Obesity, ectopic lipids, and insulin resistance

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CHAPTER 1

General introduction
THE CURRENT OBESITY EPIDEMIC

Obesity and obesity-related cardiometabolic conditions are an epidemic threat to public health [1]. Worldwide, more than 1 in 3 adults is now overweight or obese, and the prevalence of overweight and obesity continues to rise in adults and children of both developed and developing countries [2].

Obesity has a large impact on health and well-being. Its medical complications include, but are not limited to, hypertension, dyslipidemia, type 2 diabetes mellitus, atherosclerosis, non-alcoholic fatty liver disease (NAFLD), obstructive sleep apnea, osteoarthritis, and some forms of cancer [3]. In addition, obese people commonly face social stigma and experience psychological stress [4]. The Global Burden of Disease Study, an invaluable collaboration of more than 1600 scientists from 120 countries, estimated that overweight and obesity were responsible for over 3 million deaths and 4% of disability-adjusted life years (DALYs) in 2010, making it the 6th ranking (and fastest-growing) factor in global disease burden [5]. Moreover, the all-cause mortality rate is elevated in individuals who are slightly over ideal body weight [body mass index (BMI) 25.0-27.5 kg/m²], and further rises with more severe obesity [6,7].

Over the past decades, both experts and the public have become increasingly aware of the health risks associated with obesity. It is now an officially-recognized disease [8]. Researchers, clinicians, and policy makers have put in great effort to find novel ways of battling the obesity epidemic. Multidisciplinary obesity clinics have several nutritional, psychological, pharmacological, and/or surgical interventions at their disposal to address the disease [9]. Nevertheless, for most of us, gaining weight is easy, whereas losing weight can be extremely difficult. Almost all hypocaloric (negative energy balance) diets are effective for short-term weight loss, but people who lose weight often regain almost all of the lost pounds in the long term [10]. This so-called yo-yo effect is believed to make subsequent weight loss even more difficult [11]. In addition, for most obese people, the magnitude of effect of dietary weight loss interventions is not nearly enough to reach their ideal body weight [9]. However, even moderate weight loss has beneficial effects on metabolic health [12].

For now, the most effective long-term therapy for obesity is bariatric (weight loss) surgery [13]. Bariatric procedures are designed to restrict food intake and/or induce malabsorption by reducing stomach size and/or bypassing sections of the small intestine [14]. These surgeries effectively induce substantial, sustained weight loss and improve the metabolic complications associated with obesity, even after >15 years of follow-up [15,16]. In addition, the downstream savings associated with bariatric weight loss-induced health benefits are estimated to offset the initial expense of surgery within 2 to 4 years [17]. However, bariatric surgery has serious drawbacks including acute and long-term surgical complications, gastrointestinal symptoms, gall stone disease, and vitamin and mineral deficiencies. Therefore, in The Netherlands, bariatric surgery is currently recommended as a last resort for morbidly obese (BMI >40 kg/m²) patients or severely obese (BMI >35 kg/m²) patients with 1 or more obesity-related comorbidities [18].
Thus, current therapies for obesity are either insufficiently effective or unavailable for the majority of overweight or obese people, and the above-mentioned observations clearly illustrate our need for better, universally available weight loss strategies. Besides, although a reduction in the prevalence of overweight and obesity may go hand-in-hand with a reduction in comorbid diseases, obesity is not the only cause of modern non-communicable diseases, and patients will continue to present with (symptoms of) metabolic complications. An estimated 20% of obese people are metabolically healthy, whereas up to 40% of normal-weight people develop classic obesity-related metabolic complications [19]. Thus, if we want to comprehensively improve care for patients with metabolic disease, we need not only to look at the scales, but also to i) improve our understanding of the underlying mechanisms that link overeating and obesity to diabetes and other complications, ii) unravel novel targets for interventions, and iii) ascertain that new interventions are affordable and universally available.

**INSULIN ACTION AND RESISTANCE**

Humans have evolved highly efficient systems for the storage of nutrient-derived energy in times of plenty and the release of these energy stores in times of scarcity or unpredictable nutrient availability. These systems have – presumably – ensured that the high energy-demanding growing brains of early humans were continuously provided with fuel [20]. In modern societies of plenty, however, the same systems may predispose humans to the development of obesity and metabolic disease. With the balance between nutrient availability and demand dramatically shifted, modern excessive lifestyles place chronic metabolic stress on nutrient storage pathways [21]. A deeper understanding of the pathways involved in healthy and diseased nutrient handling will likely support the development of novel strategies for the prevention and/or treatment of obesity-related metabolic disease.

**Physiology**

Energy and nutrient metabolism is predominantly controlled by 1 highly conserved hormone: insulin. This peptide hormone is synthesized in the β cells of pancreatic islets and released into the portal vein under tight control to meet the metabolic demands of the body. Insulin secretion is mainly stimulated in response to increased glucose levels (for instance, after a meal), but may be augmented or diminished in response to other nutrients such as free fatty acids (FFAs) or amino acids, and hormones such as leptin, growth hormone, or glucagon-like peptide 1 (GLP1) [22].

Insulin binds to the insulin receptor (INSR) on the cell membrane of its target cells. This results in the phosphorylation of insulin receptor substrates (IRs) and activation of the insulin signaling pathway. Depending on the specific kinases and IRs that are present in the intracellular signal propagation network, insulin binding to the INSR can have different downstream effects in different cell types [23,24]. In fact, insulin affects the function of practically every tissue in the body, either directly or indirectly [25], and these tissue-specific effects of insulin together mediate the coordinated regulation of energy homeostasis.
Insulin lowers blood glucose levels by suppression of endogenous glucose production (EGP) and stimulation of glucose uptake. Endogenous glucose is obtained from glycogenolysis (breakdown of glycogen) and gluconeogenesis (synthesis of glucose from precursors) in the liver (75-90% of basal EGP) and kidneys (remaining 10-25%) [26,27]. Insulin works directly to inhibit hepatic glycogen breakdown. Through indirect effects on precursor delivery to the liver, glucagon secretion, and autonomous nervous system signals to the liver, insulin also suppresses hepatic gluconeogenesis [28]. In addition, insulin stimulates glucose uptake into skeletal muscle (80-90% of insulin-mediated glucose uptake) and adipose tissue (10-20%) via the glucose transporter (GLUT) 4 [29,30]. Glucose uptake is also promoted by intracellular activation of the glycogen synthesis and glycolysis pathways in peripheral tissues [30].

Tissue-specific effects of insulin also coordinate whole-body lipid and protein metabolism. Triglyceride storage in adipose tissue is stimulated through activation of lipoprotein lipase (LPL) in adipose tissue capillaries, inhibition of LPL in skeletal muscle capillaries (which reduces myocellular FFA uptake), stimulation of adipose tissue triglyceride esterification, and inhibition of adipose tissue lipolysis. In addition, insulin has anabolic effects on protein metabolism, stimulating protein synthesis and reducing protein breakdown [31]. These and other tissue-specific aspects of insulin action are summarized in Figure 1.1.

Figure 1.1. Tissue-specific physiological effects of insulin.
**Insulin resistance**

Obesity is associated with insulin resistance: a state of diminished biological responses to insulin [32]. Often, this refers to impaired insulin regulation of glucose metabolism, but it is now established that resistance can also develop to other biological functions of insulin. In this regard, hepatic insulin resistance may manifest as elevated EGP, altered triglyceride secretion and/or intrahepatic lipid accumulation [33]; adipose tissue insulin resistance as impaired glucose uptake and/or increased release of FFAs from lipolysis [34]; and muscle insulin resistance as impaired glucose uptake and/or intramuscular lipid accumulation [35]. As such, insulin resistance is the major contributor to hyperglycemia, dyslipidemia, and ectopic lipid accumulation, which together are the pathological hallmarks of the metabolic syndrome and type 2 diabetes [36]. Paradoxically, the metabolic syndrome and other insulin-resistant states are often characterized by resistance to some, but not to all effects of insulin. In selective hepatic insulin resistance, for instance, insulin may fail to suppress glucose production, but continue to promote lipogenesis [33,37]. This further illustrates the importance of recognizing (tissue-)specific effects and defects of insulin, and we will aim to do so throughout this thesis.

**MEASUREMENT OF INSULIN SENSITIVITY IN VIVO**

**The hyperinsulinemic-euglycemic clamp method**

Precise and reproducible quantification of insulin action is essential for research on the epidemiology, etiology, diagnosis, and treatment of insulin resistance. The hyperinsulinemic-euglycemic clamp method, which dates back to the 1970s [38], is often considered the gold standard for assessment of insulin sensitivity in vivo. Although first developed to quantify insulin-stimulated glucose uptake, the clamp method can also be employed to assess specific insulin effects on EGP, lipolysis, or energy expenditure. The method is based on i) the continuous intravenous infusion of exogenous insulin at a constant rate in order to increase plasma insulin to hyperinsulinemic levels and ii) the maintenance of plasma glucose levels at 5.0 mmol/l by variable infusion of exogenous glucose (euglycemic). Under these conditions, the glucose infusion rate that is required to maintain steady plasma glucose levels reflects the rate of glucose disposal into peripheral tissues, assuming that the level of hyperinsulinemia is sufficient to completely block EGP [39]. The concomitant infusion of stable isotope-labeled metabolic tracers allows for the direct measurement of metabolite release into [rate of appearance (Ra)] vs uptake from [rate of disappearance (Rd)] the blood, following the principles of isotope dilution analysis [40-42]. Commonly used tracers include deuterated or carbon-13-labeled glucose, glycerol, or fatty acids. Thus, the hyperinsulinemic-euglycemic clamp and isotope dilution techniques can be used to effectively quantify insulin effects on glucose production (mainly reflecting hepatic insulin sensitivity), glucose uptake (mainly reflecting muscle insulin sensitivity), and glycerol or FFA production (mainly reflecting lipolysis in adipose tissue). Therefore, in research settings where quantifying tissue-specific insulin action is a primary outcome, and the time-consuming, labor-intensive nature of these experiments is acceptable, the hyperinsulinemic-euglycemic clamp should be the method of choice.
**Other methods**

In the human studies described in this thesis, we assessed tissue-specific parameters of insulin action with the use of the clamp method. It is important to note, however, that several other methods for the direct and indirect assessment of insulin sensitivity have been developed [39]. The simplest indirect estimates of insulin sensitivity are derived from a single fasting blood sample. Under fasting conditions, the rate of whole-body glucose utilization is matched by the rate of EGP in order to maintain blood glucose levels within range [43]. With insulin resistance, suppression of EGP and glucose disposal are impaired, which drives up plasma glucose levels. This is then compensated, in full or in part, by increased insulin secretion, which raises plasma insulin levels. Fasting plasma insulin concentrations thus reflects, to some extent, the level of insulin sensitivity in the basal state. The homeostasis model assessment of insulin resistance (HOMA-IR) is calculated as the product of fasting glucose and fasting insulin levels divided by a constant, and simply assesses whether the plasma insulin concentration is appropriate (sensitive) or high (resistant), in light of the glucose concentration. These fasting indices show modest correlations with direct measurements of insulin sensitivity and may be useful in some circumstances, but limited validation and lack of consensus regarding normal vs abnormal values preclude current clinical use [44,45]. More advanced indirect methods depend on glucose and insulin measurements before and during dynamic tests such as the oral glucose tolerance test (OGTT), the mixed meal tolerance test, or the intravenous glucose tolerance test [39]. Both the Matsuda index, which reflects the average glucose and insulin levels during an OGTT, and the minimal model, which models glucose disposal relative to insulin levels, correlate well with clamp-derived parameters of insulin sensitivity [46,47]. One advantage of these tolerance test-derived methods is that they simultaneously provide information about pancreatic insulin secretion and whole-body insulin sensitivity. Nevertheless, their reproducibility may still be disturbed by differences in absorption from the gut or in the concomitant effects of other glucoregulatory/incretin hormones. Finally, the insulin suppression test directly assesses the effect of an insulin infusion on plasma glucose levels [48], thereby providing a precise and reproducible measure of the glucoregulatory properties of insulin [49]. Importantly however, only the hyperinsulinemic-euglycemic clamp technique can adequately quantify tissue-specific parameters of insulin action and resistance.

**Clinical assessment of insulin sensitivity**

Clinically, identification of insulin-resistant patients at high risk for metabolic complications is still problematic. Clamp and metabolic tracer methods are too expensive and laborious and simple assessment tools have not been sufficiently validated. There is also no consensus with respect to what level of insulin sensitivity can be considered healthy or normal [50,51]. Therefore, in Chapter 2, we propose a novel definition of normal insulin sensitivity (vs resistance) on the basis of gold-standard clamp results. In addition, we describe how insulin-resistant obese subjects can be identified from simple parameters that are readily available in clinical practice. In Chapter 3, we extend on this concept by describing and validating simplified index methods for the quantification of adipose tissue insulin resistance in humans.
Since insulin effects vary greatly across tissues, it follows that insulin resistance can manifest in various ways depending on the site of impairment. And that different features of the metabolic syndrome can be the result of different tissue-specific defects. This may have important clinical implications. For instance, some type 2 diabetes patients may benefit greatly from interventions that target muscle glucose uptake, whereas others may benefit more from interventions that target hepatic glucose production [52]. Likewise, in the context of NAFLD, ectopic lipids may derive locally from increased de novo lipogenesis (DNL) or distantly from increased lipolysis in insulin-resistant adipose tissue [53]. This kind of information is essential if we want to move towards more personalized treatment strategies. However, the relative contribution of the liver, adipose tissue, muscle, and other tissues to the pathogenesis of metabolic diseases such as prediabetes, type 2 diabetes, and NAFLD is currently only partially elucidated. Therefore, in Chapters 4 and 5, we studied these primary aspects of insulin resistance in 2 populations at high risk for diabetes: subjects with elevated fasting glucose levels in the prediabetes range, and morbidly obese men and women.

**MECHANISMS OF INSULIN RESISTANCE**

Understanding the mechanisms underlying insulin resistance will support the development of novel interventions that specifically target the root cause of type 2 diabetes. Unfortunately, the pathophysiology of insulin resistance is complex and multifactorial [21,32,54,55]. Although current preclinical evidence implicates many cellular mechanisms, a unifying model for all metabolic defects associated with insulin resistance has not yet been established. Therefore, the challenge ahead is to decipher which of the proposed mechanisms are most relevant for human insulin resistance and find the common pathway that integrates these defects.

**Fatty acid-mediated insulin resistance**

The association between obesity and insulin resistance has long been recognized, and so has the interaction between fat and insulin action. Obesity is associated with elevated plasma FFA levels due to, in part, increased adipose tissue lipolysis [56], and emerging evidence indicates that adipose tissue dysfunction and ectopic lipotoxicity are the major contributors to insulin resistance in obesity [54-60]. When adipocytes grow in size, like in obesity, their ability to store glucose and fatty acids slowly declines [61]. Fatty acids may then overspill from the saturated adipose tissue to ectopic (that is, non-adipose) tissues [62]. In fact, lipids can accumulate ectopically in sites such as muscle and liver when lipid delivery exceeds oxidation [63]. Inside myocytes and hepatocytes, the most abundantly stored lipids are triglycerides stored in inert lipid droplets, but these have been dissociated from insulin resistance [64,65]. It is now established, however, that other lipid species may also accumulate. In this regard, diacylglycerol (DAG) species are of particular interest. These consist of 2 fatty acid chains bonded to glycerol, and buildup of these molecules in myocytes has been linked to the activation of novel protein kinase C (PKC) isoforms. When activated, PKC translocates from the cytosol to the cell membrane and inhibits INSR phosphorylation of IRS1 [58,65]. Consequently, through PKC activation, intramuscular DAG species can block downstream insulin signaling.
and subsequent glucose transport into muscle. Supporting this model of lipotoxicity, intravenous lipid infusion in healthy humans causes transient muscle insulin resistance [66,67]. Moreover, lipid infusion is associated with transiently elevated intramuscular DAG content, PKC activation, and impaired post-receptor insulin signaling [66], but not with alterations in intrinsic mitochondrial function [67]. This suggests that lipids interfere with glucose transport into muscle cells rather than glucose oxidation within these cells.

**Hepatic lipid accumulation**

Skeletal muscle insulin resistance is 1 of the earliest defects in the natural course of diabetes development and may contribute to the subsequent development of other metabolic abnormalities [35]. When meal-derived glucose is not taken up by muscle, it may instead be used as substrate for DNL in the liver, thereby promoting hepatic lipid accumulation and the development of NAFLD [68]. Hepatic steatosis in the context of NAFLD is often associated with hepatic insulin resistance [69-71]. However, analogous to lipid-induced muscle insulin resistance, recent rodent studies implicate the accumulation of intrahepatic DAG lipids to be particularly harmful with respect to insulin action. Here, DAGs may activate liver-specific PKC isoforms, which inhibit proximal insulin signaling and insulin-mediated hepatic glycogen synthesis [72-74]. The human aspects of this mechanism are studied in Chapter 10.

Hepatic insulin resistance can also manifest as elevated EGP from increased gluconeogenesis, but the mechanism for this may primarily involve extrahepatic insulin resistance. Adipose tissue lipolysis and availability of gluconeogenesis substrate (glycerol and FFA) drive hepatic gluconeogenesis through the regulation of the pyruvate carboxylase flux by intrahepatic acetyl CoA, and this was recently shown to be independent of direct insulin signaling to the liver [75]. Hence, insulin resistance in muscle and insulin resistance in adipose tissue both contribute to hepatic insulin resistance via substrate-driven promotion of hepatic lipid accumulation and hepatic gluconeogenesis.

**Adipose tissue lipolysis**

Adipose tissue insulin resistance is an important feature of obesity-related metabolic disease [76]. Lipolysis in adipocytes is normally stimulated by glucagon and catecholamines and inhibited by insulin [77], but several pathophysiological factors may affect the inhibitory effect of insulin on lipolysis. Expanded adipose tissue, as in obesity, releases FFAs and chemokines, which attract and activate adipose tissue macrophages [78]. Activated macrophages, in turn, release cytokines that promote adipocyte lipolysis and chemokine release [79]. Thus, a positive feedback loop caused by adipocyte-macrophage crosstalk may keep the lipolysis program constitutively active (reviewed in [80]). Released FFA and glycerol can subsequently stimulate hepatic gluconeogenesis, further increasing plasma glucose levels [75]. In support of these and other preclinical data, human obesity is characterized by adipose tissue macrophage infiltration, the release of pro-inflammatory cytokines, and a state of low-grade systemic inflammation [79]. Insulin resistance in the brain, specifically in the hypothalamus, may also increase adipose tissue lipolysis through increased sympathetic tone and catecholamine stimu-
Nevertheless, the exact contribution of inflammation and other factors to increased lipolysis in humans is unclear and warrants further investigation.

**The importance of adipose tissue glucose disposal**

Although only a small percentage of meal-derived glucose is normally taken up by adipose tissue, adipose tissue glucose disposal may have important systemic effects. In humans with obesity or type 2 diabetes, GLUT4 expression and glucose uptake in adipose tissue are decreased [29,83], and adipocyte-specific Glut4 knockout mice develop secondary muscle and liver insulin resistance [84]. Some of these systemic effects have been attributed to the (endocrine) release of signaling molecules, such as retinol binding protein 4 [84], from adipose tissue.

In rodent adipose tissue, GLUT4 and glucose fluxes regulate expression of carbohydrate response element-binding protein (ChREBP) α and its active isoform ChREBPβ [85], a key transcription factor for the glycolysis and DNL gene programs [86,87]. Thus, ChREBPβ is activated upon glucose disposal into rodent adipose tissue and a major determinant of fatty acid synthesis [85]. Notably, adipocytes secrete lipokines, specific lipid molecules that have the ability to modulate systemic metabolism in an endocrine fashion. These include fatty acids, such as palmitoleic acid, or branched fatty acid esters of hydroxy fatty acids (FAHFAs), such as palmitic-acid-hydroxy-stearic-acid, and their synthesis may be regulated by ChREBP activity [88]. Both palmitoleic acid and FAHFAs improve insulin sensitivity and glucose tolerance in rodent models. In addition, decreased ChREBP expression in subcutaneous adipose tissue is predictive of insulin resistance in obese prediabetic humans [85,89]. In short, decreased GLUT4 expression, glucose disposal, and ChREBP activation in adipose tissue may contribute to systemic insulin resistance by modulating adipose tissue lipid synthesis, storage, and/or secretion. Some human aspects of these pathways will be addressed in Chapter 7.

Other adipocyte-derived hormones also modulate whole-body metabolism. In this regard, rodent studies have shown that adiponectin has important insulin-sensitizing effects by protecting against hepatic steatosis, suppressing hepatic glucose production, and suppressing systemic inflammation [90]. Humans with obesity or NAFLD have low adiponectin [91], and adiponectin receptor agonists may be a promising new approach to insulin resistance and NAFLD [92]. Note that many different adipokines with possible systemic effects have been described [93], but that the role of the adipose tissue endocrine functions in the pathogenesis of insulin resistance requires further evaluation.

Finally, although the glucose flux into adipose tissue is quantitatively small compared to muscle, it may be important to observe that obesity-related metabolic diseases develop over the course of many years. A small reduction in adipose tissue glucose uptake may, over time, result in the diversion of a large cumulative amount of glucose to other tissues.
**Current working model**

Defects in the nutrient fluxes and signaling pathways across insulin target tissues, give rise to the most important determinants of insulin resistance and type 2 diabetes in obesity: decreased adipose tissue storage capacity and ectopic lipid accumulation [21]. A schematic of this working model for obesity-related insulin resistance is presented in Figure 1.2. Although it goes beyond the scope of this chapter, we note that other factors including, but not limited to, gut microbes [94], incretin hormones [95], the unfolded protein response [96], and lifestyle and exercise [97] are also involved. A better understanding of how these mechanisms integrate will undoubtedly support the development of novel personalized treatments for chronic metabolic disease.

**Figure 1.2.** Schematic of the major nutrient fluxes and metabolic pathways involved in obesity-related insulin resistance.

**OUTSTANDING QUESTIONS**

More people live with diabetes now than ever before. Diabetes prevalence is estimated to double or triple by 2050 [98]. To effectively battle this ongoing epidemic with novel pharmacological agents and other interventions, some outstanding questions will have to be resolved. Muscle insulin resistance is often considered the first and primary defect in the pathogenesis of type 2 diabetes and does, in fact, precede liver insulin resistance [21] or β cell failure [35]. However, this etiological paradigm does not adequately ex-
plain the complete metabolic picture, which includes decreased adipose tissue storage capacity, increased lipolysis, and the diversion of nutrients from adipose tissue to other tissues. Chronic stimulation of adipose tissue under hypercaloric conditions likely activates pathways that ultimately lead to adipose tissue insulin resistance, which may, in series, parallel, or synergy to muscle defects, contribute to whole-body metabolic demise. It will be highly relevant to determine the molecular “hit” that initiates the adipose tissue lipolysis-FFA-inflammation feed-forward loop, and how this relates to metabolic disturbances in liver and muscle. In any case, tissue-specific defects in nutrient handling may require tissue-specific targeted interventions. To this end, novel treatment strategies for adipose tissue and muscle insulin resistance will have to be explored.

Obesity is clearly linked to diet and nutrition [99]. Since in vivo nutrient fluxes are also emerging as major determinants of insulin action, we should also consider diet and nutrition as contributors to insulin resistance. Hypocaloric diets almost invariably promote insulin sensitivity (as well as weight loss) [100], but subsequent weight regain often limits their long-term success [10]. However, independent of weight loss, the macronutrient composition and nutritional content of isocaloric diets may also affect insulin action and diabetes risk [100]. In this regard, low-carbohydrate, low-sugar, low-glycemic index, low-fructose, low-fat and/or high-protein diets have all been claimed to produce spectacular results, but many of these claims lack strong human evidence, also because these diets are difficult to maintain. On the basis of current evidence, we may recommend Mediterranean-style diets [101], limiting dietary saturated fatty acid intake [102], and increasing cereal fiber intake [103], but we should keep in mind that long-term controlled human trials are needed to determine optimal dietary strategies.

In recent years, fructose has received particular public and scientific interest as high fructose intake has been implicated in the current epidemics of obesity and insulin resistance [19,104]. Notably, we generally consume fructose together with glucose in the form of sucrose or high-fructose corn syrup. Overconsumption of sugar increases energy intake, leading to weight gain with evident health implications [105,106]. However, several lines of evidence also specifically implicate fructose in the development of insulin resistance. Fructose intake is epidemiologically associated with insulin resistance [107] and high-fructose diets consistently cause insulin resistance in animal models [108-110]. In humans, however, fructose consumption induced insulin resistance in some diet-intervention trials [111], whereas this effect could not be confirmed in other trials [112]. Thus, the precise role of fructose in the development of human insulin resistance and metabolic disease is an active interest of ongoing research. In Chapter 6, we performed a systematic review and meta-analysis of human diet-intervention trials to determine the effect of fructose consumption on insulin action, whereas, in Chapters 7 and 8, we performed a series of metabolic experiments to study human fructose metabolism.

More specific nutrition-related factors have also been suggested to play a role in insulin sensitivity. Vitamin A supplementation improves insulin sensitivity in rats [113], and low vitamin B12 levels are associated with insulin resistance in humans [114]. In addition,
vitamin C and E supplementation has been extensively studied in humans, but were recently found ineffective to improve insulin sensitivity in patients with type 2 diabetes [115]. Data on the role of vitamin D deficiency in insulin resistance have been inconsistent. Therefore, we studied the relationship between the major vitamin D isoforms and measures of glucose metabolism and insulin sensitivity in Chapter 9. The long list of nutritional modulators of insulin sensitivity also features nuts [116], vinegar [117], coffee [118], minerals [119], cinnamon [120], and other supplements [121], but overall this body of evidence should be carefully interpreted due to the low methodological quality and small sample size of most trials.

Finally, and most importantly, many of the nutritional and molecular mechanisms described here are emerging as important contributors to insulin resistance and type 2 diabetes, but translational evidence in humans is limited. Although animal studies are important to advance biomedical science, they do not always predict human outcomes [122,123]. Studies involving human subjects are essential to understand the principal mechanisms of complex human disease [124]. Hence, in Chapter 10, we translationally investigated the human relevance of basic scientific findings that may explain impaired insulin action in the context of obesity.

In summary, the overall aims of this thesis were to i) investigate tissue-specific aspects of insulin resistance in humans, ii) explore the role of specific nutritional factors in human insulin action and metabolism, and iii) determine the human relevance of emerging integrative mechanisms underlying insulin resistance in adipose tissue, muscle, and the liver.