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General introduction

Metabolic diseases

In today's world, more people die from complications of overweight than from underweight. Overweight and obesity significantly increase the risk for type 2 diabetes and cardiovascular disease (CVD). Globally, CVD is the number one cause of death (World Health Organization, March 2013). Preventing obesity-related diseases is estimated to save a substantial amount of medical care costs, but also diminish environmental, labor productivity and human capital loss [1]. Therefore, obesity is a growing societal and political point of concern. Understanding how susceptibility to obesity-related diseases arises and how to tip the balance back in favor of a more healthy energetic state is crucial.

Energy metabolism

Energy is taken up by the body by digesting energy-rich nutrients in the diet. These digestion products can be further metabolized according to the requirement at that time. Nutrients can be used either as building blocks for biomass, or oxidized for generating heat and/or the cellular chemical energy currency: Adenosine triphosphate (**ATP**). Nutrients can also be stored. The metabolic fates (oxidation, biomass and storage) of dietary protein, carbohydrates and fats are depicted in figure 1.

In the post-prandial state, dietary fats and carbohydrates are broken down in the gastrointestinal tract to fatty acids and monosaccharides, respectively. Monosaccharides such as glucose enters the blood stream directly, but fatty acids are first converted to triglycerides in the small intestine and loaded into chylomicron lipoprotein particles (built around apolipoprotein (apo) B48), used to transport water-insoluble lipids. The lipoprotein particles are then transported via lymph and the water soluble nutrients via the blood to all organs, which will take up nutrients according to their need. Chylomicrons adhere to the vascular endothelial wall to release fatty acids near the cell membrane. Adipocytes can store excess fatty acid as triglycerides. The liver can store excess glucose as glycogen.

In the fasting state, the body relies on the release of stored glycogen and triglycerides to fulfill the energy requirements of each cell. Adipocytes can hydrolyze the stored triglycerides, releasing non-esterified fatty acids (NEFA). As NEFAs are toxic, they are quickly shielded from the environment. For example, NEFAs in the blood complex with albumin. The liver can rapidly take up NEFAs from albumin complexes. The NEFAs are then esterified to glycerol to reform triglycerides. These TGs are loaded by the liver onto very low density lipoprotein particles (VLDL, built around apoB100) and secreted into the blood. Similarly, glycogen can be broken down, thus secreting glucose.

The energy-rich lipids and carbohydrates are important building materials. The cells of the body grow, divide and develop by metabolizing dietary nutrients. Lipids are important components of the cell membrane, creating a closed compartment wherein homeostasis is possible. Carbohydrates are important components for nucleotides. Amino acids are essential

for the synthesis of enzymes and other proteins.

Fatty acids and glucose are mostly used as fuel. They can both be metabolized to acetyl-CoA, which is the main input metabolite of the mitochondrial tricarboxylic acid (TCA) cycle. In the TCA cycle acetyl-CoA is oxidized in a step-wise controlled fashion generating carbon dioxide, water, but also reducing equivalents (NADH). The reducing equivalents are fed into in the mitochondrial electron transfer chain to generate a proton gradient which drives the conversion of ADP to ATP or the generation of heat. ATP is the central energy currency of the cell, needed to maintain homeostasis.

The energy buffer capacity of available intracellular ATP is generally very small. Therefore an energy buffer of triglycerides and glycogen is stored mostly in adipose tissue and liver, respectively. Since the energy density of triglycerides is about six times higher than glycogen, triglycerides are the preferred energy storage form. Glucose can be converted to fatty acids as storage, but it is impossible to convert fatty acids back to glucose. As the brain is mostly dependent on glucose, a glucose storage in the form of glycogen is vital.

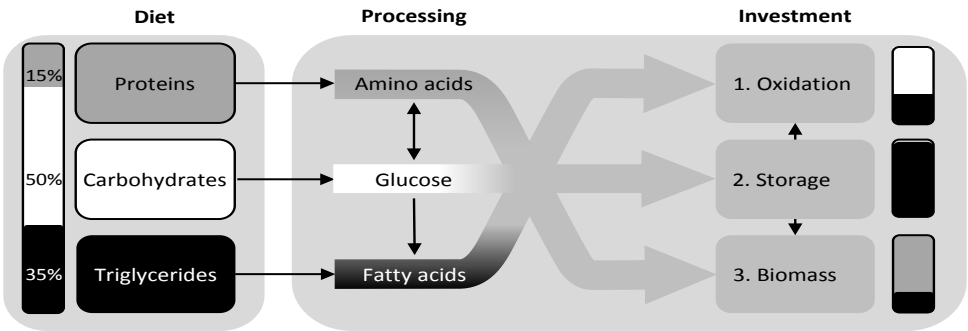

Figure 1: Macronutrient composition of the human diet, which is absorbed and processed in the body and subsequently allocated to fuel oxidation for energy, energy storage or to growth of biomass. The macronutrient composition is given as a stacked bar graph: Carbohydrates (C, White), Protein (P, Grey) and Fat (F, Black). Western Diet (Standard American Diet) composition in C/P/F = 50/15/35 kcal% [2]*. Average muscle biomass composition in C/P/F = 0/75/25 kcal%* [3] *and average human storage composition in C/P/F = 1/0/99 kcal%* [4]*. Average human oxidative respiratory quotient in C/P/F = 40/0/60 kcal%* [5, 6]*.*

Figure 1 describes the schematic roadmap that dietary nutrients follow, from nutrient intake to nutrient usage and storage. It does not describe how the decision is made to allocate certain nutrients to certain processes under certain conditions. In order to gain more insight in this allocation process, it is useful to determine the energy balance. If the balance has shifted from the homeostatic set point, nutrient allocation should be adjusted to regain balance. The energy balance describes how the energy intake from the diet compares to the energy expended in order to fuel the nutrient usage:

$$\Delta Energy\ storage(kJ/h) = Energy\ intake(kJ/h) - Energy\ expenditure(kJ/h)$$

If the energy intake of an organism over a period of time is in balance with the energy expenditure, the amount of energy storage will stay the same. In the case of a positive energy balance the energy storage will increase over time.

Balancing energy metabolism

Regulating the energy storage and release is crucial to keep up with the ATP demand of every cell in the organism. To regulate most processes in biology, negative feedback loops have evolved. The concentration of a certain metabolite is sensed by a sensor. If the concentration (input) increases beyond a threshold, the sensor will elicit a response (feedback) leading to increased consumption and/or decreased production of the metabolite, thereby bringing the input concentration back to the set-point homeostatic level. Due to its robustness, negative feedback loops are the most abundant regulatory mechanism to balance consumption and production at a homeostatic level [7].

The first step in the regulation of energy metabolism is therefore to sense the energy balance. Sensing the energy balance is done at multiple levels: The intracellular energy availability, the energy available circulating in the blood and the energy available in storage. It is important to sense the energy balance at these different levels, so that energy allocation can be adjusted to fit the local and global requirements of the organism, but also to fit the acute and long-term requirements of the organism.

In the post-prandial state, more energy intake has occurred than required for energy expenditure at that moment. When the digestion products enter the bloodstream, the concentration of glucose, amino acids and lipids will rise. Pancreatic β-cells taking up increasing levels of glucose will sense an increase in their intracellular ATP/AMP energy balance [8]. This leads to the secretion of insulin, which will bind to the insulin receptor present on the cell membranes. Insulin will signal the switch from a catabolic state (burning nutrients for fuel) to an anabolic state (use nutrients to build up storage and biomass), required during the switch from fasting to feeding. This results in an increased nutrient uptake and a decrease of the blood nutrient concentrations back to homeostatic set-point levels, at which insulin secretion is not stimulated any longer.

In contrast, in the fasting state, more energy is expended than is taken up via the intestine. This might cause cells to use up their ATP supply faster than they rebuild ATP. Each cell can sense the drop in ATP/AMP energy balance via amongst others the enzyme AMPK. Activated AMPK will locally stimulate the cell's insulin sensitivity, thereby increasing insulin-induced glucose import from the circulation into the cell [9]. AMPK signaling can also activate the production of extracellular energy demanding hormones, namely glucagon (by pancreatic α-cells), adrenaline and cortisol (by the adrenal glands). These hormones will travel via the blood to the storage cells, signaling the release of fatty acids and glucose into the blood, from where they can be processed and absorbed by the cells.

The energy balance is also sensed at the storage level. Storage cells must also be able to signal the total amount of stored nutrients present, otherwise the buffer capacity might not be sufficient for unforeseen fasting conditions. The glycogen buffer in the liver is small, but crucial. Therefore, when the glycogen content is low, hepatocytes stimulate adipocyte lipolysis forcing a switch from oxidizing low-abundant glucose to oxidizing fatty acids [10]. Reversely, when the glycogen content is high, hepatocytes stimulate adipocyte lipogenesis to convert high-abundant glucose to triglycerides [11]. The mechanism for sensing and signaling according to glycogen content are not yet completely known. Adipocytes release leptin, an adipokine, according to their lipid content. Therefore, sensing the adipocyte lipid content is possible by sensing the blood leptin concentration. When stored energy is in abundance, it can be used for growth, development and reproduction processes. These global and long-term responses are indeed stimulated by leptin [12].

The response to sensing the energy balance is mostly mediated via blood hormones, which can manipulate the cellular energy metabolism. However the response can also be mediated via neuronal signaling, which could manipulate not only energy metabolism, but also behavior [13]. Leptin for example acts as an extracellular hormone, inducing a neuronal satiety signal in the brain. Low leptin levels will not signal satiety, thereby steering behavior to finding food. High leptin levels induce satiety behavior [12]. Many different hormones have been found to manipulate neuronal satiety signaling. Even in anticipation of nutrients entering the blood, different gastro-intestinal sensory cells secrete ghrelin, cholecystokinin and peptide YY in response to nutrients, which are all stimulatory signals of satiety [13]. In response to high levels of blood nutrients insulin also acts as a stimulus of satiety. The default signal in food intake regulation is appetite signaling, which can be suppressed by for example the satiety mechanisms described above.

Negative feedback is present in many different energy balance sensing processes, ranging from neuronal and hormonal to intracellular processes. Although it is a very robust regulatory mechanism, resistance to negative feedback can occur. Resistance can be useful physiologically to adapt to a short-term overwhelming response. However, resistance can also occur if certain environmental triggers become chronic triggers. For example, in the event of a chronic overconsumption of energy-rich nutrients. Resistance to some of the regulatory hormonal, neuronal and intracellular feedback responses described above can have adverse metabolic effects, such as insulin resistance, leptin resistance and neuronal satiety resistance [14]. With negative feedback, an altered level of the input (for example a high glucose concentration) is sensed and accordingly feedback is given (for example a higher insulin concentration). With resistance to feedback, a chronically elevated level of the feedback (for example a high insulin concentration) is sensed and accordingly feedback resistance arises (for example a lower insulin receptor concentration). Therefore, resistance to negative feedback is paradoxically governed by negative feedback itself. It is an adaptation to chronically altered (environmental) feedback triggers. And it is precisely this altered 'obesogenic environment' that has led to a sudden rise in the prevalence of obesity and the metabolic syndrome since the late 1970's.

Unbalanced energy metabolism

Metabolic syndrome

Starting from the 1920's, the recognition of a particular clustering of metabolic abnormalities in patients led to a new diagnosis: Metabolic syndrome (MetS) [15]. There are multiple clinical definitions for MetS used worldwide, but MetS is predominantly characterized by increased risk for cardiometabolic diseases such as type 2 diabetes, atherosclerosis and subsequent CVD and infarction. The risk factors used in the various MetS definitions include obesity, high levels of blood glucose (hyperglycemia), abnormal levels of blood lipids (dyslipidemia), increased inflammation and high blood pressure. The utility of the diagnosis MetS is debated, as the MetS definition does not predict cardiovascular disease development any different from the sum of the individual risk factors [16]. Nonetheless, the co-occurrence of the metabolic risk factors indicates a common underlying cause or causes, most likely a combination of genetically determined susceptibility and a change in environmental factors affecting energy metabolism at large. The different risk factors and their known genetic and environmental interactions will be reviewed.

Obesity

In obesity, the adipose tissue mass expansion can be due to increased triglyceride storage in the lipid droplet of the adipocyte (hypertrophy), and/or increased formation of new adipocytes (hyperplasia). The relative importance of hypertrophy and hyperplasia in obesity has been estimated and although total adipose tissue mass predictions were highly correlated to adipocyte size, the correlation failed for morbidly obese individuals. When adipocyte number was also taken into account [17] the majority of the variability in adipose tissue mass could be explained. Therefore both hypertrophy and hyperplasia contribute to (morbid) obesity. In obesity, the absolute adipocyte number is often higher than normal weight controls, as determined by an increased hyperplasia rate before adulthood [17]. Regulation of both hypertrophy and hyperplasia occurs, most likely via negative feedback. A homeostatic set-point of adipocyte size was found in studies conducting short term food restriction or overfeeding studies and a set point of adipocyte number was found in studies towards the effects of liposuction [18, 19].

The expansion of the adipose tissue mass can occur at different adipose tissue depots. Adipose tissue surrounding the internal organs (visceral white adipose tissue, vWAT) has been shown to produce relatively more fatty acids and inflammatory compounds [20] compared to fat stored under the skin (subcutaneous, sWAT). Therefore, expanded vWAT has been associated with an increased risk of the obesity-related complications of the MetS compared to expanded sWAT [21]. Although vWAT is an important risk factor, the abdominal fat in the belly of obese men is mostly sWAT and not vWAT. Why abdominal sWAT is riskier than

sWAT located around the hips remains largely unsolved [22].

The evolutionary mechanisms that have shaped our energy balance seem to favor mechanisms which create a positive energy balance in preparation of periods of shortage. It drives our behavior to eat energy rich foods used for energy storage in order to overcome these periods. Intriguingly, a large part of the known genetic contributors to obesity are polymorphisms in genes involved in eating behavior, regulating the neuronal satiety balance between orexic and anorexic appetite signals [23, 24]. It was estimated that between 40-80% of the variation in body mass index (BMI) can be explained by heritable factors such as genetic variants involved in appetite regulation and pre-adipocyte differentiation regulation [24, 25]. The non-heritable variation is also strongly affected by variation in socio-economic status (SES), as a lower SES is generally associated with a higher body mass [26] or in the variation of individual economic insecurity and related uncertainty stress [27]. These non-heritable factors could prompt less healthy eating habits.

The prevalence of obesity is rising in a timeframe too short to be explained by changes in heritable factors. Therefore, the increased prevalence must be due to environmental changes (obesogenic environment), such as energy-dense foods becoming more widely available in bigger, cheaper portions. This has led to an increased energy intake, mainly by increased carbohydrate intake compared to the 1970s. At the same time, aerobic fitness and energy expenditure have been diminishing, as a result of a more sedentary lifestyle, by for example increased car usage and screen entertainment [28, 29]. What the relative contribution is of the two factors, energy intake and energy expenditure, to the obesity epidemic is difficult to estimate [30].

In obesity, adipocytes are chronically relaying their hypertrophic stressed state by for example high leptin secretion. Therefore, resistance to chronically high adipokine levels occurs during obesity, both in the brain and in peripheral tissues. For example obese subjects suffer from leptin resistance, where leptin can no longer efficiently inhibit hunger signaling nor can it stimulate energy expenditure [31]. Therefore, leptin resistance contributes to a vicious cycle in obesity development. Resistance to other adipokines has been observed, but their role in obesity-related diseases is not as clear as for leptin at the moment [32].

Insulin resistance

Insulin resistance is a decreased responsiveness of the body to insulin signaling. The amount of insulin secreted from the pancreatic β-cells is based on sensing the levels of nutrients, primarily blood glucose levels and secondarily NEFA and amino acid levels, such as branched chain amino acids [33]. Insulin resistance is associated with a decreased number of insulin receptors on the insulin-sensitive cell membranes. Also, the downstream signaling efficiency of the activated insulin receptor is diminished by a decreased number of (active) signal transduction proteins in the insulin pathway [33]. Once insulin resistance develops, the pancreas secretes more insulin to maintain normal blood glucose levels (~5 mM). The insulin

resistant state can deteriorate further, leading to hyperglycemia despite increased levels of insulin. This state is called type 2 diabetes mellitus (T2DM). Finally, the chronically elevated insulin production will lead to a failure of the β-cells, which will cease to produce endogenous insulin. These patients are subsequently dependent on exogenous insulin injections [33].

Insulin resistance may be due to a number of possible causes, their contribution differing widely per individual. As the risk of T2DM is strongly related to increasing BMI, obesity is an important risk factor. Therefore many explanations for T2DM and insulin resistance involve consequences of obesity [34].

Due to an overabundance of digested nutrients entering the bloodstream, adipocytes will take up more NEFAs. It is well known that acute lipid infusion and NEFA uptake in cells will cause insulin resistance on the short term [35], by an acute accumulation of the intermediates of NEFA β-oxidation in mitochondria [36]. Therefore an important early contributor to adipose tissue insulin resistance could be increased NEFA uptake and subsequent mitochondrial processing. Adipocyte-specific knockout of genes involved in mitochondrial functioning resulted in a less burdened mitochondrial membrane potential. Upon feeding a high fat diet, these adipocyte-specific knockout mice showed decreased mitochondrial stress, enhanced adipose insulin sensitivity and enhanced whole-body insulin sensitivity [37, 38].

A later stage contributor to insulin resistance originating from adipose tissue could be the increasing lipid load of adipocytes becoming hypertrophic. Lipid load is correlated to lipid hydrolysis and NEFA release, but also to the release of certain adipokines, such as leptin. Increased adipocyte lipolysis would lead to increased intracellular adipocyte NEFA concentrations, but also to increased circulating NEFA concentrations. NEFAs could become lipotoxic by affecting mitochondrial functioning in adipose tissue, but also in peripheral tissues taking up increased amounts of NEFAs [39]. Furthermore, adipocyte hypertrophy also leads to hyperleptinenemia and leptin resistance. As leptin inhibits β-cell insulin release, leptin resistance can cause systemic hyperinsulinemia [36].

Another late stage contributor to obesity related insulin resistance might be the fact that advanced adipocyte hypertrophy can induce intracellular stress [34], possibly due to: 1) reaching the maximal mechanical expandability of the adipocytes and adipose tissue within the body, 2) intracellular hindrance of the expanding lipid droplet with organelles such as mitochondria and the endoplasmic reticulum, and/or 3) reaching the maximal oxygen diffusion limit of the enlarged cells. As a consequence of adipocyte intracellular stress, serine protein kinases will be activated and will phosphorylate serine residues of insulin receptor substrate 1 (IRS1) [34]. Serine phosphorylation of IRS1 will directly interfere with the kinase activity of the activated insulin receptor on IRS1 tyrosine residues. Insulin resistant adipocytes will respond less to insulin-inhibition of lipolysis and will therefore have a higher rate of basal lipolysis and NEFA release, which then forms a vicious cycle for increased NEFAs.

The intracellular stress of hypertrophic adipocytes can become too great, leading to the secretion of immune modulating stress factors and eventually to adipocyte death. Dying adipocytes attract immune cells, which will start secreting their own immune modulating

factors [34]. This also leads to a vicious cycle of inflammation. These immune cells and immune factors will decrease insulin sensitivity locally, but will eventually spread via the circulation to peripheral tissues, thereby inducing whole-body insulin resistance. Although many important associations have been found between insulin resistance and adipose tissue related changes, the causal sequence and the relative contributions of the adipose tissue changes to adipose and whole-body insulin resistance have not been completely elucidated.

Although T2DM is strongly associated with obesity, the association does not have to be directly causative. Other causative associations exist with T2DM, unrelated to or indirectly related to obesity. For example, 38% of the variation in HOMA-insulin resistance can be explained by heritable factors in Caucasian families [40]. Genome wide association studies on T2DM have been done to identify which molecular mechanisms could be involved. These GWAS have found that most genetic variants associated to T2DM are related to pancreatic β-cell functioning, not to obesity [41, 42]. An unstable pancreatic β-cell functioning might become problematic under certain conditions, such as obesity. If the β-cell functioning of metabolically healthy obese patients is more robust, these obese patients might be more resilient to developing T2DM and will merely develop hyperinsulinemia [43]. As with many complex diseases, the discovered genetic variants are capable of explaining only a small portion of the theoretical heritability of insulin resistance. Therefore, the search for the "missing heritability" of insulin resistance will continue [25].

Dyslipidemia

Dyslipidemia is characterized by abnormal blood lipid levels, such as triglycerides (TG), phospholipids (PL), cholesterol esters (CE) and total cholesterol (TC) levels. Not only is the absolute concentration of blood lipids an important risk factor for CVD, the distribution of blood lipids over lipoprotein particles also contributes [44]. For example, cholesterol is present in both VLDL and LDL (built around apoB100) as well as in HDL (built around apoAI). The VLDL/LDL particles deliver cholesterol and the HDL particles are thought to remove cholesterol from peripheral tissues. If the relative balance between LDL-cholesterol and HDL-cholesterol has shifted towards the LDL side, reverse cholesterol transport would be lowered [45] leading to peripheral and vascular cholesterol accumulation as a potential risk factor for CVD [44]. This view has led to an interest in drugs which shift the LDL/HDL-cholesterol ratio towards the HDL side. One of these drugs is niacin [46].

Another dyslipidemic balance that has gained interest is the omega-3 over omega-6 poly unsaturated fatty acid ratio (n-3/n-6 PUFA ratio) [47]. Similar to total plasma cholesterol the absolute PUFA quantity is the most important determinant of CVD risk [48], and similar to LDL/HDL cholesterol the relative distribution of n-3/n-6 PUFA has been associated with CVD risk [49]. The quantitative intake of PUFAs is easily met in our present-day Western type diet. However, the Western dietary n-3/n-6 PUFA ratio is about 1:16. This low dietary n-3/n-6 PUFA ratio also resulted in a low n-3/n-6 PUFA ratio of the PUFAs incorporated the body's

cell membranes. A shift in the blood n-3/n-6 PUFA ratio away from n-3 PUFAs has been shown to enhance dyslipidemia and increase LDL-cholesterol levels [48]. Epidemiological studies have repeatedly found that people with a higher n-3/n-6 PUFA ratio in their blood have a reduced risk of dying from CVD [50, 51]. Also, the addition of the n-3/n-6 PUFA ratio improves CVD risk estimations on top of the classical dyslipidemia criteria [47, 52].

Susceptibility to dyslipidemia can be inherited. Variation in TG and HDL-C levels can be explained for 31% and 43% respectively by heritable factors in a Dutch population [53]. The known genetic contributors are mostly directly associated with lipid metabolism in the liver. For example, via genetic variants affecting apolipoproteins (such as apolipoprotein A5), lipoprotein particle synthesis enzymes or adaptor proteins involved in the synthesis, transport and binding of lipoprotein particles [54].

Heritable factors also explain some 24% of the biological variation in n-3/n-6 PUFA ratio [55]. Interestingly, genome wide association studies have found an association between plasma cholesterol levels and genetic variants affecting *FADS1* and *FADS2*. These genes encode for the rate-limiting enzymes in the synthesis of n-3 and n-6 PUFAs [56]. This genetic association between PUFA synthesis genes and cholesterol levels is interesting, as PUFAs can inhibit cholesterol biosynthesis [57]. This hints towards a mechanistic link between decreased (endogenously synthesized) PUFAs and the development of dyslipidemia.

The mechanisms underlying the non-heritable causes of dyslipidemia are less clear. Diet quality and increased carbohydrate intake have been associated with dyslipidemia [58]. Obesity and insulin resistance have also been implicated in the development of dyslipidemia. Obesity induces increased blood NEFA levels which are substrates for liver VLDL-TG thereby increasing VLDL production and circulating levels of these atherogenic particles. In the hepatic insulin resistant state, the liver is less responsive to insulin-mediated inhibition of VLDL production. Therefore both obesity and insulin resistance could lead to VLDL overproduction and hyperlipidemia.

Mortality of the Metabolic Syndrome

The majority of severe complications associated with the MetS are due to cardiovascular disease, although MetS also increases the risk of other complications, such as cancer. Cardiovascular diseases are mostly a consequence of atherosclerosis. Atherosclerosis is the process of the narrowing of arteries by accumulation of calcium, (modified) lipids and inflammatory cells in the arterial wall. If the narrowed artery becomes blocked, the downstream tissue becomes ischemic. If the atherosclerotic wall ruptures, the accumulated debris will initiate blood coagulation and the resulting blood clot will travel through the bloodstream. The blood clot can occlude a downstream narrower artery. Depending on the location of the occluded artery, this can result in for example a heart attack or a stroke.

Dyslipidemia is an important prerequisite for atherosclerosis development in most cases.

Dyslipidemia can shift the balance between cholesterol delivery and cholesterol removal by LDL and HDL respectively [45]. Increased levels of LDL particles will bind to proteoglycans on the arterial endothelial cells resulting in increased LDL particle transport across the arterial wall. If these lipids become oxidized and modified, this stimulates phagocytosis by macrophages [59]. and will attract more inflammatory cells to the site of early inflammation. These cells will accumulate in the arterial wall, causing atherosclerosis. If the total cholesterol over HDL-cholesterol doubles in a person between 40-65 years old from 4 to 8 this also doubles the risk to dying from a CVD [60].

The complications of insulin resistance and T2DM are characterized by glucose-induced damage to the retina, kidneys, neurons and the extremities, as specifically endothelial cells and nerve cells cannot efficiently down-regulate glucose influx during hyperglycemia [61]. The increased intracellular glucose level has been suggested to increase mitochondrial production of radical oxygen species and advanced glycosylated end-products [61]. At high concentrations, glucose will react with amino acid residues of proteins, thereby glycosylating proteins, which are an inflammatory trigger [62]. The inflamed capillary endothelial cells become damaged, which can initiate atherosclerosis [63]. About 5% of the Dutch population suffered from type 2 diabetes in 2011 and this percentage is increasing [64]. A person diagnosed with T2DM has an increased risk of dying from CVD within 10 years that is equivalent to that person aging 15 years [60].

Obesity is not lethal as such, however it significantly increases the risk for other co-morbidities which can lead to death. Increased adipose tissue mass is strongly associated with increased blood pressure [21], which can damage the arteries and the heart. In the Netherlands, 42% of the population was overweight in 2013 and 10% was obese [65]. Obese subjects have about a 1.5 time higher risk of dying than normal weight subjects [66].

Predicting the risk of dying from CVD is most effective by measuring factors closely associated to death. For example, atherosclerosis is a better risk marker then dyslipidemia, which is a better risk marker then obesity. The further away from death, the worse the factor will be at predicting the risk of death. But the further away from death, the more time there might be to intervene with the risk of dying.

Outline of this thesis

The research described in this thesis focuses on factors that modulate energy metabolism, leading to obesity and insulin resistance, with a specific focus on fatty acid metabolism. An introductory overview of this topic is presented in Chapter 1. In the subsequent chapters, we examine the role of specific genes in disturbing energy metabolism and reversely, we examine the effects of disturbed energy metabolism on adipose tissue characteristics, in the context of diet-induced obesity and insulin resistance.

GWAS have revealed numerous single nucleotide polymorphisms (SNPs) in genes associated with obesity and related metabolic disorders. However, translating this genetic

information to a mechanism by which the disease could arise has proven difficult. In order to assist scientists in reviewing possible mechanisms by which a SNP could be associated to a disease or disease-marker, we describe in Chapter 2 the application of a newly developed knowledge-based workflow for assisting users in mapping SNPs to genes. We applied this new workflow to a statistically strong, but mechanistically weak association of a SNP mapped to the *HPS5* gene, which associated with branched chain amino acid (BCAA) degradation products [67]. We found that another gene (*LDHA*) is mechanistically a more plausible candidate gene than *HPS5* and we verified this functional association experimentally. Previous research from our laboratory has shown that BCAA metabolism is affected in obese and diabetic individuals, indicating the possible relevance of the SNP mapped to *LDHA* in metabolic diseases.

In Chapter 3, we investigated the mechanism by which the *APOA5* gene, which has been shown to affect plasma triglyceride levels [68], affects energy metabolism using mice deficient for *Apoa5*. APOA5 has been proposed to function as an anchor protein on lipoprotein particles and to play a role in lipoprotein lipase mediated lipolysis [69]. Since *Apoa5* deficient mice eat more food and develop severe obesity, we investigated the mechanism underlying the hyperphagic phenotype. Our results indicate that APOA5 may play a role in the central regulation of satiety.

In the second part of this thesis, we studied the impact of a disturbed energy metabolism on adipose tissue characteristics. Niacin is a drug that has been used to treat dyslipidemia in humans. Although niacin acutely inhibits fatty acid release from adipocytes, prolonged treatment is associated with adipocyte insulin resistance. In Chapter 4, using a transgenic mouse model with a human-like lipoprotein metabolism, the underlying mechanism of prolonged niacin treatment leading to insulin resistance was investigated. Our results show that niacin induced insulin resistance is due to an adaptation of PDE3B; a central regulator of cAMP signaling.

Chapter 5 focuses on changes in PUFA metabolism in mouse adipose tissue after niacin treatment, which might play a role in the effects of niacin on CVD risk. We found that niacin increases PUFA synthesis and n-3 PUFA secretion into the circulation. These n-3 PUFAs were converted to increased levels of anti-inflammatory oxylipins, which might contribute to niacin's beneficial effects on CVD.

In Chapter 6, we examined adipose tissue from extremely obese women with and without T2DM. In previous research from our laboratory we have found that adipose tissue from obese women with T2DM is in a more pro-inflammatory state both at the cellular and at the gene expression level. In Chapter 6 the adipose tissue was analyzed for fatty acid composition. We found that obese women with T2DM have more n-3 and n-6 PUFAs in their adipose tissue and seem to have an increased conversion of PUFAs towards pro-inflammatory leukotrienes. Finally, we will discuss the results obtained from these studies in Chapter 7 and their implications in the regulation of energy and fatty acid metabolism and how they relate to the development of obesity and insulin resistance.

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