atherosclerosis (Figure 4). Approximately 30% of pre-β HDL particles are produced in the intestine and 70% in the liver[94,95]. ATP-binding cassette A1 (ABCA1) transports cholesterol and phospholipids into lipid-free apolipoproteins which is a rate-limiting step in pre-β HDL particle synthesis. Abca1 gene expression is upregulated by LXR[96] and PPARα[97] activation.

Figure 4. Sterol fluxes across the enterocyte. At the apical membrane, Free cholesterol (FC) is taken up by NPC1L1 protein and can be directed to ACAT2-mediated esterification and subsequent secretion in chylomicrons via MTP. FC can be secreted as an HDL component by the basolateral transporter ABCA1 or can be effluxed back into the intestinal lumen by the apical heterodimer ABCG5/G8. Enterocytes are also actively excreting plasma-derived cholesterol in a process named TICE, however the underlying mechanisms remain to be uncovered (adapted from:[98]).

Dietary and biliary cholesterol in the intestinal lumen is absorbed via the cholesterol transporter Niemann Pick C 1 like 1 (NPC1L1), which expression is upregulated by the NRs PPARα[99] and HNF4α[100] and downregulated by LXR[101] and PPARδ[102]. The heterodimer ATP-binding cassette (ABC) transporters G5 (ABCG5) and G8 (ABCG8) counteract the function of NPC1L1 by transporting cholesterol back into the intestinal lumen. LXR activation increases Abcg5 and Abcg8 gene expression resulting in enhanced cholesterol secretion[103]. Overall, LXR controls many genes in lipid metabolism resulting in increased cholesterol efflux, HDL plasma levels and hepatic lipogenesis upon systemic LXR activation[104]. Synthetic agonists targeting LXR are therefore putative drugs to treat atherosclerosis. However, until now no LXR agonists have reached the clinic due to serious side effects[105]. The main side effect is caused by LXR activation in
hepatocytes which induces lipogenic activity and drives the development of non-alcoholic fatty liver disease (NAFLD). Intestine-specific LXR activation could therefore be a strategy to circumvent the hepatic adverse effects while still inducing the therapeutic benefits. To study this, a villin-specific constitutively active LXRα transgenic mouse model has been developed[73]. Indeed, intestinal LXRα activation stimulated macrophage-mediated RCT, without inducing lipid accumulation in hepatocytes. Intestine-specific LXRα activation also decreased cholesterol absorption and induced HDL plasma levels via increased expression of known intestinal LXR targets Abcg5/Abcg8 and Abca1. An intestine selective activation of LXR was also observed for the selective agonist GW3965 possibly via alternative recruitment of co-activators[106]. GW3965 treatment of mice by oral gavage induced HDL-c levels in an intestine-specific, Abca1 dependent manner and this was not associated with hepatic de novo lipogenesis[96]. Intestine-specific LXR activation might thus be a great strategy to treat atherosclerosis while avoiding the development of a fatty liver.

In conclusion, understanding the molecular mechanisms by which intestine-specific NRs regulate metabolism is important to develop more effective and safer drugs while limiting off-target effects.

Aim and outline of this thesis

The general aim of this thesis was to explore the potential of intestinal NR activation in the regulation of whole-body energy homeostasis. While systemic activation of NRs has been successfully applied in the pharmaceutical industry, it often causes major adverse effects due to the pleiotropic target genes of NRs. Only recently, the importance of the gut as an endocrine organ with a major role in whole body energy homeostasis has been underscored. Selective activation of NRs in the gut might therefore prove to be an effective way of inducing beneficial effects on metabolism while avoiding, or at least minimizing, systemic toxicity.

High fructose consumption is implicated as an important factor in the development of metabolic syndrome, however, no consensus has been reached on the precise role and impact of dietary fructose. Moreover, recently it has been demonstrated that the gut has a more dominant role than the liver in determining the fate of dietary fructose[63,107]. In chapter 2 we therefore aimed to gain more insight in the specific effects of fructose on the intestine by investigating the changes in the intestinal and hepatic transcriptome after a 2-weeks high-carbohydrate challenge of dietary fructose as compared to glucose or cornstarch in mice.

The GLUT-family of facilitative hexose transporters is essential to effectively absorb simple sugars from the intestinal lumen and transport them throughout the body and to tissues where they can be utilized. To match sugar uptake with energy demand, the
expression levels of GLUT-members are tightly controlled by various regulatory mechanisms. The intestinal expression and regulation of members the GLUT-family of hexose transporters, however, is not well-studied and their contribution to the development of metabolic disorders remains largely unknown. In chapter 3 we set out to identify novel transcriptional regulators of the 14 human GLUT-members. For this, we used a high-throughput promoter reporter screen testing for regulation by all 49 members of the nuclear receptor (NR) family, a superfamily of ligand-modulated transcription factors (TFs) as well as by NR co-regulator peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1α) and glucose-activated transcription factors ChREBPα and -β. In addition we profiled the expression of members of the GLUT-family in human Caco2 cells and validated several of the newly identified regulations of intestinal GLUTs in this in vitro model of the small intestine. Fructose absorption in the small intestine is primarily mediated by GLUT5, which makes this transporter indispensable to absorb our modern high-fructose diet. The physiological function of GLUT7, a protein with 53% amino acid similarity to GLUT5, is still largely unknown. Moreover, the physiological substrate of GLUT7 is the subject of an ongoing discussion and no consensus has been reached about its ability to transport fructose and/or glucose[108–111]. The transcriptional regulation of these two fructose transporters, GLUT5 and GLUT7, was further investigated in chapters 4 and 5, respectively. Understanding how these transporters are regulated will not only enhance basal knowledge of fructose absorption and metabolism but might also help to assess the role of high fructose consumption in the development of metabolic syndrome.

In the final experimental chapter, we focused on the role of peroxisome proliferator-activated receptor δ (PPARδ) in the intestine. PPARδ is well known for its role in fatty acid oxidation in adipose tissue and skeletal muscle and improves dyslipidemia in mice and humans. Although PPARδ is abundantly expressed along the entire intestinal tract, its potential role in energy homeostasis in this organ has not been well explored. In chapter 6 we therefore investigated the role of intestinal PPARδ activation on whole body metabolism using mice with an intestinal epithelial cell-specific deletion of PPARδ (PPAR-delta^{IEC-KO}). PPARδ plays an important role in energy homeostasis; systemic PPARδ activation with the ligand GW501516 improves plasma lipid profile and protects against diet-induced obesity in mice, however, the role of intestine-specific PPARδ remained to be elucidated[112]. Here we investigated the metabolic effects of a high-fat diet and treatment with the selective PPARδ agonist GW501516 in mice with a specific deletion of PPARδ in the intestine (PPAR-delta^{IEC-KO} mice).

Finally, chapter 7 discusses the main findings of this thesis and gives recommendations for future research.
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