Modulating energy metabolism: pathophysiological aspects and novel interventions

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CHAPTER 1

General introduction and outline of this thesis

GENERAL INTRODUCTION

Obesity and cardiometabolic diseases: a global epidemic

Over the past centuries, urbanization, globalization, and technological developments have led to drastic changes in the human lifestyle. With the development of the current modern 24-hour society, humans are regularly exposed to an environment where food rich in fat and sugar is readily available, with a low demand for physical activity, and where artificial light during day and night disrupts their biological rhythms. This so-called 'obesogenic' environment has led to a disturbing increase in obesity prevalence. The World Health Organization (WHO) states that in the European region, 59% of adults are overweight (body mass index (BMI) > 25 kg/m^2) and 23% of adults are currently living with obesity (BMI > 30 kg/m^2), the latter being a relative increase of 138% since 1975 (1). Obesity is a complex disease that is characterized by excessive adiposity and is strongly associated with disturbances in glucose and lipid handling as well as local and systemic inflammation. As such, obesity is a major risk factor for the development of cardiometabolic diseases, including type 2 diabetes (T2D) and cardiovascular diseases (CVD), of which the prevalence is consequently thriving as well. Specifically, T2D and CVD are responsible for 1.5 and 17.9 million deaths per year, respectively, and together underlie 35% of all deaths worldwide (2).

Obesity arises in case of a positive energy balance, with the rate of energy intake exceeding that of energy expenditure, resulting in excessive storage of energy from sugar and lipids in the form of triglycerides (TGs) in white adipose tissue (WAT) depots, but eventually also in other metabolic organs (*i.e.*, ectopic lipid deposition), disturbing the function of those organs. To restore or maintain energy homeostasis, there is a need 1) to further unravel the physiological aspects of energy metabolism, 2) to investigate populations at risk for cardiometabolic diseases, and 3) to explore novel treatment strategies aimed at beneficially modulating energy metabolism.

Physiology of energy metabolism

White adipose tissue

WAT plays an essential role in maintaining energy homeostasis, by regulating the balance between energy storage in the form of TGs and energy mobilization in the form of free fatty acids (3). Histologically, white adipocytes, the dominant cell type in WAT, are defined as large unilocular cells, containing a single lipid droplet, with only a few mitochondria and no uncoupling protein 1 (UCP1). WAT is located at multiple locations throughout the body, but main depots are located underneath the skin (*i.e.*, subcutaneous WAT, > 80% in lean individuals) and around the internal organs (*i.e.*, visceral adipose tissue, approx. 10-20% in lean individuals) (4).

In the postprandial state (*i.e.*, after a meal), energy contained in sugar and lipids is stored in WAT in the form of TGs (**Figure 1**). The main source for these TGs are fatty acids derived from circulating TG-rich lipoproteins: *i.e.*, small intestine-derived chylomicrons and liver-derived very-low-density lipoproteins (VLDL). With the use of lipoprotein lipase (LPL), present on endothelial cells within the capillary wall, TGs in the core of these lipoproteins are hydrolyzed, after which the released fatty acids can enter the adipocytes either passively though diffusion of actively using fatty acid transporters including CD36 (3, 5). Once in the cell, the fatty acids are esterified and stored as TGs within the intracellular lipid droplet (3). To a lesser extent, the stored TGs in adipocytes can also derive from *de novo* lipogenesis from glucose that is also taken up by white adipocytes, especially in the postprandial state. In this process, circulating glucose enters the adipocyte via the insulin-sensitive glucose transporter 4 (GLUT4) or non-insulin-sensitive GLUT1 and is metabolized via glycolysis and oxidation in the tricarboxylic acid cycle to be eventually converted into fatty acids, stored as TGs (3). In addition, glucose can be converted into glycerol-3-phosphate as precursor of the glycerol backbone of TGs. The uptake and storage of fatty acids and glucose into WAT is largely regulated by insulin (6).

In times of high energy demands, such as fasting, exercise or cold exposure, fatty acids can be mobilized from WAT to provide fuel to other metabolic organs (**Figure 1**). The release of fatty acids from the stored TGs in WAT is regulated by three lipases, namely adipose TG lipase (ATGL), hormone-sensitive lipase (HSL), and monoacylglycerol lipase (MAGL). In sequence, ATGL is responsible for TG hydrolysis (retrieving one fatty acid per TG), HSL is responsible for diacylglycerol hydrolysis, and MAGL for the hydrolysis of monoacylglycerol into glycerol and a fatty acid (3). The liberated free fatty acids are released into the circulation, and, bound to albumin, mainly transported to the liver, where fatty acids cause substrate driven synthesis of TGs that are packed into VLDL, which can again provide energy-demanding metabolic organs with fatty acids. This intracellular lipolytic process in white adipocytes is controlled by catecholamines (*e.g.*, noradrenaline) that are released after activation of the sympathetic nervous system and is suppressed by insulin (7).

Brown adipose tissue

Although most of the adipose tissue in the human body is classified as WAT, mammals possess another type of adipose tissue, which is called brown adipose tissue (BAT) (8-10). BAT contributes to energy expenditure due to combustion of glucose and fatty acids into heat through a process known as thermogenesis (11). In contrast to white adipocytes, brown adipocytes are small multilocular cells, containing numerous small lipid droplets and large amounts of mitochondria with the unique presence of UCP1 in

their inner membrane (**Figure 1**). The BAT owes its name to the iron-rich mitochondria that give the adipose tissue a brownish color. BAT is highly vascularized and densely innervated by the sympathetic nervous system (12). In humans, the main BAT depots are located in the supraclavicular, cervical, and axial area (13), where the produced heat is believed to be efficiently dissipated throughout the body via convection. It is not long ago that the presence of metabolically active BAT in humans was recognized. Initially, BAT was especially known to be present in hibernating small mammals, such as rodents, and infants, in whom BAT is essential to maintain body temperature. However, accumulating evidence for the presence of active BAT in human adults appeared in 2007 (14), and two years later this was confirmed by several independent research groups (8-10). Still, humans possess considerably less relative amounts of BAT than rodents with an estimated amount of 50-150 mL of BAT (13), and therefore there is still a lot to learn about the contribution of BAT to whole-body energy homeostasis and cardiometabolic health in humans.

In humans, the most potent physiological activator of BAT is cold exposure, which is first sensed by receptors from the transient receptor potential family on the skin. From there, a signal is sent via the dorsal horn neurons towards the pro-optic area of the hypothalamus and, via various hypothalamic nuclei and signaling pathways, a sympathetic outflow is generated towards the tissue (15-18). BAT is in turn activated after binding of noradrenaline to β-adrenergic receptors on the cell membrane of the brown adipocytes (19, 20), resulting in rapid intracellular hydrolysis of TGs located in the many small lipid droplets by the use of the lipases ATGL, HSL and MAGL. Unlike in WAT, where liberated fatty acids are mainly released into the circulation, in BAT the majority of the released fatty acids are used for oxidative respiration within mitochondria (**Figure 1**). Moreover, fatty acids rapidly activate UCP1 in the inner membrane of these mitochondria. While in most tissues oxidative respiration is coupled to the production of adenosine triphosphate (ATP) that enables energy-demanding processes such as muscle contraction, in BAT UCP1 uncouples mitochondrial respiration from ATP production, leading to the generation of heat instead (11).

As a result of activated thermogenesis, the intracellular lipid droplets gradually become depleted. In order to replenish these droplets, like WAT, BAT takes up fatty acids from circulating TG-rich lipoproteins, mediated by LPL (21), and glucose, mediated by GLUT1 and GLUT4 (22). The uptake of glucose is thought to mainly feed *de novo* lipogenesis, rather than being used directly for oxidation (22). Due to the fact that activated BAT takes up large amounts of glucose, BAT can be visualized using 2-[^{18}F]fluoro-2-deoxy-D-glucose ([^{18}F]FDG) positron emission tomography-computed tomography (PET-CT) acquisitions, which is currently the most widely applied method ('gold standard') to

detect and quantify human BAT (8-10). With this method, the uptake of the glucose analogue [^{18}F]FDG by active BAT is visualized using PET acquisition, while he CT scan provides the anatomical reference and distinguishes tissues according to their radiodensity.

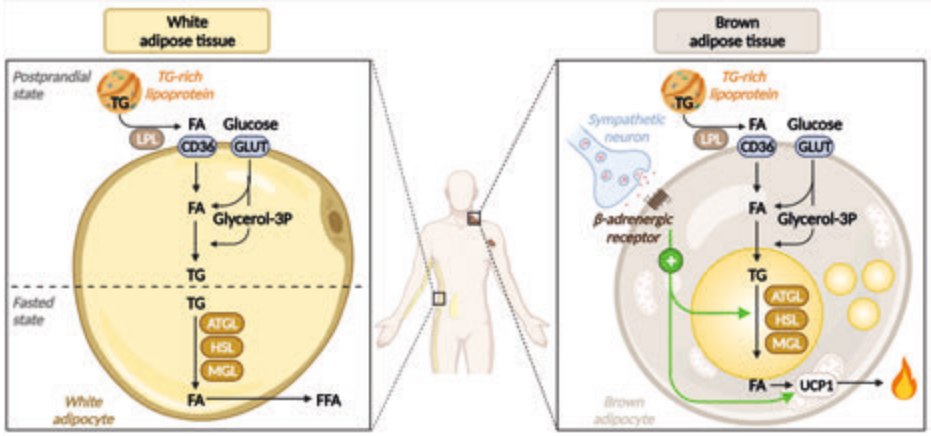

Figure 1. Location and function of white adipose tissue and brown adipose tissue. See sections 'white adipose tissue' and 'brown adipose tissue' for explanation. ATGL, adipose triglyceride lipase; CD36, cluster of differentiation 36; FA, fatty acid; FFA, free fatty acid; GLUT, glucose transporter; Glycerol-3P, glycerol-3-phosphate; HSL, hormone-sensitive lipase; LPL, lipoprotein lipase; MGL, monoacylglycerol lipase; TG, triglyceride; UCP1, uncoupling protein 1.

Beige adipose tissue

In addition to 'classical' brown adipocytes, humans possess brown-like adipocytes, also called 'beige' or 'brite' adipocytes. These adipocytes can originate from stimulation of precursor cells that are closely similar to the white adipocyte cell lineage and lead to the appearance of brown-in-white ('brite') adipocytes, or they may appear from transdifferentiation of existing white adipocytes (23). This process is known as 'browning' and can, at least in rodents, be induced in response to several stimuli including continuous sympathetic stimulation (24, 25). Histologically and functionally, beige adipocytes are comparable to brown adipocytes, as they are multilocular with a high mitochondrial content and the presence of UCP1, due to which they can contribute to thermogenesis (26, 27). However, their basal UCP1 expression is low, they lack other classical brown adipocyte transcript markers, and express transcripts that are associated with white adipocytes (28). Whether BAT depots in humans consist mainly of classical brown adipocytes, beige adipocytes, or a combination of both is still under debate (27, 29).

Liver

To maintain energy homeostasis, the different adipose tissues work closely together with the liver. In the postprandial state, surplus energy is primarily stored in WAT under the influence of insulin as explained above, although the liver is also responsible for taking up sugar and lipids after a meal. Where WAT takes up fatty acids from intestine-derived TG-rich chylomicrons, the liver takes up the resulting TG-poor chylomicron remnants. In addition, the liver takes up free fatty acids that are present in the circulation as a result of 'spillover' during LPL-mediated hydrolysis (30, 31). At the same time, the high insulin levels in the postprandial state largely inhibit the hepatic secretion of TG-rich lipoproteins (*i.e.*, very-low-density lipoproteins; VLDL). Therewith the liver acts in concert with WAT to prevent a postprandial rise in circulating TG levels (32, 33). The liver also takes up most glucose that is absorbed by the intestine and reaches the liver by the portal vein and which, stimulated by insulin, is converted into TGs by *de novo* lipogenesis, which is secreted in the postabsorptive state as VLDL (34).

In catabolic states, such as fasting, the liver takes up large amounts of the free fatty acids that are released from WAT after intracellular lipolysis as explained above. Within the liver, these fatty acids are esterified into TGs (in addition to cholesteryl esters and phospholipids), and secreted in VLDL particles to deliver fatty acids as fuel to metabolically active organs. Of note, although VLDL synthesis is particularly important during fasting, during which it is the main energy source for metabolically active tissues such as the skeletal and cardiac muscles, it is a continuous process that is also present during other metabolic states (35).

Lipoprotein metabolism

From the previous sections it becomes clear that metabolic tissues operate tightly together to process dietary nutrients in the postprandial anabolic state resulting in storage of excess energy and to deliver fuel to energy demanding tissues in catabolic states. While glucose is hydrophilic and can therefore freely circulate in the blood, lipids are hydrophobic (*i.e.* water insoluble) and are therefore packed within lipoprotein particles to allow transport between organs via the blood. As such, lipoprotein particles transport dietary cholesterol and TGs that are absorbed in the small intestine to metabolic tissues including the liver, as well as transport cholesterol and TGs between the liver and peripheral metabolic tissues. Lipoprotein particles consist of a core of TGs and esterified cholesterol (*i.e.* cholesteryl esters) that is surrounded by an amphiphilic layer of phospholipids and free cholesterol, which makes the particles soluble in blood (36). In addition, they carry apolipoproteins on their surface that are involved in lipoprotein synthesis and particle stabilization, can act as regulators of lipid transfer and lipolysis, and can interact with cell-surface receptors, therewith ensuring optimal

distribution of lipids over the various metabolic tissues depending on their metabolic need (37, 38). Dependent on the size, composition and density of the lipoprotein particles, four major subclasses can be identified: chylomicrons, VLDL, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) (39).

Chylomicrons are TG-rich lipoproteins that are formed postprandially in the so-called exogenous (intestinal) pathway (**Figure 2**). After eating a meal, dietary TGs are hydrolyzed into 2-monoglycerides and fatty acids that are all taken up by enterocytes, where they are re-esterified and packed into chylomicrons (75-1200 nm in diameter) containing a single apolipoprotein (apo)B48 on their surface. Chylomicrons are then, via the lymphatic system, released into the circulation (40, 41). In the endogenous (hepatic) pathway, TG-rich VLDL particles are formed in the liver. VLDL particles are smaller than chylomicrons (30-80 nm) and are formed by lipidation of a single molecule of apoB100 instead of apoB48. Both TG-rich lipoproteins (*i.e.*, chylomicrons and VLDL particles) are synthesized with additional apolipoproteins and /or acquire additional exchangeable apolipoproteins in the circulation, such as apoC-I, apoC-II, apoC-III, and apoE (40, 41). These apolipoproteins have distinct functions and their presence on the lipoprotein surface can vary. For instance, apoC-I, apoC-II and apoC-III affect TG hydrolysis by either inhibiting LPL (*i.e.*, apoC-I and apoC-III) or serving as co-factor for LPL (*i.e.*, apoC-II) (37). Through LPL-mediated lipolysis, fatty acids from chylomicrons and VLDL are liberated that can be taken up by adipocytes or myocytes as fuel for oxidation. By TG depletion these lipoproteins thus become smaller, relatively ore enriched in cholesteryl esters, and acquire (more) apoE. Therewith, the remnant particles can be cleared by the liver via binding of apoE to specific receptors on the hepatocytes, of which the low-density lipoprotein LDL receptor (LDLR) and LDLR-related protein 1 (LRP1) are the most important (42). Yet, a substantial part of VLDL remnants that are not taken up by the liver are catabolized further, leading to the generation of LDL as lipolytic end product. LDL particles are again smaller (19-25 nm) and highly enriched in cholesteryl esters. LDL particles have lost all exchangeable apolipoproteins but still contain apoB100 that can, although with lower affinity than apoE, bind to the LDLR on hepatocytes for removal from the circulation (39). When circulating at high concentration, TG-rich lipoprotein remnants and LDL are a risk factor for the development of CVD since they can accumulate in the vessel wall after uptake by macrophages, which initiates atherosclerotic plaque formation.

During LPL-mediated lipolysis, phospholipids from the surface of TG-rich lipoproteins are transferred to circulating lipid-poor apoA-I and/or apoA-II, which are synthesized in the liver and small intestine and released into the circulation via ABCA1. ApoA-I/II together with phospholipids and an array of additional (apolipo)proteins form a

lipoprotein particle called HDL (8-12 nm). HDL has the ability to take up cholesterol from cells within organs as well as the vessel wall, to esterify the cholesterol into cholesteryl esters by lecithin-cholesterol acyl transferase (LCAT), and to deliver those cholesteryl esters to the liver via scavenger receptor class B type 1 (SRB1). The liver uses the cholesterol for either VLDL synthesis or generation of bile acids that are secreted into the intestine. In the intestine, bile acids are involved in emulgation to facilitate lipid absorption, are mostly reabsorbed by the enterohepatic circulation and partly eliminated via the feces. The process by which cholesterol is removed from the periphery and eventually excreted via the feces, is called reverse cholesterol transport (RCT) and is regarded a beneficial property of HDL to protect against CVD (43).

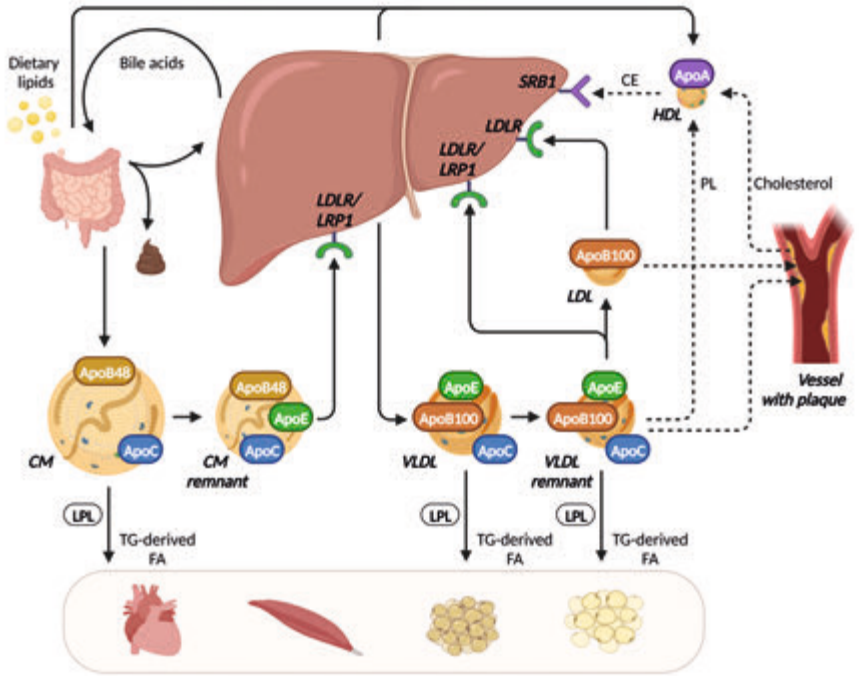

Figure 2. Lipoprotein metabolism. See section 'lipoprotein metabolism' for explanation. Apo, apolipoprotein; CE, cholesteryl ester; CM, chylomicron; FA, fatty acid; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LDLR, low-density lipoprotein receptor; LPL, lipoprotein lipase; LRP1, low-density lipoprotein receptor-related protein 1; PL, phospholipids; SRB1, scavenger receptor class B type 1; TG, triglycerides; VLDL, very-low-density lipoprotein.

Disturbed energy metabolism

In health, well maintained energy homeostasis allows the human body to fulfill its energy needs without disproportionate energy storage or combustion. However, during the development of obesity, the rate of energy intake exceeds the rate of energy expenditure, leading to a long-term positive energy balance. To accommodate the high nutrient influx, WAT depots expand to increase storage capacity via a combination of enlargement ('hypertrophy') and proliferation ('hyperplasia') of white adipocytes. Especially WAT hypertrophy, characterized by large adipocytes due to enhanced accumulation of lipids within the cells, can form a risk for cardiometabolic diseases (3). Hypertrophy within the visceral adipose tissue depot is generally considered to pose a larger risk than within the subcutaneous adipose tissue depot (44). When WAT is unable to store the excess nutrients, TG surplus will be deposited in other organs, including skeletal muscle, liver, pancreas and kidneys, the so-called 'ectopic fat deposition', which renders these organs insulin resistant and is a major risk factor for the development of T2D.

Obesity-associated inflammation

An important causal link between obesity and the development of cardiometabolic diseases is the accumulation and activation of immune cells in metabolic tissues, resulting in release of pro-inflammatory factors that lead to a chronic low-grade inflammatory state. Although various tissues can be involved in obesity-associated inflammation, most of our current knowledge stems from studies in adipose tissue, in which hypoxia is one of the main triggers inducing inflammation (45). Mechanistically, hypertrophic adipocytes have increased need for oxygen, which, together with insufficient perfusion, makes them prone to hypoxia resulting in the induction of hypoxia-inducible factor 1α (HIF1α) (46, 47). Mainly via HIF1α, hypoxia leads to the secretion of chemokines that recruit various immune cells such as T lymphocytes, B lymphocytes, and monocyte-derived macrophages (45). Accordingly, one of the hallmarks of dysfunctional adipose tissue in obesity is the progressive increase in the number of macrophages and their transformation from a predominantly anti-inflammatory to a pro-inflammatory phenotype (48). Whereas macrophages are estimated to embody less than 10% of all cell types in healthy adipose tissue, this can increase to up to 50% in hypertrophic adipose tissue in obese subjects (49). These macrophages serve to clear debris by surrounding dying adipocytes as 'crown-like structures', and secrete pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α, interleukin-6 (IL-6), and IL-1β that aggravate inflammation. Moreover, a diet rich in fat itself and damaged adipocytes may cause further strain on WAT, via the binding of dietary saturated fatty acids and damage-associated molecular patterns to pattern recognition receptors that are, amongst others, present on macrophages

(45). This chronic low-grade inflammation plays a critical role in the development and exacerbation of insulin resistance, which will be discussed in the next section.

Insulin resistance

Insulin is produced by the pancreatic β-cells in response to high blood glucose and fatty acid levels following a meal, to stimulate the uptake of glucose by GLUT4 and TG-derived fatty acids by LPL in peripheral tissues (*e.g.*, skeletal muscles and adipose tissues), to inhibit intracellular lipolysis WAT, and to additionally inhibit hepatic glucose and TG output. As such, insulin acts as an anabolic hormone that prevents excessive postprandial glucose and TG excursions. In insulin resistant states, as may develop during obesity, β-cells will initially produce more insulin to compensate for the insulin resistance of metabolic tissues. However, when this compensation fails and blood glucose levels rise, impaired glucose tolerance and eventually T2D will develop. Patients with insulin resistance not only develop elevated blood glucose levels, but generally also dyslipidemia characterized by hypertriglyceridemia that is often accompanied by low HDL levels (50).

Insulin resistance can develop when pro-inflammatory cytokines, produced by macrophages and/or lymphocytes, bind to their receptors on parenchymal cells within the metabolic tissues (*e.g.*, myocytes, adipocytes and hepatocytes) and activate intracellular signaling pathways that interact with the insulin signaling cascade (51, 52). Insulin resistant white adipocytes show enhanced intracellular lipolysis, leading to an increased release of free fatty acids (50). This increased free fatty acid flux towards the liver, together with hepatic insulin resistance, enhances hepatic VLDL-triglyceride production (53). Insulin resistance additionally lowers LPL-mediated TG hydrolysis, by attenuating the activity of LPL in peripheral tissues, therewith further increasing the circulating concentration of TG-rich lipoprotein particles (54). Eventually, WAT is unable to efficiently store the high concentration of circulating TGs, leading to ectopic fat deposition (55, 56).

Atherosclerosis

Besides contributing to T2D, increased circulating (apoB-containing) TG-rich lipoproteins also form a risk for the development of atherosclerosis, which underlies the majority of CVD (57). Atherosclerosis starts with endothelial dysfunction, mostly caused by 'shear stress' at susceptible areas of the artery including around bifurcations. TG-rich lipoprotein remnants and LDL particles are small enough to enter the endothelial barrier and, especially at high circulating concentrations, can accumulate in the intimal layer of arteries. Modifications of these particles, such as by oxidation and aggregation, activate endothelial cells and vascular smooth muscle cells, causing local inflammation

and the secretion of pro-inflammatory cytokines and chemokines that attract immune cells. Recruited monocytes differentiate into macrophages once infiltrated in the arterial wall. These macrophages ingest the modified particles, turning them into lipid-laden 'foam cells' that together form a 'fatty streak', which is the earliest lesion visible during atherosclerosis development. When lesions advance by continuous lipid pressure, they develop a fibrous cap of vascular smooth muscle cells and collagen. Over time, sustained inflammation and oxidative stress within the lesion results in macrophage apoptosis and necrosis, which is exacerbated by failure of the remaining macrophages to clear dead cells and debris in the lesion core (58). As inflammation intensifies, the necrotic core grows, and the cap gets thinner, the plaque becomes unstable and at risk for rupture. Ruptured plaques can result in formation of a thrombus in the circulation that occludes arteries and obstructs oxygen supply to distal organs, ultimately causing a cardiovascular event such as a myocardial infraction or a cerebrovascular accident (59).

Factors affecting energy metabolism

Some people are at particularly high risk to develop cardiometabolic diseases. This can be due to unhealthy behavioral factors, such as an unhealthy diet, low physical activity, and/or performing shift work, but also certain ethnicities have a high risk due to largely unknown pathophysiological mechanisms. In the next sections, I will first introduce the South Asian population as a population at risk for cardiometabolic diseases. Secondly, I will elaborate on the role of the biological clock in energy metabolism and how misalignment of our behavior with the biological clock poses a cardiometabolic health risk.

South Asian ethnicity

South Asians originate from the Indian subcontinent (generally defined as India, Pakistan, Bangladesh, Nepal, Bhutan, and Sri Lanka) and represent more than 20% of the world population. Many South Asians have migrated to Western countries during the twentieth century. Within Europe, the largest South Asian population is based in the United Kingdom (3 million) and it is roughly estimated that 160,000 South Asians are currently living in the Netherlands ('Dutch Hindustani' or in this thesis 'Dutch South Asians'). Intriguingly, both South Asians living in the Indian subcontinent as well as migrant South Asians are at a substantially higher risk to develop T2D than people with an Europid background (60, 61). Compared to people of Europid descent, the lifetime risk to develop T2D is two-to-four times higher for migrant South Asians. They develop T2D on average 5-10 years earlier than Europids and at a much lower BMI (62, 63). Moreover, although data are scarce, the progression rate of glucose intolerance to T2D seems faster among South Asians, and, once T2D has evolved, the disease progresses faster as indicated by a rapid worsening of their yearly hemoglobin A1c levels and higher

macro- and microvascular complication rates (60). Specifically, migrant South Asian patients with T2D show a higher prevalence of CVD, retinopathy, and nephropathy (63-67). Although several factors have been proposed to be involved, the exact mechanism (s) that contribute (s) to the disadvantageous metabolic phenotype of South Asians is largely unknown and is likely multifactorial.

Insulin resistance may be present at a very young age in South Asians. Already in childhood, migrant South Asians show signs of insulin resistance, with higher circulating concentrations of insulin and TGs as compared with Europids, which could not be explained by differences in adiposity (68, 69). Moreover, although possibly explained by higher maternal glucose levels, migrant South Asian neonates have higher insulin levels in umbilical cord blood (70), suggesting exposure to metabolic disturbances from a very young age on. Moreover, in adult migrant South Asians, rates of overweight and obesity are higher than Europids and, importantly, the adverse metabolic effects of overweight seem more harmful. More specifically, per unit BMI increase the risk for T2D increases more in migrant South Asians than in Europids (60). A plausible explanation could be the disadvantageous fat distribution of South Asians, with more centrally distributed fat (*i.e.*, visceral and truncal subcutaneous adipose tissue) and higher ectopic fat deposition (71-73). Mechanistically, this could be related to a lower storage capacity of the superficial subcutaneous adipose tissue, which corresponds with findings that South Asians possess a higher proportion of large abdominal subcutaneous adipocytes compared to Europids (71, 73). Other differences in body composition include a lower lean mass (74) and lower BAT volume (75), both of which probably explaining the reported lower resting energy metabolism in South Asians.

In summary, an unfavorable body composition and lower insulin sensitivity are apparent in migrant South Asians compared to Europids. However, despite those factors an excess risk remains in South Asians for the development of T2D. Inflammation is likely one of the underexplored underlying factors. Several studies reported higher concentrations of the nonspecific inflammatory marker C-reactive protein (CRP), cytokine interleukin-6 (IL-6), and TNF-α in healthy South Asians compared with Europids (76-79). As inflammation is well-recognized to be a key player in the pathogenesis of T2D and CVD, further exploration of the contribution of inflammation, as well as the precise components of the immune system that are differentially regulated, is highly needed, to identify the most promising treatment target to lower cardiometabolic risk in the South Asian population.

The biological clock

Every 24 hours, the earth rotates on its axis, generating day and night with profound differences in light and temperature. To adapt to these daily variations of the environment, organisms possess an evolutionarily preserved 'biological clock' that determines an approx. 24-hour cycling pattern in physiological processes, which is called 'circadian rhythm' (*circa* = approximately, *dies* = day). Circadian rhythms are in essence self-sustained molecular systems regulated by clock genes and clock proteins that cycle around in a robust 24-hour pattern and regulate gene expression in peripheral tissues. The environment further coordinates this rhythm, via external signals called 'zeitgebers' (which literally translates to 'time-givers'). The strongest zeitgeber is light, which is perceived via the retina by neurons in the suprachiasmatic nucleus (SCN) (80, 81). Other zeitgebers include environmental temperature, physical activity, and dietary patterns (82, 83).

In the current modern 24-hour society our behavior is often misaligned with the natural biological clock, for example during shiftwork during which people invert their sleep/wake and fasting/feeding cycles. Importantly, many processes that control metabolic homeostasis, such as lipid and glucose metabolism (84) and immune responses (85), are tightly regulated by the biological clock. Consequently, disruption of the biological clock provokes a cardiometabolic risk, which is well illustrated by the strong association between shift work and metabolic syndrome, T2D and CVD (86-89). Furthermore, circadian misalignment results in disturbed glucose homeostasis (90), elevated blood pressure and increased inflammatory markers (91). The causal role between disruption of the circadian rhythm and cardiometabolic diseases has been further evidenced in rodent studies, by manipulating their timing of food intake, sleep and/or light exposure (92). Nevertheless, the exact mechanisms and tissues involved in the link between the circadian misalignment and adverse cardiometabolic effects have not yet been fully elucidated.

Interestingly, rodent BAT activity shows a diurnal oscillation with a peak in uptake of glucose and TG-derived fatty acids at the start of the wakeful period (93, 94). Moreover, increasing the daily light exposure period in mice, therewith disrupting the circadian rhythm, attenuates the uptake of nutrients by BAT and results in weight gain (95). Such a circadian rhythm in BAT is likely also present in humans, as *in vitro* primary human brown adipocytes and BAT explants show a diurnal variation in their insulin-stimulated glucose uptake (96). The possible relevance of the circadian rhythm for human lipid metabolism is highlighted by the fact that postprandial lipid excursions are virtually absent at wakening, but increase later during the day (93). The notion that BAT is subjected to a circadian rhythm may indicate that its response to therapy, using

either cold exposure or pharmacological strategies, depends on the moment of the day and should be harmonized with the biological clock (*i.e.*, 'chronotherapy'). Therefore, more insight into the daily rhythm of human BAT activity is needed.

Modulating energy metabolism to improve cardiometabolic health

Current treatment strategies for reducing obesity and its associated cardiometabolic diseases mainly focus on lifestyle, by implementing a healthy diet or increasing physical activity. However, due to low compliance, these interventions are commonly not effective on the long-term, at least for the majority of patients living with obesity, warranting the development of alternative non-pharmacological or pharmacological treatment strategies.

Cold exposure

Since the discovery of active BAT in human adults in the beginning of this millennium, the application of cold exposure as non-pharmacological intervention to alleviate insulin resistance and dyslipidemia has attracted renewed interest. During a cold stimulus, the body aims to limit reduction of core temperature by both inducing peripheral vasoconstriction as well as by generating heat in BAT and skeletal muscles. As described previously, the generation of heat is induced by activation of the sympathetic nervous system and the binding of noradrenaline to β-adrenergic receptors and leads to an increased metabolic rate often referred to as 'cold-induced thermogenesis'. BAT and skeletal muscles are the main contributors to cold-induced thermogenesis, and are fueled by glucose and fatty acids, that are mobilized from white adipose tissue and the liver, respectively, in response to the sympathetic outflow.

By targeting multiple metabolic tissues at the same time, cold exposure elicits beneficial metabolic effects. In healthy individuals, short-term (3 hours) cold exposure improves insulin-sensitivity (19) and lowers fasting and postprandial insulin levels (97, 98). Long-term intermittent cold exposure (*e.g.*, 4-10 weeks for 2-6 hours per day) has been shown to increase BAT activity and energy expenditure (99), reduce fat mass (100) and lower fasting glucose levels (101). Moreover, 10-days daily cold exposure increases BAT activity and energy expenditure in individuals living with obesity (102) and improves peripheral insulin sensitivity in patients with T2D, at least in part by enhanced translocation of the GLUT4 in skeletal muscle (103). Whereas it is thus clear that cold exposure enhances energy expenditure and improves glycemic control in humans, the effects of cold exposure on lipid metabolism are less clear. In mice, prolonged cold exposure generally reduced TGs by accelerating the clearance of TG-rich lipoproteins from the circulation (21, 104-107). In seeming contrast, in humans, circulating TGs either do not change (108, 109) or even increase (110) in response to short-term cold exposure. Hence, the effects

of cold exposure on whole body lipid metabolism need further exploration, ideally by investigating dynamic changes during cold exposure.

It is important to note that the beneficial metabolic effects of cold exposure cannot be pinpointed to a single tissue. In addition, the relative contributions of skeletal muscles versus BAT in cold-induced thermogenesis in humans remains controversial. Although the cold-induced glucose uptake per volume of tissue is higher by BAT than by skeletal muscles (98), skeletal muscles represent approx. 42% of the total body weight versus approx. 1% for BAT. Hence, skeletal muscles seem responsible for the major part of the cold-induced thermogenesis (111) and clearance of circulating substrates (97, 98). Nevertheless, when comparing BAT positive individuals (*i.e.*, detectable [^{18}F]FDG uptake by BAT on PET scans) with BAT negative individuals after prolonged (5-8 hours) cold exposure, whole-body energy expenditure and insulin sensitivity was reported to only increase in the BAT positive group (112), underscoring a significant physiological role of BAT. The importance of BAT in cardiometabolic health is further highlighted by a recent large retrospective analysis of [^{18}F]FDG PET-CT scans of more than 50,000 patients. In this study, individuals who showed glucose uptake by BAT during thermoneutral conditions (*i.e.*, BAT positive individuals) had lower plasma TGs, higher HDL-cholesterol, lower blood glucose, and a lower prevalence of T2D and CVD (113).

Pharmacologically mimicking cold exposure to activate BAT

Unfortunately, cold exposure is fairly uncomfortable for most people potentially leading to low compliance and may not be a supreme tool for prevention and treatment of cardiometabolic diseases. A logical resolution would be to pharmacologically mimic cold exposure by directly stimulating the adrenergic receptors on BAT.

In rodents, the β3-adrenergic receptor (ADRB3) is the most potent receptor to activate BAT thermogenesis (114). In mice, targeting the ADRB3 effectively thus activates BAT and improves metabolic outcomes (115, 116). More specifically, activation of the ADRB3 reduces body weight, reduces plasma TG and cholesterol levels, and as a result attenuates atherosclerosis development (115). Hence, the ADRB3 was presumed to be also the most important activating receptor on human BAT. Indeed, the ADRB3 agonist mirabegron activates human BAT and increases whole body lipolysis and resting energy expenditure (117). However, this only occurs at a supra-pharmacological dose (*i.e.*, 200 mg versus the therapeutic dose of 50 mg) (118) together with cardiovascular side effects (19, 119, 120), suggesting cross-activation with other β-adrenergic receptors. This notion is supported by the finding that in human BAT biopsies and brown adipocytes, expression of the ADRB2 is dominant over the ADRB3 (19). Moreover, *in vitro* experiments using primary human brown adipocytes evidenced that thermogenic activation by

noradrenalin and mirabegron is primarily mediated by the ADRB2 (19). Together, these pre-clinical results warrant further exploration to test whether stimulation of the ADRB2 is indeed able to activate human BAT *in vivo* in the form of a clinical trial.

OUTLINE OF THIS THESIS

From the **current chapter** it becomes clear that our metabolic tissues work tightly together to maintain glucose and lipid homeostasis. After a meal, surplus nutrients are efficiently removed from the circulation and predominantly stored in adipose tissues so that they can later be used for oxidation during catabolic (*e.g.*, fasting) states. Nevertheless, in current modern society energy metabolism is frequently imbalanced by the rate of energy intake exceeding the rate of energy expenditure. In addition, due to our 24-hour economy our behavior is regularly misaligned with our natural biological clock, disturbing metabolic processes. As a consequence, the prevalence of obesity and its associated cardiometabolic diseases including T2D and CVD are currently increasing at an alarming rate. Although this increase is seen worldwide, especially people from South Asian descent are at high risk to develop cardiometabolic diseases due to largely unknown underlying mechanisms. Novel treatment strategies that improve cardiometabolic health by restoring glucose and lipid homeostasis are thus highly warranted. Promising approaches include the application of cold exposure, which increases whole-body metabolic rate via skeletal muscles and BAT, or directly targeting BAT by pharmacologically stimulating its β-adrenergic receptors. In this thesis, I address two key objectives: 1) to gain more insight in various pathophysiological aspects of cardiometabolic diseases including in the disease-prone South Asian population, and 2) to study the physiological effects of cold exposure and identify a novel pharmacological approach to directly target BAT.

To address key objective 1, patients with a genetic defect in genes encoding LPL or in genes encoding LPL regulators were studied. Due to the crucial role of LPL in catalyzing intravascular lipolysis of TG-rich lipoproteins, dysfunctional LPL leads to severe hypertriglyceridemia. The aim of **chapter 2** was to comprehensively characterize the (apo)lipoprotein profile in blood from patients with this genetic from of hypertriglyceridemia, by combining two novel approaches (*i.e.*, multiplex liquid chromatography–mass spectrometry and nuclear magnetic resonance spectroscopy) in comparison with normolipidemic controls. Then, focus was shifted to the involvement of inflammation in cardiometabolic diseases. In **chapter 3**, circulating mRNA transcripts of 182 immune related genes in Dutch South Asian versus Dutch Europid patients with T2D were studied, to investigate the involvement of the immune system in the disadvantageous metabolic phenotype of South Asians. Part 1 of this thesis ends with a review about another group of people at risk for metabolic disbalance, *i.e.* patients

with the rare neurological disorder narcolepsy type 1, which is characterized by a dysregulated sleep-wake cycle but also high rates of obesity. The aim of **chapter 4** was to increase the knowledge on the pathophysiology of adiposity development in patients with narcolepsy type 1 and discuss a possible mediating role for BAT.

To address key objective 2, the physiology of cold exposure was first investigated, followed by an elaboration on the relevance of rhythms in BAT and the best timing to apply cold exposure, to end with a proof-of-concept study to identify a novel pharmacological target to activate BAT. In **chapter 5**, the use of cold exposure to modulate the immune system was studied, by measuring mRNA transcript levels of immune genes in blood after short-term cold exposure of both lean Dutch South Asian and Europid individuals. Next, the aim of **chapter 6** was to investigate the effect of cold exposure on whole-body lipid metabolism, as prolonged cold exposure accelerates the clearance of TG-rich lipoproteins from the circulation in mice, therewith lowering circulating TGs, whereas controversial responses were previously observed upon short term cold exposure in humans. To this end, sequential lipidomic profiling was performed in serum at multiple time points during a 2-hour cold exposure of lean individuals. In addition, a mechanistic study was performed in mice to elucidate the role of intracellular lipolysis in the cold-induced effects in humans. In **chapter 7**, genetic, neuronal and endocrine generation of rhythms in BAT are described, and the current knowledge on the effects of disrupted or attenuated rhythms in BAT for lipid metabolism and cardiometabolic diseases are addressed. Following on the notion that BAT exhibits a pronounced diurnal rhythm in the uptake of lipids in mice, in **chapter 8** is investigated whether time of day is a critical determinant when applying cold exposure in humans. To this end, young, lean males and females were exposed to cold once in the morning and once in the evening with cold-induced thermogenesis as primary outcome. Finally, in **chapter 9** is focused on the pharmacological activation of human BAT by investigating the effect of the ADRB2 agonist salbutamol on BAT glucose uptake and whole-body energy expenditure. Lean, male individuals participated in a randomized, double-blinded crossover trial over two days with one week washout. Participants received a single intravenous bolus of salbutamol with or without the ADRB1/2 antagonist propranolol. At both study visits, BAT glucose uptake was estimated using a dynamic [^{18}F]FDG PET-CT scan.

Finally, in **chapter 10**, the most important findings of these studies and their implications are discussed.

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