Targeting inter-organ cross-talk in cardiometabolic diseases

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General introduction and outline

Cardiometabolic health is tightly controlled by highly coordinated cross-talk among various tissues and organs. In this thesis, by using dietary and pharmacological interventions, I explored the therapeutic potential of targeting inter-organ communication in cardiometabolic diseases. By the end I hope you will realize that when talking about therapeutic strategies for cardiometabolic diseases - it's all about inter-organ cross-talk.

GENERAL INTRODUCTION

Cardiometabolic health is achieved through a complex network of organ communication. Dysfunction of these lines of communication contributes to the development of cardiometabolic diseases, which are quickly becoming pressing public-health concerns globally.

1. Cardiometabolic diseases

Definition of cardiometabolic diseases. Cardiometabolic diseases are a collective term referring to metabolic disorders, non-alcoholic fatty liver disease (NAFLD) and cardiovascular diseases (CVDs). Metabolic disorders that are driven by abnormal fat expansion are a group of conditions including dyslipidemia, hyperglycemia, insulin resistance, obesity, type 2 diabetes (T2D) and hypertension, and these metabolic abnormalities are all well-documented risk factors for the development of NAFLD and CVDs [1-4]. Given the close association between NAFLD and CVD risk factors encapsulated by the metabolic disorders, it is not surprising that NAFLD was recently proposed to be associated with increased risk of CVDs. Substantial epidemiological evidence and population-based cohort studies indeed link NAFLD to subclinical atherosclerosis and a high prevalence of clinically manifest CVDs. In case control studies, NAFLD has been shown to be associated with increased artery intima-media thickness and arterial wall stiffness [5, 6]. Likewise, cohort studies in people with biopsy-proven NAFLD demonstrate that CVDs are the most common cause of death in this population [7-9]. According to these available findings, there seems little doubt that NAFLD is also a risk factor of CVDs.

NAFLD is an umbrella term for a group of liver diseases ranging from hepatic steatosis, featured by hepatocyte lipid overload, to nonalcoholic steatohepatitis (NASH) with hepatic steatosis, lobular inflammation, hepatocyte ballooning and varying degrees of fibrosis [2]. People diagnosed with NASH are predisposed to developing cirrhosis and hepatocellular carcinoma, among whom people with severe hepatic fibrosis are at highest risk of overall and liver-related mortality [10]. Currently, there are no established pharmacological agents for NASH. Rather, lifestyle interventions remain

the first-line treatment for it, despite that lifestyle changes are rarely attainable in the long term, and the liver transplantation is still the sole intervention to treat the end-stage of NASH [2, 11].

CVDs, as indicated by the name, are a broad term of diseases affecting the heart and/or blood vessels. The main cause of CVDs is atherosclerosis, which is defined by the build-up of atherogenic lipoprotein-derived cholesterol and immune cells in the vessel wall, to form so-called atherosclerotic plaques [12]. The most clinically dangerous plaques, by rupturing or eroding, can trigger occlusive luminal thrombosis, thereby causing a heart attack or stroke, depending on the specific artery that is occluded by the thrombus [12]. Of note, almost 18 million people die from CVDs each year, making it the number one cause of death globally [13]. Although alleviation of dyslipidemia largely decreases cardiovascular morbidity and mortality as consistently observed in large clinical trials, many at-risk patients either fail to reach their target lipid levels using current therapeutics like statins or are intolerant to statins owing to their adverse effects [14]. This leaves many patients with obvious residual risks.

Collectively, there is an unmet need for additional therapeutic targets and strategies that control the development of cardiometabolic diseases, in particular of NAFLD and atherosclerotic CVD, or even reverse the underlying pathophysiology.

Pathogenesis of insulin resistance and obesity. The etiology of cardiometabolic diseases is associated with a positive energy balance. In the human body, energy is stored as glycogen and, to much larger extent, as lipids, which represents long-term energy storage. In health, lipids are mainly stored as triglycerides (TGs) located within lipid droplets in white adipose tissue (WAT), and, to much lesser extent, stored ectopically in non-adipose tissues. In obesity, a dysfunction of long-term fat storage in WAT resulting from chronic overnutrition, impaired adipogenesis, restricted hyperplasia and other factors, leads to increased circulating free fatty acids (FAs) and consequently increased ectopic fat deposition in e.g. the liver and skeletal muscle. Excessive lipid accumulation in these non-adipose tissues can disturb their metabolic function, thereby promoting disease progression. FAs in the circulation are partially derived from the lipolysis of chylomicron- and very-low density lipoprotein (VLDL)-derived TGs by the action of lipoprotein lipase (LPL) [15]. Most circulating FAs are derived from TG stores in WAT, released via the action of the cyclic adenosine monophosphate (cAMP)-dependent enzymes, including adipose triglyceride lipase (ATGL), hormone sensitive lipase (HSL), and monoglyceride lipase (MGL) [16]. Insulin secreted by pancreatic β cells is crucial for inhibition of FA release from WAT; inhibition of adipose tissue lipolysis is even the most sensitive pathway of insulin action. When

insulin resistance develops, intracellular lipolysis in adipocytes increases to produce more FAs, which further inhibit the anti-lipolytic effect of insulin and thus promote additional lipolysis [1].

Pathogenesis of NAFLD. A 'two-hit' theory was posited for several years to explain NAFLD pathogenesis [17]. This theory indicates that in the setting of the first 'hit', namely steatosis alone, which is caused by a chronic lipid accumulation in hepatocytes, a second 'hit', such as endoplasmic reticulum (ER) stress, oxidant stress and inflammasome activation, is required for the development of NAFLD by activating inflammatory cascades and fibrogenesis. However, this view appeared too simplistic and is now considered outdated [18]. There are various molecular pathways that contribute to NAFLD development, and it is even uncertain whether NAFLD is always preceded by fatty liver. Therefore, a 'multi-hit' concept has recently been proposed and is currently used for understanding NAFLD pathogenesis. The 'multi-

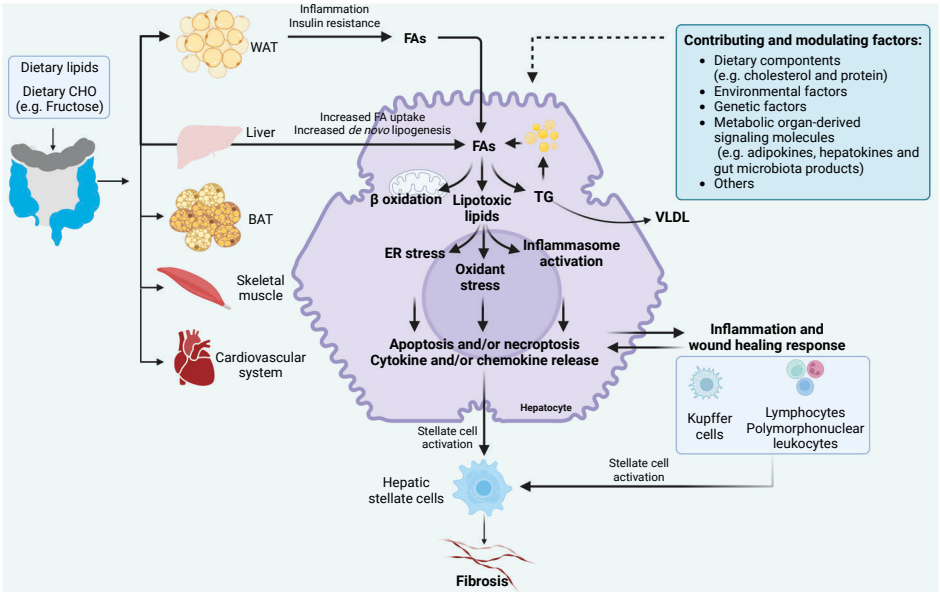

Figure 1-1. Pathogenesis of NAFLD. Fatty acids (FAs) derived from dietary lipids or white adipose tissue (WAT) circulate in the blood as bound to albumin, and can then be delivered to the liver. The liver itself can also produce FAs via *de novo* lipogenesis, the process through which hepatocytes convert excess dietary carbohydrates (CHO) like glucose to FAs. The two main fates of FAs in hepatocytes are breakdown through mitochondrial β-oxidation and re-esterification to form triglycerides (TGs) that can be stored in lipid droplets. Lipid droplet-derived TGs undergo precisely-controlled lipolysis to release FAs back into the hepatocyte FA pool. When the disposal of FAs through β-oxidation or TGs synthesis is overwhelmed, FAs can form toxic lipid species that can cause endoplasmic reticulum (ER) stress, oxidant stress and inflammasome activation. These processes can lead to hepatocellular injury, inflammation, stellate cell activation and fibrogenesis. BAT, brown adipose tissue; WAT, white adipose tissue.

hit' theory is based on the concept that various factors (e.g. dietary, environmental and genetic) can induce insulin resistance, obesity and abnormal changes of the gut microbiota, all of which are all risk factors contributing to NAFLD initiation and progression [11]. The conceptual frame work behind this theory is that the capacity of the liver to handle the primary metabolic energy substrates, e.g. FAs and carbohydrates (CHO), is overwhelmed, leading to aberrant accumulation of toxic lipid species in hepatocytes, which can induce hepatocellular stress, injury and death (**Figure 1-1**).

Pathogenesis of atherosclerosis. Atherosclerosis, the main cause of CVDs, is considered to be a lipid-driven inflammatory disease [19]. In the early stage, apolipoprotein B (ApoB)-containing lipoproteins, such as TG-rich lipoprotein (TRL) remnants and low-density lipoprotein (LDL) particles, are deposited and accumulate in the vessel wall, where these particles can undergo oxidative and other modifications that can render them pro-inflammatory and immunogenic. This is followed by the recruitment of classic monocytes into the intima of the vessel wall. Bloodstream monocytes can bind to adhesion molecules expressed by the endothelial cells. Chemokines can promote the migration of these bound monocytes into the vessel wall. Once in the intima, monocytes can differentiate into macrophages, and attain a phenotype related to the reparative macrophage population [19]. These macrophages express scavenger receptors that allow them to bind to and take up modified lipoprotein particles, which turns them into lipid-rich foam cells. At this stage, mild lesions or 'fatty streaks' are formed that are still reversible. With disease progression, these foam cells and endothelial cells can release pro-inflammatory cytokines, and T lymphocytes, despite numerically less abundant than macrophages, also enter into the intima, where they can affect the function of innate immune cells, endothelial and smooth muscle cells (SMCs) [19]. SMCs within the media are able to migrate into the intima in response to factors released by the accumulating leukocytes. These SMCs can produce extracellular matrix molecules (such as collagen), that contribute to the intimal thickening. T cell-derived factors, such as interferon γ, can dampen collagen synthesis by SMCs and impair the ability of SMCs to repair and maintain the fibrous cap that overlines the fatty streak. Foam cells and SMCs may eventually die, resulting in the formation of a necrotic core within the plaque. Furthermore, pro-inflammatory macrophages and other immune cells can increase their production of matrix metalloproteinases to degrade collagen and consequently impair fibrous cap strength. Thinning and structural weakening of the fibrous cap can upregulate the susceptibility of the plaque to rupture (**Figure 1-2**).

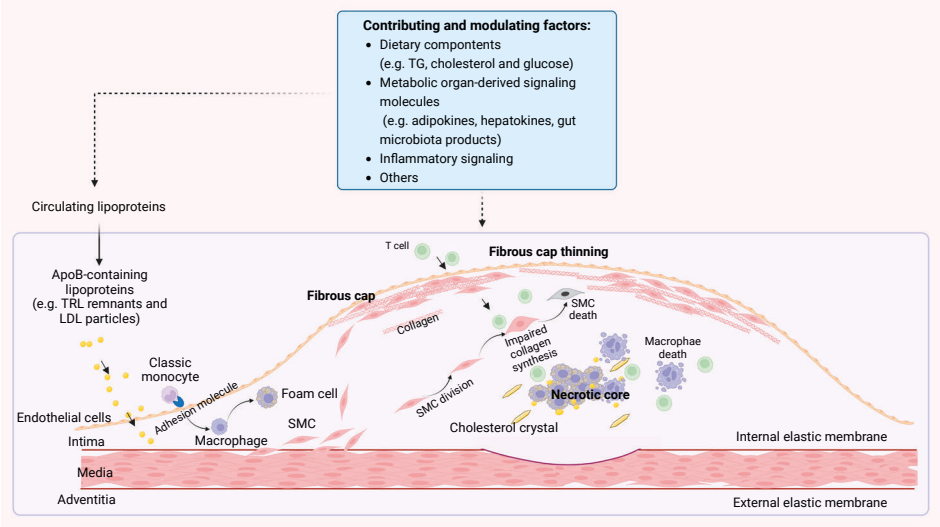

Figure 1-2. Pathogenesis of atherosclerosis. Increased circulating levels of atherogenic lipoproteins, caused by multiple factors e.g. dietary lipids and metabolic organ-associated signaling molecules, can promote atherosclerosis development. Early events of atherosclerosis include infiltration of atherogenic lipoproteins into the intima and adhesion of blood leukocytes, primarily monocytes, to the activated endothelial monolayer. This is followed by migration of monocytes into the intima and their maturation into macrophages. These macrophages can then take up large amounts of lipids to transform into lipid-laden foam cells. Lesion progression involves smooth muscle cell (SMC) migration from the media into the intima, resident intimal and media-derived SMC proliferation and extracellular matrix synthesis (e.g. collagen synthesis). Macrophages and SMCs within plaques can die in advancing lesions. Extracellular lipids derived from dead and dying cells can accumulate in the central region of a plaque, often denoted the lipid or necrotic core. TRL, triglyceride-rich lipoprotein; LDL, low-density lipoprotein.

2. Inter-organ cross-talk: a gatekeeper for cardiometabolic health

In multicellular organisms, maintenance of whole-body homeostasis and response to nutritional and environmental challenges require the coordination of different tissues and organs. To respond to metabolic demands, higher organisms have evolved multidirectional interactions among various metabolic organs and tissues in periphery and in the central nervous system (CNS). In this regard, inter-organ cross-talk represents a system of biological communication which relays important information about metabolic fluxes between physiological distant cells. Although how these metabolic organ systems interact with one another is still not completely understood, one key strategy for modulating whole-body metabolism is communication among tissues through signaling molecules including peptide/protein hormones, bioactive lipids, functional small molecules and other factors (e.g. micro-vesicles and exosomes). These molecules allow multiple organ systems to work together to absorb, store, sense and use energy, and to regulate the efficiency of energy metabolism of the body. Thus, tightly controlled inter-organ cross-talk is crucial for maintaining cardiometabolic health.

Gastrointestinal tract-derived signaling molecules. The gastrointestinal (GI) tract, the key interface between ingested dietary nutrients and the body, plays a pivotal role in regulating energy utilization. The GI tract harbors enteroendocrine cells (EECs) along its entirety. EECs are the main sensors of the ingested nutrients, and can release peptide hormones into the paracellular space where they either act locally or enter into the bloodstream and circulate to other organs [20]. Recently, it has also been identified that many EECs contain basolateral cytoplasmic elongations known as neuropods that not only contain peptide/protein hormones but also connect to nerves of the GI tract, which allows the transduction of nutrient signals from the GI tract directly to the brain [21]. Accordingly, in response to various challenges (e.g. dietary changes) within a proper range, the GI tract has the ability to maintain a homeostatic status through inter-organ cross-talk (**Figure 2-1**).

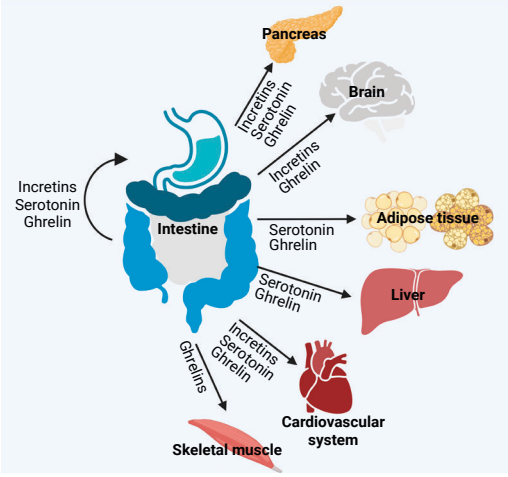

Figure 2-1. Gastrointestinal tract-derived signaling factors. Hormones secreted by the gastrointestinal (GI) tract, such as the incretin hormones GIP and GLP-1, serotonin and ghrelin, play an important role in the regulation of nutrient uptake and storage. These hormones can act on the brain to modify energy metabolism, and also have diverse metabolic effects on target organs, such as pancreas, adipose tissue, the liver, skeletal muscle and cardiovascular system (selected GI tract-derived factors and selected target organs are shown).

Among GI-derived signaling molecules, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) are the best-characterized. GLP-1 and GIP are released from enteroendocrine L and K cells, respectively, within the gut upon consumption of dietary nutrients, primarily simple carbohydrates (CHO). Upon secretion, the majority of GLP-1 is rapidly degraded by dipeptidyl peptidase-4 (DPP4); only approximately 25% reaches the hepatoportal circulation and 10% reaches the systemic circulation [22, 23]. GLP-1 exerts its function through binding to and activating its specific G-protein-coupled receptor (GPCR), e.g. the GLP-1 receptor (GLP-1R) [24]. Likewise, most of GIP is rapidly degraded by DPP4 after releasing from the gut following nutrient intake, and the remaining GIP in the circulation can act on its receptor, the GIPR, to confer its function. Since the GLP-1R and GIPR are highly expressed in pancreas, these two GI-derived peptide hormones can act on pancreatic β cells to exert insulinotropic effects in a glucose-dependent manner [25]. Functional GLP-1R and GIPR are also expressed on extra-pancreatic organs, such as the brain,

where GLP-1 and GIP can achieve the control of energy intake (e.g. food consumption) and expenditure (e.g. BAT thermogenesis) [26]. GI tract also produces serotonin. After releasing to the bloodstream, the GI-derived serotonin interacts with multiple metabolic organs (e.g. the liver and adipose tissue) to regulate whole-body metabolism [27]. Other GI-derived signaling molecules, such as ghrelin, also relay metabolic information to multiple metabolic organs to influence whole-body metabolism in response to different metabolic conditions [20, 27].

Gut microbiota-associated signaling molecules. Besides producing signaling molecules itself, the GI tract also harbors a complex and dynamic population of microorganisms, the microbiota, the density of which increases from proximal to the distal end of the intestine. The gut microbiota is dominated by bacteria, while fungi, viruses, archaea and protozoa are also present. It forms a bioreactor which is fueled by exogenous dietary components and endogenous compounds generated from microorganisms and the host to produce bioactive compounds. These gut microbiota-derived metabolites signal to various metabolic organs in the body, which adds to cross-talk between the gut and the host. The structural components of the microbes also contribute to organ cross-talk under pathological conditions. Owning to the gut microbiota-host communication, the gut microbiota plays critical roles in regulating physiological functions of the host (**Figure 2-2**).

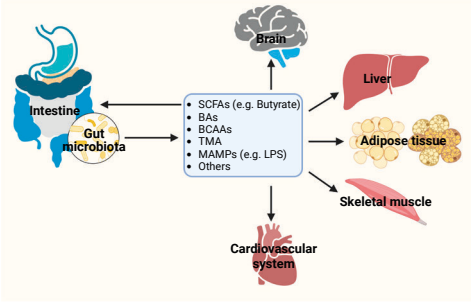

Figure 2-2. Gut microbiota-associated signaling molecules. Gut microbiota convert environmental signals and dietary molecules into signaling metabolites to communicate with the host. These gut microbiota-associated bioactive compounds, such as short chain fatty acids (SCFAs), bile acids (BAs), branch-chain amino acids (BCAAs), trimethylamine (TMA) and microbe-associated molecular patterns (MAMPs) including lipopolysaccharide (LPS), can signal to different organs and tissues (e.g. intestine, the gut microbiota, brain, liver, adipose tissue, skeletal muscle and cardiovascular system), in the host to modulate the function of these organs and tissues (selected gut microbiota-associated factors and selected target organs are shown).

Short-chain fatty acids (SCFAs) are important signaling molecules produced by the gut microbiota. They are the end products of the gut microbial fermentation of non-digestible CHO. The most abundant SCFAs in the gut are acetate, butyrate and propionate, which constitute >95% of the total SCFAs, while caproate, formate and valerate are present in substantially lower amounts and make up the remaining 5% [28]. SCFAs exert their functions partially by acting on specific GPCRs, including GPR41 and GPR43, which are widely expressed [29]. The binding of SCFAs to their receptors on EECs can trigger GLP-1 secretion, which improves pancreatic function and reduces appetite [30]. Moreover, butyrate and

acetate have been shown to act on the vagus nerve to increase energy metabolism, and acetate can also cross the blood-brain barrier and act centrally to increase satiety [31]. Propionate acts as a precursor for intestinal glucogenesis, which contributes to the inhibition hepatic glucose production and the improvement of hyperglycemia [32]. Moreover, SCFAs can act on WAT to decrease lipolysis and suppress inflammation, act on brown adipose tissue (BAT) to increase thermogenesis, and act on skeletal muscle and the liver to increase fat oxidation and decrease inflammation, thereby contributing to the whole-body metabolic homeostasis [22]. According to the available literature, SCFAs may be potential therapeutic targets for treating cardiometabolic disorders.

In addition to SCFAs, bile acids (BAs) have also been regarded as important gut microbiota-associated metabolites. BAs are metabolites of amphipathic cholesterol, and they solubilize dietary lipids by forming mixed micelles in the small intestine and facilitate the absorption of those lipids [33]. They are also hormones that regulate BA biosynthesis, lipid and glucose metabolism as well as immune signaling. The BA pool contains primary BAs, synthesized by hepatocytes, and secondary BAs, the products of primary BA metabolism by the gut microbiota. Primary BAs are conjugated in the liver with glycine/taurine in humans or primarily taurine in mice, to form bile salts that are stored in the gall bladder [33]. After being released into the small intestine upon the intake of dietary nutrients, primary BAs can be deconjugated by the gut microbes, which enables these BAs to escape reabsorption [34]. BAs have been shown to activate farnesoid X (FXR) and Takeda G-protein receptor 5 (TGR5), and the intestinal microbiota can affect the metabolism of BAs to modulate signaling mediated by these two receptors [35]. For instance, liver-derived primary BAs mainly activate FXR in enterocytes to induce the expression of fibroblast growth factor (FGF)15 that suppresses hepatic BA synthesis, and thus leads to reduced BA pool through the gut-liver feedback loop [36]. In addition to generating signaling molecules to modulate CHO and lipid metabolism, the gut microbiota can also regulate amino acid metabolism to control metabolic health. Recent clinical trials have reported that elevated circulating levels of branched-chain amino acids (BCAAs), including isoleucine, leucine and valine, are implicated in the development of cardiometabolic diseases [37]. Intriguingly, several gut microbes (e.g. *Parabacteroides merdae*) have been shown to enhance BCAA degradation, thereby reducing blood BCAA and improving cardiometabolic disorders [38].

Not all gut microbiota-derived signals exert beneficial effects to maintain metabolic health, and recent studies reported that several gut microbiota-associated signaling molecules can trigger the development of cardiometabolic diseases via communication with multiple metabolic organs [39]. A well-studied example is trimethylamine oxide (TMAO) that is derived from dietary choline [40-42]. Gut microbiota convert choline

into trimethylamine (TMA) that is delivered via the portal vein to the liver where hepatocytes rapidly oxidize TMA into TMAO by flavin monooxygenases (FMOs) [42]. Studies conducted in *Apoe*$^{-/-}$ and *Ldlr*$^{-/-}$ mice showed that TMAO aggravates atherosclerosis via various mechanisms, including promoting formation of foam cells and activating the inflammatory response [42, 43]. Besides, by feeding choline-enriched diet to C57BL/6 mice, TMAO has been reported to induce insulin resistance and adiposity, which is partially attributed to the stimulation of hepatic glucose production [39]. These available data likely indicate that TMAO-lowering interventions may have therapeutic potential to reduce cardiometabolic disease. However, *Apoe*$^{-/-}$, *Ldlr*$^{-/-}$ and C57BL/6 mice are suboptimal models for human metabolism, and studies in more humanized animal models and humans are still needed to validate TMAO as therapeutic target.

Likewise, microbe-associated molecular patterns (MAMPs), such as lipopolysaccharide (LPS), are also important bioactive molecules contributing to the development of cardiometabolic diseases. They are recognized by pattern-recognition receptors, such as Toll-like receptors (TLRs), on epithelial and immune cells [29]. Under physiological conditions, these MAMPs hardly translocate across the epithelial barrier. Occasionally, low amounts of microbial components (e.g. LPS) might reach the lymph and circulation via paracellular diffusion, transcellular transport or co-transport with chylomicrons. In pathological conditions, such as obesity, the gut-blood barrier can become leaky, thereby increasing LPS levels in the circulation, which induces local and systemic inflammation to initiate and exacerbate cardiometabolic diseases [44, 45].

Liver-derived signaling molecules. As a key regulator of energy metabolism, the liver is able to adapt quickly to various metabolic conditions. For instance, during the postprandial state, the hepatic uptake of glucose is increased in response to elevated blood glucose, and hepatocytes then convert glucose into glycogen and into TGs through *de novo* lipogenesis (DNL). Simultaneously, the influx of glucose into the liver decreases the hepatic glucose production. In the fasting state, hepatic glucose production is increased via glycogenolysis and gluconeogenesis to supply glucose as a fuel source to extra-hepatic tissues. In addition, the liver can also generate ketones from lipid oxidation, and produce very-low density lipoproteins (VLDL) to supply lipids to peripheral tissues. To maintain the balance of aforementioned metabolic processes and other pathways involved in whole-body metabolism, the liver needs to communicate with other organs in periphery and/or in the CNS via functional molecules. The term 'hepatokines' has recently been coined to indicate liver-secreted hormones, which are essential for transmitting information regarding the metabolic status of the liver to other tissues (**Figure 2-3**).

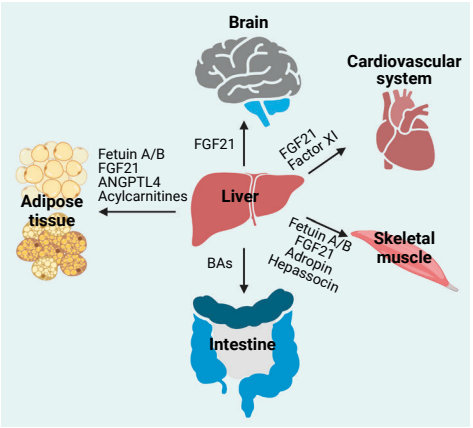

Figure 2-3. Liver-derived factors. The liver plays a central role in energy metabolism and communicates with other organ systems via many secreted signaling molecules. These factors, including hepatokines, lipids and other small molecules, have diverse effects on target organs including adipose tissues, the brain, cardiovascular system, skeletal muscle and the intestine, which help maintain energy homeostasis in response to rapidly changing nutritional states (selected liver-derived factors are shown). ANGPTL4, angiopoietin-like 4; BA, bile acids.

Fetuin-A is one of well-studied liver-derived factors proposed to regulate metabolic balance through integrated organ cross-talk. Fetuin-A is expressed predominately in the liver, but also in the placenta and the tongue to a lesser extent. It was identified as an endogenous inhibitor of the insulin receptor tyrosine kinase in the adipose tissue and skeletal muscle [46]. Another hepatokine is FGF21, an atypical member of the endocrine FGF family that lacks mitogenic activity. Although mRNA levels of FGF21 can be detected in numerous tissues, including the liver, adipose tissue, pancreas and muscle, under physiological conditions circulating FGF21 levels are known to be mainly derived from the liver. FGF21 elicits its biological effects by binding and activating a receptor complex comprised of FGF receptors (FGFRs) and its co-receptor-klotho (KLB) [47]. Whereas FGFRs exhibit a ubiquitous expression pattern, the expression of KLB is primarily restricted to specific metabolic organs (e.g. the liver and adipose tissue). Being a stress-induced hormone, hepatic FGF21 expression is markedly increased by myriad of nutritional and cellular stress signals, and FGF21 released from the liver into the circulation exerts paracrine and endocrine control of many aspects of energy metabolism in multiple tissues. For instance, FGF21 acts on the liver in an autocrine manner to protect hepatocytes from metabolic stress-induced lipid overload, and acts in an endocrine manner on WAT to increase lipid lipolysis under fasting conditions, and on BAT to increase thermogenesis upon cold exposure [48]. FGF21 also reduces sweet food intake via signaling through the paraventricular nucleus in hypothalamus [48]. In fact, most of the hepatokines are secreted in response to an adverse metabolic state like obesity, dyslipidemia and insulin resistance. In particular, liver lipid overload is the most important signal that promotes the production and release of these signaling molecules. In line with this, circulating levels of many hepatokines including FGF21 have been shown to rise in metabolically compromised states, such as obesity, NAFLD and CVDs [47-49]. The induction of FGF21 is thought to mediate a compensatory response to limit metabolic dysfunction, although the response is inadequate to

counteract the metabolically compromised state. This likely indicates that increasing FGF21 levels or activity is a potent target for combating cardiometabolic diseases. Other hepatokines (e.g. angiopoietin-like proteins and acylcarnitine) and liver-derived bioactive compounds (e.g. BAs) also participate in this inter-organ communication to maintain cardiometabolic balance [46, 50]. Therefore, a better understanding of how liver-secreted factors affect other organ systems during the development of cardiometabolic diseases may lead to new therapeutic targets and tools.

Adipose tissue-derived signaling molecules. Three main types of adipocytes (e.g. white, brown and beige) can be found in the human body. Among these adipose tissue depots, WAT is the most abundant form of adipose tissue. In general, white adipocytes contain a single, large lipid droplet occupying most of the cell, and relatively few mitochondria, and they are specialized for lipid storage and release in response to changing nutritional status. BAT is a highly vascularized organ rich in brown adipocytes, and beige adipocytes mainly reside in subcutaneous WAT. In contrast to white adipocytes, brown and beige adipocytes are characterized by multilocular lipid droplets and high mitochondria density. Brown and beige fat depots are unique to mammals and function to generate heat by metabolizing FAs and glucose, called "adaptive" (non-shivering) thermogenesis, which is associated with high expression of uncoupling protein 1 (UCP1). Upon activation, UCP-1 separates nutrient catabolism from ATP synthesis by dissipating the proton gradient in the inner mitochondria membrane, releasing potential energy in the form of heat [51, 52]. The thermogenic fat, although representing a small part of total adipose tissue, can exert a profound metabolic impact because of their capacity to engage in thermogenesis. When fully active, the thermogenic adipose tissue can upregulate whole-body energy expenditure by over 100% in mice [53, 54]. Although the abundance of these thermogenic fat depots in humans (~0.1% of body mass) is relatively smaller than that in mice (> 0.5% of body mass), it has been shown that human brown fat burns ~20% of the basal caloric need [55]. Many studies have shown the beneficial effects BAT

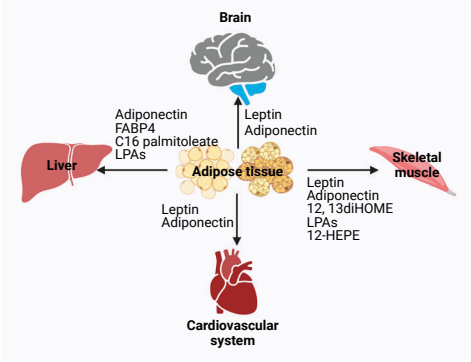

Figure 2-4. Adipose tissue-derived factors. Some adipocyte-secreted molecules signal to the brain to regulate energy metabolism. Others, including adipokines and lipokines, have diverse effects on their target organs, such as the liver, skeletal muscle and cardiovascular system (selected adipose tissue-associated factors and selected target organs are shown). FABP4, fatty-acid-binding proteins; LPA, lysophosphatidic acid.

activation and WAT browning on not only suppressing body weight gain, but also improving systemic metabolism [16, 56]. In addition to their role in lipid and glucose handling, both white adipocytes and thermogenic brown and beige adipocytes secrete bioactive molecules, which can be peptides (adipokines), lipids (lipokines) and exosomal microRNAs, which have profound impact on adjacent or remote tissues (**Figure 2-4**).

Of those factors, leptin is one of well-established endocrine hormones that acts on its receptor (also known as obesity receptor, Ob-Rs), which is a member of class I cytokine receptor family [56]. The leptin receptor is expressed in various cell types both in the brain and the periphery. In the brain, leptin signaling is important for mediating its metabolic effects (e.g. reduction of food intake), as evidenced by increased body fat and increased plasma levels of glucose and insulin in neuron-specific leptin receptor-deficient mice [57]. Leptin signaling also occurs in skeletal muscle and the heart to increase glucose and fatty acid metabolism [58, 59]. Adiponectin is another adipocyte-secreted hormone with systemic insulin-sensitizing and anti-inflammatory properties. Adiponectin signaling is initiated by binding of adiponectin to either of two associated cell surface receptors, AdipoR1 which is widely expressed or AdipoR2 which is mainly located in the livers [60]. The adiponectin signaling pathway, which can be mediated by the activation of e.g. peroxisome proliferator-activated receptor-α (PPARα), plays a pivotal role in modulating whole-body metabolism. For example, adiponectin has been shown to decrease gluconeogenesis and increase FA oxidation in the liver and skeletal muscle, and to increase glucose uptake in WAT and skeletal muscle [61]. Also, adiponectin acts on the brain to promote weight loss [62], and exerts protective effects on cardiovascular diseases [63]. Other adipokines (e.g. adipsin; FA binding protein 4, FABP; and neuregulin 4), lipokines (e.g. 12-HEPE, palmitoleate and 12,13-dihydroxy-(9Z)-octadecenoic acid) and microRNAs [56] also play important roles in regulating whole-body energy metabolism and influence cardiometabolic health by modulating organ-specific functions [64]. Overall, considering the critical role of adipose tissue and its secreted factors in metabolic health, targeting adipose tissue function and adipose tissue-derived factors may provide novel options in treating cardiometabolic diseases.

Other metabolic organ-derived signaling molecules. Muscle-secreted factors, known as myokines, have also been shown to play a role in cardiometabolic health [65]. Irisin, one of the important myokines, can increase muscle oxidative metabolism and uptake of substrates (e.g. glucose and fatty acids), and reduce hepatic glucose output. Irisin was also shown to induce WAT remodeling by increasing p38 signaling, UCP1 expression, and therefore browning [66]. The heart can also secrete signaling compounds, such as atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP). However, a wide range of cardiac hormones remain largely uncharacterized [67]. Inflammatory

mediators released by the immune system are also involved in whole-body metabolism. Tissue resident immune cells can release numerous cytokines, chemokines, and other bioactive molecules, which can trigger pro- or anti-inflammatory signaling to regulate the metabolic function of their targeted organs [68].

3. Inter-organ cross-talk: therapeutic target for cardiometabolic diseases

As aforementioned, inter-organ cross-talk is a gatekeeper for cardiometabolic health. Currently, interventions that regulate cross-talk among metabolic organ systems are being explored as promising strategies to prevent and treat a wide variety of cardiometabolic diseases.

Drugging the gut microbiota. Accumulating evidence demonstrates that the gut microbiota has profound impact on the susceptibility for cardiometabolic diseases of the host. The gut microbiota-host axis contains various layers, including dietary components, gut microbial profiles and meta-organismal pathways, and they are all potential therapeutic targets for cardiometabolic diseases. Therefore, researchers have started to develop gut microbiota-oriented strategies to combat these diseases.

The use of anti-microbial drugs, such as poorly absorbed antibiotics, has been proposed as a strategy for treating cardiometabolic diseases. Several studies indeed reported an association between the development of atherosclerosis and the presence of pathogens *Helicobacter pylori* and *Cytomegalovirus*, but many prospective randomized trials with antibiotics treatment have failed to show clinical benefits [69-71]. A recent research screened more than 1000 commonly prescribed non-antibiotic drugs for their influence on the gut microbiota. Almost 25% of these medications had antibiotic-like effects, as shown by decreasing at least one human commensal gut microbial species [72]. The anti-diabetic drug metformin, for example, improves metabolic dysfunction, including hyperglycemia, in part by decreasing the abundance of *Bacteroides fragilis* in the gut of individuals with T2D [73]. These findings thus indicate that commonly prescribed medications may exert their beneficial effects at least in part through modulating the gut microbiota. Given that drug-microbiota cross-talk varies between metabolic conditions and individuals, investigating the effects of commonly used non-antibiotic therapeutics on the gut microbiota composition and function might reveal therapeutic handles possible use in drug development and personalized medicine.

Beyond classical therapeutics, probiotics have also been proposed to alleviate cardiometabolic diseases. For example, a mouse study showed that *Akkermansia muciniphila* (*A. muciniphila*), a SCFA producer, exerts protective effects on NAFLD and atherosclerosis [74, 75]. Besides, administration of a butyrate producer *Roseburia*

intestinalis (R. intestinalis) can protect against various cardiometabolic diseases, including atherosclerosis [76]. Although probiotic treatment has shown the ability to favorably modulate metabolic phenotypes in several clinical trials, results are highly variable [77]. Moreover, the current probiotic selection is likely driven by abundance-based analyses of the microbial composition. However, the key commensal providing a crucial gain of function may be a low abundant component and is often not easily detected by the current techniques.

Dietary interventions remain the most effective strategy for modulating the gut microbiota-host interactions. This is perhaps best exemplified by production of the compound TMAO from choline-enriched diet (e.g. red meat and eggs) [40-42]. Studies in *Apoe*$^{-/-}$ and *Ldlr*$^{-/-}$ mice have shown that high circulating TMAO levels caused by high dietary choline intake can promote the development of cardiometabolic diseases [39], indicating the potential of TMAO-reducing therapies for the treatment of cardiometabolic diseases. In contrast to dietary choline, dietary fiber has been shown to alleviate cardiometabolic diseases through increasing the production of SCFAs by the gut bacteria [78]. Several studies have determined the impact of a SCFA-enriched diet on cardiometabolic health, and established a direct causal relationship between fermentation of dietary fiber and improvement of cardiometabolic health. For example, mice fed a high-fat diet (HFD) enriched in butyrate showed decreased food consumption and increased energy expenditure [79-81]. Likewise, in obese and diabetic mice, oral administration of acetate reduced weight gain and improved glucose tolerance [82]. In humans, propionate administration reduced obesity, lowered blood glucose levels and increased insulin release during an oral glucose tolerance test [83]. Thus, dietary interventions that directly or indirectly increase intestinal levels of SCFAs may be an efficient strategy to combat cardiometabolic diseases. In addition, given that the SCFAs and TMAO have opposite effects on cardiometabolic diseases, treatment with SCFAs may have the potential to protect against TMAO-induced cardiometabolic disturbance.

Activating brown fat. It has been speculated that decreased cold-induced thermogenesis, for instance due to advances in home insulation and heating, contributes to increased prevalence of obesity-related cardiometabolic diseases [84]. Indeed, in people with dyslipidemia, long-term BAT activation by cold exposure has been shown to lower plasma lipids, such as TGs and atherogenic cholesterol [85]. In fact, our research group has shown that BAT activation in *APOE*3-Leiden.CETP* mice, a well-established model with human-like lipoprotein metabolism, lowers plasma lipid levels and reduces obesity, hepatic steatosis and atherosclerosis [86]. These findings indicate BAT as a potential therapeutic target for cardiometabolic diseases.

Previous studies have reported that cold exposure to 17℃ for 2 hours per day over 6 weeks induces the detectability of BAT by [^{18}F]fluorodeoxyglucose (FDG) PET-CT scan in human adults in whom BAT was undetectable at baseline. The increased BAT volume after cold exposure was associated with reduced fat mass [87]. However, the long-term cold exposure was shown to be associated with an increased FA release from WAT in overweight/obese men [88], which could induce excess ectopic fat deposition and thus could be detrimental to cardiometabolic health. As such, although using cold exposure to recruit and activate BAT has been provided valuable insight into cardiometabolic benefits, concerns related to long-term tolerability and potential adverse effects possibly limit its use as a therapy for obesity and its related diseases.

In addition to cold exposure, researchers have been working on searching for potential targets for BAT activation in humans. For example, β3-adrenergic receptor (β3-AR) agonists (e.g. mirabegron), which are potent activators of BAT thermogenesis in mice, have been tested in humans for BAT thermogenesis. However, whether β3-AR activation stimulate human BAT thermogenesis remains controversy [89]. A study reported that β3-ARs in human brown adipocytes are required to regulate the lipolytic and thermogenic cellular processes, and that β3-AR agonists might have cardiometabolic benefits in people with obesity [90]. Another study, however, proposed that β2-adrenergic receptor (β3-AR) rather than β3-AR is the primary target for pharmacological activation of human brown adipocytes [91]. In addition to β-adrenergic receptors (β-ARs), researchers have also uncovered an alternative pathway of GPCR-mediated adipose thermogenesis via the constitutively active receptor GPR3 in mice [92]. However, the potential of targeting β-ARs and GPR3 signaling in treating cardiometabolic diseases in humans remains further investigation.

Aside from catecholamines such as norepinephrine, other hormones can act on various adipose tissue depots, including BAT, independent of adrenergic receptors. For instance, liver-derived FGF21 can act on its receptors expressed in adipose tissue to increase expression of thermogenic genes, thereby enhancing the heat-producing capacity of BAT and promoting browning of WAT [93]. In line with this, FGF21 has been shown to promote weight loss and lower systemic lipid and glucose levels in obese mice and non-human primates [93, 94]. Given that hepatokine FGF21 plays pivotal roles in mediating the cross-talk between the liver and adipose tissue and in maintaining cardiometabolic health, extensive efforts have been made with the use of recombinant FGF21 or FGF21 variants as potential therapy for obesity and associated cardiometabolic diseases [95]. While analogues FGF21 are in clinical development for obesity and T2D [48], the therapeutic potential of FGF21 in the treatment of NASH and atherosclerotic CVD remains unclear.

OUTLINE OF THIS THESIS

Chapter 1 provided an introduction on inter-organ cross-talk as a gatekeeper for cardiometabolic health. It was explained that metabolic organs can interact to regulate whole-body metabolism through signaling molecules, such as peptide/protein hormones, bioactive lipids and functional small molecules. These molecules allow various metabolic tissues and organs to work together to absorb, store, sense and use energy, and to maintain energy balance of the body. However, aberrant production of these signaling molecules is associated with energy imbalance and contributes to the development of cardiometabolic diseases, indicating inter-organ crosstalk as a therapeutic target for cardiometabolic diseases. In this thesis, by using dietary and pharmacological interventions, I explored the therapeutic potential of targeting inter-organ communication in cardiometabolic diseases, aimed to reveal novel and effective inter-organ cross-talk-directed therapeutic strategies for these diseases.

Studies in *Apoe*$^{-/-}$ and *Ldlr*$^{-/-}$ mice have linked dietary choline to the pathogenesis of cardiometabolic diseases, related to production of TMAO, which could theoretically be counteracted by the anti-inflammatory and antiatherogenic properties of butyrate. Therefore, in **Chapter 2**, we aimed to investigate whether butyrate can alleviate choline-induced atherosclerosis. To this end, we used *APOE*3-Leiden.CETP* mice, a well-established atherosclerosis-prone model with human-like lipoprotein metabolism, and fed these mice with an atherogenic diet alone or supplemented with choline, butyrate or their combination. In addtion to its atherogenic effects, dietary choline or its metabolite TMAO has also been reported to promote adiposity in diet-induced (DIO) obese and *ob*/*ob* mice. However, several clinical trials have reported anti-obesity effects of high dietary choline intake. Therefore, in **Chapter 3**, we aimed to assess the effects of dietary choline on adiposity in humanized *APOE*3-Leiden.CETP* mice exposed to a Western-type diet, and to elucidate underlying mechanisms.

Narcolepsy is a clinical condition of severely disturbed sleep that is characterized by an increase in body weight after disease onset, frequently leading to obesity. Clinical studies have reported that the narcolepsy drug γ-hydroxybutyric acid (GHB), a SCFA that is structurally similar to butyrate, promotes weight loss. Due to its central effects and its misuse-associated adverse effects, GHB is unlikely to be prescribed as anti-obesity drug. Nonetheless, elucidating the mechanisms by which GHB reduces body weight may reveal therapeutic handles for the development of effective body weight control medications. Therefore, in **Chapter 4**, we aimed to to unveil the metabolic mechanisms underlying GHB-induced weight reduction in obesity. Furthermore, we aimed to examine whether GHB administration also confers weight loss during

the development of obesity. To this end, C57BL/6J mice that were lean and fed HFD to induce obesity, or that were obese due to previous HFD feeding, were orally administered with GHB.

Besides the SCFAs butyrate and GHB, FGF21 is another promising therapeutic tool for treatment of cardiometabolic diseases. Currently, analogues of FGF21 are in clinical development for treatment of obesity and type 2 diabetes. Although their glucose-lowering and insulin-sensitizing effects have been largely unraveled, the mechanisms by which they alleviate liver injury have only been scarcely addressed. In **Chapter 5**, we thus aimed to unveil the mechanisms underlying FGF21-mediated improvement of NASH, in particular with respect to steatohepatitis and fibrogenesis. To this end, liver-specific FGF21 overexpression was achieved in *APOE*3-Leiden.CETP* mice, followed by administration of a high-fat high-cholesterol diet (HFCD). Finally, in **Chapter 6**, we investigated the importance of FGF21 in other aspects of cardiometabolic health, particularly in lipoprotein metabolism in relation to atherogenesis, by administration of long-acting recombinant FGF21 to *APOE*3-Leiden.CETP* mice fed an atherogenic diet.

In the last part of this thesis, **Chapter 7**, the findings of these experimental studies and their implications for future research will be discussed.

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