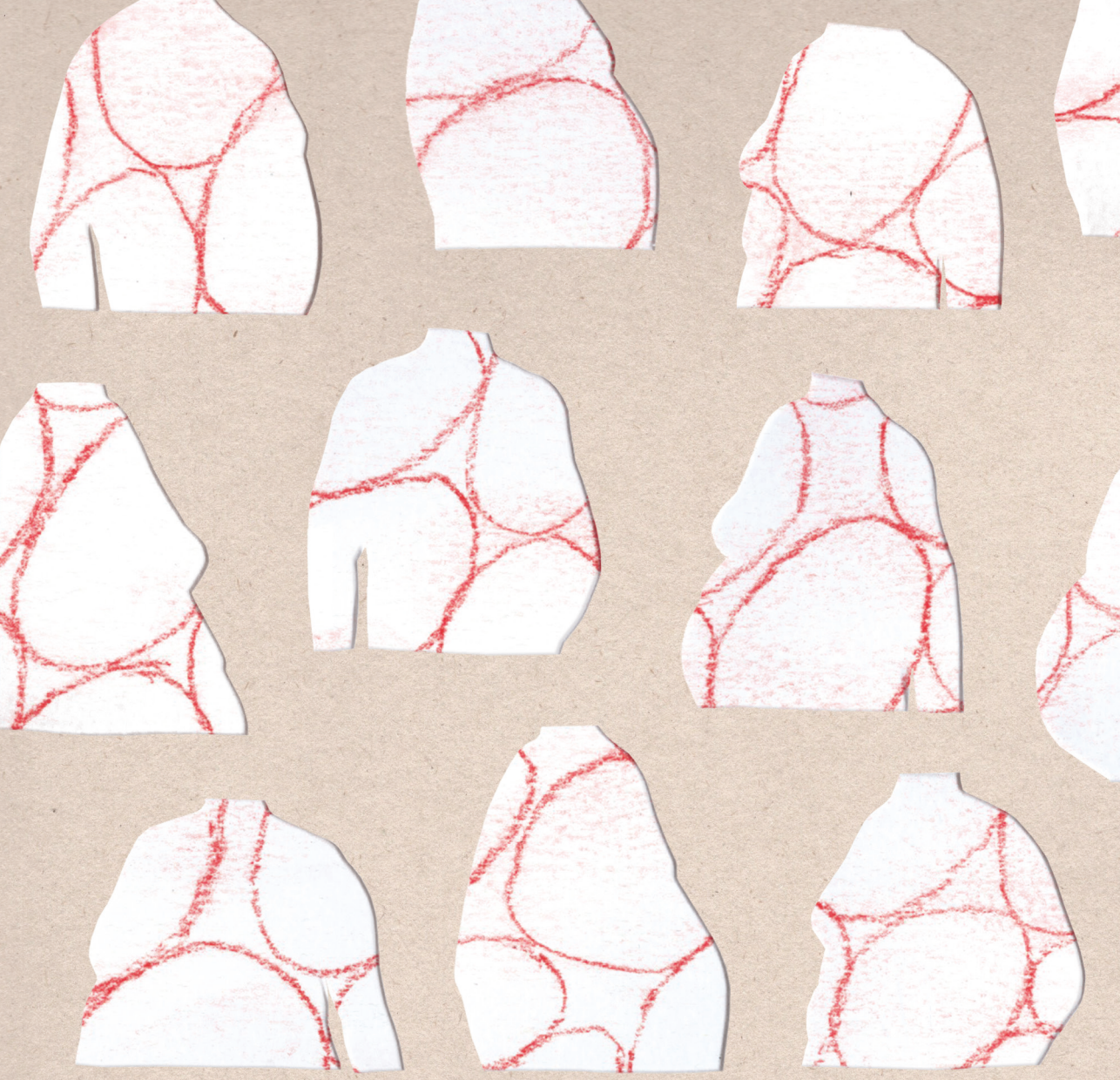

Distinct metabolic phenotypes towards cardiometabolic diseases

implications for precision nutrition strategies

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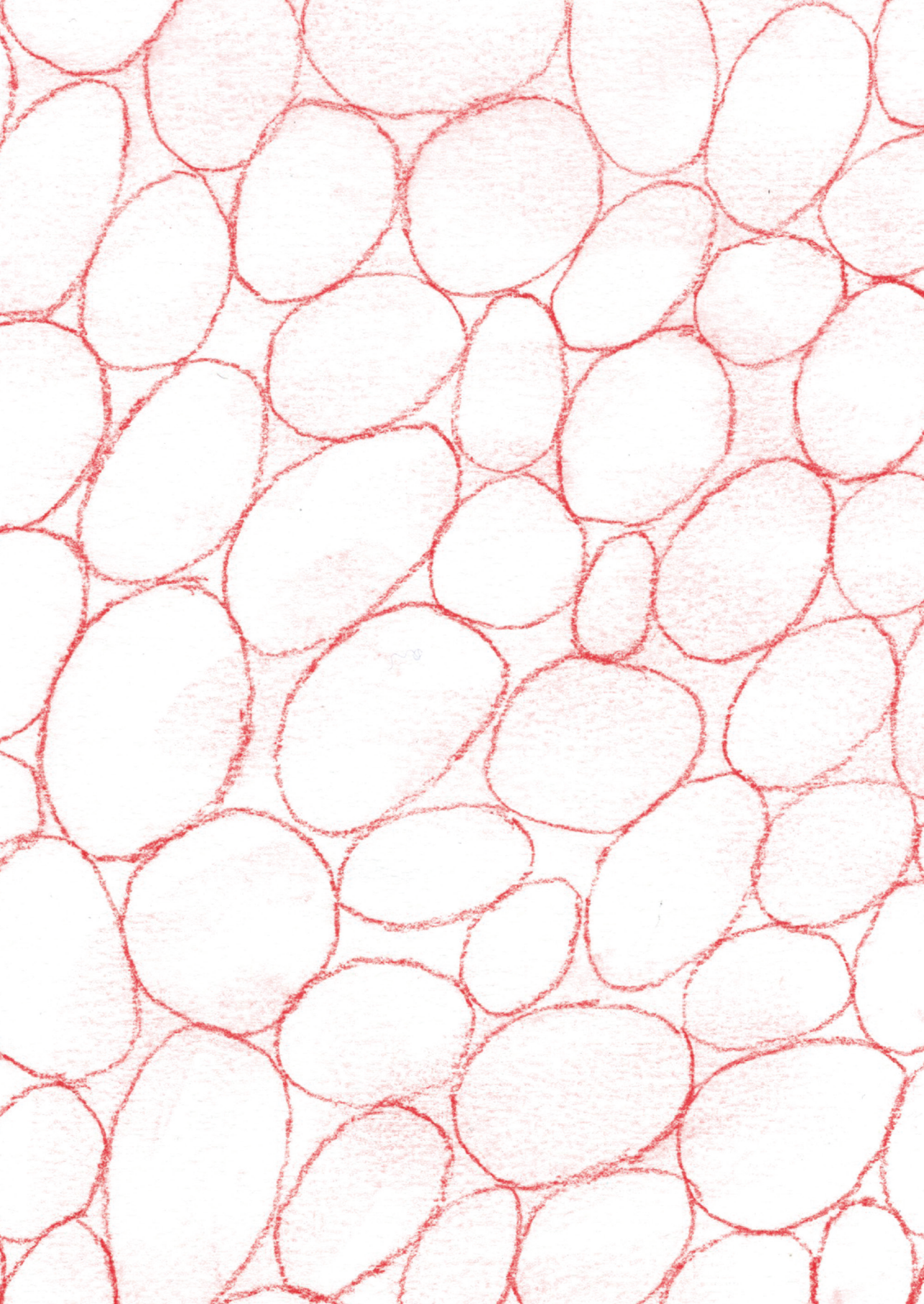

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Distinct metabolic phenotypes towards cardiometabolic diseases: implications for precision nutrition strategies

PROEFSCHRIFT

ter verkrijging van de graad van doctor aan de Universiteit Maastricht, op gezag van de Rector Magnificus, Prof. Dr. Pamela Habibović volgens het besluit van het College van Decanen, in het openbaar te verdedigen op donderdag 2 februari 2023 om 10:00 uur

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Chapter 1

General Introduction

Obesity is a complex multifactorial chronic disease, defined as abnormal or excessive accumulation of fat that presents a risk to health. In May 2022, the World Health Organization (WHO) reported that almost 60% of the adult population, and nearly one in three children, in the WHO European region was affected by overweight or obesity (1). The obesity prevalence is about threefold higher compared to four decades ago (2). Alarming predictions indicate that this number is expected to rise even further in the next decades in both the developed, and the developing world (2). Overweight and obesity are a consequence of a chronic disbalance between energy intake (i.e., intake of energy dense food) and energy expenditure (i.e. physical inactivity), and is dependent on the complex interplay between genetic, biological, behavioral, social, and environmental factors (3). Overweight and obesity increase the risk for the development of other chronic noncommunicable diseases, including cardiovascular disease (CVD), musculoskeletal disorders, certain types of cancer, and type 2 diabetes (T2D), and is currently causing more deaths globally compared to undernutrition and underweight (1). In addition to reducing quality of life, obesity poses a major burden on the healthcare system, with an estimated 25% greater annual healthcare expenditure for an individual living with obesity compared to someone of normal weight (4). ddddadasd

Overweight and obesity are diagnosed by determining the Body Mass Index (BMI). BMI is calculated by dividing total body weight in kilograms by height in meters squared (kg/m^2). Individuals with BMI ranging between 25 and 30 kg/m^2 are considered overweight, while a BMI above 30 kg/m^2 is defined as obesity. On a population level, BMI is associated with decreased life expectancy (5) and obesity-related metabolic complications (6), making BMI a good proxy for population-level risk assessment. Nevertheless, BMI predicts disease risk less well on an individual level, implying that body weight and height per se do not reflect the complexity of the disease (7-9). Obesity is a highly complex, multifactorial, relapsing adiposity-based chronic disease (ABCD) (10-12). A better insight into the underlying metabolic disturbances in obesity is essential to understand the complex relationship between excess body weight and metabolic complications.

Obesity and metabolic complications

Obesity is often accompanied by impaired glucose homeostasis and insulin resistance. Insulin resistance is defined as the impaired ability of insulin to act on insulin sensitive organs, e.g., adipose tissue, liver, skeletal muscle, pancreas, heart, and brain, thereby impairing tissue- specific glucose and lipid metabolism. The adipose tissue, liver, and skeletal muscle are the three major organs involved in regulation of whole-body glucose and lipid metabolism in fasted, fed and exercise conditions (13). The complex involvement

of metabolic organs in whole-body glucose and lipid metabolism is illustrated in Figure 1 and further discussed in the following sections.

Impaired glucose homeostasis and insulin resistance are important risk factors for the development of cardiovascular diseases and T2D. T2D is characterized by elevated glucose levels (hyperglycemia) resulting from a combined process of whole-body insulin resistance and relative deficiency in insulin secretion by pancreatic β-cells (13). Insulin resistance often precedes the development of hyperglycemia and is reflected by compensatory hyperinsulinemia to maintain normoglycemia (13). With the development towards T2D, progressive β-cell failure and hence impaired insulin secretion may result in insufficient compensation for insulin resistance. Indeed, in the prediabetic state, glucose levels are already moderately elevated. Clinical criteria for the prediabetic state are the presence of impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT), where fasting plasma glucose levels (>6.0 mmol/L) and/or 2hr plasma glucose levels (>7.8 mmol/L) are elevated, respectively. In T2D, glucose levels are even further elevated (fasting>7.0 mmol/L, 2-hr>11.0 mmol/L), often together with a (relatively) decreased insulin secretion as a result of progressive pancreatic β-cell dysfunction (13).

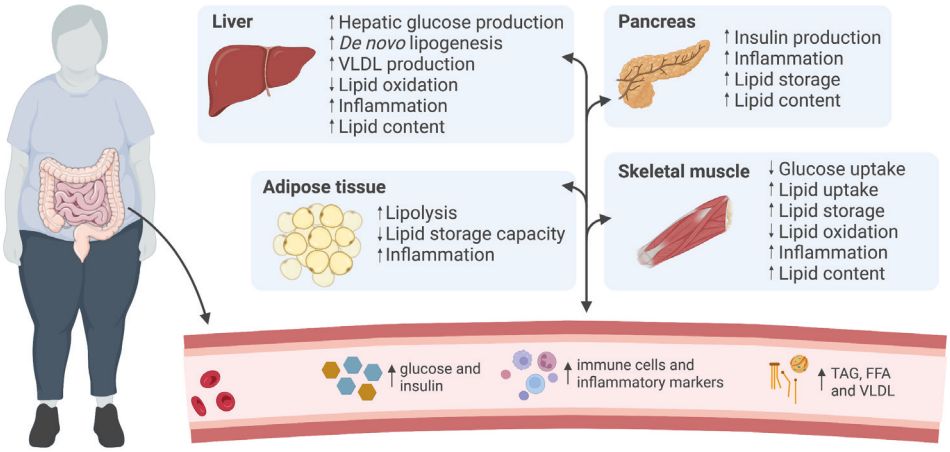

Fig 1. Inter-organ crosstalk in obesity and obesity-associated insulin resistance. Food intake results in increased nutrient (e.g., glucose and lipid) uptake by the intestine and release into the circulation. The pancreas secretes insulin to maintain metabolic homeostasis. However, a chronic disbalance between energy intake and expenditure in the context of obesity can lead to tissue-specific and whole-body insulin resistance and impaired

glucose homeostasis. In this context, adipocytes in the adipose tissue expand through hypertrophy and can become dysfunctional, characterized by a decreased lipid storage capacity, decreased glucose uptake, decreased insulin- mediated suppression of lipolysis, and low-grade inflammation. The liver is characterized by decreased suppression of insulin-mediated glucose production and decreased and incomplete breakdown of lipids. Hyperinsulinemia stimulates hepatic de novo lipogenesis resulting in an increased lipid storage and hepatic lipid output. Furthermore, skeletal muscle's ability to take up glucose and lipids is attenuated, and lipid oxidation is decreased. An increased lipid supply as well as disturbances in lipid metabolism in different tissues may result in increased ectopic fat storage, which can interfere with local insulin signaling. Importantly, lipids, immune cells, and inflammatory markers from different metabolic tissues can spillover to the circulation and interfere with glucose and lipid metabolism in other tissues, highlighting the complex inter-organ crosstalk in obesity (as reflected by the bidirectional arrows from, and to, the different tissues and the circulation). FFA = free fatty acid, TAG = triacylglycerol, VLDL = very-low density lipoprotein.

Pre-diabetes and early stages of T2D are reversible conditions. Early intervention can improve glucose homeostasis, thereby reversing towards non-diabetic conditions and/or preventing worsening of T2D (14-17). As insulin resistance and impaired insulin secretion can be observed already before the onset of (pre-)diabetes (13, 18), it is apparent that a great number of individuals that are at risk for the development of T2D which can be identified in an early disease risk stage and may benefit from lifestyle prevention strategies (19). Understanding the etiology of insulin resistance and impaired insulin secretion is therefore crucial in the prevention of obesity-related metabolic complications. The role of the adipose tissue, the liver, and the skeletal muscle in the pathophysiology of insulin resistance will be described in the next sections.

Adipose tissue mass, distribution, and function in obesity

Excess energy in obesity is largely stored in the body as lipids (triacylglycerols (TAGs)) and lipid components within adipocytes in the adipose tissue. The adipose tissue can expand upon increased energy supply, resulting in body weight gain. Due to the large storage capacity of healthy adipose tissue, excessive lipid storage in non-adipose tissue such as liver or skeletal muscle is prevented (20, 21). Adipose tissue can increase in size through increases in adipocyte size (hyperplasia) or adipocyte number (hyperplasia) (7).

In obesity, disturbances in adipose tissue remodeling are present, characterized by decreased recruitment and differentiation of adipocytes, limiting hyperplasia, and promoting hypertrophy of adipocytes (22). Adipocyte hypertrophy is a key characteristic of adipose tissue dysfunction, which displays marked metabolic features including disturbed mitochondrial function, decreased capillary density, higher number of death or dying adipocytes, immune cells and inflammatory markers, and insulin resistance (7, 23-25). Thus, the adipose tissue is not merely a storage tissue but rather a metabolically active organ involved in many regulatory bodily processes, including appetite regulation, immunity and glucose and lipid metabolism (7, 20, 24-26). Total fat mass, body fat distribution and adipose tissue (dys)function and inflammation all play an important role in relation obesity-related metabolic complications (7) and are discussed in more detail below.

Body fat distribution

Accumulation of fat in the upper body (abdominal region) is linked to increased risk of developing obesity-related metabolic complications, while accumulation of body fat in the lower region (gluteofemoral region) shows opposite associations with metabolic complications and is even considered to be protective as it can act as a "metabolic sink" (27, 28). In line, the inability to expand lower body fat is associated with increased metabolic risk (29). Gluteofemoral fat shows functional differences to abdominal fat, having a greater capacity to store lipids, to recruit additional adipocytes upon weight gain (via hyperplasia), and may display lower inflammatory potential (27). Additional to upper- and lower body fat, both subcutaneous (SAT) and visceral adipose tissue (VAT) can be identified. The latter is located in the abdominal cavity surrounding abdominal organs while SAT can be found directly under the skin. VAT drains largely into the portal vein and higher VAT mass has therefore been linked to greater free fatty acid (FFA) supply to the liver (30). Additionally, VAT is more vascularized, innervated, shows lower capacity for adipocyte differentiation, contains a greater number of immune cells, and is more insulin resistant compared to SAT (31). VAT, in comparison to SAT, is especially linked to higher risk for cardiometabolic complications (31-34). Nevertheless, VAT generally contributes to a significant smaller proportion of total fat mass compared to SAT. Small metabolic changes in the larger SAT depot can therefore already lead to significant whole- body metabolic changes (34). Importantly, distinct sex differences are present in relation to body fat distribution, especially in pre-menopausal women compared to men of similar age. Women generally have less abdominal fat, and less VAT compared to men, resulting in substantial differences in metabolic risk between men and women (35, 36).

Adipose tissue metabolic (dys)function

Adipose tissue metabolic dysfunction and insulin resistance are important links between obesity and obesity-related metabolic complications (37). In healthy conditions, insulin acts on adipocytes by binding to the insulin receptor present on the adipocyte cell membrane, thereby initiating a downstream insulin signaling pathway. This pathway promotes inhibition of lipolysis as well as translocation of glucose transporter-4 (GLUT-4) to the cell membrane to facilitate glucose uptake into adipose tissue (38). Postprandially, insulin activates lipoprotein lipase (LPL). The endogenously-derived very-low density lipoprotein (VLDL)-TAG and exogenously- derived chylomicron-TAG are hydrolyzed by LPL to facilitate plasma FFA clearance by adipocytes (39, 40). In insulin resistant and obese states, LPL activity is reduced, resulting in reduced TAG clearance by adipose tissue (40-42). Additionally, adipose tissue of individuals with obesity is characterized by decreased adipose tissue blood flow and angiogenesis, increased tissue fibrosis, increased inflammation, and a lower capacity to recruit adipocyte precursor cells, thereby limiting the ability to expand through hyperplasia (42-44). Together, dysfunctional hypertrophic adipocytes in obesity show a lower storage capacity for lipids compared to adipocytes in individuals of normal weight (26, 44). Decreased lipid storage capacity leads to a reduced TAG clearance, and thus increased TAG and FFA spillover, resulting in increased lipid supply to other organs, thus contributing to ectopic fat deposition and insulin resistance (41, 42).

Insulin-stimulated lipolysis within the adipocyte is also impacted by insulin resistance, but due to relative hyperinsulinemia in the insulin resistant state hyperinsulinemia may compensate for the reduced insulin sensitivity of lipolysis (45). In line, hyperinsulinemia is negatively associated with the protein expression of adipose triglyceride lipase and hormone sensitivity lipase, independent of fat mass (46). These findings support the notion that per unit fat mass, FFA release as a result of lipolysis is decreased in individuals with normal weight compared to individuals with overweight or obese (45). However, due to the larger absolute fat mass, FFA concentrations may be similar or slightly elevated in individuals with obesity compared to individuals of normal weight (45). Furthermore, adipose tissue insulin resistance results in decreased adipocyte glucose uptake per unit fat mass (13) but may not contribute substantially to reduced insulin-stimulated whole-body glucose disposal in obesity, also due to the larger absolute fat mass (42, 47). Therefore, adipose tissue insulin resistance is generally defined by increased FFA levels per unit of insulin, resulting from a decreased antilipolytic action of insulin (48).

Adipose tissue inflammation

Obesity is characterized by systemic chronic low-grade inflammation, which seems to be mediated by a pro-inflammatory phenotype of dysfunctional adipose tissue (49-52). Adipose tissue with hypertrophic adipocytes is characterized by an increased number of pro- inflammatory immune cells and altered adipokine secretion. The secretion of many adipokines, including macrophage chemoattractants like monocyte chemoattractant protein-1 (MPC-1), is elevated in obesity (38). MCP-1 recruit monocytes to the adipose tissue, which can differentiate into pro-inflammatory macrophages that can remove harmful products (i.e. adipocyte debris) in the adipose tissue. These pro-inflammatory macrophages are often observed surrounding dead or dying adipocytes as "crown-like structures" (52). Together with natural killer (NK) cells, macrophages can produce pro-inflammatory cytokines (including interleuking-6 (IL-6) and tumor necrosis factor-α (TNF-α)) that promote further immune cell infiltration, including recruitment of neutrophils, mast cells, B- and T-cells (52). Together, these factors contribute to a pro-inflammatory adipose tissue microenvironment in obesity (38, 51). Per gram tissue, inflammation is especially present in the VAT, but can also be observed in SAT. Alterations in adipose tissue immune cell populations in obesity have been linked to mitochondrial dysfunction, disturbances in lipid metabolism and insulin resistance (52-55). Adipose tissue inflammation can impact insulin sensitivity locally but can also interfere with metabolic processes in other metabolic tissues in a tissue-specific manner by the release of immune cells and inflammatory markers into the circulation (51, 56, 57). Pro-inflammatory factors may inhibit insulin signaling by several processes including, among others, the activation of Jun N-terminal kinase (JNK) and nuclear factor (NF)-κβ (38, 58), suppression of AMPK activity (59), and downregulation of PPAR-γ (38). However, seemingly contradictory, recruited immune cells may also appear during prolonged fasting or following weight loss to support adipocyte remodeling (49, 52), highlighting the complex relationship between inflammation and metabolic health.

Liver metabolism in obesity

The liver is an insulin-sensitive organ, responsible for glycogen storage and the production and release of glucose upon increased energy demand. Insulin suppresses hepatic glucose production and release into the circulation by suppressing gluconeogenesis and glycogenolysis (60). The liver also plays a key role in whole-body lipid metabolism, as it is involved in lipid uptake from the circulation, synthesis of lipids from carbohydrates and protein (de novo lipogenesis), and lipoprotein synthesis (including synthesis of VLDL-TAG) (26, 60). Insulin suppresses lipid oxidation, and promotes the synthesis and storage of lipids, contributing to whole-body glucose and lipid homeostasis (61, 62).

Metabolic pathways in the liver are often disturbed in individuals with obesity, including a decreased insulin-mediated suppression of hepatic glucose production, leading to increased hepatic glucose output (63). Insulin resistance in the liver in relation to glucose homeostasis is especially evident in the fasting and early postprandial phase (0-30 minutes) when insulin- stimulated suppression of hepatic glucose production is decreased, and skeletal muscle glucose uptake is only slightly increased (64). Furthermore, insulin resistance in the liver is marked by excess hepatic de novo lipogeneses and increased hepatic VLDL-TAG output resulting from elevated insulin levels (63, 65, 66). Also, impaired lipolytic and lipogenic pathways can contribute to the incomplete breakdown or incomplete formation of lipids. As a result, lipids and lipotoxic lipid-intermediates such as diacylglycerol (DAG) and ceramides accumulate in the liver (67). Especially the intake of saturated fatty acids, opposed to the intake of unsaturated fatty acids, contribute to impaired lipid synthesis rates and impaired mitochondrial oxidative capacity (68, 69). This in turn may contribute to the formation of lipotoxic lipid-intermediates, which can interfere with insulin signaling, further promoting the development of insulin resistance in the liver (62, 70, 71). In line, the saturated fatty acid lipid fraction in the liver is associated with increased de novo lipogenesis and hepatic insulin resistance (72). Additionally, the accumulation of lipids within the liver is linked to higher endoplasmic reticulum and mitochondrial stress, impaired autophagy, and inflammation (73, 74). Excessive lipid accumulation in the liver can ultimately lead to the development of non- alcoholic fatty liver disease (NAFLD) (63, 75, 76). Metabolic disturbances and fat accumulation in the liver may at least partly be a result of increased FFA supply to the liver, either derived from the VAT ("portal vein" hypothesis) or SAT (30, 77), resulting from an excess release, and decreased uptake, of FFA's by the adipose tissue (75). Interestingly, the contribution of the VAT to hepatic FFA delivery increased with increasing VAT volume (30). Altogether, the liver is an important metabolic organ and disturbances as often present in individuals with obesity can lead to increased glucose and lipid output, thereby impacting peripheral tissues, including skeletal muscle and adipose tissue (78, 79).

Skeletal muscle metabolism in obesity

The skeletal muscle is largely involved in maintaining whole-body glucose homeostasis as it is responsible for the majority of whole-body glucose disposal, both via insulin-dependent and insulin-independent mechanisms (80, 81). In postprandial conditions, when insulin levels are elevated, the vast majority of glucose uptake in the skeletal muscle is insulin-stimulated, highlighting the important role of insulin in whole-body glucose disposal (13, 61). Additionally, the skeletal muscle synthesizes and oxidizes lipids, and clears lipids from the circulation, importantly contributing to whole-body lipid metabolism (82).

Whole-body glucose disposal is markedly decreased in individuals with obesity and T2D as a result of decreased insulin-stimulated glucose uptake and decreased non-oxidative glucose disposal (likely glycogen synthesis) in the skeletal muscle (81). Insulin resistance in the skeletal muscle is reflected by increased systemic postprandial glucose and insulin levels (64). The insulin resistant muscle shows attenuated insulin receptor phosphorylation, affecting downstream signaling pathways, including decreased insulin-dependent GLUT-4 translocation to facilitate glucose uptake, and impaired glycogen synthase (61). Additionally, insulin resistance and impaired glucose metabolism in the skeletal muscle are closely linked to disturbances in nutritive blood flow and capillarization, lipid turnover, lipid droplet dynamics, and mitochondrial function and oxidative capacity (83-86). The rate of intramyocellular lipid oxidation is dependent on the mitochondrial oxidative capacity and the availability of fatty acids (87). Interestingly, lower skeletal muscle lipid oxidative capacity is observed in individuals with obesity compared to individuals of normal weight (83). Additionally, greater postprandial TAG extraction is observed in individuals with impaired glucose metabolism (88). Together with an oversupply of lipids from adipose tissue and liver, these factors can contribute to the accumulation of lipid intermediates such as DAGs, ceramides, and long-chain fatty acyl-CoAs (LCFA-CoAs) in the skeletal muscle in obese, insulin resistant states (26, 89). These lipotoxic intermediates can interfere with insulin signaling downstream pathways via the action of protein kinase C (PKC) and Akt, further contributing to the metabolic inflexibility to adapt to lipid supply and contributing to local insulin resistance in skeletal muscle (81, 82, 89).

Heterogeneity in the etiology of obesity-related complications: tissue-specific insulin resistance

Pathophysiological heterogeneity exists already before the development of obesity-related complications (90-92) and may be associated with different long-term disease risk outcomes (93, 94). Recently, researchers have identified six pathophysiological sub-phenotypes within individuals at risk for developing T2D (94). These clusters differed from each other related to insulin secretion (β-cell function), glycemia, liver fat content, genetics, body composition, insulin resistance and other clinical parameters. Importantly, long term follow-up of individuals in these metabolic clusters revealed distinct risk of developing T2D, cardiovascular disease, kidney disease and all-cause mortality. Also, within individuals with newly diagnosed T2D, different clusters of the disease progression were identified combining data including insulin sensitivity and secretion, age of diagnosis, and BMI (93). The identified subgroups revealed differences in risk towards kidney-disease and all-cause mortality. The identification of metabolic subgroups in the population may help to target nutritional interventions to improve efficacy for improving metabolic health.

Since insulin resistance is an important mediator in the etiology of obesity-related complications, heterogeneity in insulin resistance is a clinically relevant topic and may give leads for targeted intervention strategies. Interestingly, insulin resistance does not necessarily develop simultaneously in different tissues, and individuals can develop adipose tissue, liver, or muscle insulin resistance (ATIR, LIR and MIR, respectively) separately or with different severity. To illustrate, in a population of adults with overweight and obesity, the majority (66%) of the insulin resistant individuals was either predominant LIR or MIR, while only one third of the insulin resistant population showed a combined MIR and LIR phenotype (95). Furthermore, the discordance between the presence of ATIR and MIR (unpublished data), and discordance between the presence of fasting insulin resistance assessed by the HOMA-IR and ATIR have been described (96). Interestingly, the latter shows the presence of discordance in about a quarter of an overweight but otherwise healthy population.

Discordant insulin resistant subgroups are linked to distinct metabolic features, representing different etiologies towards obesity-related metabolic complications (57, 97). For example, discordance in HOMA-IR and ATIR was linked to distinct VAT mass, fasting TAG concentrations, and basal metabolic rate (96). Furthermore, the MIR but not LIR phenotype showed lower plasma lysophpsphatidylcholine concentrations, while people with LIR but not with MIR had higher TAG and DAG concentrations in women (95). In addition, inflammatory gene expression in the SAT and inflammatory markers in the circulation are associated with insulin resistance in the muscle, but not the liver, while gene expression profiles related to extracellular matrix (ECM) remodeling were higher expressed in individuals with LIR (57). Another study observed distinct differences in individuals with MIR versus LIR regarding plasma metabolic profiles, with the liver insulin resistance being associated with lower ketone bodies and elevated ketogenic amino acids (97). Finally, higher expression of inflammatory and ECM genes, together with a worse metabolic and inflammatory profile was observed in individuals with ATIR, independent of the presence of MIR (unpublished data). Together, these data support the presence of distinct insulin resistant phenotypes, which are relevant in relation to risk factors for obesity-related metabolic complications, as well as provide leads for personalized intervention strategies. Nevertheless, knowledge on the complex (metabolic) physiology of these different phenotypes is still limited and requires further investigation.

Measurement of (tissue-specific) insulin resistance

Quantification of insulin sensitivity is essential in order to study its response to dietary interventions. There are different methods available to determine insulin sensitivity,

which differ in feasibility, invasiveness, costs, and validity. The differences between some of these methods will be discussed in more detail in the General Discussion (Chapter 8) of this thesis. In short, the gold standard to measure insulin sensitivity is the hyperinsulinemic-euglycemic clamp. This technique can be used to measure whole-body insulin sensitivity (1-step clamp using high-insulin infusion only) and tissue-specific insulin resistance (2-step clamp using both low-insulin and high-insulin infusion) (98, 99). As the clamp technique is an invasive, labor- intensive, and expensive method, insulin sensitivity is often quantified using data from a fasting blood sample and/or from an oral glucose tolerance test (OGTT). For example, the HOMA-IR (100) and Matsuda index (101) are widely used to determine whole-body insulin sensitivity. More recently, the disposition index has been used as a combined measure of insulin sensitivity and insulin secretion (102). Nevertheless, the latter indices do not provide insight into tissue-specific insulin sensitivity (i.e., liver, skeletal muscle, and adipose tissue insulin sensitivity). The hepatic insulin resistance index (HIRI), muscle insulin sensitivity index (MISI), and adipose tissue insulin resistance index (ATIRI) quantify the level of insulin resistance in the liver, skeletal muscle, and adipose tissue, respectively. These indices have been validated against the gold standard two-step hyperinsulinemic-euglycemic clamp technique (48, 64, 103). Figure 2 represents the measurement of HIRI (panel A) and MISI (panel B) in two representative individuals with predominant LIR and MIR, respectively.

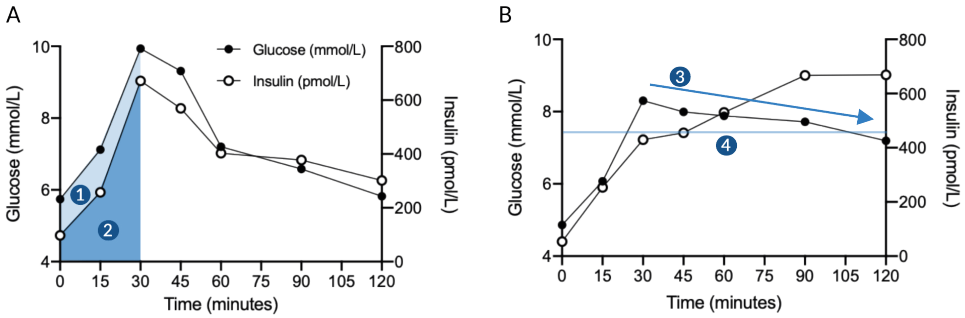

Figure 2. The measurement of tissue-specific insulin sensitivity. Two representative examples of responses to a 75-gram oral glucose tolerance test (OGTT) of an individual with predominant liver insulin resistance (LIR) (A) and muscle insulin resistance (MIR) (B). LIR is characterized by elevated fasting and increased glucose and insulin values in the early postprandial phase, while MIR is characterized by high glucose and insulin values in the late postprandial phase. The hepatic insulin resistance index (HIRI) is calculated by multiplying the area under the curve (AUC) of glucose (1) and

insulin (2) of the first 30 minutes of an oral glucose tolerance test (OGTT). A higher HIRI indicates greater insulin resistance in the liver. The muscle insulin sensitivity index (MISI) is calculated by dividing the decrease in glucose from the peak to nadir (3) by the mean insulin concentration throughout the 120 minutes of the OGTT (4). A lower MISI indicates greater insulin resistance in the muscle.

Interventions targeting obesity-related metabolic complications

From a societal perspective, understanding the etiology of obesity-related metabolic diseases is relevant to develop effective intervention strategies. A broad range of interventions for the prevention of T2D and cardiometabolic have already been developed, ranging from lifestyle interventions (including diet and physical activity) to medication. Dietary interventions may be focused on energy restriction to promote weight loss or focused on a healthy diet with an optimal nutrient composition, focused on improving metabolic health in general (104).

Dietary interventions with or without weight loss

Energy restricted diets have consistently shown to be very effective in promoting weight loss and improving metabolic parameters in individuals at risk for developing cardiometabolic diseases, thereby delaying the onset of T2D (105-110). Dietary interventions resulting in weight loss generally report greater improvements in metabolic health in the short term compared to diets focusing on diet composition per se (111, 112). Despite the promising effects of energy restricted diets, weight loss is often difficult to maintain in the long term (113- 115). Energy restricted diets typically result in relatively fast weight loss, followed by a period of weight maintenance, and/or progressive weight regain (113). Indeed, partial, or complete weight regain is generally observed following weight loss and results in deterioration of the initially improved metabolic parameters (116-118). Minimal long-term weight maintenance may be attributed to low long-term adherence to energy restricted diets as well as metabolic adaptations to weight loss (119-121).

However, major weight loss does not seem essential in the prevention of obesity-related metabolic complications, as clinically relevant improvements in metabolic markers may already be achieved following diets achieving moderate weight loss (<5%) (16, 122-125), or even in the absence of weight loss (17, 124). Indeed, 5% weight loss resulted in improved insulin sensitivity in liver, muscle and adipose tissue, and other metabolic markers in individuals with obesity (122). Furthermore, moderate weight loss resulted in about 65% lower incidence of T2D in adults with IGT after one year in the EDIPS study (16). The latter

study also highlights the importance of maintaining the weight that was lost, as this resulted in sustained metabolic improvements and a further reduction in T2D incidence (16). Finally, results from the SLIM study, investigating the effects of a lifestyle intervention without the presence of weight loss on metabolic syndrome, demonstrated that the intervention group had a four-time lower risk of developing the metabolic syndrome compared to the control group (17). These studies highlight that diet composition, irrespective of weight loss, is important in reducing the risk of cardiometabolic disease (126-128). In view of the relatively poor long-term weight maintenance following weight loss, the focus of lifestyle intervention may be more on improving health through lifestyle changes including a healthy diet, instead of the focus on weight loss perse.

Dietary interventions focused on macronutrient quality and quantity

Several diets within the general guidelines of healthy nutrition, differing in macronutrients quality and quantity, have been demonstrated to effectively prevent, or decrease the risk of, the onset of cardiometabolic diseases in individuals with obesity at risk of developing T2D (111). The Mediterranean diet, typically high in mono- and poly-unsaturated fatty acids, and a low-fat diet, are often observed to improve insulin sensitivity and overall cardiometabolic health in individuals at risk for T2D (111, 128-130). The PREDIMED study observed that a Mediterranean diet was more successful in improving metabolic syndrome parameters and delaying the onset of T2D in the absence of weight loss, compared to a low-fat diet with similar complex carbohydrates content (129), although superiority of diet above the other is not consistently reported in relation to cardiometabolic health (111, 130, 131). Nevertheless, these diets illustrate that manipulation of dietary fat quality and quantity can be an important strategy in reducing cardiometabolic disease risk. Indeed, replacement of saturated fatty acids by unsaturated fatty acids, can improve markers of the metabolic syndrome (132, 133) and improve skeletal muscle metabolism and insulin sensitivity (134). Nevertheless, the effects of dietary fat quality may be overridden when fat content in the diet is too high (>37% of energy intake) (135).

Besides dietary fats, dietary carbohydrate quality and quantity, which may be defined as the glycemic index and glycemic load of a diet, are clear predictors of T2D risk (128, 136-138). In fact, reduction of dietary sugars, and substitution of sugars for more complex carbohydrates, including dietary fibers, reduces the risk for T2D in both men and women (136). Furthermore, the combination of a Mediterranean diet with a low-glycemic index, in contrast to a Mediterranean diet with a high-glycemic index, decreased average glucose concentrations and glycemic variability in adults at risk of developing T2D (139). Furthermore, high intake of dietary fibers (>30 g/day) have consistently shown to reduce

insulin resistance and improve glycemic control, thereby reducing the risk of developing T2D (140-142). Importantly, dietary fibers may also regulate satiety hormones, which leads to increased perceived satiety and reduced food intake, thereby contributing to body weight control (143). Finally, high plant- and animal- source protein reduce liver fat and inflammation and have also been implicated to improve insulin sensitivity (144-146). Evidence of the latter is however mainly based on prospective observational studies, and thus requires further investigation (111).

Inter-individual variation in response to dietary intervention

Despite the promising effect of a healthy diet on cardiometabolic health, great inter-individual variation in metabolic response to diet, in dietary intervention outcome, and in dietary adherence is often observed (147-153). Although metabolic health-related improvements following a dietary intervention may be observed on a population level, not all individuals respond to dietary intervention. For example, in the large European DiOGenes study, about half of 383 individuals with obesity that lost 28% of their body weight, did not improve in insulin sensitivity and glycemic control, and this effect was maintained one year after intervention (148, 149). Partially, this inter-individual variation in dietary response can be explained by heterogeneity in both endogenous and exogenous factors including anthropometrics, sex, metabolic phenotype, demographics, food preferences, behavioral factors, and microbial phenotype (149-155). Illustrative is the LIPGENE study, which is a large randomized controlled trial, including individuals with metabolic syndrome, investigating the effect of four diets differing in dietary fat quantity and quality on metabolic syndrome risk factors (156). Reducing saturated fatty acid content, and substitution of saturated by unsaturated fatty acids, led to a reduction in insulin resistance and other metabolic risk factors. Interestingly however, individuals with insulin resistance were more susceptible to the beneficial effect of substituting saturated fatty acids for mono- or polyunsaturated fatty acids, while individuals without insulin resistance were more sensitive to the detrimental effects of high saturated fatty acid intake (156). These data suggest that baseline metabolic status is a determinant for health outcomes following dietary intervention. In another study investigating the effect of weight loss on metabolic health revealed that despite similar weight loss, great variation in improvements in HOMA-IR as marker of insulin sensitivity was observed in individuals with obesity, which appeared to be dependent on baseline metabolic health status (157). Besides, previous studies have identified a variety of baseline characteristics, including gut microbial composition (158), insulin and glucose markers (156, 159), and urinary metabolites (154), that can determine the metabolic response to a dietary intervention at least to some extent.

Also, on an a single-meal level, the presence of inter-individual variation in metabolic response is largely acknowledged. Zeevi and colleagues (151) observed that food products that are considered healthy (for example bread), can elicit up to a 10-fold difference in postprandial glucose response between individuals. Several attempts have been made to predict postprandial glucose responses by means of machine-learning algorithms, integrating information on clinical, microbial, and lifestyle parameters (150, 151, 160). Prediction of glycemic responses appeared relatively accurate, with approximately 70% of the glucose variation explained, with similar accuracy when the model was tested on an independent validation cohort (151). Furthermore, in the PREDICT study, a machine-learning model was developed to predict postprandial lipid and glycemic response and reported that factors including microbial profile, meal timing and physical activity were highly predictive for the metabolic response (150). Importantly, the authors therefore conclude that variation in dietary response is not solely explained by differences in dietary adherence (150). Therefore, these data suggest that "one size does not fit all" and that individuals may benefit from more personalized dietary recommendations (150, 161, 162), also referred to as precision nutrition.

Precision nutrition

Interestingly, diets based on these machine-learning-predicted dietary responses have shown to successfully improve parameters of metabolic health in individuals as compared to general dietary guidelines (151, 163, 164). Nevertheless, the large amount of data required for developing such a machine-learning-based personalized dietary recommendation makes implementation into health care practice highly challenging. Furthermore, the machine- learning models do not allow for physiological understanding of mechanisms involved in dietary response to a more personalized diet. The latter may be especially important for the successful replication of findings in other cohorts. An alternative approach of precision nutrition, by clustering individuals by more detailed clinical characteristics, may be practically more feasible to implement into health care practice to improve obesity-related metabolic complications. Furthermore, this strategy provides more insight into the underlying pathophysiology towards cardiometabolic disease compared to machine-learning models.

One way to group individuals is by their tissue-specific insulin resistance phenotype, as these are distinct metabolic subgroups and targeting a dietary intervention based on these subgroups may therefore be a promising strategy to improve metabolic health in individuals at risk of developing obesity-related metabolic complications (92, 165, 166). Indeed, a *post-hoc* analysis of the CORDIOPREV-DIAB study revealed that LIR and MIR

phenotypes responded differentially to dietary intervention (166). The LIR phenotype responded most favorably to a diet low in fat and higher in carbohydrates, while the MIR phenotype benefitted most from a Mediterranean diet. However, prospective evidence on the efficacy of subgroup-based precision nutrition in the prevention of obesity-related cardiometabolic diseases is largely lacking and therefore prospective randomized research is warranted.

Outline of the thesis

The research in this thesis is focused on different metabolic phenotypes towards cardiometabolic diseases in individuals with overweight and obesity. The different metabolic phenotypes may respond differentially to diet and give therefore implications for precision nutrition strategies.

Chapter 2 provides a comprehensive review of the current literature on ectopic fat accumulation in tissue-specific insulin resistance. Precision nutrition may provide a strategy to improve ectopic fat accumulation and metabolic health. Available evidence of the effect isocaloric diets on ectopic fat and insulin resistance are described in this chapter.

As indicated in this introduction, men and women show apparent metabolic differences, leading to different risk profiles for the development of obesity-related metabolic complications. Interestingly, men and women also respond differentially to weight loss interventions, and the following weight maintenance phase. In Chapter 3, a *post-hoc* analysis within the Diet, Obesity, and Genes (DiOGenes) study is performed on the difference between men and women in relation to weight changes and metabolic parameters following weight loss.

Following, Chapter 4, 5, 6, and 7 are related to the Personalized Glucose Optimization Through Intervention (PERSON) study. The PERSON study is a two-center randomized dietary intervention study investigating the efficacy of modulation of dietary macronutrient according to tissue-specific insulin resistant phenotypes (MIR and LIR) on the disposition index and other parameters related to glucose homeostasis, insulin resistance, and cardiometabolic disease risk. The rationale and study-design, and preliminary screening results of the PERSON study are described in Chapter 4.

Previous research indicates that MIR and LIR are distinct metabolic phenotypes, also in relation inflammatory profile. However, the relationship between adipose tissue and systemic immune cells and inflammation with tissue-specific insulin resistant phenotypes

remains to be established. In Chapter 5, in a cross-sectional analysis of the baseline (pre-intervention) data of the PERSON study, and data from The Maastricht Study, the relationship between circulating and abdominal SAT immune cells, circulating inflammatory markers, and MIR and LIR is assessed. Furthermore, in Chapter 8, we comment on a published paper that suggests adipose tissue inflammation not to be related to adipose tissue insulin resistance.

The primary aim of the PERSON study was to assess whether a precision nutrition approach by modulating dietary macronutrients according to tissue-specific insulin resistance phenotypes, within the context of guidelines healthy nutrition, could enhance improvements in cardiometabolic health. The effects of the intervention on parameters of glucose homeostasis, cardiometabolic risk markers, and perceived well-being are described in Chapter 6.

The final chapter describing results of the PERSON study, Chapter 7, reports whether body composition and body fat distribution differ in MIR and LIR phenotypes. Besides a cross- sectional analysis, the effects of two healthy isocaloric diets that were followed for 12 weeks on parameters of body composition and (tissue-specific) insulin resistance are described. Data is collected with a state-of-the-art whole-body magnetic resonance imaging technique.

Finally, in Chapter 9, the main findings of the individual chapters are integrated and discussed in a broader perspective, along with suggestions for future research.

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Chapter 2

Ectopic fat accumulation in distinct insulin resistant phenotypes; targets for personalized nutritional interventions

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ABSTRACT

Cardiometabolic diseases are one of the leading causes for disability and mortality in the Western world. The prevalence of these chronic diseases is expected to rise even further in the next decades. Insulin resistance (IR) and related metabolic disturbances are linked to ectopic fat deposition, which is the storage of excess lipids in metabolic organs such as liver and muscle. Notably, a vicious circle exists between IR and ectopic fat, together increasing the risk for the development of cardiometabolic diseases. Nutrition is a key-determining factor for both IR and ectopic fat deposition. The macronutrient composition of the diet may impact metabolic processes related to ectopic fat accumulation and IR. Interestingly, however, the metabolic phenotype of an individual may determine the response to a certain diet. Therefore, population-based nutritional interventions may not always lead to the most optimal (cardiometabolic) outcomes at the individual level, and differences in the metabolic phenotype may underlie conflicting findings related to IR and ectopic fat in dietary intervention studies. Detailed metabolic phenotyping will help to better understand the complex relationship between diet and metabolic regulation, and to optimize intervention outcomes. A subgroup- based approach that integrates, among others, tissue-specific IR, cardiometabolic parameters, anthropometrics, gut microbiota, age, sex, ethnicity, and psychological factors may thereby increase the efficacy of dietary interventions. Nevertheless, the implementation of more personalized nutrition may be complex, costly and time consuming. Future studies are urgently warranted to obtain insight into a more personalized approach to nutritional interventions, taking into account the metabolic phenotype to ultimately improve insulin sensitivity and reduce the risk for cardiometabolic diseases.

INTRODUCTION

Cardiometabolic diseases such as obesity, diabetes mellitus type 2 (T2DM) and cardiovascular diseases are the leading causes of death and disability in the Western world. In 2014, T2DM affected about 422 million adults worldwide, and its prevalence is expected to rise even further in the next decades (1), representing a large burden for society. Although different etiologies precede the development of cardiometabolic diseases, insulin resistance (IR) is a major contributing risk factor and is therefore a relevant target for preventive healthcare.

IR represents a physiological state in which the action of the hormone insulin is impaired. As a consequence, the body is not effectively able to adapt to its metabolic or energy demands, also described as metabolic inflexibility (2, 3). IR can develop simultaneously in multiple organs and the IR severity may vary between different organs. More specifically, the regulation of metabolic processes such as glucose uptake and oxidation, glycogen synthesis and breakdown, lipid storage and lipolysis may be disturbed. A wide range of literature has linked these metabolic disturbances to accumulation of excess lipids in organs such as the liver, skeletal muscle, pancreas, and heart, also referred to as ectopic fat deposition (4, 5). The link between ectopic fat, IR, and metabolic inflexibility, however, is multifaceted. The type of tissue (e.g. liver or skeletal muscle), the accumulation of lipids (e.g. triacylglcyerol, diacylglycerol) and lipid metabolites (e.g. ceramides and acylcarnitines) as well as composition, turnover and localization of lipids have all been shown to play important roles in the metabolic consequences of ectopic fat accumulation (6, 7). More globally, age, sex, lifestyle and (epi)genetics, as well as microbial composition and functionality, may also play an important role in ectopic fat accumulation (5, 8, 9). These factors impact the development of distinct IR phenotypes, and should be considered when targeting ectopic fat and IR.

Diet and physical activity are key modifiable risk factors that play a significant role in the development, progression and reversal of IR and ectopic fat accumulation (5, 10). Related to the diet, not only caloric intake, but also the macronutrient quantity and quality of the diet may affect insulin sensitivity, which may be mediated by changes in ectopic fat accumulation (11-14). Interestingly, however, recent evidence has shown that the metabolic phenotype of an individual seems to contribute to the inter-individual differences in response to dietary intervention (13, 15). Therefore, interventions to prevent or reverse IR may be optimized using a more subgroup-based or personalized approach.

In this review, we will provide an overview of the mechanisms that play a role in ectopic fat storage and its relationship to distinct IR phenotypes. Additionally, it will be discussed how dietary macronutrient quality and quantity may affect ectopic fat and, consequently, IR in individuals with different metabolic phenotypes.

ECTOPIC FAT AND INSULIN RESISTANCE: PATHOPHYSIOLOGY AND INTER-RELATIONSHIPS

Cellular mechanism leading to ectopic fat and IR

IR conditions are often accompanied by a systemic overflow of lipids, which is mainly the result of disturbances in adipose tissue lipid handling, in combination with a chronic excess energy intake (2). Disturbances in adipose tissue function are, beside an impaired lipid storage capacity, characterized by an increased infiltration of macrophages and other immune cells leading to a state of chronic low-grade inflammation in obesity (2). Adipose tissue inflammation may induce alterations in adipose tissue metabolism (i.e., lipid metabolism/lipolysis) and the storage capacity of dietary lipid in adipose tissue (2, 16). The excessive flux of lipids towards multiple oxidative tissues, including the liver and skeletal muscle, has marked effects on lipid uptake and metabolism in these tissues, thereby contributing to the development of ectopic fat deposition and IR. Furthermore, the increased secretion of inflammatory factors by adipose tissue may lead to systemic inflammation, which in turn may affect insulin signaling and metabolism of non-adipose tissues, like the liver and skeletal muscle, contributing to the accumulation of ectopic fat and development of IR (2). The mechanisms by which ectopic fat accumulates and how this accumulation affects IR are, however, different between tissues, and has been extensively studied for the skeletal muscle (17). Importantly, the link between ectopic fat and IR is not a one-directional pathway. Accumulation of lipids within the liver, for example, may also be driven by increased de novo lipogenesis due to hyperinsulinemia, as present in IR conditions, and by increased NEFA supply from the adipose tissue (18, 19). Additionally, a reduced capacity to oxidize fatty acids upon increased supply in skeletal muscle (metabolic inflexibility) is often observed in obese insulin resistant conditions, which may be accompanied by mitochondrial dysfunction (2). Hence, a vicious cycle exists between the progression of IR and ectopic fat deposition. In the following sections, we will provide an overview of the mechanisms underlying the accumulation of ectopic fat in the liver and skeletal muscle and its effect on IR.

Disturbances in liver and skeletal muscle lipid uptake

IR individuals are characterized by an altered plasma lipid profile (20). In fasted and

postprandial IR conditions, (slightly) elevated levels of non-esterified fatty acids (NEFA), very- low-density lipoprotein (VLDL)-TAG (21), and dietary-derived chylomicron-TAG (22) are observed, although data is not entirely consistent (23, 24). Notably, NEFA concentrations may not be elevated in proportion to the increased fat mass, since NEFA concentrations per unit fat mass are down regulated in individuals with excess adipose tissue mass (24). Elevated levels of circulating TAG concentrations may be attributed to an increased VLDL-TAG production by the liver or reduced clearance of TAG by adipose tissue (2). Indeed, the removal of TAG across adipose tissue was found to be impaired in obesity, insulin resistance and T2DM due to a reduced insulin-mediated stimulation of lipoprotein lipase (LPL) activity (23, 25-29), suggesting less efficient removal of dietary lipids by adipose tissue in these subjects. Notably, LPL in AT macrophages has been found to increase AT lipid storage in obese mice, (30, 31). In line, studies in high-fat-diet-fed mice showed local lipid fluxes are central regulators of AT macrophage recruitment and that once recruited these macrophages can buffer local increases in lipid concentrations (30). These data suggest that AT macrophages may compensate for the insufficient lipid storage in adipocytes in the obese insulin resistant state, reversing thereby glucose intolerance and insulin resistance.

Despite slightly elevated plasma NEFA concentrations in T2DM as compared to healthy control subjects, a similar postprandial NEFA uptake in skeletal muscle was observed in both groups (32). Likewise, we have recently demonstrated a comparable skeletal muscle uptake of circulating NEFA in overweight and obese subjects with mild versus more severe IR (33). Elevated plasma NEFA levels may, however, lead to increased uptake of NEFA into the liver (34). An increased supply of NEFA to the liver leads to an increased partitioning of NEFA towards VLDL-TAG synthesis and may be accompanied by a decreased insulin-mediated suppression of VLDL-TAG synthesis (35). Both processes contribute to elevated postprandial VLDL-TAG levels in IR. Interestingly, skeletal muscle extraction of VLDL-TAG in postprandial conditions is higher in more severe versus mild IR individuals, despite similar VLDL-TAG supply (7, 33). In accordance with these findings, IR subjects show higher skeletal muscle VLDL-TAG extraction than normal glucose tolerant (NGT) individuals (36). Together, these findings indicate that disturbances in lipid supply and/or uptake in both liver and skeletal muscle may contribute to the pathogenesis of ectopic fat deposition and IR.

Plasma lipids can be transported into the liver or skeletal muscle cell by receptor-mediated transport, as well as passive diffusion via the cell membrane (37). The rate of lipid uptake is dependent on the type of tissue, type, and activity of transporter proteins

on the cell membrane and is a key factor in lipid handling by the liver or skeletal muscle cells. LPL in skeletal muscle and the membrane carrier proteins (specifically CD36) and fatty acid transport proteins (FATP) in liver and skeletal muscle are, therefore, an integral part of this process. LPL is, next to its large abundance in adipose tissue, expressed in endothelial cells near skeletal muscle and is an important facilitator of chylomicron- and VLDL-TAG extraction across the endothelium into the skeletal muscle cell (38). LPL has the capacity to bind and hydrolyze TAG on the surface of the capillary lumen, thereby releasing NEFA which can be taken up by the tissue (39). FATPs are involved in the transport of long-chain fatty acids across the membrane. Different members of the FATP family are expressed in the liver (FATP3,5) and skeletal muscle (FATP3,4), but may have similar properties related to lipid uptake (40). CD36 is a well- characterized protein that is also responsible for the uptake of a variety of lipids in the cell. Expression of FATPs and CD36 in liver and skeletal muscle is often dependent on the metabolic condition. Overexpression of these transport proteins is often seen in response to hyperinsulinemia and may lead to an increased uptake of lipids in IR conditions, which in turn contributes to ectopic fat accumulation in these tissues and associated metabolic impairments (38, 41-44). Interestingly, this may not be the case for LPL in skeletal muscle since insulin seems to stimulate LPL activity in adipose tissue, but not in skeletal muscle (45).

Altogether, human data suggest that merely TAG rather than NEFA uptake into the skeletal muscle may be a putative target in the prevention and/or treatment of ectopic fat deposition and IR in individuals with an impaired oxidative capacity. The role of disturbances in lipid uptake by the liver in relation to liver fat accumulation and hepatic IR is less established.

Disturbances in intramyocellular lipid metabolism

In addition to lipid uptake, insulin sensitivity is strongly related to the oxidative capacity of key metabolic organs such as the liver and the skeletal muscle. The high oxidative capacity represents the ability of the body to adapt to the appropriate metabolic condition, also described as metabolic flexibility (46). Following uptake from the circulation, lipids can either be oxidized or stored for later use. In healthy, insulin sensitive individuals, lipid accumulation within the cell is limited, albeit not absent, as an elevated influx of lipids into the cell triggers the conversion of lipids to long-chain fatty acyl-CoA (LC-CoA), which is transported into the mitochondria and, consequently, degraded by beta-oxidation.

In contrast, IR conditions are characterized by disturbed skeletal muscle lipid metabolism (2). In obese individuals with more severe as compared to mild IR the following

has been reported: a higher saturation of skeletal muscle NEFA and DAG (in particular membrane-bound DAG), a tendency towards a lower fractional synthesis of TAG, and reduced gene expression of oxidative metabolism markers (33, 36, 47). In line, in overweight and obese adults, more severe IR individuals showed lower re-esterification of intramuscular NEFA into TAG, and decreased expression of several genes encoding for proteins involved in TAG synthesis as compared to individuals with mild IR (7). Additionally, in individuals with long-term diagnosed T2DM, a blunted insulin-mediated suppression of skeletal muscle lipolysis was reported, which was related to accumulation of more saturated DAG at the skeletal muscle membrane as compared to normal glucose tolerant (NGT) individuals (47).

These disturbances reflect, at least partly, a decreased capacity of the skeletal muscle to oxidize lipids (48) and a decreased ability to switch from carbohydrate to fat oxidation (49). The decreased skeletal muscle oxidative capacity may be a result of reduced mitochondrial activity, content and/or plasticity, leading to an imbalance between lipid supply, lipid oxidation and conversion into TAG (50). Furthermore, decreased mitochondrial function may lead to overproduction of reactive oxygen species (ROS), which are reactive molecules and free radicals, further contributing to impaired mitochondrial function and oxidative stress (51). The reduced oxidative capacity may contribute to lipid peroxidation whereby lipotoxic compounds are formed in the skeletal muscle, including diacylglycerol (DAG) and ceramides (48, 52). DAG and ceramides may have lipotoxic effects on mitochondrial DNA, RNA and proteins, thereby promoting a further decline in mitochondrial bioenergetics (48). In addition, DAG are responsible for the activation of protein kinase C (PKC) isoforms. PKCs are important signaling molecules involved in insulin-stimulated glucose uptake, since PKC inhibits several steps of the insulin signaling pathway (53). As a consequence, PKC activation by lipid-intermediates interferes with insulin signaling, resulting in a reduced skeletal muscle glucose uptake, as extensively reviewed elsewhere (5). The relevance of ceramide accumulation in muscle insulin resistance in humans is still unclear (17). These findings illustrate the important role of lipotoxic lipid-intermediates in the development and progression of IR in the skeletal muscle.

Disturbances in intrahepatocellular lipid metabolism

As discussed, lipid accumulation within the liver has been linked to IR. The mechanisms within hepatocytes that link lipid metabolism to IR are, however, less understood. It has been shown that insulin increases hepatic (de novo) lipogenesis, TAG storage, as well as the production of VLDL-TAG in the liver in IR conditions (17). Moreover, similar to skeletal muscle, an impaired mitochondrial function in the liver has been found in obese IR

individuals (17). The link between mitochondrial dysfunction in the liver and IR may be explained by excess lipid supply, as often seen in IR conditions. The chronic increased activation of beta-oxidation due to excess lipid supply may stimulate oxidative stress and lipotoxicity, in turn resulting in impairments in mitochondrial oxidative capacity (54). In line, *in vivo* MRS measurements showed that IR individuals exhibited lower hepatic ATP levels, which related to liver-IR and partly accounted for hepatic lipid accumulation (55). However, a causal relationship between mitochondrial function and hepatic IR has yet to be confirmed.

Impaired mitochondrial function in the liver may lead to reduced oxidation of lipids, resulting in the formation of different lipid moieties such as ceramides (54). The accumulation of both total DAG and cytosolic DAG in the liver have been linked to HOMA-IR as well as to liver-IR, as determined by insulin-induced suppression of hepatic glucose output (56, 57). Peterson et al. (58) hypothesized that DAG mediates lipid-induced IR in the liver by inhibition of insulin signaling in a similar manner as occurs in skeletal muscle. As discussed earlier, DAG may activate several PKC isoforms, thereby inhibiting several steps of the insulin signaling pathway. Furthermore, DAG accumulation in the liver could result in decreased mitochondrial function, inflammation, and increased VLDL-TAG production, which may affect IR on a local (liver-IR) and systemic level. Consequently, DAG accumulation in the liver may contribute to liver-IR and, consequently, to decreased glycogen synthesis and increased hepatic gluconeogenesis (2, 17, 58). In humans, there is currently no evidence for a relationship between ceramide accumulation and impaired hepatic insulin action (17, 56, 57). It must be noted that the number of studies related to lipid metabolism in the liver and IR are limited, studies are predominantly cross-sectional, or *in vitro* models have been used, which makes it difficult to understand the molecular mechanisms involved in the putative relationship between disturbances in hepatic lipid metabolism and liver IR.

To summarize, cellular mechanisms responsible for IR and lipid accumulation in both liver and skeletal muscle are related to altered lipid uptake into the cell as well as to an impaired intracellular regulation of lipid metabolism. Together, these impairments may cause an excess accumulation of lipotoxic metabolites if oxidative capacity is insufficient, which in turn interferes with insulin signaling.

Ectopic fat and liver- and muscle-IR

As mentioned earlier, IR can occur in multiple key metabolic organs such as the liver and skeletal muscle. Nevertheless, insulin sensitivity and lipid metabolism may

substantially differ between organs within an individual (59). Moreover, the extent to which IR is present in these distinct organs may vary among individuals. These inter-individual differences in tissue- specific insulin sensitivity may partly be explained by the location where excess lipids are stored. Ectopic fat and lipotoxic intermediates affect metabolism on a local tissue level, as described above. Thus, intrahepatocellular lipids (IHCL) and intramyocellular lipids (IMCL) are therefore often linked to the development of liver-IR and muscle-IR, respectively (5). Nonetheless, ectopic fat is not only related to site-specific disturbances in glucose homeostasis and IR, but also to more systemic disturbances (40). It could be speculated that tissue specific- IR may lead to a redistribution of substrates from the IR tissue towards other tissues during postprandial conditions (60). For example, muscle-IR may promote postprandial glucose supply to the liver, leading to increased de novo lipogenesis and hepatic steatosis (61, 62). This highlights the complex relationship between ectopic fat deposition and tissue-specific IR. Also, it should be noted that liver- and muscle-IR often coexist, which may bi-directionally affect metabolic perturbations in both organs. However, irrespective of the relationship between liver and skeletal muscle fat accumulation and liver- and muscle-IR, both IHCL and IMCL seem to play an important role in the progression towards T2DM (2). Clearly, multiple factors play a role in the complex relationship between ectopic fat deposition and tissue specific-IR, and careful interpretation of research findings is therefore needed.

Interestingly, plasma lipid profiles and lipid handling seem to differ between various IR-states, contributing to ectopic fat accumulation in both liver and skeletal muscle (33, 63). More specifically, higher fasting and postprandial plasma TAG, FFA and VLDL-TAG concentrations have been observed in individuals with IGT as compared to impaired fasting glucose (IFG) (63-66), and in T2DM versus non-diabetic individuals (67). Furthermore, an early postprandial increase in TAG concentration and higher skeletal muscle uptake TAG was observed in both IFG and IGT as compared to NGT (63). Additionally, IGT individuals showed higher skeletal muscle uptake of VLDL-TAG, higher intramuscular TAG content and higher intramuscular saturation of FFA compared to IFG individuals (33).

Interestingly, although IGT and IFG are both IR-states, these seem to differ in their tissue- specific IR, as liver-IR and muscle-IR seem to be the primary disorders in IFG and IGT, respectively (10, 68-70). Consistently, IGT is characterized by a decreased peripheral glucose disposal (71), whereas IFG is characterized by impaired insulin-induced suppression of liver gluconeogenesis and glycogenolysis (69). Not surprisingly, both IFG and IGT have been linked to the accumulation of ectopic fat in liver and skeletal muscle

(72-74). Interestingly however, liver-IR is often considered to be a more severe IR-state as compared to muscle-IR. To exemplify, liver-IR seems to be accompanied with elevated postprandial levels of TAG and VLDL compared to muscle-IR (67) and has been linked to more severe cardiometabolic complications (75). This seems opposite to the finding that IGT is accompanied by higher fasting and postprandial plasma TAG and VLDL-TAG levels (63-66). These findings emphasize that the relationship between ectopic fat deposition, glucometabolic status (i.e., NGT, IFG, IGT, T2DM) and IR is complex, and suggest that these inter-relationships are dependent on certain exogenous (e.g. sex, ethnicity and age) and/or endogenous factors (e.g. physical activity and diet). A better understanding of the different factors involved in these metabolic perturbations is needed to develop more personalized strategies to prevent or reverse IR, as discussed below.

ENDOGENOUS AND EXOGENOUS FACTORS AFFECT ECTOPIC FAT AND LIVER- AND MUSCLE-IR

Endogenous factors

Sex-differences in health and disease is a well-recognized concept in literature (76-78). More specifically, sex differences have been linked to ectopic fat accumulation and IR (9). Difference between sexes may be partly related to differences in body fat distribution between men and women. Generally, women have a higher percentage of subcutaneous adipose tissue (SAT) as compared to BMI-matched men, who generally have more visceral adipose tissue (VAT) (79, 80). Interestingly, the expandability of subcutaneous adipose tissue seems to be a critical factor in the development of insulin resistance (81, 82). Lipids may be predominantly stored in SAT before marked VAT expansion occurs (81, 83). VAT has been linked to higher levels of inflammatory markers, insulin resistance and other cardiometabolic complications (83, 84). Moreover, in general women accumulate more adipose tissue in the gluteofemoral as compared to the abdominal fat depot. Abdominal obesity is associated with an increased risk of developing type 2 diabetes and cardiovascular diseases (85). In contrast, lower body fat has protective properties that are associated with an improved cardiometabolic risk profile in men and women (86, 87). These metabolic differences in adipose tissue depots may partially explain why women are relatively protected against metabolic diseases compared to men with the same BMI (84).

Furthermore, although not completely elucidated, the metabolic differences between men and women are, at least partly, a consequence of differences in hormonal status. Estrogen has been shown to reduce IHCL accumulation and IR in both sexes (77). Until

menopause, women have a lower risk for developing fatty liver, whereas post-menopausal women have a similar risk compared to age-matched males. The increased risk after menopause may be the result of the marked drop in estrogen concentrations during the menopausal phase (88). Indeed, estrogen treatment in the menopausal phase is protective for the development of non-alcoholic fatty liver (89). Furthermore, BMI was reported to be independently related to IHCL content in both men and women, whereas postprandial glucose level was independently related to IHCL content in women only (90). In contrast to IHCL content, some studies reported that IMCL content was significantly lower in both lean and overweight men as compared to BMI matched pre- and postmenopausal women (91-93), although conflicting data have also been reported (94). Noteworthy, for a given BMI, men generally have more muscle mass as compared to women, which may at least partly explain the latter findings (8). Interestingly, despite a higher IMCL content in women, insulin sensitivity and plasma lipid levels were similar between men and women, suggesting that women are relatively protected against lipid-induced IR (93). The relative protection against IMCL was, however, not observed in women using oral contraceptives (93). Interestingly, higher IMCL, lower fractional synthesis rate (FSR) and lower oxidative capacity was observed in prediabetic versus NGT men, while no differences were observed in these parameters in prediabetes versus NGT age and anthropometry matched post-menopausal obese women (95). Together, these findings suggest sex-specific differences in both IHCL and IMCL content. Whether these sex-specific differences in ectopic fat are dependent or independently related to IR, and which mechanisms are involved, is not yet fully understood and requires further investigation (59).

The relationship between ectopic fat storage, IR and sex seems to be largely dependent on the population. IMCL was significantly associated with IR in European Americans, while in African Americans, IMCL varied independent of IR (96). Another study reported a stronger association between IHCL and IR in individuals born in Ira*q* as compared to individuals born in Sweden (97). Moreover, it is well-established that South-Asians are more likely to develop IR and have more ectopic fat (both IHCL and IMCL) when compared to BMI-matched Caucasians (98-101). A possible explanation for this could be that South-Asians have a decreased lipid storage capacity in subcutaneous adipose tissue, leading to an excess flux of lipids towards other tissues such as visceral adipose tissue, the liver, pancreas, heart and skeletal muscle (102). Interestingly, when IHCL and IMCL content in South-Asian and Caucasian men were adjusted for whole-body insulin sensitivity, IMCL was similar, while Asian men maintained a 2-fold higher IHCL content, compared to Caucasian men (100). These findings suggest that the amount of IHCL, in contrast to IMCL content, varied independent of whole-body insulin sensitivity in South-Asians. In a

study of Goedecke et al. (103), IHCL content was lower and IMCL content was higher in pre-menopausal black versus white women. Interestingly, this same study reported that the association between ectopic fat (both IHCL and IMCL) and IR was only present in black women (103). Noteworthy, the total amount of lipids rather than the concentration of toxic lipid-intermediates was measured in these studies. Therefore, it remains uncertain whether the relationship between lipid-intermediates in liver and skeletal muscle and insulin sensitivity depends on ethnicity. Differences between ectopic fat and IR in these populations may be the result of certain genetic polymorphisms. For example, polymorphism of the ApoC3 and PNPLA3 gene have been linked to alterations in IHCL and are more prevalent in South-Asian and Hispanic individuals, respectively (52, 104, 105). To conclude, the studies described above illustrate that the amount of ectopic fat may not always be directly related to IR and seems to differ between populations.

Age seems to be a very relevant factor in the etiology and pathophysiology of IR and seems to be positively related to ectopic fat accumulation (8, 106). Interestingly, there also appears to be an interaction between sex and age, especially related to the pre- or post-menopausal state of women, as already indicated above. More specific, pre-menopausal women are as insulin sensitive as age-matched men, despite higher IMCL content, while this relative protection against IR disappears after menopause (93). However, whether relationships between ectopic fat, IR, age and sex remain after adjustment for visceral fat or total fat mass is not fully known, but has been extensively reviewed elsewhere (8).

Exogenous factors

Lifestyle factors, such as unhealthy diet and a lack of physical activity, are key factors for the accumulation of ectopic fat and the development of IR (5, 10). Physical activity, with its different modalities such as resistance, endurance, or concurrent exercise, exerts several metabolic advantages. For example, improvements not only in cardiovascular fitness but also ectopic fat and insulin sensitivity are often observed (5). Furthermore, dietary intake is an important exogenous factor in relation to ectopic fat and IR. Hypocaloric diets, with resulting weight loss, will lead to a loss of body weight and fat mass, which is accompanied by a decrease in ectopic fat storage (5, 107, 108); metabolic improvements are often observed (5). In addition, the individual macronutrients in the diet, fats, carbohydrates, and proteins, seem to determine metabolic adaptations that have been related to both ectopic fat and IR. In the next section, we will focus on the relationship between macronutrient composition of the diet, ectopic fat storage and IR.

Macronutrient quality and quantity impact ectopic fat and IR

Ample data is available on the effect of different types of isocaloric diets with varying macronutrient composition on IR. Nevertheless, fewer studies are available that report the effects of a specific diet on both IR and ectopic fat accumulation. As discussed, the accumulation of ectopic fat is a major factor in the development and progression of IR. To select relevant studies, a semi-systematic literature search was performed. A literature search was performed in January 2018 using the databases PubMed and Google Scholar. The search strategy consisted of a combination of the following search terms using the Boolean operator "AND" and "OR": "Insulin resistance"[MeSH], "choristoma"[MeSH], "fatty liver"[MeSH], "Non-alcoholic Fatty Liver Disease"[MeSH], "liver steatosis" [TIAB], "ectopic fat"[TIAB], "intramuscular fat"[TIAB], "intramuscular adipose tissue"[TIAB], "muscle fat"[TIAB], "Glucose tolerance"[TIAB], "impaired fasting glucose"[TIAB], "impaired glucose tolerance"[TIAB], "energy metabolism"[Mesh], "metabolic flexibility"[TIAB], "liver insulin resistance"[TIAB], "hepatic insulin resistance"[TIAB], "muscle insulin resistance"[TIAB], "peripheral insulin resistance"[TIAB], "liver insulin sensitivity"[TIAB], "hepatic insulin sensitivity"[TIAB], "muscle insulin sensitivity"[TIAB], "peripheral insulin sensitivity"[TIAB], "dietary proteins "[Mesh], "dietary carbohydrates "[Mesh], "dietary fiber"[Mesh], "dietary fats "[Mesh], "diet therapy"[Mesh], "diet"[Mesh].

Articles included in Table 1 are selected with the following inclusion and exclusion criteria: articles written in both English and Dutch languages were included. Articles were excluded if *in vitro* or animal models were used. Studies examining the effects of single meals (acute setting) rather than dietary interventions, studies investigating hypo- or hypercaloric diets, and review articles were excluded. Endnote X7 was used for the management and selection of articles. The search yielded a total of 729 papers. The majority of the studies were excluded based on abstract (n=694), and others after having read the full text (n=25), resulting in a final selection of 10 original articles (Table 1).

Carbohydrate/fat ratio of the diet

Four different studies included in Table 1 investigated the effect of a low-fat high-complex carbohydrate (LFHCC) diet on ectopic fat and IR in overweight or obese individuals. The studies investigated the effect of diets that were slightly different in macronutrient composition and study duration (ranging between 3-12 weeks) (Table 1). However, these studies consistently reported no significant effects of a LFHCC or a low-fat high- carbohydrate (LFHC) diet on IR, determined by either HOMA-IR or the M-value (12, 109-111). Study outcomes were less consistent with respect to ectopic fat content. The study of Van Herpen et al. (110) reported a significant decrease in IHCL following 3

weeks of a LFHC diet (21 energy percent (E%) fat, 49 E% carbohydrate, 24 E% protein), while Ryan et al. (109) did not report changes in IHCL after a 6-week LFHCC diet (20 E% fat, 65 E% carbohydrate, 15 E% protein). Two studies including LFHC or LFHCC diets did not report changes in IMCL in overweight and obese individuals after 3 or 12 weeks of dietary intervention (12, 110), while the study by Parente et al. (111) showed a 49% increase in IMCL content after a 4-week LFHC diet. It was not clear whether the carbohydrates were mainly complex or not. These discrepant findings may partly be explained by differences in the metabolic phenotype of the study participants. For example, it has been found that the intake of a carbohydrate-rich meal resulted in more than 2–fold increase in de novo lipogenesis and TAG synthesis leading to a significant increase in IHCL content in the liver in IR individuals as compared to insulin sensitive individuals (61). Interestingly, in the latter study, no changes were observed in IMCL content between the groups. However, the proportion of saturated (SFA), mono-unsaturated (MUFA) and poly-unsaturated (PUFA) fatty acids in these diets was either not comparable or not described, making it difficult to compare these studies.

Dietary fat quality

There is substantial evidence that dietary fat quality is related to ectopic fat storage. In an *in vivo* study in rats and an *in vitro* study in myotubes, it was reported that SFA preferentially accumulate as DAG in skeletal muscle and may thereby potentially interfere with insulin signaling, whereas MUFA and PUFA more readily convert to TAG (112, 113). Also, after incubation of human skeletal muscle cells with either the MUFA oleic acid or the SFA palmitic acid, higher lipolytic rates were observed with oleic acid (114). Furthermore, the glucose and insulin responses to a high SFA meal was greater when compared to a high PUFA meal in IR men, indicating an impaired postprandial insulin sensitivity, which was accompanied by a decreased skeletal muscle lipid turnover and FSR of TAG and DAG after the SFA meal (11).

These data are supported by longer-term human dietary intervention studies. The study of Jans et al. (12) reported no significant change in IMCL following a 12-week LFHCC diet in obese older adults but reported a significant reduction in IMCL when this diet was supplemented with 1.24 gr/day of PUFA omega-3 (LFHCC+n3 diet, Table 1). Interestingly, the latter study also reported that a 12-week diet rich in MUFA, defined as 20E%, had the tendency to decrease skeletal muscle FSR into DAG and TAG compared to pre-intervention (12). Both the MUFA diet and LFHCC+n-3 diet decreased the expression of lipogenic genes, although this was not reflected in IMCL changes in participants after completing the MUFA diet (12). Nonetheless, it shows that the

LFHCC+n3 and MUFA diets have the potential to decrease skeletal muscle lipid (intermediate) accumulation, which in turn may affect insulin signaling. However, neither of the diets showed that this effect was related to changes in IR (12).

Clearly, dietary fat quality may have significant effects on ectopic fat and potentially also on insulin sensitivity. Significant reductions in IHCL content and improvements in insulin sensitivity were found following a MUFA diet (5 weeks) (115), a diet supplemented with omega-3 (6 months) (116) and a Mediterranean diet (high in MUFA and PUFA) (6 weeks) (109), see also Table 1. Interestingly however, excessive intake of fat (>55E% for 3 weeks) in the diet (including high PUFA and MUFA) resulted in a 17% increase in IHCL content in overweight men but did not change IMCL (110). Whether these changes in IHCL content are also related to changes in insulin sensitivity remains to be elucidated. Several longer-term (12 to 24 weeks) large dietary intervention studies that investigated the effects of isoenergetic replacement of SFA with MUFA and/or PUFA on insulin sensitivity either did not report significant improvements in insulin sensitivity following the different diets (117, 118), or only reported significance when absolute intake of fat was not high (<37E%) (119).

Carbohydrate quality

The metabolic implications of dietary carbohydrates are largely dependent on the type of carbohydrate. The intake of simple carbohydrates has especially been linked to metabolic disorders (120). A diet low in glycemic index (GI), which is a relative ranking of foods and their effect on blood glucose values, may therefore be beneficial in relation to insulin sensitivity, and has also been linked to decreased IHCL accumulation (120). Indeed, an isocaloric low-GI diet for 4 weeks in overweight older adults led to a significant decrease in IHCL and improvement in insulin sensitivity (Matsuda index) compared to a 4-week isocaloric high-GI diet (121) (Table 1).

Individual carbohydrates and their effect on insulin sensitivity and ectopic fat have been studied. For example, excessive dietary fructose, as present in sugar-sweetened beverages, is thought to promote hepatic de novo lipogenesis, IHCL and IMCL content, and has accordingly been correlated to the development of cardiometabolic diseases (122-124), although This has been confirmed in animal models (125, 126). However, a 4-week isocaloric high-fructose diet (1.5g/kg/body weight) in healthy men resulted in an increase in fasting plasma concentrations of TAG, VLDL-TAG and glucose, but did not significantly alter ectopic fat content and insulin sensitivity in the liver and skeletal muscle (127). Furthermore, another study measured fat content in liver or skeletal muscle by computed

Table 1. Effect of isocaloric diets on insulin resistance and liver and muscle fat content in populations at risk for T2DM.

Ref.	Diet (E%fat (F); E%carbohydrate (C); E%protein (P))	Duration	Participants	N	% Females	Age (yrs)	IR measure	Statistical significance	Ectopic fat measure	Statistical significance
(12)	SFA diet: (38F (SFA: 16); ?C; ?P) MUFA diet (38F (MUFA: 20); ?C; ?P) LF+HCC diet (28F; ?C; ?P) LF+HCC+1.24g/d PUFA) (28F; ?C; ?P)	12 weeks	Obese	24 26 16 18	50 50 50 50	56 58 57 58	HOMA-IR	N.S. N.S. N.S. N.S.	Skeletal muscle biopsy	Muscle: N.S. Muscle: N.S. Muscle: N.S. Muscle: -45%, P<0.05
(109)	MED diet (44F (MUFA: 23F); 34C; 16P) LF+HCC diet (21F (MUFA: 8); 49C; 24P)	6 weeks	Obese NAFLD	12	50	55	M-value	-1.7, P<0.01 N.S.	MRS	Liver: -39%, P<0.05 Liver: N.S.
(110)	HF+LC diet (55F; 30C; 15P) LF+HC diet (20F; 65C; 15P)	3 weeks	Overweight	10	0	56	M-value	N.S.	MRS	Liver: +17%, P<0.05 Muscle: N.S. Liver: -13%, P<0.05 Muscle: N.S.
(111)	HF+LC diet (60F; 10C; 30P) LF+HC diet (30F; 50C; 20P)	4 weeks	Overweight	11 11	100 100	31 32	HOMA-IR	N.S. N.S.	MRS	Muscle: N.S. Muscle: +49%, P<0.05
(115)	HP+MUFA (40F (MUFA: 20.5); 30C; 30P)	5 weeks	Obese	10	100	?	HOMA-IR	-0.74, P<0.01	MRS	Liver: -49%, P<0.01 Muscle: N.S.
(116)	DG diet (30F; 50C; 20P) DG+n-3 diet (1g/d PUFA) (30F; 50C; 20P)	6 months	Obese NAFLD	16 16	56 39	51 50	HOMA-IR	N.S. -0.73, P<0.05	Abdominal ultrasound	Liver: N.S. Liver: -25%, P<0.01
(121)	Low-GI+LF diet, GI<55 (23F (SFA: 7); 57C; 17P) High-GI+HF diet, GI>70 (43F (SFA: 24); 38C; 16P)	4 weeks	Overweight	20 15	65 60	69 69	Matsuda HOMA-IR Matsuda HOMA-IR	0.6, P<0.05 N.S. N.S. N.S.	MRS	Liver: -2.2%, P<0.01 Liver: N.S.
(130)	60 g/d whey supplementation	4 weeks	Obese	11	100	38	HOMA-IR	N.S.	MRS	Liver: -21%, P<0.05
(13)	HP(plant) diet: (30F; 40C; 30P) HP(animal) diet: (30F; 40C; 30P)	6 weeks	Obese T2DM	19 18	37 33	65 65	AT-IR	-0.37, P<0.05 -3.38, P<0.05	MRS	Liver: -36%, P<0.001 Liver: -48%, P<0.001
(14)	MUFA diet (46F (22 MUFA); 40C; 14P) High fiber diet (20g fiber/100kcal) (28F; 55C; 17P) DG diet (34F; 49C; 17P)	12 weeks	Obese	15 15 13	47 53 23	62 63 60	DI	N.S. N.S. N.S.	MRS	Liver: -17%, P<0.001 Liver: N.S. Liver: N.S.

IR=insulin resistance, IS=insulin sensitivity, DI=disposition index, HOMA-IR=Homeostatic model assessment insulin resistance, AT-IR=adipose tissue IR (fasting plasma glucose *fasting free fatty acids), LF=low fat, HF=high fat, MUFA=mono-unsaturated fatty acid, n-3=omega 3 fatty acid, SFA=saturated fatty acid, LC=low carbohydrate, H(C)C=high (complex) carbohydrate, HP=high protein, GI=glycemic index, MED=Mediterranean, DG=dietary guidelines, MRS=magnetic resonance spectroscopy, N.S.=not significant

tomography (CT) and found no differences between a 10-week isocaloric diet high in fructose and an isocaloric diet high in sucrose (123). Unfortunately, insulin sensitivity was not reported in the latter study. Finally, a study including both NGT as well as IGT individuals compared the effect of 50 grams of added carbohydrate to the diet from either honey, sucrose, or high fructose for two weeks (128). Insulin sensitivity and lipid levels were not different between the different types of carbohydrates after two weeks of dietary intervention (128). Although the complexity of dietary carbohydrates may be important in relation to insulin sensitivity and ectopic fat, simple carbohydrates, such as fructose and sucrose, when consumed in iso-energetic exchange for other CHO sources may not be directly related to differences in insulin sensitivity and ectopic fat (129).

Protein intake

The amount of protein in the diet also seems to be important in relation to IR and ectopic fat. Markova et al. (13) showed that a 6-week diet high in protein (30 E%, either high in plant- or animal-source protein) reduced IHCL content in individuals with T2DM, as measured by MRS. Remarkably, these diets induced pronounced changes in IHCL, ranging from approximately - 36% for the plant protein diet to -48% for the animal protein diet. Additionally, the reduction in IHCL content correlated with several parameters related to glucose homeostasis, including decreased fasting glucose levels, improved whole-body insulin sensitivity and decreased de novo lipogenesis. A decreased IHCL content following a high protein diet was also reported in a study supplementing whey protein (60 g/d) for 4 weeks in obese individuals (130). However, the latter study was not controlled. The effect on IMCL was not reported for either of the above- mentioned studies. The number of studies describing the effect of protein quality and quantity is limited; however, it seems that protein quality and quantity in the diet may have a significant effect on ectopic fat accumulation in the liver.

Overall, drawing conclusions on diets that are most effective in improving ectopic fat is highly challenging. Comparing dietary intervention studies is very difficult as characteristics between populations vary with age, sex, BMI, amongst others. Moreover, the duration of the intervention and macronutrient composition between studies differs considerably. Also, the majority of studies measure either IHCL or IMCL, but rarely do they assess both. Moreover, measures of IR (i.e., HOMA-IR, Matsuda Index, M-value) differ between studies and may reflect different etiologies of IR (131, 132). In addition, lipid-intermediates were not assessed in most of these studies, and the majority of the studies performed to date did not take liver- and muscle-IR into account when assessing the effectiveness of interventions. Finally, the baseline metabolic phenotype of individuals may

be an important determinant on the effectiveness of dietary interventions, as discussed in more detail in the next section.

Differential effect of macronutrients on ectopic fat in distinct IR phenotypes; towards personalized nutrition

For several decades, many studies have focused on diets either low in carbohydrate or in fat as the most effective approach to improve glucose homeostasis and manage T2DM (133). Interestingly, however, not all individuals may benefit from the same diet. Recent studies have shown large inter-individual variation in response to a meal (134), which may also explain why individuals respond differently to the same dietary intervention. Indeed, in recent years, several studies have demonstrated that the macronutrient quality and quantity in the diet can lead to a differential response, depending on the individual's metabolic phenotype (11, 12, 14, 75, 135, 136). More specifically, this response may be dependent on liver- or muscle-IR, ectopic fat content, age, ethnicity, sex, and likely other factors that have not been studied extensively thus far.

There has been a debate on different dietary approaches in the prevention of T2DM, one is the Mediterranean diet, rich in olive oil, which may provide cardiovascular benefits. Second, diets low in fat and high in complex carbohydrates with increased fiber content (within the context of a lifestyle intervention) may decrease the cumulative incidence of diabetes by more than 50% over 3–6 years (137-139). In the CORDIOPREV-DIAB study, the Mediterranean (high in MUFAs) and the LFHCC dietary patterns were compared with respect to tissue-specific insulin resistance and beta-cell function in cardiovascular patients not treated for diabetes (n=642, analysis at baseline and at 2 years follow-up) (75). Interestingly, the change in disposition index (DI), which is a composite score of insulin sensitivity and secretion, after a long-term dietary intervention, was related to the tissue-specific IR phenotype of the participants at baseline. The tissue-specific IR phenotypes were defined as no-IR, muscle-IR, liver-IR or combined muscle and liver-IR, based on tertiles of the muscle insulin sensitivity index (MISI) and hepatic insulin resistance index (HIRI), which was modeled from an OGTT (140). Interestingly, the Mediterranean diet was reported to be most beneficial for individuals with muscle-IR, while the LFHCC diet was most beneficial for the liver-IR phenotype (75). In addition, a recent *post-hoc* analysis in the European project LIPGENE, which focused on the effects of dietary fat quantity and quality in the metabolic syndrome (MetS), found that IR MetS individuals were more sensitive to health effects from the substitution of a high-saturated fat diet by either high-MUFA or high (complex)-carbohydrates (with added n-3 PUFA) diets (15). On the contrary, the individuals with higher insulin sensitivity were more susceptible to the

detrimental effects of SFA. Furthermore, Guess et al. (141) recently reported that 6 weeks of supplementation with the dietary fiber inulin (30 g/d) decreased HOMA-IR in IFG but not in IGT individuals. These results may indicate that inulin has beneficial effects in subjects with liver- IR rather than muscle-IR, as IFG individuals have often a more pronounced liver-IR.

Mediterranean or LFHC high-fiber diets may be healthy for all, however, these studies indicate that dietary prevention or treatment may require a more subgroup-based or personalized approach to optimize the effects of dietary interventions. Indeed, a recent study by Zeevi et al. (134) demonstrated that personalized diets seem more effective in lowering postprandial blood glucose responses compared to diets based on expert advice. In this study, a machine- learning approach was used to predict blood glucose responses, using parameters such as dietary habits, physical activity, gut microbiota composition and anthropometrics, thereby developing the most optimal diet for each individual. The studies that are mentioned in this section are illustrative of the importance of personalized nutrition to optimize the effect of dietary interventions in IR individuals. Targeting tissue-specific IR phenotypes, such as liver- and muscle-IR, might prove to be an effective strategy to optimize metabolic outcomes of dietary interventions (Figure 1).

Nonetheless, the available literature to date does not provide sufficient evidence from prospective studies into the optimal quantity and quality of macronutrients in the diet based on metabolic phenotype. The tissue-specific IR phenotypes are related to a variety of different characteristics, such as ectopic fat, glucose control, gut microbiota, sex, age and others, and are likely not isolated entities. Defining metabolic phenotypes is difficult and may therefore not always do justice to its complexity. Moreover, although not surprising, the studies mentioned in this review only focus on a specific phenotype, such as muscle- versus liver-IR or mild- versus severe-IR, and mainly focus on Caucasian, middle-aged populations. Therefore, trying to understand an optimal diet for other ethnicities, age groups, or other metabolic (sub-)phenotypes such as IFG versus IGT is difficult. Recommendations or conclusions on the optimization of diets for each metabolic phenotype are therefore not yet available (Figure 1).

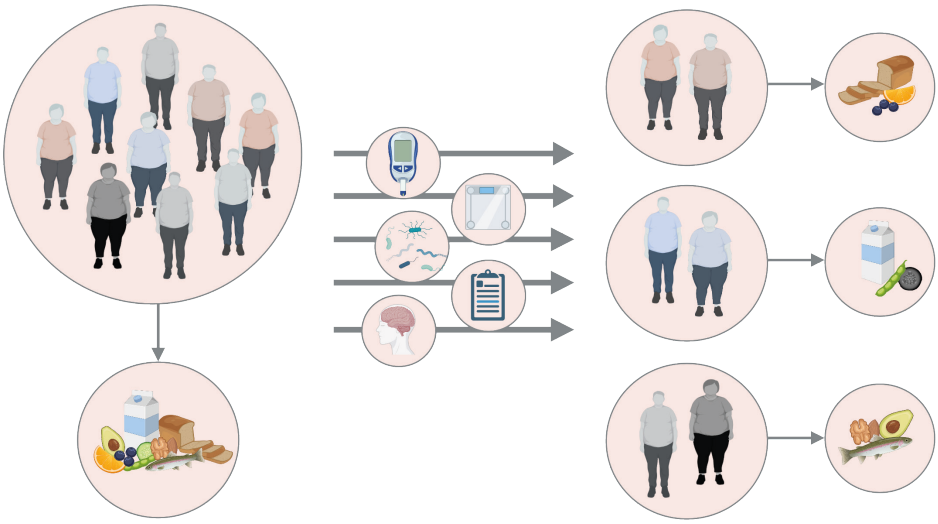

Figure 1. Population-based versus subgroup-based nutrition.
Large inter-individual variation can be seen in the response to dietary interventions. This implies that population-based nutritional interventions may not always lead to the most optimal (metabolic) outcomes for each individual. A subgroup-based approach that integrates among others cardiometabolic parameters, anthropometrics, gut microbiota, mental status, age, sex and ethnicity, may increase the efficacy of dietary interventions. Future studies should elucidate the most optimal diet for a certain (metabolic) phenotype.

Available evidence clearly indicates that there is great potential to optimize the effectiveness of dietary interventions on glucose homeostasis by, for example, targeting liver- and muscle-IR phenotypes. A first step toward the development of more personalized nutrition could be to investigate the role of tissue-specific IR and related ectopic fat content in the effectiveness of dietary interventions, in particular when studying the effect of manipulation of the macronutrient composition of the diet. Macronutrient composition, both quality and quality, is highly important and should be taken into account for these types of intervention studies. To develop effective personalized dietary interventions, it seems necessary to perform extensive phenotyping of individuals to understand the complexity of metabolic regulation and its target for different macronutrients, as illustrated

in Figure 1. Here, a schematic overview is given of the concept of subgroup-based or more personalized nutrition. Based on the baseline metabolic phenotype, a specific dietary intervention may be initiated to optimize intervention outcomes.

In conclusion, to understand the complexity of the interaction of an individual's metabolic phenotype and the response to diet, a detailed physiological phenotyping including the use of advanced -omics methodologies like (epi)genomics, metagenomics, and metabolomics is required. Additionally, mobile apps and wearable devices may facilitate real-time assessment of dietary intake and physical activity and may provide individual feedback to optimize personalization of advice. By integrating these technologies with big data analytics, personalized nutrition has the potential to provide targeted nutrition and lifestyle guidance for more effective prevention and management of type 2 diabetes and related chronic diseases. Despite all technological advances, the step towards implementation into a public health and clinical setting is still more remote and includes other factors like dietary preferences, the socio- economic context, and behavioral factors. Evidence is required to demonstrate the efficacy, cost-effectiveness as well as additional benefits of a personalized or subgroup-based approach beyond a traditional approach before these nutritional interventions can be implemented in daily practice in the future.

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Sexual dimorphism in body weight loss, improvements in cardiometabolic risk factors and maintenance of beneficial effects 6 months after a low-calorie diet: results from the randomized controlled DiOGenes trial

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ABSTRACT

A low-calorie diet (LCD) is an effective strategy to lose weight and improve cardiometabolic risk factors, however, sexual dimorphism may be present. This study aims to investigate sexual dimorphism in cardiometabolic risk factors following weight loss and after weight maintenance. 782 overweight/obese participants (65% women) of the DiOGenes trial followed an 8-week LCD (~800 kcal/day), with a 6-months follow-up weight maintenance period on ad libitum diets varying in protein content and glycemic index. Men lost more body weight during the LCD period (−12.8 ± 3.9 vs. −10.1 ± 2.8 kg, respectively, $p < 0.001$), but regained more weight during the follow-up period than women (1.5 ± 5.4 vs. −0.5 ± 5.5 kg, respectively, $p < 0.001$). Even though beneficial LCD-induced changes in cardiometabolic risk factors were found for both sexes, improvements in HOMA-IR, muscle and hepatic insulin sensitivity, triacylglycerol, HDL−, LDL− and total cholesterol, diastolic blood pressure, cholesterol esters, sphingomyelins and adiponectin were more pronounced in men than women (std. ß range: 0.073–0.144, all $q < 0.05$), after adjustment for weight change. During follow-up, women demonstrated a lower rebound in HDL-cholesterol, triacylglycerol, and diacylglycerol (std. ß range: 0.114–0.164, all $q < 0.05$), independent of changes in body weight. Overall, we demonstrated sexual dimorphism in LCD-induced changes in body weight and cardiometabolic risk profile, which may be attributed to differences in body fat distribution and metabolic status.

INTRODUCTION

Obesity is as a metabolic disease with major implications for the development and progression of several chronic diseases, including cardiovascular diseases and type 2 diabetes mellitus (1). With the increasing worldwide prevalence of obesity, interventions targeting obesity and its cardiometabolic complications are increasingly warranted. Weight loss resulting from a low- calorie diet (LCD) is seen as an effective strategy to improve obesity and its related metabolic risk factors (2). Nevertheless, weight maintenance after successful weight loss is one of the biggest challenges in the management of obesity, while it is of utmost importance to maintain improvements in cardiometabolic risk factors (3). Interestingly, men and women show distinct responses to a LCD, both in relation to the amount of weight loss, the associated improvements in cardiometabolic risk factors and also how well these improvements are maintained during follow-up (2,4).

Firstly, distinct sex steroid hormone concentrations may contribute to sex-specific differences in glucose homeostasis, body composition and energy balance, as evidenced by the deleterious impact of the decreasing estrogen concentrations during menopause on cardiometabolic risk factors (4, 5). Furthermore, women generally have a greater subcutaneous lipid storage capacity as reflected by the larger gluteal-femoral subcutaneous adipose tissue (SAT) depot compared to men (6), which seems protective against the development of several cardiometabolic diseases (7). In line, differences in plasma lipid(ome) profile are present between overweight and obese men and women (8, 9). In recent years, it has become clearer that these differences may reflect tissue-specific and/or whole-body lipid metabolism (9). Altogether, these differences appear to be important determinants of sexual dimorphisms in weight loss and its associated metabolic adaptations (10-12) and may explain the distinct responses following LCDs that have been reported between men and women (12- 14). However, often it is not clear whether sex-specific metabolic responses to LCDs are independent of difference in weight loss or weight regain, or whether differences can be ascribed to sexual dimorphism in baseline (metabolic) characteristics.

A better understanding of the complex interplay between sex and changes in cardiometabolic risk profile following weight loss and during weight maintenance may contribute to the development of more targeted dietary interventions to prevent and treat overweight/obesity and related cardiometabolic complications (4, 15). In the present study, we therefore investigated sex-specific differences in diet-induced weight loss and

cardiometabolic risk parameters, including plasma lipidome profile and indicators of insulin sensitivity, in overweight and obese individuals in the large pan-European multicenter DiOGenes study.

MATERIALS AND METHODS

Study design and population

The DiOGenes study is a large randomized controlled dietary intervention study executed in 8 different European cities. Detailed description of the specific study objective, methods and sample size calculation are described elsewhere (16, 17). Briefly, 938 participants followed an 8-week LCD consisting of ~800 kcal/d (Modifast®, Nutrition et Santé, Belgium) to achieve weight loss. When ≥8% of the initial body weight was lost during the weight loss phase, the participant was randomly assigned to follow one of 5 ad libitum diets, differing in protein content and glycemic index, for 6 months (n = 555) (Figure 1). The latter period was aimed at weight maintenance. Weight stable adults with a BMI of ≥27 kg/m^2 and ≤40 kg/m^2 and <65 years of age and fasting blood glucose concentrations <6.1 mmol/L without history of cardiovascular disease or diseases affecting body weight control were recruited. Local ethics committees approved the study (MEC 05–097), and all participants gave written informed consent before participation in the study. The study was carried out in accordance with the principles of the Declaration of Helsinki. The study is registered under the ClinicalTrials.gov Identifier: NCT00390637.

Clinical visits

Data of three clinical investigation days (CIDs) were included in this study. CID1 was performed before the start of the 8-week weight loss phase, CID2 after the weight loss phase and CID3 after the 6-month weight maintenance phase. During all CIDs weight and height, waist and hip circumference and blood pressure were determined. Furthermore, after an overnight fast, participants underwent an oral glucose tolerance test (OGTT). Venous blood was sampled before (t = 0 min) and at 30, 60, 90, 120 min after ingestion of the glucose drink. Physical activity was assessed using the validated BAECKE questionnaire, including domains of work, leisure, and sports (18).

Biochemical analysis

Venous plasma and serum samples were stored at −80 °C and analyzed. Both fasting and postprandial glucose and insulin and fasting lipid parameters (free fatty acids (FFA), triacylglycerol (total TAG), HDL-cholesterol, LDL-cholesterol, and total cholesterol), C-reactive protein (CRP) and adiponectin were analyzed. Furthermore, plasma lipidome

profiles were analyzed (see 'Plasma lipidomics' below for method of analysis). All samples were sent to a central laboratory, depending on the type of analysis.

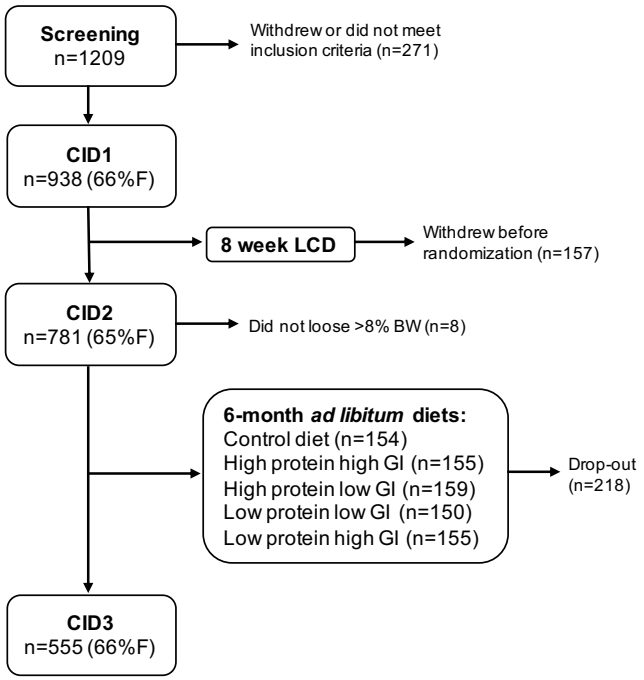

Figure 1. Schematic overview of participant inclusion and study design of the DiOGenes study. %F = percentage of females.

Insulin sensitivity

Whole-body insulin sensitivity was estimated using HOMA-IR, which was calculated using the following formula: (glucose t=0 (mmol/L) × insulin t=0 (mU/L))/22.5. Furthermore, tissue-specific insulin resistance was estimated according to the method of Abdul-Ghani et al. (19) and validated against the hyperinsulinemic euglycemic clamp technique. Previous studies have demonstrated that, using these indices, muscle, liver and adipose tissue insulin resistance are related to distinct cardiometabolic disease risk profiles (for example a distinct lipidome profile) (9, 20, 21) and have been found to be different in men and women (9, 20, 22).

Hepatic IR and muscle insulin sensitivity can be estimated based on glucose and insulin values during a 5-point OGTT, using the hepatic insulin resistance index (HIRI) and muscle insulin sensitivity index (MISI), respectively [10,19,20]. HIRI was estimated using the square root of the product of the area under curves (AUCs) for glucose and insulin during the first 30 min of the OGTT using the formula: HIRI = √(glucose0-30 [AUC] * insulin0-30 [AUC]). MISI was calculated using the following formula: MISI = (dG/dt)/mean insulin during the OGTT. Here, dG/dt is the rate of decay of plasma glucose concentrations during the OGTT, calculated as the slope of the least square fit to the decline in plasma glucose concentration from peak to nadir. MISI and HIRI have been validated against the gold standard hyperinsulinemic- euglycemic clamp, where MISI was validated against the glucose disposal rate and HIRI with the endogenous glucose production (19). The adipose tissue insulin resistance index (ATIRI) was calculated as fasting insulin (μU/L) × fasting non-esterified fatty acids (μmol/L)/1000.

Plasma lipidomics

Fasting plasma lipidome analysis was performed for all three clinical visits using liquid chromatography-mass spectrometry as described previously (11). Briefly, a mixture of internal standards and calibration standards were added to each sample. Then, liquid-liquid extraction was performed using a dichloromethane-methanol (2:1) mixture. Following, lipids were separated on a Ascentis Express C8 2.1 9 150-mm (2.7-μm particle size) column (Sigma- Aldrich) with the use of an Acquity UPLC system (Waters) and visualized and quantified using quadrupole time-of-flight mass spectrometry (Agilent Technologies) (11). A total of 140 lipids were detected based on the actual presence of target compounds in a LC-MS raw data file. The target compounds are previously identified by LC-high resolution MS, using the FT-ICR- MS and (relative) retention time (23), resulting in a high degree of certainty (24).

The lipids were grouped into 11 lipid groups species according to Lipid Maps nomenclature (http://www.lipidmaps.org (accessed on 5 April 2020)). The lipid groups are defined as follows: TAG (n = 55), diacylglycerols (DAG; n = 2), cholesterol esters (CholE; n = 3), lysophosphatidylcholine (LPC; n = 11), lysoalkylphosphatidylcholine (LPCO; n = 2), lysophosphatidylethanolamine (LPE; n = 1), phosphatidylcholine (PC; n = 25), alkyl-phosphatidylcholine (PCO; n = 15), phosphatidylethanolamine (PE; n = 3), alkylphosphatidylethanolamine (PEO; n = 3) and sphingomyelins (SM; n = 20). The TAG species was further sub-divided into potential metabolically relevant sub-groups based on composition, saturation and chain length of TAGs as they have been shown to be differentially related to cardio-metabolic risk factors in men and women (9).

Statistical analysis

In this exploratory analysis, differences in characteristics between men and women were assessed using an independent sample t-test. Linear mixed-model analyses were performed to assess the differences in change in cardiometabolic risk parameters between men and women following weight loss (from CID1 to CID2) and after weight maintenance (from CID2 to CID3). The linear mixed-model was performed with cardiometabolic risk parameters as the dependent variable, sex as fixed effect and study center as random effect. Age, body weight at baseline, weight loss and baseline value of the dependent variable were included as covariates in the analyses for the change following weight loss (from CID1 to CID2). For the weight maintenance phase (from CID2 to CID3), age, baseline weight, baseline value of the dependent variable, change of the dependent variable during weight loss, weight change during the weight loss phase and weight maintenance phase, and diet were included as covariates. Since physical activity level was not different between men and women, and was not significantly altered during the intervention, physical activity was not included in the analyses. In case data were not normally distributed, data were Ln-transformed to approximate normality. Z-scores were calculated in order to standardize effect sizes, allowing direct comparison of different variables. To correct for multiple comparisons and limit false positive outcomes, *p*-values were corrected for False Discovery Rate (FDR) using the Benjamini-Hochberg method. FDR-adjusted *p*-Values (*q*-Values) were used to describe the data with a significance set at $q < 0.05$. Only results of the fully adjusted models are shown (depicted as 'adj.'). The statistical analyses were performed using the IBM SPSS Statistics software (version 25).

RESULTS

Clinical characteristics of the study population

782 participants were included in the analysis of the weight loss phase, of which 555 also participated in the weight maintenance phase. Of the total population, 506 (=64.7%) were women. A detailed overview of the clinical characteristics of the men and women in the study are listed in Table 1. Briefly, the average age of men in the study was 42.5 ± 6.0 years (mean ± standard deviation (SD)), compared to 41.0 ± 6.3 years in women (significant different, $p = 0.001$). At baseline, women had lower values for waist-hip ratio, systolic (SBP) and diastolic blood pressure (DBP), fasting glucose, 2-hr glucose, fasting insulin, HOMA-IR, HIRI, total cholesterol, LDL-cholesterol, triglycerides and adiponectin compared to men while HDL- cholesterol, FFA and CRP were higher in women (all $p < 0.01$). BMI, physical activity, 2-hr glucose, MISI and ATIRI, were not significantly different between men and women. The clinical characteristics of the 555 participants participating in the weight

maintenance phase are comparable with the complete population characteristics (data not shown).

Table 1. Participant characteristics.

	n	Men	Women	Total Group	*p*-value
Sex (%)	782	35.3	64.7	100	**<0.001**
Age (years)	782	42.5 ± 6.0	41.0 ± 6.3	41.5 ± 6.26	**0.001**
BMI (kg/m^2)	782	34.3 ± 0.5	34.5 ± 5.0	34.4 ± 4.9	0.108
Bodyweight (kg)					
CID1	782	109.3 ± 17.4	95.0 ± 15.6	100.1 ± 17.6	**<0.001**
Weight loss CID2-CID1	782	−12.8 ± 3.9	−10.1 ± 2.8	−11.0 ± 3.50	**<0.001**
Weight maintenance CID3-CID2	555	1.6 ± 5.3	−0.5 ± 5.5	0.5 ± 5.48	**<0.001**
Physical activity (Baecke)	718	7.9 ± 0.9	8.0 ± 0.9	8.0 ± 0.9	0.090
Waist:hip ratio	769	1.01 ± 0.05	0.88 ± 0.07	0.92 ± 0.09	**<0.001**
Systolic BP (mmHg)	758	132.0 ± 12.8	121.9 ± 14.4	118.2 ± 13.3	**<0.001**
Diastolic BP (mmHg)	758	81.4 ± 10.6	75.9 ± 10.6	72.49 ± 9.72	**<0.001**
Fasting glucose(mmol/L)	749	5.27 ± 0.62	5.00 ± 0.66	5.10 ± 0.66	**<0.001**
2 hr glucose (mmol/L)	741	6.58 ± 2.37	6.74 ± 2.02	6.68 ± 2.16	0.333
Fasting insulin (µIU/mL)	723	13.9 ± 11.3	10.5 ± 9.4	11.7 ± 10.3	**<0.001**
HOMA-IR (A.U.)	733	3.87 ± 3.23	2.77 ± 2.64	3.16 ± 2.91	**<0.001**
MISI (A.U.)	649	0.063 ± 0.052	0.060 ± 0.052	0.061 ± 0.052	0.488
HIRI (A.U.)	686	144.8 ± 101.8	98.9 ± 76.0	115.9 ± 89.1	**<0.001**
ATIRI (A.U.)	720	7.92 ± 8.95	7.69 ± 8.38	7.72 ± 8.61	0.625
Total cholesterol (mmol/L)	768	5.05 ± 1.08	4.84 ± 0.96	4.92 ± 1.01	0.008
HDL cholesterol (mmol/L)	770	1.07 ± 0.28	1.29 ± 0.33	1.21 ± 0.33	**<0.001**
LDL cholesterol (mmol/L)	764	3.24 ± 0.93	3.00 ± 0.84	3.08 ± 0.88	**<0.001**
TAG (mmol/L)	759	1.60 ± 0.70	1.23 ± 0.57	1.36 ± 0.64	**<0.001**
FFA (µmol/L)	672	552.3 ± 281.6	711.54 ± 326.7	646.8 ± 318.7	**<0.001**
C-reactive protein (mg/L)	747	3.78 ± 3.18	4.54 ± 4.13	4.12 ± 3.86	**<0.001**
Adiponectin (µg/ml)	768	7.46 ± 3.17	9.96 ± 4.71	9.08 ± 4.40	**<0.001**

Values are represented as mean ± standard deviation (SD) unless otherwise indicated. *p*-values <0.05 are highlighted in bold. CID: clinical investigation day, CID1: baseline measurements, CID2: measurements following the weight loss phase, CID3 measurements following the weight maintenance, BP = blood pressure, HOMA-IR = homeostatic model assessment for insulin resistance, MISI = muscle insulin sensitivity index, HIRI = hepatic insulin resistance index, ATIRI = adipose tissue insulin resistance index, TAG = triacylglycerol, FFA = free fatty acid.

Weight changes following LCD

On average, men lost significantly more body weight following the weight loss phase than women (12.8 ± 3.9 vs. 10.1 ± 2.8 kg or 11.7 ± 3.0 vs. 10.6 ± 2.5%, both $p < 0.001$, respectively) (Figure 2A). This difference remained significant after adjustment for age and body weight at baseline (adj. $p < 0.001$). Men regained on average 1.6 ± 5.3 kg following the weight maintenance phase whereas women lost −0.5 ± 5.5 kg. The difference in weight change during the weight maintenance phase remained significant after adjustment for age, weight at baseline and weight lost during the weight loss phase (adj. $p < 0.001$).

Changes in insulin sensitivity following weight loss

Several differential changes in glucose homeostasis parameters between men and women were observed following the weight loss phase in the fully adjusted model (see 'Statistical analysis' for details). For both HOMA-IR, HIRI and MISI, men significantly improved more following the weight loss phase compared to women (adj. stdβ = 0.104, 0.073 and −0.116, $q = 0.008$, $q = 0.028$ and $q = 0.015$, respectively) (Figure 2D–F). There was a significant decrease in fasting glucose, 2hr glucose and ATIRI in both men and women following LCD-induced weight loss with no differences between sexes (Figure 2B,C,G). During the weight maintenance phase, no significant differences were found between men and women regarding parameters related to glucose homeostasis (Figure 2B–G).

Changes in lipid profile and blood pressure following weight loss

Following LCD-induced weight loss, men demonstrated a more pronounced decrease in TAG (adj. stdβ = 0.105, $q < 0.001$), total cholesterol (adj. stdβ = 0.083, $q = 0.030$), HDL-cholesterol (adj. stdβ = −0.135, $q < 0.001$), LDL-cholesterol (adj. stdβ = 0.088, $q = 0.028$), adiponectin (adj. stdβ = 0.083, $q = 0.030$) and DBP (adj. stdβ = 0.091, $q = 0.025$), and a greater increase in HDL-cholesterol (adj. stdβ = −0.135, $q < 0.001$) compared to women (Figure 2H,J,K,N,P). In contrast, following the weight maintenance phase, in the fully adjusted model, a greater worsening in HDL-cholesterol (adj. stdβ = 0.164, $q < 0.001$) and a trend for greater worsening in HIRI (adj. stdβ = −0.125, $q = 0.075$), TAG (adj. stdβ = −0.096, $q = 0.066$) and SBP (adj. stdβ = −0.097, $q = 0.060$) were observed in men compared to women (Figure 2E,H,K,O). CRP concentrations decrease more in the weight maintenance phase in men compared to women (adj. stdβ = 0.139, $q = 0.008$). The changes in FFA, total cholesterol, LDL-cholesterol, adiponectin and DBP did not significantly differ between men and women during the weight maintenance phase (Figure 2I–K,P).

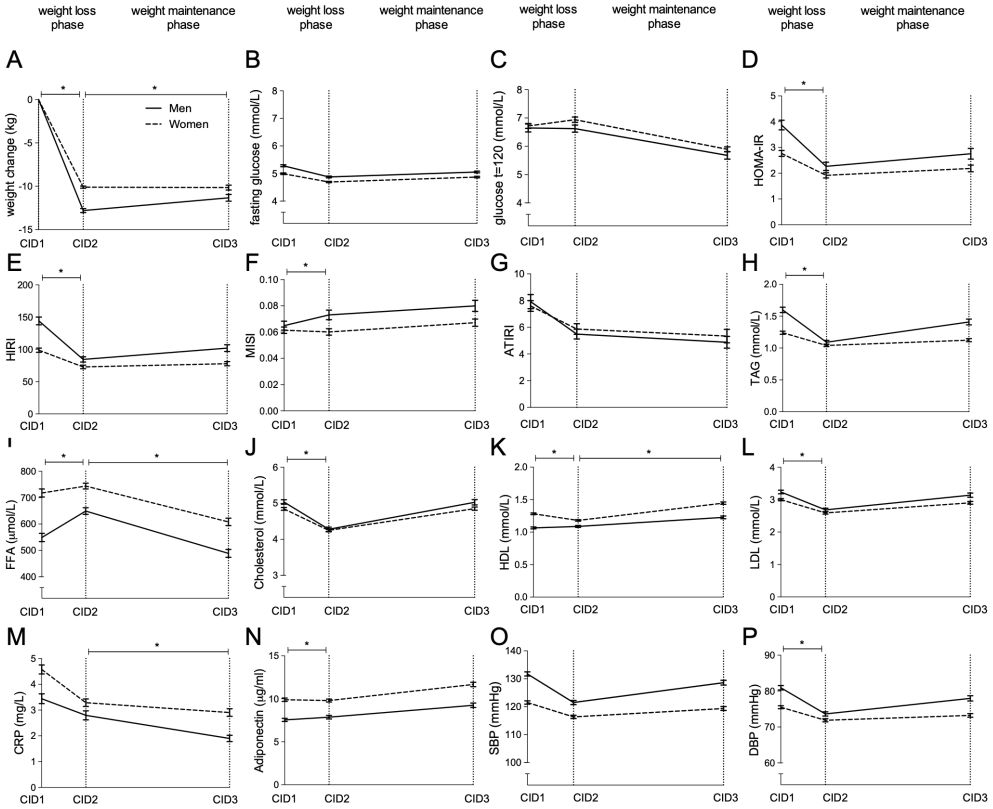

Figure 2. Mean metabolic changes in men and women following an 8-week weight loss phase (from CID1 to CID2) and after 6 months of weight maintenance (CID2 to CID3) ± standard error. Change in weight (A), fasting glucose (B), glucose t=120, |(C), HOMA-IR (D), HIRI (E), MISI (F), ATIRI (G), TAG (H), FFA (I), cholesterol (J), HDL (K), LDL (L), CRP (M), adiponectin (N), SBP (O) and DBP(P) are presented. The black line represents men, and the dotted line represents women. * Indicates a significant difference in change between men and women ($q < 0.05$) in a linear mixed model corrected for age, body weight (changes) and baseline differences.

Changes in plasma lipidome profile following weight loss

Before the start of the LCD (CID1), men showed significantly higher relative abundance of TAG, DAG and PEO, but lower relative abundance (expressed as percentage of total plasma lipids) of CholE, LPE, PC, PCO, PE and SM than women (Table 2). Furthermore, higher relative abundance of saturated, even, and very long TAG species were observed at

CID1 in men. Sex-specific changes in the sum scores of plasma lipids were found during the weight loss as well as weight maintenance phase. In the fully adjusted model, men showed a greater decrease in TAG (adj. stdβ = 0.077, q = 0.031), CholE (adj. stdβ = 0.113, q = 0.008) and SM (adj. stdβ = 0.119, q = 0.008) following LCD-induced weight loss compared to women (Figure 3A). In contrast, women demonstrated a greater decrease in LPC (adj. stdβ = −0.162, q < 0.001), LPCO (adj. stdβ = −0.140, q < 0.001) and a trend for greater decrease in LPE (adj. stdβ = −0.075, q = 0.073) following the weight loss phase than men (Figure 3A). For the weight maintenance phase, women showed a less pronounced increase in TAG (adj. stdβ = −0.153, q < 0.001), DAG (adj. stdβ = −0.114, q = 0.006) and LPC (adj. stdβ = −0.186, q < 0.001) compared to men, whilst men showed a smaller increase in SM (adj. stdβ = 0.122, q = 0.004) (Figure 3B). Standardized betas for the link between sex and individual lipid species of the lipids within a statistically significant lipid subclass are depicted in Supplemental Table S1.

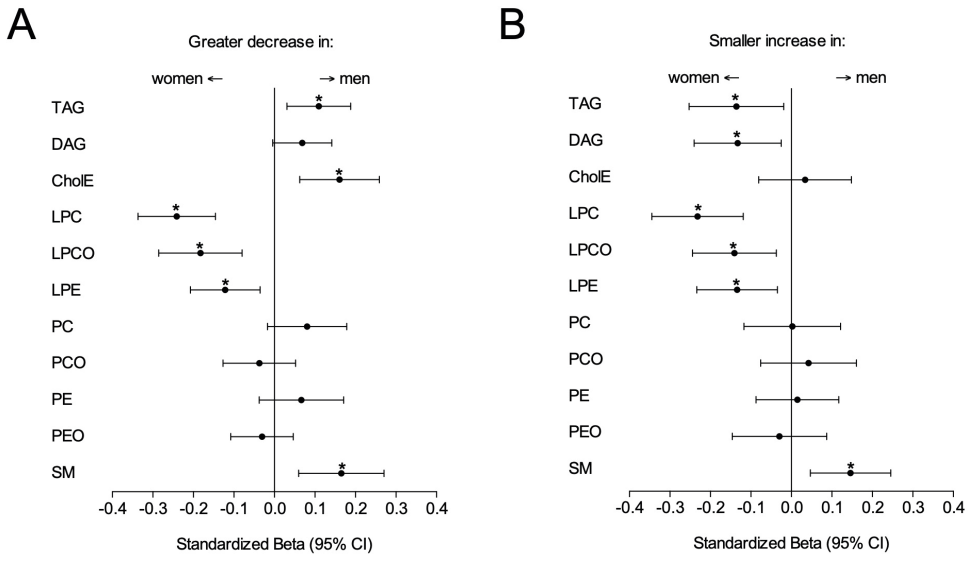

Figure 3. Standardized Beta's ± 95% confidence interval (CI) for the association between sex and the change in lipid groups following weight loss (from CID1 to CID2) (A) and after 6 months of weight maintenance (from CID2 to CID3) (B). A negative Standardized Beta indicates a greater decrease (A) or smaller increase in women (B) compared to men and vice versa. * Indicates a significant difference in change between men and women (q < 0.05 (=False Discovery Rate ***p***-Value)) in a linear mixed model corrected for age, body weight (changes) and baseline differences.

Table 2. Relative abundance individual lipid groups at baseline (CID1).

	Men (n=258)	Women (n=474)	TotalGroup (n=732)	*p*-value
TAG	50.9 ± 8.2	44.8 ± 8.9	47.0 ± 9.2	**<0.001**
Odd	5.7 ± 1.1	6.4 ± 1.3	6.2 ± 1.3	**<0.001**
Even	94.3 ± 1.1	93.6 ± 1.3	93.8 ± 1.3	**<0.001**
Sat	2.5 ± 1.1	2.3 ± 1.0	2.4 ± 1.07	**0.022**
Usat	76.0 ± 5.2	76.2 ± 5.6	76.1 ± 5.4	0.649
Psat	21.5 ± 5.7	21.5 ± 6.0	21.5 ± 5.9	0.992
Long	95.6 ± 1.2	95.9 ± 1.3	95.8 ± 1.3	**0.004**
Very long	4.4 ± 1.2	4.1 ± 1.3	4.2 ± 1.3	**0.004**
DAG	0.040 ± 0.014	0.037 ± 0.015	0.038 ± 0.015	**0.047**
CholE	0.13 ± 0.04	0.15 ± 0.04	0.14 ± 0.04	**<0.001**
LPC	5.7 ± 1.5	5.8 ± 1.8	5.8 ± 1.7	0.758
LPCO	0.035 ± 0.011	0.037 ± 0.014	0.036 ± 0.013	0.098
LPE	0.033 ± 0.014	0.037 ± 0.018	0.036 ± 0.013	**0.003**
PC	34.1 ± 5.4	38.6 ± 5.9	37.0 ± 6.1	**<0.001**
PCO	1.33 ± 0.42	1.45 ± 0.43	1.40 ± 0.43	**<0.001**
PE	1.73 ± 0.43	2.08 ± 0.50	1.95 ± 0.51	**<0.001**
PEO	0.37 ± 0.15	0.34 ± 0.12	0.35 ± 0.13	**0.047**
SM	5.6 ± 1.4	6.6 ± 1.7	6.3 ± 1.6	**<0.001**

Lipid groups are represented as percentage of all lipids ± standard deviation (SD) except for TAG subgroups who are expressed as percentage of total TAG ± SD. *P*-values<0.05 are highlighted in bold. TAG = triacylglycerol (n = 55), TAGodd = with an odd number of carbon atoms, TAGeven = with an even number of carbon atoms, TAGsat = without double bonds, TAGusat = with 1–3 double bonds, TAGpsat = with ≥4 double bonds, TAGlong = with <56 carbon atoms, TAGverylong = with ≥56 carbon atoms, DAG = diacylglycerols (n = 2), CholE = cholesterol esters (n = 3), LPC = lysophosphatidylcholine (n = 11), LPCO = lysoalkylphosphatidylcholine (n = 2), LPE = lysophosphatidylethanolamine (n = 1), PC = phosphatidylcholine (n = 25), PCO = alkyl-phosphatidylcholine (n = 15), PE = phosphatidylethanolamine (n = 3), PEO = alkylphosphatidylethanolamine (n = 3) and SM = sphingomyelins (n = 20).

Lipid groups are represented as percentage of all lipids ± standard deviation (SD) except for TAG subgroups who are expressed as percentage of total TAG ± SD. *P*-values<0.05 are highlighted in bold. TAG = triacylglycerol (n = 55), TAGodd = with an odd number of carbon atoms, TAGeven = with an even number of carbon atoms, TAGsat = without double bonds, TAGusat = with 1–3 double bonds, TAGpsat = with ≥4 double bonds, TAGlong = with <56 carbon atoms, TAGverylong = with ≥56 carbon atoms, DAG = diacylglycerols (n = 2), CholE = cholesterol esters (n = 3), LPC = lysophosphatidylcholine (n = 11), LPCO = lysoalkylphosphatidylcholine (n = 2), LPE = lysophosphatidylethanolamine (n = 1), PC = phosphatidylcholine (n = 25), PCO = alkyl-phosphatidylcholine (n = 15), PE =

phosphatidylethanolamine (n = 3), PEO = alkylphosphatidylethanolamine (n = 3) and SM = sphingomyelins (n = 20).

DISCUSSION

The present study demonstrates sexual dimorphism in LCD-induced body weight loss, improvements in cardiometabolic risk factors, and maintenance of beneficial effects 6 months after weight loss in the large pan-European DiOGenes trial. More specific, men showed greater weight loss and greater improvements in several cardiometabolic risk factors following an 8- week LCD (~800 kcal/day), whereas women demonstrated lower regain of weight, and smaller deterioration of several cardiometabolic risk factors, independent of weight change and diet, during the weight maintenance period 6 months after the weight loss. Interestingly, plasma lipidomic analysis revealed that several lipid species respond in a sex-specific manner to weight loss and weight maintenance, independent from changes in body weight. These findings may have important implications for the development of more targeted dietary interventions to prevent or delay the progression of cardiometabolic complications associated with obesity.

We found more pronounced weight loss following the LCD in men compared to women. One could argue that this may be attributed to a greater energy restriction in men, given the on average greater energy requirements than women. Interestingly, however, men improved more in HOMA-IR, HIRI, MISI, TAG, HDL-, LDL- and total cholesterol, diastolic blood pressure, triglycerides, cholesterol esters, sphingomyelins and adiponectin compared to women, independent of body weight loss. Importantly, the observed differences are also independent of the more beneficial cardiometabolic risk profile at baseline as observed in women compared to men, which is reflected in greater (whole body and liver) insulin sensitivity, higher HDL- cholesterol concentration, lower blood pressure and lower concentration of several lipid species including (saturated and even chain) TAG and DAG. Furthermore, during the 6-month weight maintenance phase, HDL-cholesterol, TAG and DAG had a lower rebound in women, independent of body weight change and diet. These findings suggest that other factors than weight change per se are important determinants of the cardiometabolic changes that occur during weight loss and weight maintenance in men and women.

Based on the present findings, women appear to be less responsive to the dietary intervention with respect to cardiometabolic outcomes. These findings are in line with several previous studies, showing that following weight loss, men lose more weight and

improve more in amongst others blood pressure, cholesterol concentration, plasma TAG and HbA1c compared to women (12-14, 25, 26). It is tempting to postulate that the smaller fluctuations in cardiometabolic risk factors in premenopausal women may be due to sex-differences in metabolic homeostasis and result in a relative protection against metabolic perturbations. Indeed, for a given BMI, women are relatively protected against lipid-induced insulin resistance (27, 28) and show higher HDL-cholesterol and lower TAG concentration compared to men (29). This relative metabolic protection may relate to differences in body fat distribution and ectopic fat storage as a result of differences in substrate supply and utilization, and storage and mobilization of excess lipids, as reviewed elsewhere (4). Following weight loss, more pronounced changes in intra-abdominal or visceral adipose tissue (VAT) are generally observed in men, whereas women show a more pronounced decrease in (lower body) SAT (30-32). In line, VAT area increased more with weight regain in young overweight men compared to women when expressed as percentage weight regain (33). VAT mass is positively associated with cardiometabolic risk factors such as insulin resistance and dyslipidemia (34, 35), while the expandability of SAT is a critical factor in the prevention of cardiometabolic diseases and seems to act as an important 'metabolic lipid sink' (6, 36). Unfortunately, we have no data on changes in VAT mass available in the current study.

Sexual dimorphism in metabolic homeostasis is also observed in relation to (tissue-specific) insulin sensitivity and glucose homeostasis (4, 9). In the present study, women showed lower whole-body and liver insulin resistance, with similar muscle insulin sensitivity compared to men. Interestingly, women responded less favorable to weight loss with regard to HOMA-IR, HIRI and MISI (independent of baseline insulin sensitivity). It can be speculated that a greater VAT mass loss in men following weight loss, as previously reported, could explain the observed differences in tissue-specific insulin resistance. Insulin signaling in VAT is generally more disturbed compared to SAT (37) and greater VAT mass is linked to the development of liver insulin resistance via FFA release from VAT into the portal vein, affecting glucose and lipid metabolism within the liver (38).

The present plasma lipidomic analyses revealed that LPC decreased more pronounced following weight loss and increased less during weight maintenance in women compared to men. Previously, reduced concentration of LPC have been linked to (muscle) insulin resistance and type 2 diabetes mellitus (9, 39). Moreover, baseline SM concentrations were higher, and SM concentrations decreased less during weight loss and increased more during follow-up in women than men. In contrast to LPC, higher concentrations of SM seem to be related to a more detrimental metabolic health profile including insulin

resistance (40, 41). These observations seem to contrast to the more beneficial cardiometabolic profile observed in women. An explanation for this is currently lacking but may possibly reflect a sexual dimorphism in the relationship of these lipidome components and insulin resistance and/or cardiometabolic risk, as previously reported for relationship of TAG and DAG species and hepatic insulin resistance (9). Interestingly, also diet composition seems to mediate the effect on certain lipid species in both sexes, with the high-protein diets inducing more pronounced increases in PCO and PEO. Further research should elucidate the relationship between LPC and SM and cardiometabolic disease risk in men and women, as well as the impact of diet composition.

To our knowledge, this is the first study to report sex-specific changes in plasma lipid species and tissue-specific insulin resistance following weight loss and during follow-up weight maintenance, independent of the differences in weight change. More detailed measures of body composition are needed to elucidate whether the observed sex-specific changes are at least partly driven by differences in (changes in) body fat distribution. Additionally, sex hormones may affect differences in lipid handling and insulin sensitivity. In the present study, we could not directly explain our results by hormonal status, since most women were in the premenopausal state. Additionally, more subtle effects of sex hormones could not be determined since no data was available on sex hormones or phase of menstrual cycle. Nevertheless, the present findings contribute to a better understanding of the metabolic underpinnings of sex-specific changes following weight loss and weight maintenance.

In conclusion, in this study we observed that overweight or obese men lose more weight and improve more in several cardiometabolic risk parameters following a low-calorie diet but are less able to maintain several of the improvements after 6 months of follow up compared to premenopausal women. Furthermore, several plasma lipid species changed in a sex-specific manner, independently of the direction of the weight loss, which may have implications for the sex-specific development of cardiometabolic diseases. Altogether, these findings may provide directions for more sex-targeted dietary interventions in the prevention of cardiometabolic diseases.

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SUPPLEMENTARY MATERIAL

Table S1. Standardized beta's of the link between sex and the changes in individual lipid species following loss and weight maintenance

	Change from CID1-2		Change from CID2-3	
Lipid species	**Std. beta ± SE**	***q*-value**	**Std. beta ± SE**	***q*-value**
TAG (46:2)	-0.023 ± 0.018	0.290	0.023 ± 0.044	0.599
TAG (48:0)	-0.038 ± 0.021	0.159	-0.070 ± 0.047	0.154
TAG (48:1)	-0.010 ± 0.022	0.764	-0.099 ± 0.046	0.041
TAG (48:2)	-0.004 ± 0.023	0.910	-0.072 ± 0.044	0.114
TAG (48:3)	-0.002 ± 0.024	0.950	-0.024 ± 0.041	0.570
TAG (48:4)	-0.057 ± 0.026	0.078	0.045 ± 0.042	0.299
TAG (50:0)	-0.038 ± 0.022	0.169	-0.140 ± 0.047	0.005
TAG (50:1)	0.009 ± 0.028	0.831	-0.119 ± 0.046	0.015
TAG (50:2)	0.070 ± 0.029	0.046	-0.132 ± 0.045	0.005
TAG (50:3)	0.068 ± 0.030	0.061	-0.099 ± 0.040	0.020
TAG (50:4)	0.047 ± 0.029	0.182	-0.075 ± 0.038	0.065
TAG (50:5)	0.031 ± 0.025	0.295	-0.056 ± 0.041	0.182
TAG (51:1)	-0.005 ± 0.024	0.874	-0.160 ± 0.045	<0.001
TAG (51:2)	-0.008 ± 0.026	0.831	-0.070 ± 0.043	0.119
TAG (51:3)	0.042 ± 0.031	0.255	-0.108 ± 0.037	0.006
TAG (51:4)	0.046 ± 0.030	0.220	-0.097 ± 0.038	0.016
TAG (52:0)	-0.037 ± 0.017	0.068	-0.210 ± 0.047	<0.001
TAG (52:1)	-0.001 ± 0.025	0.958	-0.177 ± 0.046	<0.001
TAG (52:2)	0.065 ± 0.036	0.158	-0.135 ± 0.043	0.004
TAG (52:3)	0.105 ± 0.034	0.010	-0.098 ± 0.041	0.024
TAG (52:4)	0.118 ± 0.034	<0.001	-0.094 ± 0.040	0.024
TAG (52:5)	0.067 ± 0.027	0.0428	-0.098 ± 0.039	0.017
TAG (52:6)	0.037 ± 0.022	0.174	-0.110 ± 0.040	0.011
TAG (53:1)	-0.037 ± 0.027	0.253	-0.053 ± 0.045	0.259
TAG (54:0)	0.014 ± 0.009	0.215	0.072 ± 0.044	0.118
TAG (54:0)	-0.034 ± 0.023	0.228	-0.151 ± 0.041	<0.001
TAG (54:1)	-0.025 ± 0.018	0.248	-0.235 ± 0.047	<0.001
TAG (54:2)	0.043 ± 0.031	0.248	-0.193 ± 0.043	<0.001
TAG (54:3)	0.050 ± 0.036	0.248	-0.126 ± 0.041	0.004
TAG (54:4)	0.065 ± 0.034	0.136	-0.115 ± 0.039	0.006

TAG (54:5)	0.094 ± 0.032	0.018	-0.147 ± 0.040	<0.001
TAG (54:6)	0.078 ± 0.029	0.024	-0.152 ± 0.041	<0.001
TAG (54:7)	0.045 ± 0.026	0.163	-0.158 ± 0.042	<0.001
TAG (55:1)	-0.029 ± 0.026	0.344	-0.184 ± 0.047	<0.001
TAG (55:2)	0.011 ± 0.035	0.831	-0.077 ± 0.043	0.094
TAG (55:3)	0.064 ± 0.035	0.158	-0.076 ± 0.043	0.094
TAG (56:1)	-0.057 ± 0.013	<0.001	-0.215 ± 0.047	<0.001
TAG (56:2)	-0.043 ± 0.015	0.024	-0.251 ± 0.048	<0.001
TAG (56:3)	-0.014 ± 0.028	0.760	-0.198 ± 0.043	<0.001
TAG (56:4)	0.014 ± 0.030	0.764	-0.090 ± 0.040	0.033
TAG (56:5)	0.028 ± 0.032	0.478	-0.108 ± 0.037	0.006
TAG (56:6)	0.105 ± 0.032	0.007	-0.139 ± 0.040	<0.001
TAG (56:7)	0.077 ± 0.030	0.029	-0.177 ± 0.043	<0.001
TAG (56:8)	0.093 ± 0.030	0.010	-0.184 ± 0.047	<0.001
TAG (57:1)	-0.029 ± 0.025	0.317	-0.216 ± 0.045	<0.001
TAG (57:2)	0.014 ± 0.033	0.786	-0.198 ± 0.042	<0.001
TAG (58:1)	-0.057 ± 0.015	<0.001	-0.184 ± 0.049	<0.001
TAG (58:2)	-0.059 ± 0.014	<0.001	-0.185 ± 0.049	<0.001
TAG (58:3)	-0.045 ± 0.014	0.009	-0.198 ± 0.049	<0.001
TAG (58:6)	0.080 ± 0.030	0.024	-0.131 ± 0.040	0.002
TAG (58:8)	0.086 ± 0.031	0.024	-0.156 ± 0.046	0.002
TAG (58:9)	0.103 ± 0.031	0.007	-0.175 ± 0.045	<0.001
TAG (58:10)	0.098 ± 0.031	0.010	-0.149 ± 0.047	0.004
TAG (59:0)	-0.031 ± 0.023	0.258	-0.123 ± 0.040	0.004
TAG (60:2)	-0.056 ± 0.013	<0.001	-0.197 ± 0.050	<0.001
DAG (36:2)			-0.139 ± 0.039	<0.001
DAG (36:3)			-0.082 ± 0.035	0.020
CholE02	0.038 ± 0.033	0.250		
CholE05	0.208 ± 0.039	<0.001		
CholE06	0.208 ± 0.039	<0.001		
LPC (14:0)	-0.066 ± 0.033	0.069	0.031 ± 0.041	0.460
LPC (16:0)	-0.072 ± 0.039	0.081	-0,178 ± 0.041	<0.002
LPC (16:1)	-0.010 ± 0.034	0.776	-0.061 ± 0.043	0.176
LPC (18:0)	-0.042 ± 0.039	0.308	-0.102 ± 0.040	0.017
LPC (18:1)	-0.275 ± 0.037	<0.001	-0.159 ± 0.047	0.002
LPC (18:2)	-0.235 ± 0.040	<0.001	-0.146 ± 0.040	<0.001

LPC (18:3)	-0.171 ± 0.029	<0.001	-0.097 ± 0.043	0.032
LPC (20:3)	-0.198 ± 0.036	<0.001	-0.108 ± 0.042	0.016
LPC (20:4)	-0.127 ± 0.040	0.003	-0.180 ± 0.044	<0.001
LPC (20:5)	-0.138 ± 0.033	<0.001	-0.194 ± 0.048	<0.001
LPC (22:6)	-0.198 ± 0.038	<0.001	-0.142 ± 0.047	0.005
LPCO (16:1)	-0.177 ± 0.039	<0.001		
LPCO (18:1)	-0.068 ± 0.039	0.085		
SM (d18:1/14:0)	0.094 ± 0.036	0.018	0.092 ± 0.038	0.027
SM (d18:1/15:0)	0.140 ± 0.041	0.003	0.091 ± 0.039	0.031
SM (d18:1/16:0)	0.103 ± 0.040	0.018	0.074 ± 0.037	0.059
SM (d18:1/16:1)	0.185 ± 0.044	<0.001	0.122 ± 0.039	0.007
SM (d18:1/17:0)	0.201 ± 0.040	<0.001	0.027 ± 0.037	0.528
SM (d18:1/18:0)	0.284 ± 0.040	<0.001	0.045 ± 0.030	0.162
SM (d18:1/18:1)	0.255 ± 0.041	<0.001	0.070 ± 0.031	0.033
SM (d18:1/18:2)	0.289 ± 0.040	<0.001	0.097 ± 0.037	0.016
SM (d18:1/20:0)	-0.054 ± 0.033	0.147	0.104 ± 0.036	0.009
SM (d18:1/20:1)	0.008 ± 0.040	0.888	0.144 ± 0.040	<0.001
SM (d18:1/21:0)	-0.028 ± 0.033	0.467	0.126 ± 0.037	0.004
SM (d18:1/22:0)	-0.049 ± 0.035	0.211	0.143 ± 0.040	<0.001
SM (d18:1/22:1)	-0.005 ± 0.038	0.898	0.217 ± 0.040	<0.001
SM (d18:1/23:0)	-0.041 ± 0.034	0.271	0.108 ± 0.036	0.008
SM (d18:1/23:1)	0.017 ± 0.038	0.724	0.152 ± 0.041	<0.002
SM (d18:1/24:0)	-0.073 ± 0.034	0.052	0.115 ± 0.041	0.012
SM (d18:1/24:1)	0.111 ± 0.041	0.016	0.082 ± 0.036	0.031
SM (d18:1/24:2)	0.150 ± 0.041	<0.001	0.112 ± 0.038	0.008
SM (d18:1/25:0)	0.054 ± 0.033	0.147	0.024 ± 0.039	0.574
SM (d18:1/25:1)	0.197 ± 0.041	<0.001	-0.016 ± 0.038	0.677

Standardized beta ± standard error in linear mixed model with the individual lipid specie as dependent variable, sex as fixed effect and study center as random effect. Adjusted for age, body weight at baseline, weight loss and baseline value of the dependent variable in the weight loss phase (from CID1 to CID2) and adjusted for age, baseline weight, baseline value of the dependent variable, change of the dependent variable during weight loss, weight change during the weight loss phase and weight maintenance phase and diet in the weight maintenance phase (from CID2 to CID3). *q*-value = False Discovery Rate adjusted *p*-value. Individual lipid species only reported when the change in total lipid group revealed statistically significant association with sex (Figure 2).

Chapter 4

The PERSonalized glucose Optimization through Nutritional intervention (PERSON) study: rationale, design, and preliminary screening results

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ABSTRACT

It is well established that the etiology of type 2 diabetes differs between individuals. Insulin resistance (IR) may develop in different tissues, but the severity of IR may differ in key metabolic organs such as the liver and skeletal muscle. Recent evidence suggests that these distinct tissue-specific IR phenotypes may also respond differentially to dietary macronutrient composition with respect to improvements in glucose metabolism. The main objective of the PERSON study is to investigate the effects of an optimal versus suboptimal dietary macronutrient intervention according to tissue-specific IR phenotype on glucose metabolism and other health outcomes. In total, 240 overweight/obese (BMI 25-40 kg/m^2) men and women (age 40-75y) with either skeletal muscle insulin resistance (MIR) or hepatic insulin resistance (LIR) will participate in a two-center, randomized, double-blind, parallel, 12-week dietary intervention study. At screening, participants undergo a 7-point oral glucose tolerance test (OGTT) to determine the hepatic insulin resistance index (HIRI) and muscle insulin sensitivity index (MISI), classifying each participant as either 'No MIR/LIR', 'MIR', 'LIR', or 'combined MIR/LIR'. Individuals with MIR or LIR are randomized to follow one of two isocaloric diets varying in macronutrient content and quality, that is hypothesized to be either an optimal or suboptimal diet, depending on their tissue-specific IR phenotype (MIR/LIR). Extensive measurements in a controlled laboratory setting as well as phenotyping in daily life are performed before and after the intervention. The primary study outcome is the difference in change in disposition index, which is the product of insulin sensitivity and first-phase insulin secretion, between participants who received their hypothesized optimal or suboptimal diet. The PERSON study is one of the first randomized clinical trials in the field of precision nutrition to test effects of a more personalized dietary intervention based on IR phenotype. The results of the PERSON study will contribute knowledge on the effectiveness of targeted nutritional strategies to the emerging field of precision nutrition, and improve our understanding of the complex pathophysiology of whole body and tissue-specific IR.

INTRODUCTION

The prevalence of overweight and related metabolic disturbances, including impaired glucose homeostasis, is rising at an alarming rate, thereby increasing the risk for type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD) (1). Dietary modulation can effectively lower blood glucose levels and reduce the risk of chronic metabolic diseases, independent of weight loss (2, 3). Interestingly, there is great heterogeneity in individuals' metabolic response to dietary interventions (4, 5). Part of this heterogeneity may be attributed to differences in adherence, but recent findings of large inter-individual variation in postprandial responses to standardized meals indicate that individuals actually respond differently to food (6, 7). This inter-individual variation in response to food has complex underpinnings that include biological (including genetic), environmental, and lifestyle factors, and may partly explain the differential metabolic impact of dietary interventions (4-9).

Whole-body insulin resistance (IR) reflects defective insulin action in tissues such as skeletal muscle, liver, adipose tissue, gut, and brain, and is a major risk factor for T2DM and CVD. IR can develop concurrently in different tissues, but the severity of IR may vary between tissues (10, 11). Individuals may, for example, have IR predominantly in the liver or skeletal muscle (10). Liver insulin resistance (LIR) is manifested by impaired insulin-mediated suppression of hepatic glucose production (HGP), while muscle insulin resistance (MIR) is characterized by decreased insulin-mediated glucose disposal (11). The gold-standard method to quantify LIR and MIR is the two-step hyperinsulinemic-euglycemic clamp (11). Tissue-specific IR can also be modelled based on glucose and insulin responses during an oral glucose tolerance test (OGTT), which has been validated against the clamp technique (10, 12).

These tissue-specific IR phenotypes have previously been linked to distinct metabolic profiles, representing different etiologies towards T2DM and CVD (11, 13-15). More specifically, greater disturbances in lipidome (13) and metabolome profiles (14) have been found in individuals with more pronounced LIR as compared to individuals with more pronounced MIR. Additionally, in individuals with LIR, abdominal subcutaneous adipose tissue (scAT) has been characterized by higher expression of genes related to extracellular modelling, whilst MIR has been associated with higher expression of genes related to inflammation in scAT, as well as higher levels of circulating plasma markers of systemic low-grade inflammation (16).

Recent findings indicate that these distinct metabolic phenotypes may respond differently to dietary macronutrient manipulation with regard to outcomes of glucose homeostasis, ectopic fat deposition, and tissue-specific lipid metabolism amongst others (15, 17). Indeed, a post- hoc analysis of the CORDIOPREV-DIAB study has indicated that a low-fat, high-complex carbohydrate diet may be particularly beneficial with respect to improvement in glucose metabolism for individuals with predominant LIR, while individuals with predominant MIR seem to benefit more from a Mediterranean diet high in monounsaturated fatty acids (MUFA) (18). Therefore, further characterization of these IR phenotypes as well as studying these metabolic phenotypes in relation to dietary intervention outcomes may be a promising strategy to develop more personalized dietary interventions. In addition, improvement of glycemic control by more personalized dietary interventions may enhance mood, self-control, and cognitive function (1, 19-21). Such short-term benefits may in turn increase adherence to a healthy diet.

Importantly, prospective randomized controlled trials with a pre-specified hypothesis on differential metabolic responses to diets based on (metabolic) phenotype are largely lacking in the emerging field of precision nutrition. The PERSonalized glucose Optimization through Nutritional intervention (PERSON) study was designed to investigate the effects of an optimal compared to a suboptimal dietary intervention according to tissue-specific IR phenotype on glucose metabolism and other metabolic health outcomes. This two-center, 12-week dietary intervention study with a, randomized, double-blind, controlled, parallel design, aims to enroll a total of 240 individuals with either LIR or MIR. Individuals are randomized to follow one of two diets that are hypothesized to target one of the two tissue-specific IR phenotypes.

Before and after the 12-week dietary intervention, individuals are extensively phenotyped both in laboratory settings and in daily life, which includes measures of whole-body and tissue- specific insulin sensitivity and glucose homeostasis, fasting and postprandial metabolic profile, vascular health, fecal microbiota composition and functionality, body fat distribution, ectopic fat accumulation, adipose tissue morphology and transcriptome, cognitive performance, and perceived well-being. The extensive phenotyping performed in this unique clinical trial allows for a comprehensive study of both the complex metabolic and lifestyle determinants of glucose homeostasis, as well as the dietary intervention effects on metabolic health and its metabolic underpinnings. In the present article, we describe the study design and measurements in detail, and present preliminary results of the screening population.

METHODS

Study design

The PERSON study is a two-center 12-week dietary intervention study with a randomized, double-blind, controlled, parallel design, carried out at Maastricht University Medical Center+ (MUMC+) and Wageningen University & Research (WUR), the Netherlands (Fig. 1). The protocol was approved by the Medical Ethics Committee of MUMC+ (NL63768.068.17) and registered at ClinicalTrials.gov (identifier NCT03708419). The study is conducted according to the principles of the Declaration of Helsinki (revised version, 2013, Fortaleza, Brazil), and all subjects provide written informed consent before the start of the study.

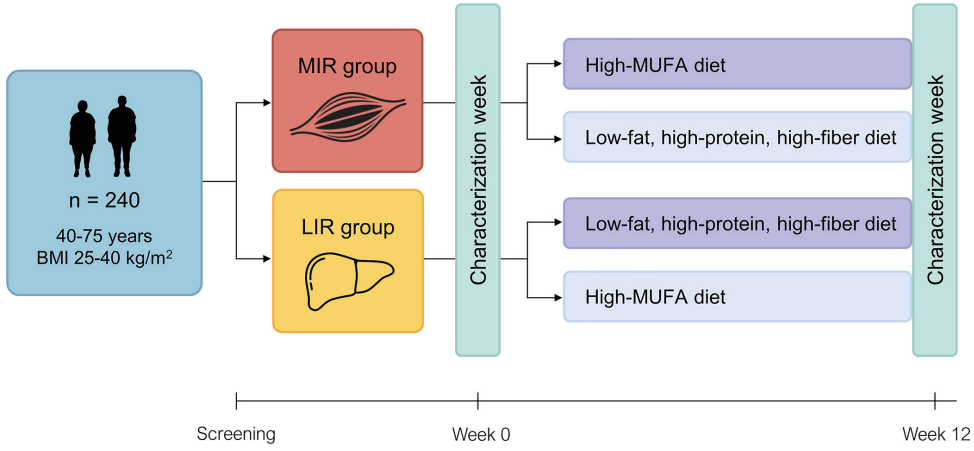

Figure 1. Study design of the PERSON study. Tissue-specific insulin resistance (MIR, muscle insulin resistance; LIR, liver muscle insulin resistance) is assessed at screening using a 7-point oral glucose tolerance test and eligible participants with MIR or LIR are randomized to follow either their hypothesized optimal (dark purple) or suboptimal (light purple) diet for 12 weeks. Before and after the intervention, participants are extensively phenotyped during a 'characterization week' in a controlled laboratory setting as well as in daily life. BMI, body mass index; MUFA, monounsaturated fatty acid.

Study participants

From May 2018 onwards, subjects have been recruited via a volunteer database, flyers, and advertisements in local and online media. Inclusion criteria are age 40-75 years, body

mass index (BMI) 25-40 kg/m^2, body weight stability for at least 3 months (no weight gain or loss >3kg), and tissue-specific IR, characterized as predominant LIR or MIR, as assessed by a 7- point OGTT (see "Screening"). Exclusion criteria include among others pre-diagnosis of T2DM, diseases or use of medication that affect glucose and/or lipid metabolism, major gastrointestinal diseases, history of major abdominal surgery, uncontrolled hypertension, smoking, alcohol consumption >14 units/wk, and >4h/wk moderate-to-vigorous physical activity (see Table S1 for the extensive list of exclusion criteria).

Screening

Eligibility is assessed during a screening visit. Subjects are asked to refrain from alcohol and vigorous physical activity 24 hours prior to the visit and arrive in the morning after a >10h overnight fast. Body weight and height are measured in duplicate without shoes and heavy clothing to the nearest 0.1 kg and 0.1 cm, respectively. Waist and hip circumference are measured in duplicate to the nearest 0.1 cm using a non-flexible measuring tape. Blood pressure is measured in triplicate on the non-dominant arm with an automated sphygmomanometer after a 5-minute rest with the subject in a supine position. The first measurement is used to acclimatize the subject to the measurements, and therefore omitted from the data.

Tissue-specific insulin resistance is assessed based on the glucose and insulin responses during a 7-point oral glucose tolerance test (OGTT). Subjects ingest 200 ml of a ready-to-use 75g glucose solution (Novolab) within 5 minutes, and blood samples are collected from the antecubital vein via an intravenous cannula under fasting conditions (t=0 min) and after ingestion of the glucose drink (t=15, 30, 45, 60, 90 and 120 min) for determination of plasma glucose and insulin concentrations. Hepatic IR and muscle insulin sensitivity are estimated using the calculations of Abdul-Ghani and colleagues (10). We have recently optimized the MISI calculator using the cubic spline method (12). The hepatic IR index (HIRI) and muscle insulin sensitivity index (MISI) were calculated according to the following formulas:

HIRI = glucose 0-30 [AUC in mmol/L x h] x insulin 0-30 [AUC in pmol/L x h]
MISI = (dGlucose/dt) / insulin [mean during OGTT in pmol/L]

In the formula for MISI, dG/dt is the rate of decay of plasma glucose concentration (mmol/L) during the OGTT, calculated as the slope of the least square fit to the decline in plasma glucose concentration from peak to nadir (10).

Glucose curves that are flagged by the calculator, because MISI calculation is not possible or possibly not biologically meaningful due to either a peak at 120 min, a 'flat' curve, or non- negligible rebound (12), are visually inspected for classification of MIR and LIR. Both indices were developed and validated against gold standard measurements of tissue-specific IR by a hyperinsulinemic-euglycemic clamp (10, 12). To obtain study groups that are predominant LIR or MIR, subjects are classified as 'No MIR/LIR', 'MIR', 'LIR', or 'combined MIR/LIR', using tertile cutoffs for MISI and HIRI. The lowest tertile of MISI represents individuals with MIR, while the highest tertile of HIRI represents individuals with LIR. The cutoffs for these tertiles are based on values of a selected study population of The Maastricht Study (DMS) (22), which resembles the target population of the PERSON study. Since the prevalence of LIR seems lower in the PERSON study as compared to DMS after inclusion of n = 163 individuals, the median HIRI value in the PERSON study population will be used for classification of individuals that will be recruited for the remainder of the study.

The homeostasis model assessment of insulin resistance (HOMA-IR) is calculated as (fasting glucose [mmol/L] × fasting insulin [mU/L])/22.5 (23). HOMA of β-cell function (HOMA-β) is calculated as (20 × fasting insulin [mU/L])/(fasting glucose [mmol/L] – 3.5). Matsuda index is defined as: [10,000 ÷ square root of (fasting plasma glucose (mmol/l) x fasting insulin (pmol/l)) x (mean glucose (mmol/l) x mean insulin (pmol/l))], using glucose and insulin values of time points 0, 30, 60, 90, and 120 min (24). Disposition index is calculated as: [Matsuda index * (AUC30 min insulin / AUC30 min glucose)], where AUC30 min is the area under the curve between baseline and 30 minutes of the OGTT for insulin (pmol/l) and glucose (mmol/l) as calculated using the trapezoidal method, respectively. Glucose status was defined according to WHO criteria (25): normal glucose tolerance (NGT), fasting glucose <5.6 mmol/L and 120- min glucose <7.8 mmol/L; impaired fasting glucose (IFG), fasting glucose 5.6-6.9 mmol/L and 120-min glucose <7.8 mmol/L; impaired glucose tolerance (IGT), fasting glucose <5.6 mmol/L and 120-min glucose 7.8-11.0 mmol/L; combined IFG/IGT, fasting glucose 5.6-6.9 mmol/L and 120-min glucose 7.8-11.0 mmol/L; T2DM, fasting glucose ≥7.0 mmol/L and/or 120-min glucose ≥11.1 mmol/L.

Hb and the parameters of hepatic and renal function alanine aminotransferase (ALT), aspartate aminotransferase (AST), and creatinine are determined in fasting blood samples by the hospital laboratories of MUMC+ and Ziekenhuis Gelderse Vallei, Ede, the Netherlands. Habitual dietary intake is estimated by a validated 163-item semiquantitative food frequency questionnaire (FFQ) (26). Dietary misreporting is evaluated by Goldberg's

method, using the ratio of daily energy intake (EI) to estimated basal metabolic rate (BMR) (27, 28). Energy under- (EI/BMR <0.87) and overreporters (EI/BMR>2.75) are excluded from data analyses. Data on demographics, medical history, family history of DM (≥1 first-degree relative with DM), medication use and lifestyle are collected by questionnaire. Education level is categorized into low (no education, primary education, lower or preparatory vocational education, lower general secondary education), medium (intermediate vocational education, higher general senior secondary education or pre-university secondary education) and high (higher vocational education, university). Perceived chronic stress is assessed with the Long-term Difficulties Inventory (29) and mental well-being with the RAND 36-Item Short Form Health Survey (RAND-36) (30) and the Social Production Function Instrument for the Level of Well-being (31).

Randomization procedure

Eligible subjects are randomly allocated to either their hypothesized optimal or suboptimal diet by an independent analyst using center-specific minimization (32, 33) with randomization factors of 1.0 for the LIR/MIR phenotype, and 0.8 for age and sex, and a base probability of 0.7 by means of biased-coin (34). Both researchers and participants are blinded to the participants' metabolic phenotype, and thus blinded to whether participants are allocated to their hypothesized optimal or suboptimal diet. Participants start the study within 3 months of the screening visit.

Table 1. Targeted nutrient composition of the HMUFA and LFHP diet

	HMUFA	LFHP
Fat (en%)	38	28
Monounsaturated fat	20	10
Polyunsaturated fat	8	8
Saturated fat	8	8
Protein (en%)	14	24
Animal-based, % of total protein	45	60
Plant-based, % of total protein	55	40
Carbohydrates (en%)	42	42
Mono- and disaccharides	12	12
Polysaccharides	30	30
Fiber, g/MJ	3	>4
Alcohol	<3	<3

en%, energy percentage of total energy intake; MJ, megajoule

Dietary intervention

The hypothesized optimal diet for MIR is a moderate-fat diet high in MUFA (HMUFA) with a targeted macronutrient composition of 38% of energy from fat (20% MUFA, 8% PUFA, 8% SFA), 48% of energy from carbohydrates (CHO) (30% polysaccharides; 3 g/MJ fiber), and 14% of energy from protein (Table 1). The hypothesized optimal diet for LIR is low in fat, and high in protein (LFHP) and fiber. Energy from CHO is similar between diets. The targeted macronutrient composition of the LFHP diet is composed of 28% of energy from fat (10% MUFA, 8% PUFA, 8% SFA), 48% of energy from CHO high in fiber (30% polysaccharides; >4 g/MJ fiber), and 24% of energy from protein (Table 1).

Individual energy requirements are estimated by averaging self-reported energy intake from the FFQ with the product of the predicted BMR, as calculated with Schofield equations (35), and self-reported physical activity level. Each participant receives dietary guidelines according to their assigned energy group (eight groups with ranges between 6-13 MJ/d), including example menus with types and amounts of food products study participants are required to consume to meet the targeted nutrient composition of the assigned diet. Importantly, to be able to assess the effects of the dietary intervention on metabolic health parameters, independent of changes in body weight, we aim to keep participants in energy balance to maintain body weight throughout the study. Key products that largely distinguish the two diets with regards to macronutrient composition are provided. For the HMUFA diet, key products include olive oil, olives, olive tapenade, and low-fat margarine with olive oil. Key products for the LFHP diet include low-fat yoghurt and quark, reduced-fat cheese, very low-fat spread, pumpkin seeds, baking margarine, and a dietary fiber supplement (2 g β-glucan per 6g, PromOat®, DSM Nutritional Products, Basel, Switzerland) providing 6-12 g of additional fiber per day. Apart from the fiber supplement, all products are commercially available. Alcohol consumption is restricted to ≤1 glass/day, in agreement with the current Dutch dietary guidelines (36).

Throughout the intervention period, participants visit the research facilities weekly for an individual dietary counseling session with a dietitian or trained researcher to monitor diet adherence and body weight. In case of weight loss or gain, participants are reassigned to a higher or lower energy group to prevent further weight change. During the COVID-19 restrictions, the weekly on-site visits are replaced by telephone or video-call consultations, key products are home-delivered by courier, and participants weigh themselves at home.

Dietary compliance is assessed by three unannounced 1-day food records (FR) with the mobile app 'Traqq' (37) on two weekdays and one weekend day. Participants that do

not have a smartphone complete the FRs on paper, which are later entered in the app by the researcher.

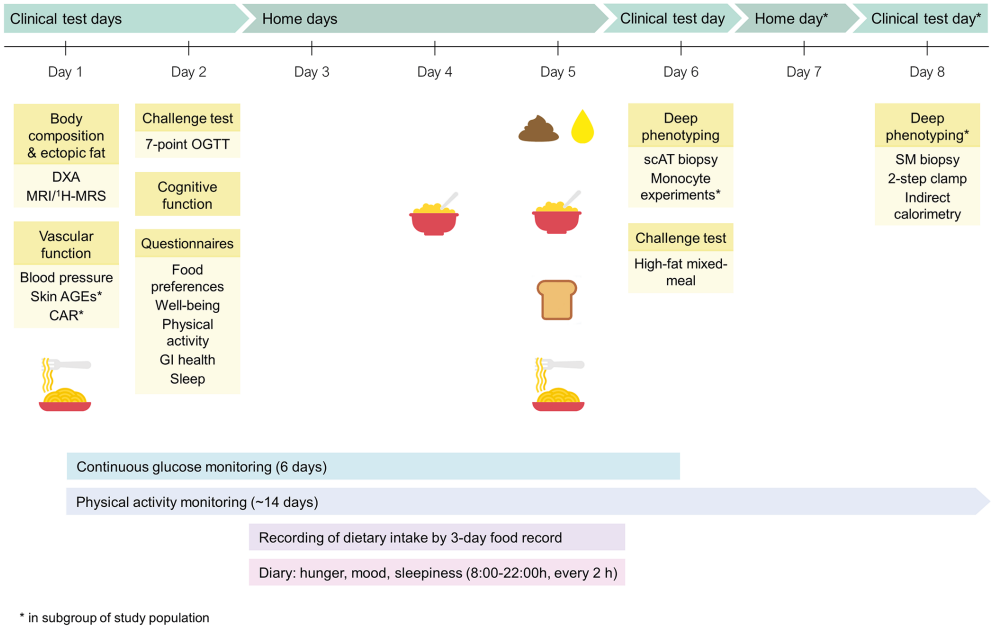

Figure 2. Graphical overview of the pre- and post-intervention characterization week. The characterization week contains multiple clinical test days, during which participants are extensively phenotyped. In addition, blood glucose and physical activity are continuously monitored. At home, participants record their dietary intake and feelings of well-being, collect a fecal sample and 24h urine, and consume standardized meals. In a subgroup of the study population, additional measurements are performed. DXA, dual-energy X-ray absorptiometry; MRI, magnetic resonance imaging; 1H-MRS, proton magnetic resonance spectroscopy; AGEs, advanced glycation endproducts; CAR, carotid artery reactivity; OGTT, oral glucose tolerance test; GI, gastrointestinal; scAT, subcutaneous adipose tissue; SM, skeletal muscle.

Measurements

In the week before start of the intervention and in the last week of the 12-week intervention, participants are extensively phenotyped during a 'characterization week' (Fig. 2). This week includes three or four (depending on study center and participation in additional subgroup measurements) clinical test days and three at-home days. Participants wear a continuous glucose monitor (CGM) and activity monitor throughout the

characterization week. During the clinical test days, participants undergo extensive laboratory testing, which includes challenge tests, body composition analysis, vascular measurements, tissue biopsies, a cognitive test, and questionnaires. During the at-home days, participants record dietary intake and feelings of wellbeing, consume various standardized meals, and collect feces and urine. An overview of all measurements can be found in Figures 2 and 3 and are described in more detail below. On the clinical test days, participants are instructed to travel to the facility by car or public transport. The day prior to and during the characterization weeks, participants are requested to refrain from alcohol and vigorous physical activity. In the week before the baseline characterization week, participants record their dietary intake for three random days (two weekdays and one weekend day) using the mobile app 'Traqq' (37).

Laboratory challenge tests

A 7-point OGTT is performed according to the same procedures used at screening (see 'Screening') (Fig. 3, Fig. 4). Participants consume a standardized low-fat macaroni meal (30% of energy intake [en%] fat, 49 en% CHO, 21 en% protein; 1560-2460 kJ, depending on energy group) the evening before the OGTT, after which they remain fasted until the OGTT. A fasting blood sample is drawn for determination of glycated hemoglobin (HbA1c) by the hospital laboratories of MUMC+ and Ziekenhuis Gelderse Vallei, Ede, the Netherlands.

On a separate clinical test day, a high-fat mixed-meal (HFMM) challenge test is performed after a 12-hour overnight fast (Fig 3, Fig. 4). Participants again consume the standardized low- fat macaroni meal the evening before the test. An intravenous cannula is inserted in the antecubital vein for blood sampling. At least 30 min following insertion of the catheter, a fasting blood sample is drawn (t=0 min). Subsequently, participants are asked to consume the HFMM (350 g containing 2.8 MJ, 49 g [64 en%] fat, 48 g [29 en%] carbohydrate, 12 g [7 en%] protein) within 5 minutes and postprandial blood samples are drawn at t=30, 60, 90, 120, 180, and 240 min for determination of glucose, insulin, free fatty acids (FFA), triacylglycerol (TAG), glucagon-like peptide 1 (GLP-1), peptide YY (PYY), and bile acids (Fig. 4). Total cholesterol and HDL cholesterol are determined in fasting serum. Extensive plasma metabolite profiling is performed in samples from T=0, 30, 60, 120 and 180 min by high-throughput nuclear magnetic resonance (NMR) metabolomics (Nightingale Health Ltd., Helsinki, Finland) (38).

Buffy coat is collected from fasting blood for later DNA isolation and genotyping. At each blood drawing, participants rate their hunger, fullness, satiety, thirst, and desire to eat on a 100-mm Visual Analogue Scale (VAS), anchored at the extremes 'not at all' to 'extremely'.

	Method	Main parameters	Study time point			
			SCR	CW1	DIW	CW2
Laboratory challenge tests	Oral glucose tolerance test	Glucose homeostasis parameters	●	●		●
	High-fat mixed-meal test	Fasting and postprandial metabolic profile		●		●
Body composition, fat distribution and ectopic fat	Digital weighing scale	Body weight	●	●	●	●
	Measuring tape	Waist and hip circumference	●	●		●
	Magnetic resonance imaging (MRI)	Abdominal subcutaneous adipose tissue, visceral adipose tissue, intrahepatic lipid content*, muscle fat infiltration*		●		●
	Proton magnetic resonance spectroscopy (^{1}H-MRS)*	Intrahepatic lipid content		●		●
	Dual-energy X-ray absorptiometry (DXA)	Whole-body and regional fat mass, lean body mass, body fat percentage, bone mineral density		●		●
Cardiovascular measurements	Automated sphygmomanometer	Systolic and diastolic blood pressure	●	●		●
	Carotid artery diameter responses to a cold pressor test*	Carotid artery reactivity		●		●
	AGE-reader*	Skin accumulation of advanced glycation end-products		●		●
Dietary intake	Food frequency questionnaire	Habitual dietary intake	●			
	3x 1-day food record	Actual dietary intake		●	●	●
Microbiota composition and functionality	Feces collection	Fecal microbiota composition, fecal SCFA		●		●
	Bristol stool chart	Self-reported stool consistency		●		●
	Rome III criteria	Self-reported gastrointestinal health		●		●
	Oral sample collection	Oral microbiota composition		●		●
Deep laboratory phenotyping	Abdominal subcutaneous adipose tissue biopsy	Gene and protein expression, adipocyte size, immune cell composition*		●		●
	Skeletal muscle biopsy in m. vastus lateralis*	Gene and protein expression		●		●
	2-step hyperinsulinemic-euglycemic clamp*	Whole-body and tissue-specific insulin resistance, substrate oxidation		●		●
	Ex vivo monocyte experiments*	Fasting immune metabolism	●	●		●
	24-hour urine collection	Urinary metabolites		●		●
Measurement in daily life	Continuous glucose monitoring (6 days)	Glycemic variability, glycemic response to standardized meals		●		●
	Physical activity monitoring (~14 days)	Sedentary and physical activity parameters		●		●
	Likert scales (every 2 hrs for 3 days)	Self-reported mood, hunger, and sleepiness		●		●
Cognitive performance	Cambridge Neuropsychological Test Automated Battery (CANTAB)	Executive function, memory, attention & psychomotor speed		●		●
Questionnaires	General questionnaire	Demographics, medical history, medication use, lifestyle	●			
	Long-term Difficulties Inventory	Perceived chronic stress	●			
	Social Production Function Instrument for the Level of Well-being	Mental well-being	●			
	RAND 36-item Health Survey	Mental well-being	●	●		●
	Perceived Stress Scale-10, Chalder Fatigue scale	Mental well-being		●		●
	PSQI, Munich ChronoType, Epworth sleepiness scale	Sleep quality and characteristics		●		●
	Baecke questionnaire, AQuAA	Self-reported physical (in)activity		●		●

Figure 3. Overview of all measurements performed within the PERSON study.
* Performed in a subgroup of the study population. SCR, screening visit; CW, characterization week; DIW, dietary intervention week

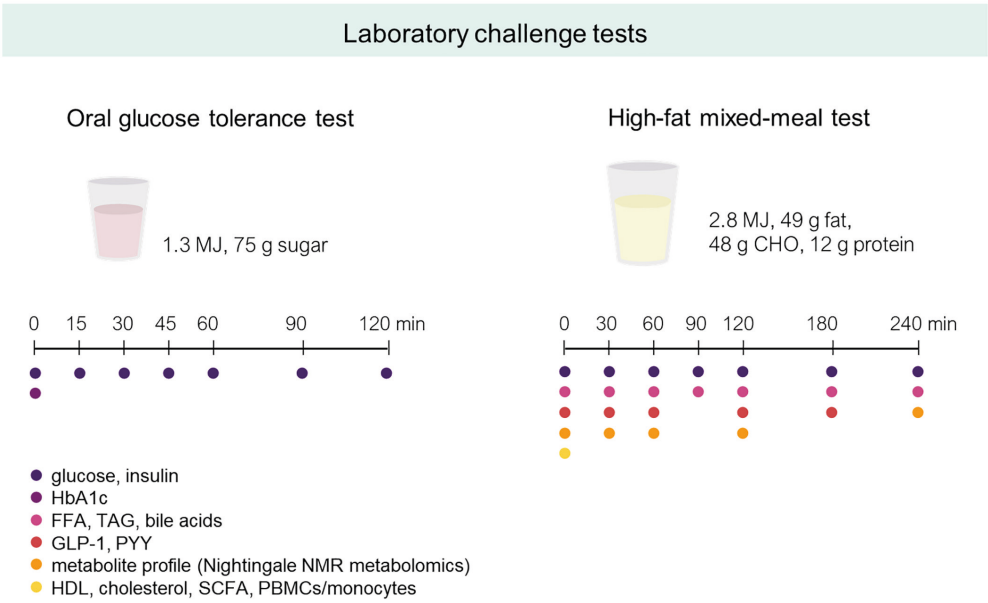

Figure 4. Graphical overview of the oral glucose tolerance test and the high-fat mixed-meal (HFMM) test that are performed during the pre- and post-intervention characterization week. Participants are instructed to drink the glucose drink or HFMM within 5 minutes, and fasting and postprandial blood samples are drawn at the indicated timepoints for determination of the indicated metabolites. CHO, carbohydrates; HbA1c, hemoglobin A1c; FFA, free fatty acids; TAG, triglycerides; GLP-1, glucagon-like peptide 1; PYY, peptide YY; NMR, nuclear magnetic resonance; HDL, high-density lipoprotein; SCFA, short-chain fatty acids; PBMCs, peripheral blood mononuclear cells.

Cardiovascular markers

Blood pressure is assessed according to the same procedures used at screening. In a subgroup of participants, vascular function is assessed by measuring carotid artery reactivity (CAR) to a cold pressor test (CPT) (39). After 10 min of rest in supine position, the participant's left hand is submerged in a bucket of icy water (≤4°C) for three minutes. The diameter of the left common carotid artery is monitored during a 1-min baseline assessment and continuously during the 3-min CPT using ultrasound (Terason uSmart 3300, Burlington, Massachusetts, USA). Wall-tracking and edge-detecting software is used to calculate the diameter after completion of the test. To confirm sympathetic stimulation, blood pressure is measured after the supine rest, 1-min and 2- min after the start of the CPT, and directly after completion of the CPT (Omron M6 Comfort, Omron healthcare Co., Ltd., Kyoto, Japan).

In a subgroup, skin accumulation of advanced glycation end-products (AGE) is measured by skin autofluorescence (AF) using the automated AGE reader (DiagnOptics Technologies B.V., Groningen, the Netherlands). Skin AF is measured at three slightly different places on the volar side of the dominant arm, avoiding impurities of the skin such as scars and birthmarks. Participants are instructed to not apply any creams, lotions, or sunscreen on their arms on the day of the measurement.

Body composition, fat distribution, and ectopic fat deposition

Body weight is measured in underwear, and waist and hip circumference are measured according to the procedures described earlier (see 'Screening'). Whole-body and regional fat mass, fat percentage, lean body mass, and bone mineral density are assessed using dual- energy X-ray absorptiometry (DXA), while participants are fasted for ≥2h (MUMC+, Discovery A, Hologic; WUR, Lunar Prodigy, GE Healthcare) (Fig. 3).

At MUMC+, a whole-body scan is made after a ≥2h fast with a 3T magnetic resonance imaging (MRI) scanner (3T MAGNETOM Prisma fit, Siemens Healthcare), using a radiofrequency transmit/receive body coil at Scannexus, Maastricht, the Netherlands. Analyses are performed using a computational modeling method (AMRA Medical AB, Linköping, Sweden (40)) for quantification of abdominal subcutaneous adipose tissue (ASAT), visceral adipose tissue (VAT), thigh muscle volume, intrahepatic lipid content (IHL), and muscle fat infiltration (MFI) in the anterior thighs (Fig. 3).

At WUR, IHL and abdominal fat distribution are assessed with proton magnetic resonance spectroscopy (1H-MRS) and MRI, respectively, on a 3T whole-body scanner (Siemens, Munich, Germany; Philips Healthcare, Best, the Netherlands from November 2020 onwards). MRI measurements are performed after a ≥2h fast at hospital Gelderse Vallei, Ede, the Netherlands. Spectra for determination of IHL are obtained from a 30 x 30 x 20 mm voxel placed in the right lobe of the liver, avoiding blood vessels and bile ducts. Participants are instructed to hold their breath when spectra are acquired to reduce respiratory motion artefacts. Spectra are post-processed and analyzed using the AMARES algorithm in jMRUI software. Abdominal fat distribution is evaluated as subcutaneous (ASAT) and visceral adipose tissue (VAT) areas in the abdomen, which are quantified in singles-slice axial T1-weighted spin echo transverse images at the inter-vertebral space L3-L4 using the semi-automatic software program HippoFatTM (41).

Microbiota composition and functionality

During one of the at-home days in the characterization week, participants collect fecal

samples (Fig. 2, Fig. 3). The samples are stored in the participants' home freezer for maximal 72 hours before the visit to the research facilities. Participants rate stool consistency of the sample using the Bristol stool scale (42). Fecal microbiota composition is determined by 16S rRNA sequencing as described elsewhere (43).

During the HFMM challenge test, fasting and postprandial blood samples are collected for determination of plasma concentrations of GLP-1, PYY, and bile acids (Fig. 3). Fecal concentrations and fasting plasma levels of gut microbiota-derived short-chain fatty acids (SCFA) acetate, propionate and butyrate are determined using optimized LC-MS protocols (44).

Data on self-reported gastrointestinal health are collected by a questionnaire based on the Rome III criteria (45). The questionnaire includes questions on presence of gastrointestinal complaints (i.e. abdominal pain, obstipation, bloating), defecation frequency, and stool consistency (Fig. 3).

In addition, oral samples are collected for microbiological and metabolite analyses. Participants are asked to rinse the oral cavity thoroughly for 30 seconds with 10 ml of sterile 0.9% saline and expectorate the rinse in a tube. The tube is kept on ice, vortexed and the rinse is aliquoted, snap-frozen in liquid nitrogen and stored at −80 °C for later analysis. Participants are instructed to refrain from oral hygiene in the morning of the sampling day. The composition of the oral microbiome is determined by 16S rRNA sequencing (46).

Abdominal subcutaneous adipose tissue biopsy

On the morning of the HFMM, an abdominal SAT biopsy is collected 6-10 cm lateral from the umbilicus under local anesthesia (1% lidocaine) by needle biopsy . The samples are washed with saline to remove blood clots. A portion of tissue is fixed overnight at 4°C in 4% paraformaldehyde and embedded in paraffin for histological sections to determine adipocyte morphology. In a subgroup of participants, at baseline only, ~0.7g of fresh AT is used for fluorescence activated cell sorting (FACS) analysis. In short, the stromal vascular fraction is isolated from the AT and stained with a cocktail of antibodies for flow cytometry for identification of immune cells (47). The remaining tissue is snap-frozen in liquid nitrogen and stored at −80 °C for later analyses of targeted gene and protein expression.

Skeletal muscle biopsy

In a subgroup of participants, a skeletal muscle (SM) biopsy is collected and a two-step hyperinsulinemic-euglycemic clamp is performed on a separate clinical test day at the end

of the characterization week (Fig. 3). The skeletal muscle biopsy is taken from the m. vastus lateralis under local anaesthesia using the Bergström biopsy needle method (48). After removal of blood and fat tissue, a portion of the biopsy is snap-frozen in melting isopentane and stored at −80 °C for biochemical analyses. The remaining tissue is snap-frozen in liquid nitrogen and stored at −80 °C for later gene and protein expression analyses.

Two-step hyperinsulinemic-euglycemic clamp

After the SM biopsy, whole-body and tissue-specific insulin sensitivity are assessed by the gold standard two-step hyperinsulinemic-euglycemic clamp (49). At t=-120 min, primed D-[6.6- $^{2}H^{2}$] glucose tracer is started and infused continuously at 0.04 mg/kg/min, to allow calculations of rates of endogenous glucose production (EGP), glucose appearance (Ra), and glucose disposal at basal conditions. At t=0, a low primed constant co-infusion of insulin at 10 mU/m^2/min is started for 3 hours for determination of hepatic insulin sensitivity. At t=180 min, the primed constant infusion of insulin is increased to 40 mU/m^2/min for 2.5 hours to inhibit EGP and measure muscle insulin sensitivity. Arterialized blood is frequently sampled from the superficial dorsal hand vein during the insulin infusion to measure glucose concentrations, which are maintained at ~5.0 mmol/L by a co-infusion of 20% glucose at variable rate (GIR). Substrate utilization is measured for 30 min during the basal, low insulin, and high insulin infusion using indirect calorimetry by ventilated hood (Omnical, Maastricht Instruments, Maastricht). Resting metabolic rate (RMR), fat and carbohydrate oxidation are calculated according to the equations of Weir and Frayn (50, 51). The clamp is performed after an overnight (≥12h hour) fast and participants consume the standardized macaroni meal the evening before the clamp.

Fasting immune metabolism

In a different subgroup of participants, circulating peripheral blood mononuclear cells (PBMCs) are isolated from fasted blood samples collected at both the screening visit and the HFMM test (Fig. 3). PBMCs are isolated by density gradient isolation using CPT tubes (BD vacutainer, cat. no. 362753). Monocytes are subsequently obtained by MACS (magnetic activated cell sorting) positive selection using CD14 MicroBeads (Miltenyi Biotec, cat no. 130-050-201). Part of the monocytes are exposed overnight (24h) to the inflammatory stimuli lipopolysaccharide (LPS) (10 ng/mL, sigma, cat. no L6529) and P3C (10 ug/mL, EMC collections, cat. no. L2000). Functional properties of monocytes are determined after treatment by measuring the release of cytokines including IL-6, IL-1b and CXCL8 (R&D DuoSet ELISA, cat. no. DY206; DY201; DY208). The metabolic potential of monocytes is measured in real-time experiments (inflammatory cell activation

test and glycolytic stress test) using the Seahorse apparatus (Agilent Technologies) in screening samples only.

24-h urine collection

Participants collect 24-h urine in 2-3 liter containers containing 5 ml/L of 4 mM hydrochloric acid (HCl). Urine collection starts after the first voiding on the morning of the home-day with only standardized meals and finishes 24 hours later on the morning of the HFMM. Participants are asked to store the containers in a cool place, preferably a refrigerator, and bring the containers to the facilities on the day of the HFMM. The urine of each participant is mixed, weighted, aliquoted, and stored at −80 °C for later analysis.

Continuous glucose monitoring (CGM)

At the start of the characterization week, a CGM (Medtronic iPro2 with Enlite sensor) is placed lateral to the umbilicus for 6 days of continuous interstitial fluid glucose measurements (Fig 2.). The CGM data are calibrated according to the manufacturer's instructions with four daily capillary glucose self-measurements using a blood glucose meter (Contour XT, Ascensia Diabetes Care).

Physical activity assessment

Physical activity is continuously monitored for ~14 days - either starting with the characterization week at baseline or ending with the characterization week in week 12 (Fig 2.) - using a triaxial accelerometer (activPAL3™ micro, PAL Technologies Ltd., Glasgow, Scotland, UK) attached to the middle of the right thigh. Participants keep a diary to record the times they go to sleep and wake up while wearing the monitor. Sedentary and physical activity parameters are quantified with a modified version of the script of Winkler et al. (52), using the sleeping and waking times as input.

Dietary intake, hunger, mood, and sleepiness

During the three at-home days, participants record their dietary intake using the mobile app 'Traqq' (37). In addition, participants are asked to report on hunger, mood, and sleepiness every two hours from 8:00h to 22:00h (Fig 2.). Hunger is rated on an 11-point Likert scale ranging from 'not hungry' to 'very hungry'. Self-reported mood is assessed with an adapted form of the Multidimensional Mood Questionnaire (MDMQ) (53). The 7-point scale consists of six bipolar items to assess the three basic dimensions of mood valence, calmness, and energetic arousal: tired/awake, satisfied/dissatisfied, agitated/calm, full of energy/without energy, unwell/well, and relaxed/tense. Sleepiness is rated on

the 9-point Karolinska Sleepiness Scale, with labels ranging from 'extremely alert' to 'very sleepy, great effort keeping awake, fighting sleep' (54, 55).

On one of the home-days, participants consume a standardized breakfast, and on another home-day, participants have a full day of standardized meals and snacks, including the standardized breakfast (Fig 2.). Participants are instructed to consume the meals according to standardized instructions including time frames. Participants are instructed to fast for 2 hours after the breakfast, and to only drink water alongside the standardized meals.

Cognitive performance

Cognitive performance is assessed in the domains of executive function, memory, and attention & psychomotor speed using the Cambridge Neuropsychological Test Automated Battery (CANTAB) (56). Executive function is evaluated with the multitasking test and spatial span test; memory with the delayed matching to sample test and paired associates learning test; and attention and psychomotor speed is assessed with the motor screening task and reaction time task. Each test is preceded by standardized instructions and a practice round for familiarization. Participants consume a standardized brunch containing of bread with cheese and/or ham and a caffeine-free drink before performing the test battery.

Self-reported food preferences, eating rate, sleep, well-being, and physical (in)-activity

After the CANTAB, participants complete the computer-based Macronutrient and Taste Preference Ranking Task (MTPRT) for assessment of food preferences (57). The task assesses liking and ranking for 32 food products that are categorized as high in carbohydrates, high in fat, high in protein, or low-calorie, as well as either sweet or savory. Furthermore, participants rate their eating rate in comparison to others on a 5-point Likert scale with labels ranging from 'very slow' to 'very fast' (Fig. 3).

In addition, during one of the clinical test days, participants provide information on general well-being, sleep characteristics, and physical (in)activity by questionnaire (Fig. 3). Mental well-being is assessed using the RAND-36 (30) and perceived stress is measured with the 10-item Perceived Stress Scale (PSS-10) (58). Physical and mental fatigue are assessed using the 14-item Chalder fatigue scale (59). Sleep quality is assessed with the 10-item Pittsburgh Sleep Quality Index (60) and sleep duration and chronotype are derived from the Munich ChronoType Questionnaire (61). Daytime

sleepiness is assessed with the 8-item Epworth Sleepiness scale (62) (Fig. 3).

Self-reported habitual physical activity and sedentary behaviour are assessed using the Baecke questionnaire (63) and the Activity Questionnaire for Adults and Adolescents (AQuAA) subscale 'sedentary leisure time activities' (64), respectively. In addition, physical activity self- efficacy is measured with 5 items from a health specific self-efficacy scale (65) and physical inactivity temptations are assessed using the 5-item subscale 'competing demands' from the Temptation to not Exercise Scale (66), extended with the item "How tempted are you not to exercise and be sedentary while being on a business trip?".

Biochemical analyses and biobanking

A wide range of biological samples are collected in the present study, including blood plasma and serum, SAT, SM tissue, feces, urine, saliva, and PBMCs. EDTA (Becton Dickinson, Eysins, Switzerland) tubes are centrifuged at 1200 g, 4 °C for 10 minutes and plasma is aliquoted subsequently. Serum tubes are left at room temperature for at least 30 minutes to allow clotting after sampling and centrifuged at 1200 g, 20 °C for 10 minutes before aliquoting of serum. All biological samples are snap-frozen in liquid nitrogen and stored at −80 °C until analysis. Samples from both centers are analyzed at central laboratories. Plasma glucose, insulin, and FFA are measured on a Cobas Pentra C400 using ABX Pentra Glucose HK CP reagens (Horiba ABX Diagnostics, Montpellier, France), ELISA (Meso Scale Discovery, Gaithersburg, USA), and NEFA HR (2) reagens (2) (Wako chemicals, Neuss, Germany), respectively. Serum TAG, total cholesterol, and HDL cholesterol are measured on a Cobas Pentra C400 using ABX Pentra Triglycerides HK CP reagens, ABX Pentra Cholesterol CP reagens, and ABX Pentra HDL Direct, respectively. During the HFMM challenge test, fasting and postprandial blood samples are collected in EDTA tubes and aprotinin tubes containing dipeptidyl peptidase-IV inhibitor (Milipore Merck, Billerica, MA, USA) for determination of plasma GLP-1 and PYY, respectively. Total GLP-1 immunoreactivity is assessed using an antiserum that reacts equally with intact GLP-1 and the primary (N-terminally truncated) metabolite as previously described (67).

Data management

Data are collected on paper case report forms (CRF) and are entered in an electronic CRF designed for the study, using a the web-based data capturing platform Caster EDC (68) that is compliant with good clinical practice (GCP) requirements. All relevant raw and processed data (e.g. from blood analyses, DXA scan) are also added to the eCRF in

Castor EDC. Data entered in the eCRF is checked against the paper CRF by a study team member that did not enter the data. Data are collected and stored according to the FAIR (Findability, Accessibility, Interoperability, and Reusability) principles (69). A central data manager monitors data entry of both centers, performs data cleaning, and ensures that inaccurate or missing data are addressed.

Sample size calculation

Based on previous data, we expect a greater improvement in disposition index in participants receiving their hypothesized optimal diet compared to those receiving their hypothesized suboptimal diet (10). Data from the previously published DiOGenes study (18) as well as the CORDIOPREV-DIAB Study (18) were used to calculate an average standardized effect size from the difference in outcome values between the optimal and suboptimal diets in those studies. For DiOGenes, the low vs high GI diets during the weight regain period were used and for CORDIOPREV the Mediterranean vs low fat-high complex carbohydrate diets were used, in interaction with either MIR or LIR. With a power of 90%, two-sided alpha of 5% and a standardized effect size of 0.46, a total sample size of 202 was calculated using the statistical analysis software R. Taking into account a drop-out rate of 15%, 240 subjects will be included.

Statistical analyses

In this paper, preliminary screening data from May 2018 to March 2020 are included. Baseline characteristics were compared between the four IR phenotypes (No MIR/LIR, MIR, LIR, combined MIR/LIR), using one-way ANOVA with Bonferroni *post-hoc* pairwise comparisons for numerical data (mean ± SD), and using Fisher's exact test for categorical data (%). Parameters of glucose homeostasis from the OGTT and dietary intake data from the FFQ were log-transformed due to non-normality, and differences between the IR phenotypes were tested using ANCOVA with adjustment for sex and Bonferroni *post-hoc* pairwise comparisons. Statistical analyses were performed in SPSS (version 25.0). Differences in glucose and insulin responses following the OGTT between the IR phenotypes were tested using linear mixed- effects models (LMM) with Bonferroni *post-hoc* pairwise comparisons. The time courses of glucose and insulin were modelled with third-order (cubic) orthogonal polynomials. The effect of IR phenotype on all time terms and sex were included as fixed effects with participant random effects on all time terms. The adequacy of the higher order polynomials was assessed with a likelihood-ratio test between nested models. The covariance matrix of the residuals was modelled as an unstructured matrix and model parameters were estimated using maximum likelihood estimation in all models. Estimated marginal means (EMM) with the degrees of freedom

and corresponding *p*-values were estimated using Satterthwaite's method. All mixed-effects models were implemented using the 'lmer' function of the lme4 package and EMMs were computed using the emmeans package in R (version 3.3.3, The R foundation for Statistical Computing, http://www.r-project.org/).

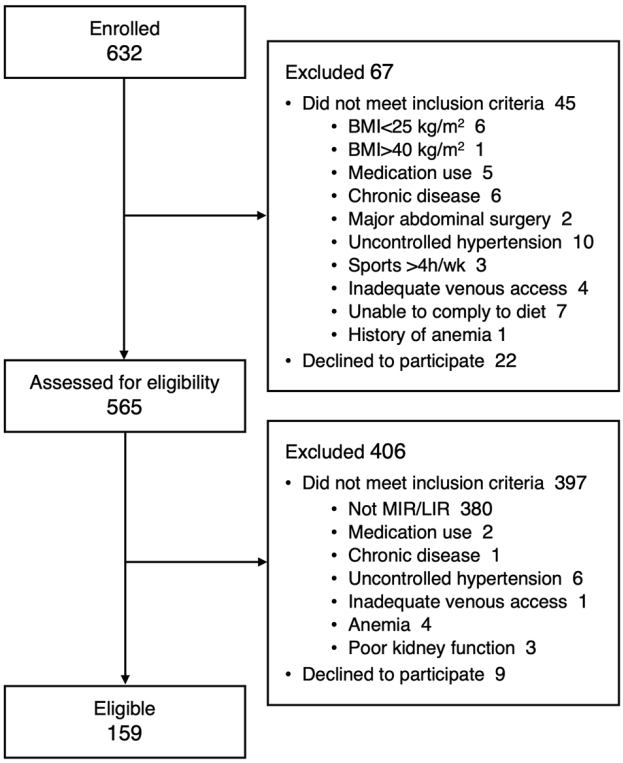

Figure 5. Flowchart of participant enrollment and eligibility from March 2018 to March 2020.

RESULTS

Between May 2018 and March 2020, 632 individuals were enrolled, of whom 565 were fully screened for eligibility (Fig. 5). In total, 40.2% of fully screened individuals were classified as No MIR/LIR, 21.4% as MIR, 10.8% as LIR, and 27.6% as combined MIR/LIR. Here, we present the characteristics of the study participants that have thus far been screened in the present ongoing clinical trial.

Table 2. Characteristics of screened participants according to insulin resistance phenotype

	No MIR/LIR (n=227)	MIR (n=121)	LIR (n=61)	Combined MIR/LIR (n=156)	*p*-value
Age (years)	61 ± 9	60 ± 9	61 ± 8	62 ± 8	0.627
Women (%)	59.9	69.4	54.1	52.6†	0.031
Weight (kg)	86.6 ± 13.1	86.0 ± 11.2	86.4 ± 11.8	94.0 ± 14.7§†‡	<0.001
BMI (kg/m^2)	29.2 ± 3.3	29.8 ± 3.1	29.6 ± 3.2	32.2 ± 4.1§†‡	<0.001
Waist circumference (cm)	98.5 ± 10.6	100.4 ± 9.5	99.9 ± 9.5	106.3 ± 11.0§†‡	<0.001
Waist-to-hip ratio	0.91 ± 0.09	0.92 ± 0.08	0.94 ± 0.09	0.95 ± 0.09§†	<0.001
SBP (mmHg)	132 ± 17	132 ± 13	132 ± 16	137 ± 16§	0.015
DBP (mmHg)	80 ± 11	80 ± 10	80 ± 11	85 ± 10§†‡	<0.001
Hemoglobin (mmol/L)	8.8 ± 0.7	8.7 ± 0.7	8.8 ± 0.7	9.0 ± 0.8§†	0.003
Creatinine (µmol/L)	75.0 ± 14.1	73.8 ± 14.1	76.0 ± 16.2	78.4 ± 14.7	0.051
ALT (IU/L)	23 ± 10	27 ± 12	25 ± 9	31 ± 14§†‡	<0.001
AST (IU/L)	22 ± 6	22 ± 7	23 ± 6	25 ± 8§†	0.002
Use of statins (%)	9.7	6.6	11.5	13.5	0.293
Use of antihypertensives (%)	17.2	15.7	14.8	28.2	0.022
Family history of diabetes (%)	24.7	21.5	18.3	25.2	0.685
Glucose status (%)					<0.001
NGT	78.4	71.1	75.4	62.2	
IFG	6.2	0.8	11.5	2.6	
IGT	7.0	18.2	6.6	16.7	
Combined IFG/IGT	1.8	1.7	6.6	7.7	
T2DM	6.6	8.3	0.0	10.9	
Employment status (%)					0.429
Paid job	44.6	49.2	43.3	36.8	
Retired	39.7	37.5	36.7	42.6	
Other	15.6	13.3	20.0	17.0	
Education level (%)					0.257
Low	17.9	11.8	25.0	21.7	
Intermediate	31.8	39.5	28.3	30.9	
High	50.2	48.7	46.7	47.4	

Differences between tissue-specific IR groups were assessed using one-way ANOVA with Bonferroni *post-hoc* pairwise comparisons for numerical data (mean ± SD), and using Fisher's exact test for categorial data (%).
§ significantly different from No MIR/LIR ($p<0.05$)
† significantly different from MIR ($p<0.05$)
‡ significantly different from LIR ($p<0.05$)
BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; ALT, alanine transaminase; AST, aspartate aminotransferase; NGT, normal glucose tolerant; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; T2DM, type 2 diabetes mellitus.

Baseline characteristics

Baseline characteristics of all participants that completed screening are reported according to IR phenotype in Table 2. Mean age of the four groups (60-62 yrs) was comparable. The proportion of women in the total study population was 59% and was higher in the MIR group (69%) compared to the other groups, but only statistically significantly different from the combined MIR/LIR group. Individuals with combined MIR/LIR had higher BMI, waist circumference, blood pressure, ALT levels, and use of antihypertensive medication compared to the No MIR/LIR, MIR, and LIR groups. Anthropometric and clinical characteristics were similar between the MIR and LIR group. IFG was most prevalent in the LIR group (11.5%), while IGT was most prevalent in the MIR group (18.2%) and the combined MIR/LIR group (16.7%). The prevalence of newly diagnosed T2DM was 6.6%, 8.3%, 0.0%, and 10.9% in the No MIR/LIR, MIR, LIR, and combined MIR/LIR group, respectively.

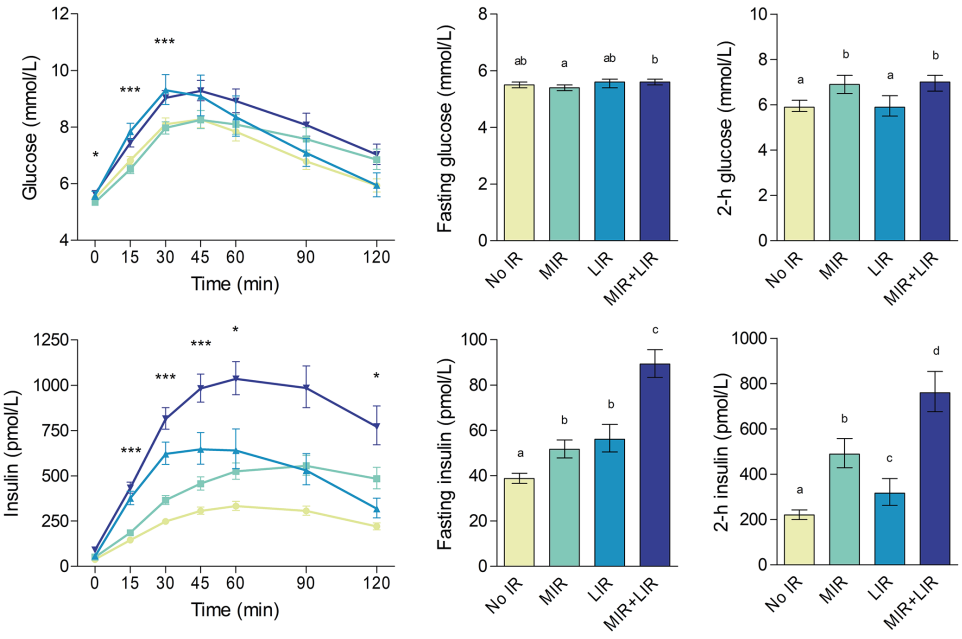

Figure 6. Plasma glucose (A-C) and insulin (D-F) concentrations during an oral glucose tolerance test according to insulin resistance phenotype. (A, D): data are geometric means with 95% confidence intervals; significant differences for MIR vs. LIR as analyzed using estimated marginal means from linear mixed-effects models with adjustment for sex and Bonferroni *post-hoc* pairwise comparisons are denoted with * (p<0.05) or *** (p<0.001). (B, C, E, F): Data are adjusted geometric means

with 95% confidence intervals. Different letters (a, b, c, d) indicate significant differences (p<0.05) between IR phenotypes, as tested using ANCOVA with adjustment for sex and Bonferroni *post-hoc* pairwise comparisons.

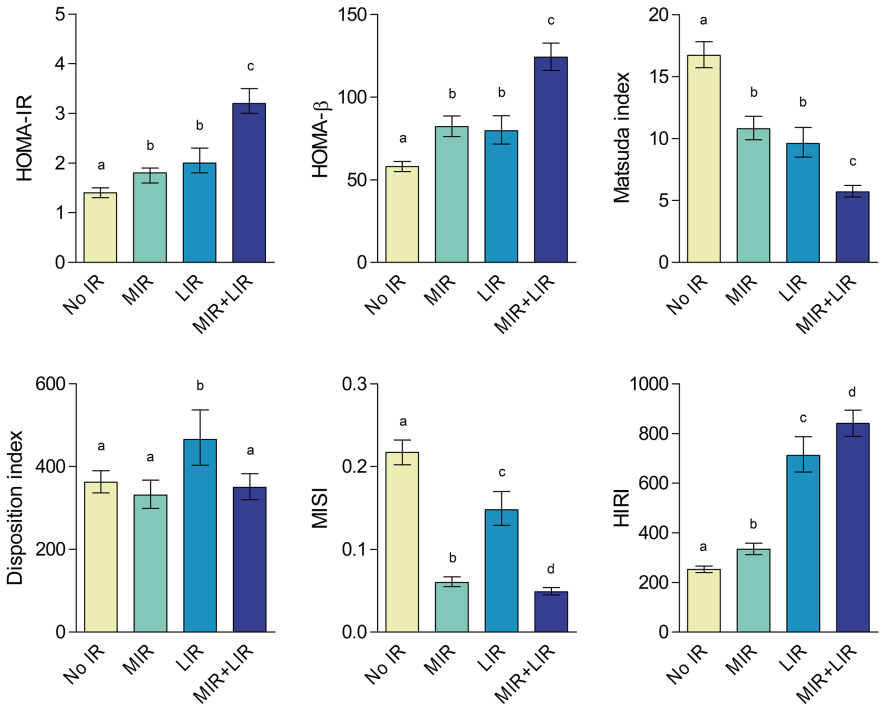

Figure 7. HOMA-IR (A), HOMA-β (B), Matsuda index (C), disposition index (D), muscle insulin sensitivity index (E), and hepatic insulin resistance index (F) according to insulin resistance (IR) phenotype. Data are adjusted geometric means with 95% confidence intervals. Different letters (a, b, c, d) indicate significant differences (p<0.05) between IR phenotypes, as tested using ANCOVA with adjustment for sex and Bonferroni *post-hoc* pairwise comparisons.

Glucose homeostasis

By definition, both plasma glucose and insulin curves throughout the OGTT differed between the IR groups (p<0.001 for both; Fig. 6). Throughout the first 30 minutes of the OGTT, plasma glucose concentrations were higher in the LIR group compared to the MIR group (Fig. 6A). Plasma insulin concentrations were higher in the LIR group compared to the MIR group at timepoints 15-60 minutes, whereas at 120 minutes, insulin was lower in

LIR compared to MIR (Fig. 6D). HOMA-IR was lowest in the No MIR/LIR group, highest in the MIR/LIR group, and similar in the MIR and LIR group (overall $p<0.001$; Fig. 7A), as was HOMA-β ($p<0.001$; Fig. 7B). Similarly, Matsuda index was highest in the No MIR/LIR group, lowest in the combined MIR/LIR group, and comparable between the MIR and LIR group (overall $p<0.001$; Fig. 7C). Disposition index was higher in the LIR group compared to the other groups (overall $p=0.002$; Fig. 7D). Furthermore, by definition, MISI was lowest in the combined MIR/LIR and the MIR group (overall $p<0.001$; Fig. 7E) and HIRI was highest in the combined MIR/LIR and LIR group (overall $p<0.001$; Fig. 7F). All analyses were adjusted for sex. Values of these glucose homeostasis parameters derived from OGTT are reported in Table S2.

Habitual dietary intake

FFQ data were available from 549 participants. After exclusion of data from 84 and 4 individuals due to energy under- and overreporting, respectively, data from 461 participants were included in the analyses. The proportion of misreporters did not differ between the

Table 3. Habitual dietary intake from FFQ according to insulin resistance phenotype

	No MIR/LIR (n=227)	MIR (n=121)	LIR (n=61)	Combined MIR/LIR (n=156)	*p*-value
Energy (MJ)[a]	9.5 ± 1.0	8.8 ± 1.0	9.5 ± 1.0	9.6 ± 1.0	0.062
Fat (en%)	37.6 ± 0.4	36.8 ± 0.6	37.3 ± 0.8	38.5 ± 0.5	0.127
Monounsaturated fat	13.5 ± 0.2	13.1 ± 0.2	13.3 ± 0.3	13.6 ± 0.2	0.551
Polyunsaturated fat	7.2 ± 0.1	7.1 ± 0.2	7.1 ± 0.2	7.1 ± 0.1	0.946
Saturated fat	13.8 ± 0.2	13.4 ± 0.3	13.8 ± 0.4	14.5 ± 0.3[†]	0.024
Carbohydrates (en%)	41.1 ± 0.5	42.6 ± 0.6	42.1 ± 0.8	40.9 ± 0.5	0.137
Mono- and disaccharides	19.0 ± 0.4	20.0 ± 0.6	19.8 ± 0.8	18.4 ± 0.5	0.150
Polysaccharides	22.1 ± 0.3	22.6 ± 0.4	22.3 ± 0.6	22.5 ± 0.4	0.748
Fiber (g/MJ)	2.6 ± 0.0	2.6 ± 0.1	2.6 ± 0.1	2.5 ± 0.1	0.538
Alcohol (en%)[b]	2.3 ± 0.1	1.8 ± 0.1	2.0 ± 0.1	1.6 ± 0.1[§]	0.011
Protein (en%)	15.7 ± 0.2	15.7 ± 0.2	15.2 ± 0.3	15.8 ± 0.2	0.401
Animal-based, % of total	58.4 ± 0.7	58.4 ± 1.0	57.1 ± 1.3	59.5 ± 0.8	0.475
Plant-based, % of total	41.6 ± 0.7	41.6 ± 1.0	42.9 ± 1.3	40.5 ± 0.8	0.481

Differences between tissue-specific IR groups were assessed using ANCOVA with adjustment for sex and with Bonferroni *post-hoc* pairwise comparisons (adjusted mean ± SE).

[a] data were logtransformed to improve normality and reported as geometric means

[b] a constant was added before logtransformation to eliminate zero values

[§] significantly different from No MIR/LIR ($p<0.05$)

[†] significantly different from MIR ($p<0.05$)

FFQ, Food Frequency Questionnaire; MJ, megajoule; en%, energy percentage of total energy intake.

IR phenotypes (p=0.411). Energy intake tended to be lower in the MIR group compared to the other groups when adjusted for sex (Table 3; p=0.062). Intake of energy from saturated fat was highest in the combined MIR/LIR group, although only statistically significantly higher compared to the MIR group. Other components of macronutrient composition of habitual dietary intake, expressed as en%, did not differ between the IR phenotypes when adjusted for sex. Alcohol consumption was lower in the combined MIR/LIR group compared to No MIR/LIR (overall p=0.011).

DISCUSSION

The purpose of the present article was to describe the study design of the PERSON study and to present preliminary screening results. In the PERSON study, individuals are classified based on IR phenotype at baseline, and randomized to follow a hypothesized optimal or suboptimal diet according to their metabolic phenotype. This study is one of the first randomized double- blind controlled trials in the field of precision nutrition to investigate whether a dietary intervention based on tissue-specific insulin sensitivity improves metabolic health to a greater extent compared to a hypothesized suboptimal diet.

Dietary intervention

Both intervention diets prescribed in this study are largely in line with the Dutch dietary guidelines of the Health Council of the Netherlands (36). Data from the FFQ indicated that the habitual dietary intake of our study population did not meet these guidelines. In particular, average fiber intake (2.6 g/MJ) was well below the recommended 3.4 g/MJ, and lower than the targeted fiber intake of 3 g/MJ and 4 g/MJ in the HMUFA and LFHP interventions diets, respectively. In addition, average intake of calories from saturated fat (14 en%) exceeded the <10 en% that is recommended. In our study, prescribed intake of saturated fat and mono- and disaccharides, which is similar between the two interventions diets, is lower than the average habitual intake. Therefore, we expect that on average, participants will benefit from both dietary interventions, regardless of their IR phenotype. Nevertheless, we hypothesize to find greater improvements in glucose homeostasis and related outcomes in study participants that follow the anticipated optimal compared to suboptimal diet.

The hypothesis that dietary macronutrient composition interacts with tissue-specific IR is supported by findings from recent studies. A *post-hoc* analysis of the CORDIOPREV-DIAB study indicated that individuals with predominant MIR had a greater improvement

in disposition index on a 2-year Mediterranean diet, while individuals with predominant LIR benefitted more from a diet high in complex carbohydrates and low in fat (18). In addition, individuals with LIR have been shown to have a more detrimental fasting plasma lipid profile (13) and impaired postprandial lipoprotein metabolism following high-fat meals (70) compared to individuals with MIR, which suggests that a low-fat diet may be especially beneficial for individuals with LIR (71). Furthermore, findings from other studies indicate that a high protein diet and high fiber diet may have beneficial effects for individuals with LIR, as both high protein and high fiber diets have been shown to successfully reduce liver fat content (72-75). Liver fat accumulation is linked to decreased suppression of hepatic glucose production in some studies (74, 76), linking liver fat to LIR, although the cause-effect relationship remains to be established. Moreover, increased fiber intake has been shown to improve insulin sensitivity in individuals with IFG but not IGT (77). IFG is characterized mainly by impaired hepatic insulin sensitivity, (78, 79), which is in line with observations in our study that individuals with IFG are most often characterized as LIR.

In addition, dietary fat quality may impact skeletal muscle lipid handling. In an acute study, meals high in saturated fat resulted in increased postprandial skeletal muscle fatty TAG extraction and/or reduced intramyocellular lipid turnover compared to meals high in unsaturated FAs in insulin resistant individuals, which was accompanied by a lower postprandial insulin sensitivity (80). Taken together, a “one-size-fits-all” approach with population-wide dietary guidelines may not be optimal for metabolic health for all individuals. A diet targeting tissue-specific IR is expected to increase the effectiveness of dietary interventions with respect to improvements in glucose homeostasis.

Changes in macronutrient composition within the context of an isocaloric diet can improve risk factors for cardiometabolic diseases, independent of weight loss (81). The two diets implemented in the PERSON study differ in macronutrient composition and are both matched to the participants’ individual energy requirements in order to maintain weight stability during the dietary intervention. Throughout the study, participants’ body weight is monitored weekly, and adjustments in absolute energy intake, but not diet composition, are made if needed to maintain body weight. We provide key food products, perform unannounced food records, and conduct weekly check-ins with skilled dieticians and researchers, together increasing the incentive to adhere to the diet and the possibility to assess dietary compliance.

Extensive and detailed phenotyping

A strength of the PERSON-study is the extensive and detailed phenotyping of the study

participants before and after the dietary intervention. This allows us to comprehensively study the metabolic underpinnings of the metabolic response to the dietary intervention. Next to performing highly standardized metabolic phenotyping in a laboratory setting, we also collect data in free-living conditions. Furthermore, in a subgroup of the study population several additional measurements such as the gold-standard hyperinsuliemic-euglycemic clamp are performed, which allows us to investigate the mechanisms involved in the pathophysiology of tissue-specific IR as well as how these may be affected by the dietary intervention.

Next to detailed metabolic phenotyping, we also collect data on mood, perceived well-being, food preferences and cognitive function. There are indications that blood glucose levels may be an important determinant of mood and cognitive function (19, 21, 82, 83). Additionally, gut microbial profile, which can be modulated by dietary intake, is linked to cognitive function and mood via the gut-brain axis (84, 85). Hence, by improving glucose homeostasis and metabolic health with a dietary intervention, individuals may also experience short-term benefits related to mental and emotional well-being and performance. Such directly perceivable benefits are expected to motivate individuals to better adhere to dietary advice.

In addition, the large amount of collected data will allow for the application of computational techniques to elucidate the inter-individual differences in glucose homeostasis and derive new functional insights. Both mechanistic and data-driven computational modelling approaches have been employed to expand on the physiological properties underlying meal responses (6, 7, 86). The frequently-sampled time series of metabolites (e.g. glucose, insulin) from the OGTT and continuous glucose monitoring will be used to construct models of short-term postprandial dynamics, facilitating the assessment of individuals' capacity to regulate glucose levels in response to a meal. The detailed phenotypic information can be integrated using machine- learning models to derive a comprehensive model of glucose homeostasis. The data generated in the PERSON study will enable such computational methods to progress the field of precision nutrition.

Preliminary screening data

Tissue-specific or whole-body IR (either MIR, LIR or combined) was prevalent in ~60% of the population, which is similar to the reported prevalence of 65% in DMS (16). The prevalence of LIR in this study was lower as compared to DMS (11% vs. 17%, respectively). This can possibly be partly explained by the higher proportion of women in the PERSON study compared to DMS (59% vs. 44%, respectively), since LIR is less prevalent in women

than men. Sexual dimorphism in glucose homeostasis and IR is well-recognized and has been linked to differences in relation to hormonal status, lipid handling and inflammatory profile (87), but does require further investigation. These data emphasize that future analyses within the PERSON study should also take sex-specific effects into account.

As expected based on the formulas used to classify MIR and LIR, our preliminary screening data confirmed that both MIR and LIR are related to worse glucose homeostasis compared to individuals without MIR or LIR, in line with observations from DiOGenes and DMS (16, 22). Interestingly, however, the majority of individuals with MIR and LIR (71-75%) were classified as normal glucose tolerant. Classical cutoff values only including plasma glucose levels may fail to detect important metabolic impairments related to insulin action, especially in early stages of disease development, while these disturbances are well known to be highly predictive for the development of cardiometabolic diseases later in life (88, 89). Identification of metabolic impairments at an early stage before the onset of dysglycemia creates an important window of opportunity to use lifestyle interventions such as dietary modulation in order to delay or prevent further glycemic deterioration and progression to cardiometabolic disease.

CONCLUSION

The PERSON study is one of the first double-blind, randomized trials in the field of precision nutrition to investigate the effects of a more personalized dietary intervention based on tissue- specific insulin resistance phenotype, on metabolic health outcomes at the functional and molecular level, mental performance and perceived well-being. The high prevalence of tissue- specific IR in adults with overweight and obesity highlights the relevance of investigating the effects of targeted dietary approaches in order to define more optimal diets to improve glucose homeostasis, thereby preventing or delaying the development of cardiometabolic diseases. The PERSON study is expected to contribute knowledge on the effectiveness of targeted nutritional strategies to the emerging field of precision nutrition and enhance the understanding of the complex etiology of generalized and tissue-specific IR.

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SUPPLEMENTARY MATERIAL

Table S1. Inclusion and exclusion criteria for the PERSON study

Inclusion criteria Men and women aged 40-75 y BMI 25-40 kg/m2 Body weight stability for at least 3 months (no weight change >3kg) Predominantly muscle (MIR) or liver (LIR) insulin resistant
Exclusion criteria <u>Diseases</u> • Pre-diagnosis of type 1 or type 2 diabetes mellitus • Renal or hepatic malfunctioning (pre-diagnosis or determined based on alanine aminotransferase [ALAT], aspartate aminotransferase [ASAT] and creatinine values) • Major gastrointestinal diseases or major abdominal surgery • Cardiovascular diseases (e.g. heart failure) or cancer • High blood pressure (untreated >160/100 mmHg, drug-regulated >140/90 mmHg) • Diseases affecting glucose and/or lipid metabolism (e.g. pheochromocytoma, Cushing's syndrome, acromegaly) • Anemia defined as hemoglobin (Hb) men <8.5 and women <7.5 mmol/l • Diseases with a life expectation shorter than 5 years • Major mental disorders • Drug treated thyroid diseases (well-substituted hypothyroidism is allowed) <u>Medication</u> • Medication known to interfere with study outcomes (e.g. peroxisome proliferator-activated receptor-α [PPAR-α] or PPAR-γ agonists [fibrates], sulfonylureas, biguanides, α-glucosidase inhibitors, thiazolidinediones, repaglinide, nateglinide and insulin, chronic use of NSAIDs) • Use of anticoagulants other than acetylsalicyclic acid • Use of antidepressants (stable use ≥3 months prior to and during the study is allowed) • Use of statins (stable use ≥3 months prior to and during study allowed) • Use of β-blockers (only for the extensive phenotyping participants)

- Chronic corticosteroids treatment (>7 consecutive days of treatment)
- Use of antibiotics within 3 months prior to the study

Lifestyle
- Participation in regular sports activities (>4 hours per week)
- Abuse of alcohol (alcohol consumption >14 units/week) and/or drugs (cannabis included)
- Regular smoking (including use of e-cigarettes)

Other
- Pregnant or lactating women who are planning to become pregnant
- Inability to comply with the study diet

Table S2. Ingredients and macronutrient composition of the high-fat mixed meal

	Full-fat milk	Whipped cream	Sugar	Whipped ice cream	Total per meal
Amount per meal (g)	125	70	5	150	350
Energy (kJ)	347.5	973.0	85	1387.5	2793.0
Protein (g)	4.5	1.5	0	5.6	11.6
Fat (g)	4.5	24.6	0	19.5	48.6
Saturated fat (g)	3.1	17.5	0	12.8	33.4
Carbohydrates (g)	5.9	2.2	5	34.5	47.5
Sugar (g)	5.9	2.2	5	31.5	44.5

Table S3. Overview of standardized products provided during at-home days and amounts provided per energy group.

		Nutrients per 100 g						Amounts (g) provided per energy group		
Meal moment (time frame)	**Product**	**Energy (kJ)**	**Fat (g)**	**Saturated fat (g)**	**Protein (g)**	**Carbohydrates (g)**	**Sugars (g)**	**6-8 MJ**	**9-11 MJ**	**12-13 MJ**
Breakfast (7am-9am)	Drink yogurt	247	0.8	0.5	3.4	8.1	7.3	400	400	400
	Gingerbread	1304	1.1	0.4	2.9	69.6	37.1	28	28	28
Snack (10am-11am)	Raisin cake	1785	21.3	6.8	6.3	51.7	35.0	60	60	60
	Banana	401	0.3	0.1	1.1	20.6	15.5	130	130	130
	Apple juice	194	0.0	0.0	0.1	11.2	10.5	200	200	200
	Wheat bread	1000	1.8	0.4	9.8	42.9	2.0	56	84	112
	Cream cheese	1540	33.3	10.7	14.0	2.7	2.0	15	30	30
Lunch	Hazelnut spread	2347	35.3	9.3	6.0	54.0	50.0	15	15	30
	Semi-skimmed milk	192	1.5	1.0	3.4	4.7	4.7	200	200	200
	Yogurt with strawberry sauce	368	2.0	1.3	4.0	13.0	11.0	190	190	190
Snack (3pm-4pm)	Apple	254	0.2	0.0	0.3	13.0	10.4	135	135	135
	Potato chips	2261	33.2	5.7	6.4	52.5	1.4	28	28	28
	Lemonade	170	0.1	0.0	0.1	9.7	9.6	200	200	VZ
Dinner (6pm-7pm)	Macaroni meal	447	3.6	1.2	5.6	12.1	2.0	350	450	550

Nutrient composition was calculated using the 2016 Dutch Food Composition Table kJ, kilojoule; MJ, megajoule

Table S4. Macronutrient composition of standardized meal moments during home-days per energy group

Energy group	Meal moment (time frame)	Energy (kJ)	Fat (g)	Saturated fat (g)	Protein (g)	Carbohydrates (g)	Sugars (g)
6-8 MJ	Breakfast (7am-9am)	1353	3.5	2.1	14.4	51.9	39.6
	Snack (10am-11am)	1980	13.2	4.2	5.4	80.2	62.2
	Lunch (12am-1pm)	2226	18.1	7.7	22.8	66.6	39.2
	Snack (3pm-4pm)	1316	9.8	1.6	2.4	51.6	33.7
	Dinner (6pm-7pm)	1565	12.7	4.2	19.7	42.2	6.9
	Total	**8440**	**57.3**	**19.8**	**64.7**	**292.5**	**181.6**
9-11 MJ	Breakfast (7am-9am)	1353	3.5	2.1	14.4	51.9	39.6
	Snack (10am-11am)	1980	13.2	4.2	5.4	80.2	62.2
	Lunch (12am-1pm)	2738	23.6	9.4	27.6	79.1	40.1
	Snack (3pm-4pm)	1316	9.8	1.6	2.4	51.6	33.7
	Dinner (6pm-7pm)	2012	16.3	5.4	25.3	54.3	8.9
	Total	**9399**	**66.4**	**22.7**	**75.1**	**317.1**	**184.5**
12-13 MJ	Breakfast (7am-9am)	1353	3.5	2.1	14.4	51.9	39.6
	Snack (10am-11am)	1980	13.2	4.2	5.4	80.2	62.2
	Lunch (12am-1pm)	3369	29.4	10.9	31.2	99.2	48.2
	Snack (3pm-4pm)	1316	9.8	1.6	2.4	51.6	33.7
	Dinner (6pm-7pm)	2459	19.9	6.6	30.9	66.3	10.9
	Total	**10477**	**75.8**	**25.4**	**84.3**	**349.2**	**194.6**

Nutrient composition was calculated using the 2016 Dutch Food Composition Table kJ, kilojoule; MJ, megajoule

Table S5. Glucose homeostasis parameters derived from OGTT according to insulin resistance phenotype

	No MIR/LIR (n=227)	MIR (n=121)	LIR (n=61)	Combined MIR/LIR (n=156)	*p*-value
Fasting glucose (mmol/L)	5.5 (5.4, 5.6)	5.4 (5.3, 5.5)	5.6 (5.4, 5.7)	5.6 (5.5, 5.7)†	0.005
Fasting insulin (pmol/L)	38.7 (36.6, 41.0)	51.6 (47.8, 55.8)§	56.1 (50.4, 62.6)§	89.3 (83.4, 95.6)§†‡	<0.001
2-hr glucose (mmol/L)	5.9 (5.7, 6.2)	6.9 (6.5, 7.3)§‡	5.9 (5.5, 6.4)†	7.0 (6.6, 7.3)§‡	<0.001
2-hr insulin (pmol/L)	220.6 (200.3, 242.9)	488.8 (428.0, 557.9)§‡	316.3 (262.7, 381.0)§†	759.6 (676.2, 853.7)§†‡	<0.001
iAUC glucose (AU)	203 (187, 219)	242 (217, 270)	255 (219, 297)	285 (259, 314)§	<0.001
iAUC insulin (AU)	27612 (25918, 29409)	47512 (43522, 51822)§	56855 (50334, 64236)§	92554 (85757, 99915)§†‡	<0.001
HOMA-IR (AU)	1.4 (1.3, 1.5)	1.8 (1.6, 1.9)§	2.0 (1.8, 2.3)§	3.2 (3.0, 3.5)§†‡	<0.001
HOMA-β (AU)	58.0 (54.9, 61.2)	82.1 (76.2, 88.6)§	79.7 (71.7, 88.7)§	124.2 (116.2, 132.7)§†‡	<0.001
Matsuda index (AU)	16.7 (15.7, 17.8)	10.8 (9.9, 11.8)§	9.6 (8.5, 10.9)§	5.7 (5.3, 6.2)§†‡	<0.001
Disposition index (AU)	362 (336, 390)	331 (299, 367)‡	465 (403, 537)§†	350 (320, 383))‡	0.002
MISI (AU)	0.217 (0.202, 0.232)	0.060 (0.055, 0.067)§‡	0.148 (0.129, 0.170)§†	0.049 (0.045, 0.054)§†‡	<0.001
HIRI (AU)	253 (240, 266)	334 (312, 359)§‡	712 (645, 787)§†	840 (789, 894)§†‡	<0.001

Differences between tissue-specific IR groups were assessed using ANCOVA with adjustment for sex and Bonferroni *post-hoc* pairwise comparisons. Data were logtransformed to improve normality and reported as adjusted geometric means with 95% confidence interval.

§ significantly different from No MIR/LIR ($p<0.05$)

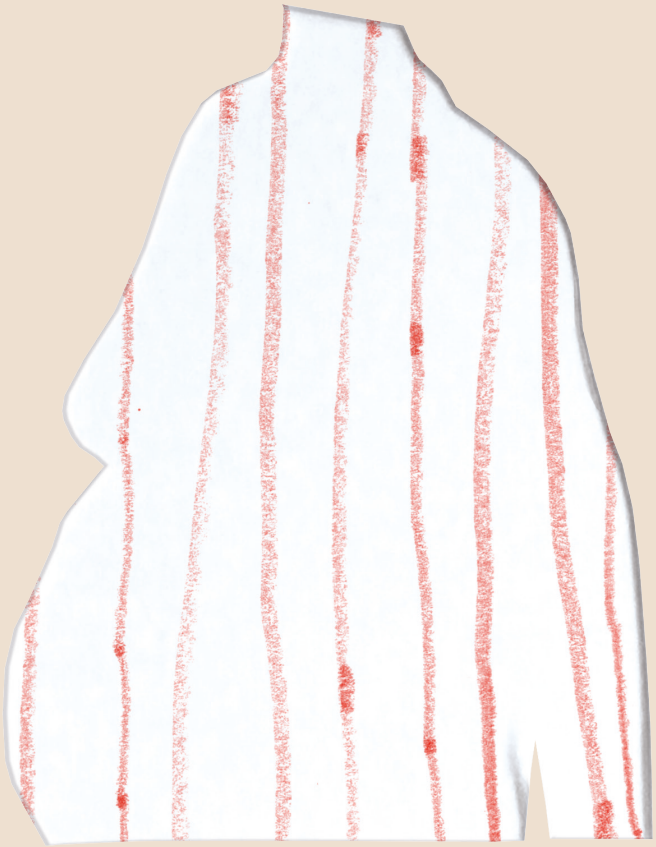
† significantly different from MIR ($p<0.05$)

‡ significantly different from LIR ($p<0.05$)

OGTT, oral glucose tolerance test; iAUC, incremental area under the curve; AU, arbitrary units; HOMA-IR, homeostasis model assessment of insulin resistance; HOMA-β, homeostasis model assessment of β-cell function; MISI, muscle insulin sensitivity; HIRI, hepatic insulin resistance.

Chapter 5

Circulating and adipose tissue immune cells in tissue-specific insulin resistance in humans with overweight and obesity



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ABSTRACT

The development of obesity is accompanied by dynamic changes in immune cell populations in adipose tissue (AT), resulting in a pro-inflammatory AT microenvironment that may contribute to obesity-related cardiometabolic diseases. These perturbations in AT may have differential effects on liver and skeletal muscle insulin sensitivity. We investigated the relationship between abdominal subcutaneous AT (aSAT) and circulating immune cells (n=81), aSAT gene expression (n=91), plasma inflammatory markers, and liver and skeletal muscle insulin sensitivity (n=219) in individuals with overweight and obesity in the PERSON study. The relative abundances of circulating T cells, non-classical monocytes, classical monocytes and CD56dimCD16+ NK cells were inversely associated with liver, but not skeletal muscle insulin sensitivity. The inverse association between the abundance of circulating (classical) monocytes and liver insulin sensitivity was confirmed in The Maastricht Study (n=273). aSAT immune cell populations were not associated with tissue-specific insulin sensitivity. However, aSAT gene expression of IL-6 and CD14 was positively associated with muscle, but not liver, insulin sensitivity. No consistent associations were observed between plasma inflammatory markers and tissue-specific insulin sensitivity. The present findings demonstrate that circulating immune cell populations as well as inflammatory gene expression in aSAT show distinct associations with liver and muscle insulin sensitivity.

INTRODUCTION

Obesity is closely associated with the development of chronic diseases such as cardiovascular disease, type 2 diabetes (T2D), and certain types of cancer (1). The excessive adipose tissue (AT) mass in obesity, resulting from a prolonged positive energy balance, is often accompanied by AT dysfunction (2-4). AT dysfunction in obesity is characterized by a pro-inflammatory phenotype of both subcutaneous AT (SAT) and visceral AT (VAT), which may contribute to insulin resistance and cardiometabolic complications (2, 5).

AT dysfunction is characterized by increased production of pro-inflammatory factors by the enlarged adipocytes as well as by dynamic changes in AT immune cell populations, together contributing to the shift from an anti-inflammatory towards a more pro-inflammatory AT microenvironment during the development of obesity (6, 7). Indeed, a growing repertoire of innate and adaptive immune cells such as monocytes, macrophages, B and T cells, natural killer (NK) cells, and their subtypes, have been reported to populate metabolic organs, including AT, in obesity (4, 8). Previous studies in rodents and humans have shown that pro- inflammatory factors produced both by adipocytes and by resident and recruited immune cells in the enlarged AT impair metabolic pathways within the AT, which is linked to low-grade systemic inflammation and impaired lipid and glucose metabolism in organs such as the liver and skeletal muscle (2, 3, 5). Thus, perturbations in the inflammatory AT phenotype may impair whole-body metabolic homeostasis in obesity, thereby contributing to the development of insulin resistance, cardiovascular diseases, and T2D (8-11).

It is well established that insulin resistance can develop simultaneously in multiple organs, but the severity may vary between organs (12-15). Likewise, AT inflammation may have distinct effects on insulin sensitivity in different metabolic tissues (11, 14). Indeed, recent findings from our laboratory demonstrated that individuals with predominant skeletal muscle insulin resistance (MIR) displayed higher inflammatory gene expression in abdominal SAT (aSAT) compared to individuals with primarily liver insulin resistance (LIR) (14). In line, in two independent cohorts, plasma markers of low-grade inflammation were associated with MIR, but not with LIR (14). Importantly, however, aSAT and circulating immune cells were not determined in the latter study. Thus, the relationship between immune cell subsets in aSAT and in the circulation, and tissue-specific insulin resistance in individuals with overweight or obesity remains elusive.

In the present study, we investigated the relationship between aSAT and circulating immune cell populations, aSAT inflammation, low-grade systemic inflammation, and liver and skeletal muscle insulin sensitivity in individuals with overweight and obesity from the two-center PERSonalized Glucose Optimization Through Nutritional Intervention (PERSON) study (16). Moreover, we aimed to replicate our findings by performing complementary analyses in a large population-based cohort, The Maastricht Study (17).

RESEARCH DESIGN AND METHODS

Study design and participants

Data was collected from the two-center, randomized dietary intervention trial, the PERSonalized Glucose Optimization Through Nutritional Intervention (PERSON) study (16). An extensive description of the PERSON study design, objectives, and methods are described elsewhere (16). We analyzed cross-sectional data collected at baseline from 219 individuals who participated in the PERSON study. Individuals (40-75 years) with overweight or obesity (Body Mass Index (BMI): 25-40 kg/m^2) and no weight gain or loss >3 kg prior 3 months of inclusion were included. Based on an oral glucose tolerance test (OGTT) performed at screening, individuals with either predominant LIR or MIR were included. Exclusion criteria included pre-diagnoses of diabetes, major cardiovascular disease, liver or kidney disease, medication affecting glucose and/or lipid metabolism, major gastrointestinal disease, uncontrolled hypertension, alcohol abuse (>14 glasses/week), smoking, and dietary restrictions interfering with the dietary study protocol. The PERSON study was approved by the Medical Ethics Committee of MUMC+ (NL63768.068.17), registered at ClinicalTrials.gov (identifier: NCT03708419). The study was carried out in accordance with the principles of the Declaration of Helsinki.

To validate findings in the PERSON study population, we used data from the population-based cohort The Maastricht Study. The Maastricht Study focuses on the etiology of T2D, its classic complications, and its emerging comorbidities. The methodology and rationale for this study have been described previously (17). The Maastricht study was approved by the institutional Medical Ethical committee (NL31329.068.10) and the Netherlands Health Council (Permit 131088-105234-PG). We selected participants from The Maastricht Study aged between 40- 75 years and with a BMI between 25-40 kg/m^2, without diabetes, who did not use glucose- lowering medication and who did not smoke. The present analysis included 1256 participants from The Maastricht Study for whom data on tissue-specific insulin sensitivity and plasma inflammatory markers were available, of whom 273 participants with additional data on

immune cells in whole blood, as described in more detail below.

Anthropometrics and body composition

Body weight (kg), height (cm), and waist and hip circumferences (cm) were determined in duplicate to the closest 0.1 unit. A dual-energy X-ray absorptiometry was performed in both studies to assess whole-body fat percentage.

Tissue-specific insulin sensitivity

A 7-point OGTT (75 g glucose 200 ml solution (Novolab)) was performed after an overnight fast (>10h). Venous blood was drawn at t = 0, 15, 30, 45, 60, 90, 120 minutes, and plasma glucose and insulin concentrations were determined for all time points. Tissue-specific insulin sensitivity was estimated using the HIRI and MISI for quantification of LIR and MIR, respectively. HIRI was calculated by the following formula: glucose 0-30 [AUC in mmol/L x h] x insulin 0-30 [AUC in pmol/L x h]. MISI was calculated as follows: (dGlucose/dt) / insulin [mean during OGTT in pmol/L]. In the calculation for MISI, dGlucose/dt is the rate of decay of plasma glucose concentration (mmol/L) during the OGTT, calculated as the slope of the least square fit to the decline in plasma glucose concentration from peak to nadir (18). The MISI calculation was optimized using the cubic spline method (19). Importantly, a higher HIRI indicates worse hepatic insulin sensitivity, while a higher MISI indicates better skeletal muscle insulin sensitivity.

Abdominal subcutaneous adipose tissue biopsy

After an overnight fast, an aSAT biopsy (~1.5 g) was collected 6–10 cm lateral from the umbilicus under local anesthesia (lidocaine 1%, without adrenaline) in the PERSON study only. The tissue samples were immediately rinsed with sterile saline. A minimum of 0.7 g of tissue was placed in 10 ml Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (DMEM/F-12) solution for flow cytometry analysis, and another part was snap-frozen in liquid nitrogen and stored in -80°C until analysis of aSAT gene expression.

Flow cytometry analysis of abdominal subcutaneous adipose tissue and whole blood
Flow cytometry was performed in aSAT for the identification of immune cells by isolation of the stromal vascular fraction (SVF) in the PERSON study (n=81). Fasted whole-blood was used for analysis of circulating immune cells in both the PERSON study and The Maastricht Study. The panels of antibodies used in the PERSON study are reported in Supplementary Table 1. The gating strategies for flow cytometry of whole-blood and aSAT are reported in Supplementary Figure 1 and 2, respectively. The antibodies and gating strategy for flow cytometry for The Maastricht Study were similar, as reported elsewhere

(20). All samples were measured with a FACS-Canto II (BD Biosciences) and analyzed with FACSdiva software (BD Biosciences). Fluorescence minus one controls were performed during panel design and an auto-fluorescence control was performed for each sample. Data are expressed as percentage of live immune cells.

Gene expression in abdominal subcutaneous adipose tissue in the PERSON study

aSAT gene expression analysis (n=91) was performed in the PERSON study using RT-qPCR, as described previously (21). In short, RNA was precipitated and purified and subsequently cDNA was synthesized and quantified by RT-qPCR using an iCycler (Bio-Rad). Gene expression was normalized to 18S and the delta CT method was used for calculating relative expression. Gene expression of several adipokines (adiponectin, DPP4, IL-6, leptin, PAI-I, TNF-α), lipolytic markers (ABHD5/CGI-58, ATGL, GOS2, HSL, PLIN1), oxidative metabolism markers (NDUFB5, NDUFA1, NDUFB3, NDUFC2, ATP6V1A, ATP6V1H, ACADM, ACADVL, ACADL, CPT2, CS, UCP2) and immune cell markers (CD11b, CD11c, hMR, CD14, CD64, CD68) were determined. Primer sequences are provided in Supplementary Table 2.

Plasma inflammatory markers

In the PERSON study participants, the plasma inflammatory markers C-reactive protein (CRP), serum amyloid A (SAA), cluster of differentiation 163 (CD163), plasminogen activator inhibitor- 1 (PAI-1), and tumor necrosis factor ligand superfamily member 12 (TNFSF12) were quantified using Luminex immunoassays (performed by DSM Nutritional Products, Kaiseraugst, Switzerland). In The Maastricht Study participants, circulating CRP and SAA concentrations were determined using Mesoscale discovery multiplex assay.

Statistical analysis

Participant characteristics are presented as mean ± standard deviation (SD) for normally distributed values. Not normally distributed values are reported as median ± interquartile range (IQR). Multiple linear regression analyses were performed to assess the association between immune cell populations, inflammatory markers, or aSAT gene expression (independent variables) with liver and skeletal muscle insulin sensitivity (as continuous dependent variables). The fully adjusted models are reported with adjustment for sex, age, BMI, and use of lipid lowering medication, antihypertensives, and antidepressants. We performed a sensitivity analysis in The Maastricht Study to further adjust for potential differences between the PERSON study and The Maastricht Study population. We additionally adjusted for the presence of major cardiovascular disease, inflammatory bowel disease, chronic respiratory diseases, and cancer. Visual inspection

of residual diagnostics was performed, and non- normality was corrected with log10 transformation. The independent variables were standardized by calculating a Z-score to allow for direct comparison of effect sizes. Standardized betas (Std. β) with 95% confidence intervals (CIs) are reported. The statistical analyses were performed using the IBM SPSS Statistics software (version 25). Significance is set at $P < 0.05$.

RESULTS

Participant characteristics

From the PERSON study, data on plasma inflammatory markers were available in 219 participants and additional data on aSAT and circulating immune cell populations were available in a subgroup of 81 participants (Table 1). Due to the relatively large difference in group size, participant characteristics of both of these groups are reported. The subgroup showed comparable population characteristics as the total population, except for having a slightly higher BMI (31.6 ± 3.7 vs. 30.0 ± 3.5 kg/m^2, respectively). Mean age of the total PERSON study population was 60.1 ± 7.8 years. The majority of participants were women (57%). The majority of study participants (76%) was characterized as normal glucose tolerant according to the WHO criteria (fasting glucose <6.1 mmol/L and 2-hr glucose <7.8 mmol/L) (22).

Association between adipose tissue and circulating immune cells and tissue-specific insulin sensitivity

The aSAT immune cell populations were not associated with either HIRI or MISI (all $P > 0.05$) in the PERSON study (Table 2). The relative abundances of several circulating immune cells, specifically T cells (Std. β with 95% CI: 0.28 [0.06; 0.49]), non-classical monocytes (0.38 [0.17; 0.60]), classical monocytes (0.40 [0.17; 0.64]), and CD56dimCD16+ NK cells (0.28 [0.04; 0.51]) were positively associated with HIRI in the PERSON study. These associations indicate that a higher relative abundance of these immune cells in whole-blood was related to more pronounced hepatic insulin resistance (i.e. worse liver insulin sensitivity). Blood granulocytes were inversely associated with HIRI (i.e., a higher abundance of blood granulocytes was related to less liver insulin resistance and hence to better liver insulin sensitivity). Furthermore, a positive association between relative abundance of granulocytes and MISI (0.41 [0.16; 0.66]) was observed, while no significant associations were observed for any of the other blood immune cells and MISI. Further adjustment for MISI (in the model with HIRI as the dependent variable) and HIRI (in the model with MISI as the dependent variable) yielded similar associations (data not reported).

Table 1. Participant characteristics of the PERSON study and The Maastricht Study

	PERSON study		The Maastricht Study	
	Total group (n=219)	Subgroup with additional immune cell data (n=81)	Total group (n=1256)	Subgroup with additional immune cell data (n=273)
Women (n, %)	124 (57%)	48 (59%)	511 (41%)	129 (47%)
Age (years)	60.1 ± 7.8	59.6 ± 7.6	60.7 ± 8.0	61.3 ± 8.2
BMI (kg/m$^{2)}$	30.0 ± 3.5	31.6 ± 3.7	28.9 ± 3.1	29.2 ± 3.3
Body fat (%)	37.2 ± 7.5	38.7 ± 7.3	34.7 ± 7.0	35.6 ± 6.6
Waist (cm)	102.3 ± 9.5	103.6 ± 11.3	101.0 ± 10.0	101.2 ± 10.7
Waist-to-hip (ratio)	0.939 ± 0.088	0.935 ± 0.103	0.965 ± 0.085	0.960 ± 0.092
Medication use (n, %)				
Lipid lowering	15 (7%)	1 (1%)	494 (39%)	116 (43%)
Antihypertensives	37 (18%)	14 (17%)	525 (43%)	123 (45%)
Antidepressants	14 (6%)	8 (10%)	66 (5%)	15 (6%)
Glucose status (n, %)				
NGT	166 (76%)	55 (68%)	940 (75%)	213 (78%)
IFG	9 (4%)	2 (3%)	62 (5%)	8 (3%)
IGT	30 (13%)	15 (19%)	154 (12%)	33 (12%)
T2D – without GL medication	14 (6%)	9 (11%)	100 (8%)	19 (7%)
Fasting glucose (mmol/L)	5.3 [5.0-5.7]	5.5 [5.2-5.9]	5.7 [5.2-6.6]	5.7 [5.3-6.8]
Fasting insulin (pmol/L)	51.2 [39.0-63.8]	54.7 [39.9-67.5]	74.1 [52.1-108.9]	78.0 [53.4-124.6]
HIRI (A.U.)	389 [289-553]	408 [308-539]	458 [311-674]	504 [330-754]
MISI (A.U.)	0.121 [0.084-0.190]	0.126 [0.088-0.190]	0.118 [0.072-0.197]	0.108 [0.068-0.184]

Data are mean ± standard deviations for normally distributed values, median [IQR] for skewed variables, or n (%) for categorical data. Population characteristics are shown separately for the total groups and for individuals in which immune cells were available due to the large difference in population size. Glucose status classification was based on WHO criteria (22), NGT, normal glucose tolerance; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; T2D, type 2 diabetes.

Table 2. Adjusted associations between blood and abdominal subcutaneous adipose tissue immune cell populations and tissue-specific insulin sensitivity in the PERSON study and The Maastricht Study.

	HIRI				MISI			
	PERSON study		The Maastricht Study		PERSON study		The Maastricht Study	
	Std β (95% CI)	*P*-value	Std β (95% CI)	*P*-value	Std β (95% CI)	*P*-value	Std β (95% CI)	*P*-value
Blood immune cells								
Granulocytes	-0.229 (-0.448; -0.010)	**0.041**	0.024 (-0.108; 0.155)	0.723	0.412 (0.164; 0.660)	**0.002**	-0.051 (-0.215; 0.114)	0.546
B cells	0.196 (-0.032; 0.424)	0.090	-0.148 (-0.270; -0.026)	**0.018**	-0.114 (-0.390; 0.163)	0.414	0.000 (-0.163; 0.163)	0.998
T cells	0.275 (0.062; 0.488)	**0.012**	-0.105 (-0.246; 0.035)	**0.141**	-0.198 (-0.461; 0.065)	0.138	0.079 (-0.113; 0.270)	0.419
Total monocytes	0.378 (0.144; 0.613)	**0.002**	0.154 (0.031; 0.276)	0.014	-0.029 (-0.332; 0.274)	0.850	-0.038 (-0.196; 0.119)	0.631
Non-classical monocytes	0.383 (0.168; 0.598)	**0.001**	0.102 (-0.029; 0.233)	0.127	-0.055 (-0.338; 0.229)	0.702	-0.013 (-0.179; 0.152)	0.874
Intermediate monocytes	0.197 (-0.037; 0.432)	0.098	0.112 (-0.008; 0.232)	0.067	0.044 (-0.246; 0.333)	0.762	-0.016 (-0.174; 0.142)	0.842
Classical monocytes	0.403 (0.168; 0.638)	**0.001**	0.131 (0.004; 0.257)	**0.044**	0.029 (-0.279; 0.337)	0.852	-0.044 (-0.205; 0.117)	0.590
NK cells	0.241 (0.014; 0.469)	**0.038**	0.034 (-0.081; 0.149)	0.560	-0.233 (-0.507; 0.042)	0.096	-0.054 (-0.203; 0.095)	0.474
CD56brightCD16- NK cells	0.102 (-0.127; 0.331)	0.376	0.000 (-0.123; 0.123)	1.000	0.039 (-0.236; 0.313)	0.779	-0.027 (-0.182; 0.128)	0.731
CD56dimCD16+ NK cells	0.276 (0.037; 0.514)	**0.024**	0.004 (-0.122; 0.131)	0.945	-0.228 (-0.519; 0.064)	0.124	-0.031 (-0.193; 0.131)	0.706
aSAT immune cells								
Total macrophages + monocytes	0.222 (-0.032; 0.476)	0.085			-0.135 (-0.398; 0.129)	0.311		
CD11C-CD206+ macrophages	0.021 (-0.252; 0.295)	0.876			-0.066 (-0.353; 0.221)	0.648		
CD11C+CD206+ macrophages	-0.058 (-0.306; 0.191)	0.645			-0.032 (-0.290; 0.227)	0.806		
NK cells	0.089 (-0.164; 0.342)	0.487			0.073 (-0.186; 0.333)	0.575		

Standardized Beta's (95% confidence intervals) are reported for the association between immune cells expressed as % of live cells and tissue-specific insulin sensitivity (n=81). A linear regression analysis was performed with adjustment for age, sex, body mass index, and medication use (lipid lowering, antihypertensives, and antidepressants). HIRI, hepatic insulin resistance index; MISI, muscle insulin sensitivity index; NK, natural killer; CD, cluster of differentiation; aSAT, abdominal subcutaneous adipose tissue. Significant *p*-values (<0.05) are highlighted in bold.

In The Maastricht Study, we also observed a positive association between circulating monocytes (0.15 [0.03; 0.28]), specifically classical monocytes (0.13 [0.00; 0.26]), and HIRI (Table 2), thus confirming our findings in the PERSON study population. In contrast, the relative abundance of circulating B cells was inversely associated with HIRI (-0.15 [-0.27; - 0.03]), indicating that a higher abundance of B cells was related to better liver insulin sensitivity. Other circulating immune cells were not associated with HIRI. Additionally, no significant associations were observed between the relative abundances of immune cells in whole-blood and MISI (all $P > 0.05$).

Abdominal subcutaneous adipose tissue gene expression is related to muscle, but not liver, insulin sensitivity in the PERSON study Gene expression of adipokines, lipolytic, oxidative metabolism, and immune cell markers in aSAT was determined in the PERSON study. In the fully adjusted model, we found inverse associations between aSAT expression of the pro-inflammatory marker IL-6 (-0.23 [-0.45; - 0.01], $P = 0.043$) and the immune cell marker CD14 (-0.27 [-0.50; -0.04], $P = 0.022$) with MISI (Fig. 1). These associations indicate that higher expression of these genes is associated with lower skeletal muscle insulin sensitivity. In contrast, none of the genes determined in aSAT were associated with HIRI.

Association between plasma inflammatory markers and tissue-specific insulin sensitivity

Plasma concentration of the inflammatory marker PAI-1 was positively associated with HIRI (0.14 [0.00; 0.28]). Plasma SAA (-0.18 [-0.31: -0.05]) was inversely associated with HIRI, while and a trend for inverse associations between plasma CRP (-0.14 [-0.28; 0.00]) and TNFSF12 (-0.13 [-0.26; 0.00]) concentrations, and HIRI was observed (Table 3). In The Maastricht Study, however, we did not observe significant associations between plasma SAA and CRP, and HIRI None of the plasma inflammatory markers were associated with MISI in either the PERSON study or The Maastricht Study.

DISCUSSION

In the present study, we investigated the relationship between systemic and aSAT inflammation in relation to liver and skeletal muscle insulin sensitivity in individuals with overweight and obesity. The present findings demonstrate that a higher relative abundance of several circulating immune cell populations, specifically classical monocytes, were indicative of worse liver but not skeletal muscle insulin sensitivity. Furthermore, we found that inflammatory gene expression in aSAT was related to skeletal muscle insulin

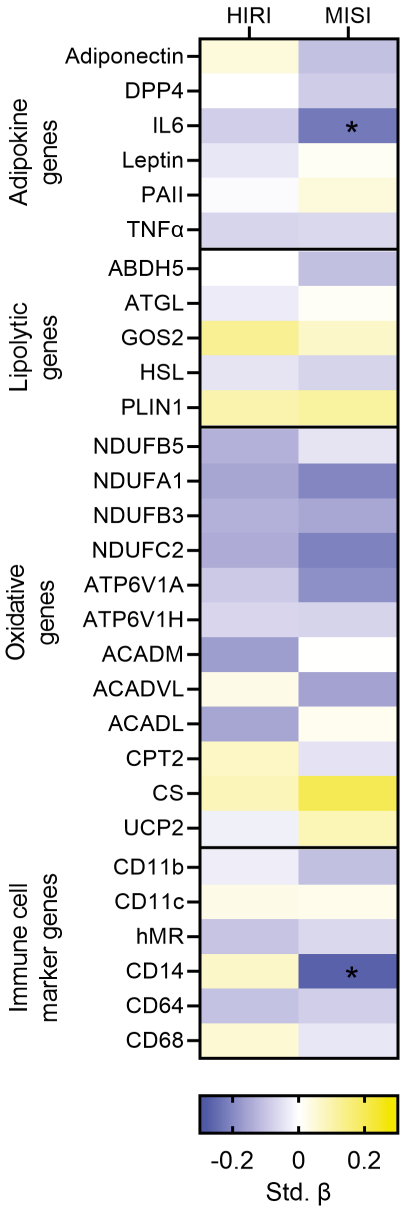

Figure 1. Associations between abdominal subcutaneous adipose tissue gene expression and the hepatic insulin resistance index (HIRI) and muscle insulin sensitivity index (MISI) (n = 91) in the PERSON study. Higher HIRI indicates lower liver insulin sensitivity, while lower MISI indicates lower muscle insulin sensitivity. Yellow indicates a positive standardized beta while blue indicates a negative standardized . Gene expression is categorized into the expression of adipokines, lipolytic, oxidative metabolism, and immune cell markers. Data is adjusted for age, sex, body mass index, and medication use (lipid lowering, antihypertensives, and antidepressants). * p<0.05.

Table 3. Associations between inflammatory markers and tissue-specific insulin sensitivity in the PERSON study and The Maastricht Study

	HIRI				MISI			
	PERSON study		The Maastricht Study		PERSON study		The Maastricht Study	
	Std β (95% CI)	*P*-value	Std β (95% CI)	*P*-value	Std β (95% CI)	*P*-value	Std β (95% CI)	*P*-value
CRP	-0.137 (-0.279; 0.004)	0.056	0.030 (-0.099; 0.159)	0.646	-0.109 (-0.251; 0.034)	0.134	-0.065 (-0.233; 0.102)	0.443
SAA	-0.183 (-0.312: -0.053)	**0.006**	0.062 (-0.072; 0.196)	0.361	-0.052 (-0.188; 0.085)	0.457	-0.145 (-0.320; 0.030)	0.105
TNFSF12	-0.127 (-0.258; 0.004)	0.057			-0.068 (-0.205; 0.068)	0.325		
CD163	0.058 (-0.075; 0.191)	0.393			0.087 (-0.052; 0.226)	0.221		
PAI1	0.141 (0.006; 0.276)	**0.041**			-0.057 (-0.198; 0.083)	0.422		

Standardized effect sizes (95% confidence intervals) are reported. A linear regression analysis was performed with adjustment for age, sex, body mass index, and medication use (lipid lowering, antihypertensives, antidepressants, and glucose lowering medication (Maastricht Study)) PERSON study, n=219; The Maastricht Study, n=1566). HIRI, hepatic insulin resistance index; MISI, muscle insulin sensitivity index. Significant *p*-values (<0.05) are highlighted in bold.

sensitivity, while aSAT immune cell populations were not associated with liver and skeletal muscle insulin sensitivity. Finally, plasma inflammatory markers showed distinct associations with liver and skeletal muscle insulin sensitivity, although this could not be validated in The Maastricht Study. Taken together, the present findings demonstrate that circulating immune cell populations as well as inflammatory gene expression in aSAT show distinct associations with liver and skeletal muscle insulin sensitivity, which may have implications for strategies (i.e. precision nutrition and pharmacological interventions) to prevent and/or treat obesity-related complications.

Circulating T cells, non-classical monocytes, classical monocytes and CD56dimCD16+ NK cells were associated with worse liver, but not skeletal muscle insulin sensitivity, in the PERSON study. We were able to confirm the association of monocytes, specifically classical monocytes, with worse liver insulin resistance in The Maastricht Study. Yet, we could not confirm the associations between other immune cell populations and liver insulin sensitivity. The reason for the latter remains to be established but may related to inherent differences between a randomized trial (PERSON study) and a population-based cohort (The Maastricht Study), resulting in differences in participant selection. Although the individuals from The Maastricht Study included in the present analysis were selected to resemble the PERSON study participants with respect to age, BMI, non-smoking, and no use of glucose lowering medication, and we adjusted for medication use, the participants of The Maastricht Study still had a slightly unhealthier metabolic profile. A sensitivity analysis in The Maastricht Study to further adjust for history of major cardiovascular disease, inflammatory bowel disease, chronic respiratory disease, and cancer did not alter the conclusion. The reason for these partly discrepant findings remains to be established but may relate to inherent differences between the study populations and potential residual confounding when comparing a randomized trial (PERSON study) and a population-based cohort (The Maastricht Study).

Although we found associations between circulating immune cells and liver insulin sensitivity, aSAT immune cell populations were not associated with liver and/or skeletal muscle insulin sensitivity. The latter seems in line with the observation that immune cells in VAT, but not aSAT, were associated with whole-body insulin resistance and impaired glucose homeostasis (23, 24), and the higher abundance of M1 macrophages and NK cells in VAT, but not aSAT, of individuals with obesity compared to normal weight (25). Importantly, the liver may be impacted to a larger extent by VAT than the skeletal muscle. More specifically, in contrast to skeletal muscle, which predominantly drains from the systemic circulation and is largely affected by SAT, the liver drains directly from the portal

vein. Therefore, the liver is directly impacted by products released from VAT and the gut (26). However, whether the (relative abundance of) immune cells in VAT rather than SAT are more closely linked to impaired liver insulin sensitivity remains elusive. Furthermore, some circulating immune cells (i.e., non- classical monocytes and surface markers on NK cells) have been associated with their counterparts in VAT in previous studies (25, 27). This may suggest that certain circulating immune cells included in the present analysis predominantly reflect VAT immune cells, which might explain the association between circulating immune cells and liver insulin sensitivity in the present study. Unfortunately, data on VAT immune cells are not available to confirm this hypothesis. Thus, although the underlying mechanism remains to be established, we show that circulating, but not aSAT, immune cells are specifically linked to liver, but not muscle, insulin resistance.

Another explanation for the observed associations between circulating immune cells, in particular classical monocytes, and impaired liver insulin sensitivity may be that the (fatty) liver, just like the AT, skeletal muscle, pancreas, and gut, may directly contribute to the composition and quantity of circulating immune cells as well as low-grade systemic inflammation in obesity (2, 8, 11). Liver resident macrophages (Kupffer cells) often become activated when obesity develops, thereby activating inflammatory pathways in the liver that may in turn exert autocrine, paracrine, and/or endocrine effects (28). Liver inflammation may be the result of AT inflammation or lipotoxicity in the liver (29). Notably, these processes are closely related to liver insulin resistance (11, 28, 30). Together, these findings suggest that multiple (patho)physiological processes that can affect circulating immune cells, systemic low-grade inflammation, hepatic lipid accumulation and inflammation and (liver) insulin sensitivity operate simultaneously. Therefore, we cannot exclude that the associations between circulating immune cells and liver insulin resistance that we found in the present analyses may at least partly be due to liver inflammation.

Interestingly, we found that gene expression of IL-6 and CD14 in aSAT was positively associated with muscle but not liver insulin sensitivity in the PERSON study. These findings are in agreement with results from the Diet, Obesity, and Genes (DiOGenes) study, showing that a pro-inflammatory gene expression profile in aSAT was present in individuals with MIR, but not with LIR, compared to individuals with normal insulin sensitivity (14). Furthermore, we subsequently confirmed these findings in a *post-hoc* analysis within the Cohort on Diabetes and Atherosclerosis Maastricht (CODAM) and The Maastricht Study demonstrating that a low- grade inflammatory Z-score (based on plasma IL-6, IL-8, TNF-α, SAA, sICAM, CRP, haptoglobin, and ceruloplasmin concentrations) was inversely associated with muscle, but not liver, insulin sensitivity (14). We could not confirm an

association of plasma inflammatory markers with tissue-specific insulin sensitivity in the present analyses, which may be a result of inclusion of different individual inflammatory markers and the smaller sample size of the PERSON study. Our findings, together with previous observations, indicate that aSAT inflammation is more strongly related to muscle, rather than liver, insulin sensitivity. This may be related to the blood supply of skeletal muscle, which drains to a large extent from the systemic circulation, which in turn is largely affected by the aSAT. Additionally, tissue-specific differences in molecular pathways affecting insulin signaling may determine to what extent inflammation affects insulin sensitivity. Indeed, obesity-related low-grade inflammation may impact insulin sensitivity via various mechanisms, including inhibition of insulin signaling by among others, the activation of Jun N-terminal kinase (JNK) and nuclear factor (NF)-κβ (31, 32), suppression of AMPK activity (33), and downregulation of PPAR-γ (31). It is tempting to speculate that SAT-derived pro-inflammatory factors may have more pronounced effects on insulin signaling in skeletal muscle as compared to the liver. Clearly, further studies are warranted to examine this in more detail.

The strengths of the present study are that PERSON study and The Maastricht Study participants have been phenotyped in detail, allowing for detailed investigation of tissue-specific insulin sensitivity and the inflammatory phenotype. Furthermore, next to data from the PERSON study, we used a replication cohort (The Maastricht Study) to investigate the associations between circulating immune cells, systemic low-grade inflammation, and tissue- specific insulin sensitivity, and were able to confirm the inverse associations between classical monocytes and liver insulin sensitivity in The Maastricht Study. The present study also has some limitations. Since the weight of the AT biopsies was not available, we were not able to quantify the absolute number of immune cells in aSAT yet were able to determine the relative abundances of immune cells. Nevertheless, quantitative expression of immune cells (i.e., per gram of AT) may lead to inaccuracies in determining immune cell numbers due to (differences in) adipocyte size. Secondly, we did not collect VAT samples due to the invasiveness of this procedure and related medical-ethical issues. Future studies are warranted to explore the relationship between immune cells in VAT, circulating immune cells and tissue-specific insulin sensitivity. Finally, HIRI and MISI are OGTT-derived measures. Although HIRI and MISI have been validated against the gold-standard two-step hyperinsulinemic-euglycemic clamp, the postprandial glucose and insulin responses are also affected by inter-individual differences in gastro-intestinal factors, including the rate of glucose absorption and the incretin response (34).

In conclusion, we demonstrated that circulating immune cell populations, specifically the abundance of (classical) monocytes, were associated with worse liver insulin sensitivity in individuals with overweight and obesity. No associations were found between immune cell populations in aSAT and tissue-specific insulin resistance. Moreover, pro-inflammatory aSAT gene expression was inversely associated with muscle but not liver insulin sensitivity. Collectively, the present findings show distinct associations between immune cells and inflammation with liver and skeletal muscle insulin sensitivity, which may have implications for more personalized lifestyle and pharmacological interventions.

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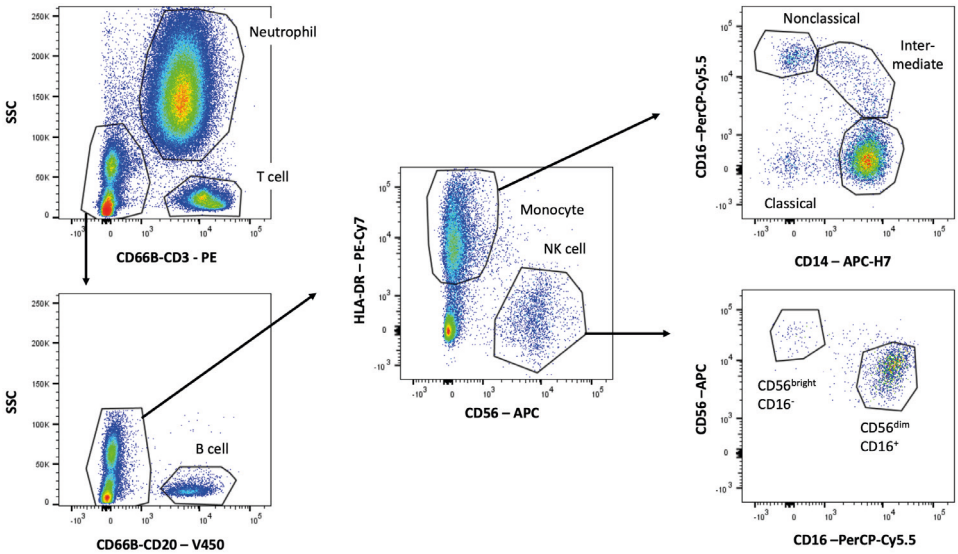
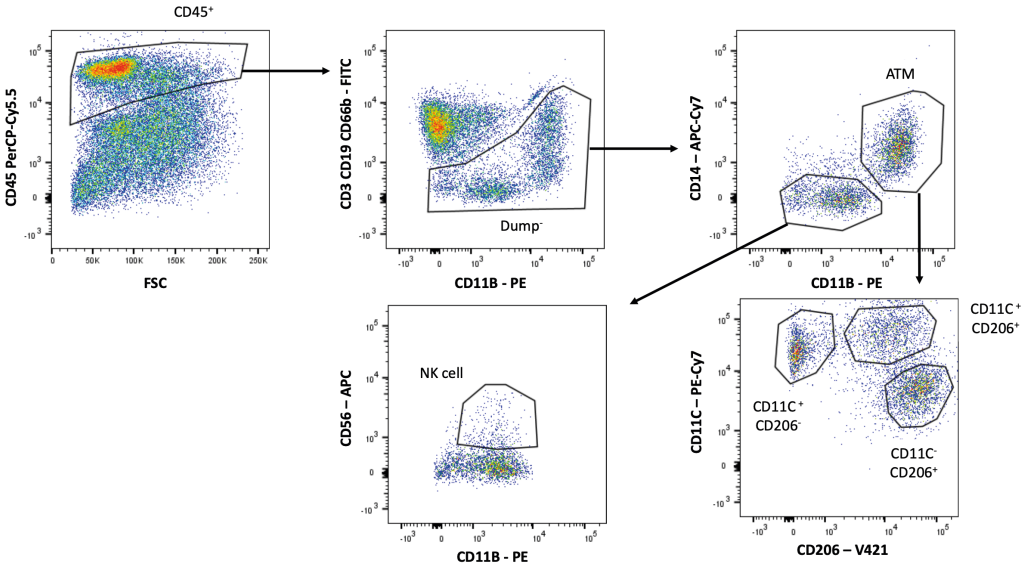
SUPPLEMENTARY MATERIAL

Supplementary Figure 1. Gating strategies Whole Blood starting from single, live cells.



Supplementary Figure 2. Gating strategy abdominal subcutaneous adipose tissue, starting from single, live cells.



Supplementary Table 1. Panel of antibodies for whole blood and subcutaneous adipose tissue in the PERSON study

	Antibody	Label	Clone	Supplier
Whole blood	CD3	PE	UCHT1	BD
	CD66b	PE	G10F5	BD
	CD16	PerCP-Cy5.5	3G8	BD
	HLA-DR	PE-Cy7	G46-6	BD
	CD56	APC	HCD56	Biolegend
	CD14	APC-H7	MPHIP9	BD
	CD20	V450	L27	BD
	Viability	V500		
Subcutaneous adipose tissue	CD3	FITC	UCHT1	BD
	CD66B	FITC	G10F5	BD
	CD19	FITC	HIB19	BD
	CD11B	PE	ICRF44	BD
	CD45	PerCP-Cy5.5	HI30	BD
	CD14	APC-H7	MPHIP9	BD
	CD11C	PE-Cy7	B-ly6	BD
	CD206	BV421	19.2	BD
	CD56	APC	HCD56	Biolegend

Supplementary Table 2. Forward and reverse primers of abdominal subcutaneous adipose tissue genes measured with RT-qPCR

Gene	Forward primer (5' => 3')	Reverse primer (5' => 3')
18S	AGTTAGCATGCCAGAGTCTCG	TGCATGGCCGTTCTTAGTTG
Adiponectin	TGGTGAGAAGGGTGAGAA	AGATCTTGGTAAAGCGAATG
DPP4	AGTGGCGTGTTCAAGTGTGG	CAAGGTTGTCTTCTGGAGTTGG
IL-6	AAATTCGGTACATCCTCGACGG	GGAAGGTTCAGGTTGTTTTCTGC
Leptin	GCTGTGCCCATCCAAAAAGTCC	CCCAGGAATGAAGTCCAAACCG
PAI-1	TCGTCCAGCGGGATCTGAA	GCCGTTGAAGTAGAGGGCATT
TNF-α	CCGAGTGACAAGCCTGTAGC	GAGGACCTGGGAGTAGATGAG
ABHD5	CAGCATCCAGTCCTTACGACCA	GTTCAGTCCACAGTGTCGCAGA
ATGL	GTGTCAGACGGCGAGAATG	TGGAGGGAGGGAGGGATG
GOS2	CGCCGTGCCACTAAGGTC	GCACACAGTCTCCATCAGGC
HSL	GCGGATCACACAGAACCTGG	AGCAGGCGGCTTACCCT
PLIN1	CTCTCGATACACCGTGCAGA	TGGTCCTCATGATCCTCCTC
NDUFB5	GCTGCTCCTGTTCGACACA	CTGCTAGTTCAGCTTGACCAAT
NDUFA1	GGACTGGCTACTGCGTACATC	GCGCCTATCTCTTTCCATCAGA
NDUFB3	GCTGGCTGCAAAAGGGCTA	CTCCTACAGCTACCACAAATGC
NDUFC2	TGTATGCTGTGAGGGACCGTGA	ACAGCAGGTATCAGTGAAACTGG
ATP6V1A	GAGATCCTGTACTTCGCACTGG	GGGGATGTAGATGCTTTGGGT
ATP6V1H	CAGAAGTTCGTGCAAACAAAGTC	TCAGGGCTTCGTTTCATTTCAA
ACADM	TGGATAACCAACGGAGGAAAAG	CTGGGGTATCTGCTTCCACA
ACADVL	ACAGATCAGGTGTTCCCATACC	CTTGGCGGGATCGTTCACT
ACADL	AGGGGATCTGTACTCCGCAG	CTCTGTCATTGCTATTGCACCA
CPT2	CATACAAGCTACATTTCGGGACC	AGCCCGGAGTGTCTTCAGAA
CS	TGCTTCCTCCACGAATTTGAAA	CCACCATACATCATGTCCACAG
UCP2	GGAGGTGGTCGGAGATACCAA	ACAATGGCATTACGAGCAACAT
CD11b	AGAGCCATCAATCAAGAAGGC	CTTGCAGTGAGAACACGTATG
CD11c	TGTACCTCACCGGACTCTG	GAACTGGCTTATCACAGCTCT
hMR	CGATCCGACCCTTCCTTGAC	TGTCTCCGCTTCATGCCATT
CD14	GCAGCCGAAGAGTTCACAAG	CAGCAGCAACAAGCAGGAC
CD64	GGGTTATACTGGTGCGAGGC	GACATGAAACCAGACAGGAGT
CD68	CTGGGGCAGAGCTTCAGTT	GATGAGAGGCAGCAAGATGG

Supplementary Table 3. Extended baseline characteristics. Relative immune cells presence between tissue- specific insulin resistant phenotypes in the PERSON study

	PERSON study	The Maastricht Study
Blood immune cells		
Granulocytes	51.6 [35.8; 59.5]	57.2 [52.2; 63.9]
B cells	3.15 [2.09; 4.85]	6.18 [4.30; 8.09]
T cells	29.2 [22.2; 38.4]	25.1 [19.7; 30.7]
Total monocytes	8.64 [6.71; 10.67]	5.42 [4.59; 6.28]
Non-classical monocytes	0.75 [0.49; 1.25]	0.75 [0.57; 1.06]
Intermediate monocytes	0.29 [0.19; 0.47]	0.36 [0.27; 0.51]
Classical monocytes	6.54 [6.16; 8.42]	4.16 [3.57; 4.97]
NK cells	3.02 [2.17; 4.39]	3.82 [2.66; 5.16]
CD56brightCD16- NK cells	0.14 [0.10; 0.23]	0.12 [0.09; 0.17]
CD56dimCD16+ NK cells	2.64 [1.79; 3.94]	3.58 [2.37; 4.81]
aSAT immune cells		
Total macrophages + monocytes	13.5 [10.5; 18.5]	
CD11C-CD206+ macrophages	1.26 [0.52; 3.02]	
CD11C+CD206+ macrophages	2.32 [1.07; 4.04]	
NK cells	2.17 [1.47; 3.31]	

Data are expressed a median % of live cells [IQR]. NK, natural killer; CD, cluster of differentiation; aSAT, abdominal subcutaneous adipose tissue.

Chapter 6

Cardiometabolic health improvements upon dietary intervention are driven by tissue-specific insulin resistance phenotype: a precision nutrition trial

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ABSTRACT

Precision nutrition based on metabolic phenotype may increase the effectiveness of interventions. In this proof-of-concept study, we investigated the effect of modulating dietary macronutrient composition according to muscle (MIR) or liver insulin resistant (LIR) phenotypes on cardiometabolic health. Individuals with MIR or LIR (n = 242) were randomized to Phenotype Diet (PhenoDiet) group A or B. PhenoDiet group A included individuals with MIR following a 12-week high-monounsaturated fatty acid diet (HMUFA) and individuals with LIR following a low-fat, high-protein, high-fiber diet (LFHP). PhenoDiet group B included individuals with MIR on LFHP and individuals with LIR on HMUFA. PhenoDiet group B showed no significant improvements in the disposition index, but greater improvements in insulin sensitivity, glucose homeostasis, serum triacylglycerol, and C-reactive protein compared to PhenoDiet group A. We demonstrate for the first time that modulation of macronutrient composition within the dietary guidelines based on tissue-specific IR phenotype enhances cardiometabolic health improvements.

INTRODUCTION

The unprecedented prevalence of obesity and related cardiometabolic disturbances calls for effective prevention strategies. A well-known strategy to improve cardiometabolic health is healthy nutrition, even in the absence of weight loss (1,2). Nevertheless, a considerable proportion of individuals does not show clinically relevant improvements upon a dietary intervention (3-5). These differential responses to diet may be explained by inter-individual heterogeneity in both exogenous and endogenous factors such as sex, dietary habits, gut microbiota composition, and metabolic phenotype (6, 7). Precision nutrition based on individual traits may increase the effectiveness of dietary interventions to improve metabolic health (8).

There are indications that parameters related to glucose metabolism and insulin action or resistance, such as plasma glucose and insulin concentrations and indices based on these concentrations, may predict the response to dietary modification (5, 9, 10). Importantly, insulin resistance (IR) can develop separately in insulin-sensitive tissues such as skeletal muscle and the liver, representing different etiologies towards cardiometabolic diseases. We have recently shown that individuals with more pronounced liver IR (LIR) have a distinct metabolome (11), lipidome (12), adipose tissue transcriptome (13), and systemic inflammatory profile (13) compared to individuals with more pronounced muscle IR (MIR). Therefore, individuals with these distinct tissue-specific IR phenotypes may respond differentially to dietary intervention.

Indeed, in a *post-hoc* analysis of the CORDIOPREV-DIAB study, individuals with predominant MIR responded more favorably to a diet high in monounsaturated fatty acids (MUFAs), while individuals with predominant LIR responded more favorably to a low-fat, high-complex carbohydrate diet with regard to the disposition index, a composite marker of whole-body insulin sensitivity and insulin secretion (14). In addition, both high-protein (15-17) and high-fiber diets (18), as well as the Mediterranean diet (19, 20), have been shown to reduce liver fat content, which in turn may improve hepatic insulin sensitivity (21, 22). Furthermore, dietary fat quality may specifically impact skeletal muscle lipid metabolism and peripheral insulin sensitivity (23). Importantly, however, well-designed, prospective, randomized, isocaloric dietary intervention trials to test the effectiveness of precision nutrition based on tissue-specific IR phenotype are currently lacking.

In the present PERSonalized Glucose Optimization Through Nutritional Intervention (PERSON) study (24), we investigated the efficacy of modulation of dietary macronutrient

composition according to MIR and LIR phenotypes on parameters of glucose homeostasis, cardiometabolic health, health-related quality of life, and perceived well-being. We hypothesized that individuals with the MIR phenotype would benefit most from a diet rich in MUFA, and individuals with the LIR phenotype from a diet low in fat and rich in protein and fiber. Interestingly, the present findings demonstrate that individuals with the MIR phenotype showed a more pronounced cardiometabolic health improvement upon a low-fat, high-protein, and high-fiber (LFHP) diet, while individuals with the LIR phenotype had the greatest cardiometabolic health benefit from a high-monounsaturated fatty acid (HMUFA) diet. Although not in concert with the initial hypothesis, these findings for the first time provide the proof-of-concept that modulating dietary macronutrient composition based on tissue-specific IR phenotype with healthy, isocaloric diets can induce more pronounced, clinically relevant improvements in cardiometabolic health, independent of changes in body weight.

RESULTS

Study design and participant characteristics

Between May 2018 and November 2021, 990 men and women aged 40-75 years and with a body mass index (BMI) 25-40 kg/m^2 were enrolled, of whom 877 were fully screened for eligibility (Fig. S1). At screening, tissue-specific insulin resistance was assessed using the muscle insulin sensitivity index (MISI) and hepatic insulin resistance index (HIRI), which were calculated from the plasma glucose and insulin responses during a 7-point OGTT (25, 26). Tertile cut-offs for MISI and HIRI from a previous study (the Maastricht study (11, 27)) were used to identify individuals with predominant MIR or LIR.

In total, 242 participants (123 at Maastricht University Medical Center+ [MUMC+] and 119 at Wageningen University [WUR]) were included and randomized to PhenoDiet group A or B (n = 121 in both groups). PhenoDiet group A included individuals with MIR following a high-monounsaturated fatty acid (HMUFA) diet and individuals with LIR following a low-fat, high-protein, and high-fiber (LFHP) diet. PhenoDiet group B included individuals with MIR following a LFHP diet and individuals with LIR following a HMUFA diet. The targeted macronutrient composition of both diets is described in Table S1. The dietary intervention strategy was based on weekly dietary counselling and provision of key products. Both diets were in line with the Dutch dietary guidelines, and we aimed for both diets to be eucaloric to keep participants on a stable body weight throughout the study. At baseline (week 0) and after 12 weeks of dietary intervention, participants underwent extensive metabolic phenotyping in a characterization week (Fig. 1).

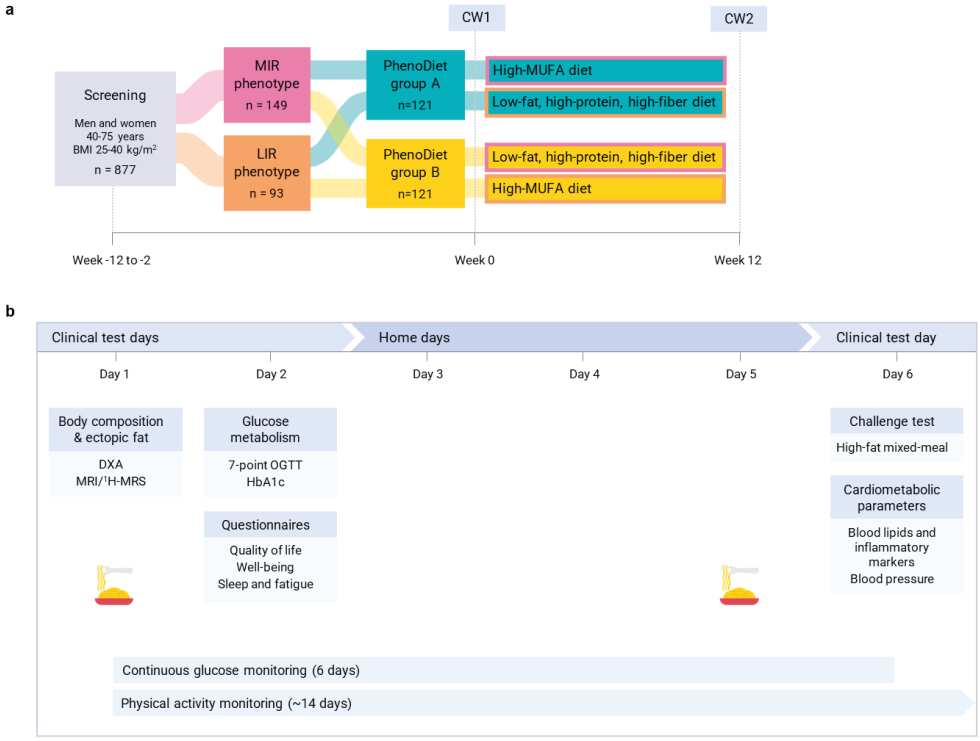

Fig 1. Study design of the PERSON study. Tissue-specific insulin resistance was assessed at screening using a 7-point oral glucose tolerance test. Individuals with predominant muscle insulin resistance (MIR) or liver insulin resistance (LIR) were randomized to a Phenotype Diet (PhenoDiet) group A or B (A). PhenoDiet group A consisted of individuals with MIR following a high-monounsaturated fatty acids (HMUFA), and individuals with LIR following a low-fat, high-protein, and high-fiber (LFHP) diet. PhenoDiet group B consisted of individuals with LIR following a HMUFA diet and MIR following a LFHP diet. In clinical investigation week (CIW) 1 and 2, in week 0 and 12, respectively, participants underwent several clinical, and at-home measurements (B).

Overall, 58% of the randomized participants were women, mean age was 60 years and mean BMI 29.9 kg/m². Baseline characteristics were well balanced in the two groups (Table 1). The majority of participants (76%) was considered normal glucose-tolerant at baseline according to fasting and 2-hour glucose levels in response to an oral glucose tolerance test (OGTT). Baseline characteristics with stratification for IR phenotype and diet intervention are described in Table S2. BMI was slightly higher in individuals with the

Table 1. Baseline characteristics of participants allocated to PhenoDiet group A or B

	PhenoDiet group A (n = 121)	**PhenoDiet group B** (n = 121)
MIR/LIR phenotype, n	76/45	73/48
Age, years	60 ± 8	60 ± 8
Women, n (%)	66 (54.5%)	75 (62.0%)
BMI, kg/m^2	30.1 ± 3.5	29.8 ± 3.5
Medication use, n (%)		
Antidepressants	5 (4.1%)	12 (9.9%)
Antihypertensives	27 (22.3%)	16 (13.2%)
Anti-inflammatory medication	14 (11.6%)	9 (7.4%)
Statins	9 (7.4%)	7 (5.8%)
Other	42 (34.7%)	37 (30.6%)
Family history of diabetes (%)	22 (18.2%)	32 (26.4%)
Glucose status (%) (n = 240)		
NGT	94 (79.0%)	88 (72.7%)
IFG	5 (4.2%)	4 (3.3%)
IGT	12 (10.1%)	16 (13.2%)
Combined IFG/IGT	3 (2.5%)	4 (3.3%)
T2D	5 (4.2%)	9 (7.4%)
Habitual physical activity, Baecke score	8.4 ± 1.2	8.3 ± 1.2
Employment status (%) (n = 236)		
Paid job	69 (59.5%)	55 (45.8%)
Retired	34 (29.3%)	43 (35.8%)
Other	13 (11.2%)	22 (18.3%)
Education level (%) (n = 235)		
Low	17 (14.7%)	18 (15.1%)
Intermediate	44 (37.9%)	48 (40.3%)
High	55 (47.4%)	53 (44.5%)

Values are n (%), mean ± SD or median [IQR] if not normally distributed.
MIR, muscle insulin resistance; LIR, liver insulin resistance; BMI, body mass index; NGT, normal glucose tolerance; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; T2D, type 2 diabetes.

LIR compared to MIR phenotype (P MIR vs. LIR = 0.037) and use of anti-inflammatory medication was higher in MIR compared to LIR (P MIR vs. LIR = 0.041).

In PhenoDiet group A, 94% (n =114 of 121) and in PhenoDiet group B 88% (n =107 of 121) completed the study (Fig. S1). Twenty-two participants (13 in PhenoDiet group A, 9 in PhenoDiet group B) completed the study according to an adjusted protocol employed during the COVID-19 lockdown (only limited post-intervention measurements; See Methods). No major difference between the characteristics of completers and drop-outs were observed at baseline (Table S3).

Habitual dietary intake at baseline was comparable between PhenoDiet group A and B

Self-reported habitual dietary intake before start of the intervention was assessed with a food frequency questionnaire (FFQ). After exclusion of data due to energy under- (n = 27) and overreporting (n = 1), FFQ data from 213 participants were included in these analyses. Habitual dietary intake was comparable between the groups, except for energy intake, which was higher in PhenoDiet group A (median [IQR]; 9.6 [7.8, 10.9] MJ) compared to PhenoDiet group B (8.6 [7.4, 10.6] MJ) (Table S4). Average intakes of calories from fat, protein, and carbohydrates were 37.7%, 15.6%, and 41.5%, respectively.

Adherence to the MHUFA and LFHP diets was high, with no differences between PhenoDiet group A and B

Compliance to the dietary interventions was evaluated with three 1-day food records that were randomly requested for throughout weeks 2-11 of the intervention via a mobile app (28), as well as with pre- and post-measurement of plasma fatty acid profile. After exclusion of data from 20 participants (MIR – HMUFA n = 10; LIR – LFHP n = 2; LIR – HMUFA n = 4; MIR – LHFP n = 4) due to energy underreporting, food record data from 206 participants were included in these analyses. Advised macronutrient composition of the two intervention diets and reported intake can be found in Table S5. Macronutrient composition of the two different intervention diets were comparable in PhenoDiet group A and B. Individuals randomized to the HMUFA diet reported higher intake of calories from total fat and MUFA, lower intake of protein and fiber, and similar intake of saturated fat (SFA) and carbohydrates compared to those on the LFHP diet. The contribution of MUFA to total plasma fatty acid concentrations increased in individuals on the HMUFA diet, while it decreased in those on the LFHP diet (Table S6). Plasma SFA concentrations were reduced after both diets.

Table 2. Primary and secondary outcomes at baseline and after 12 weeks in PhenoDiet groups A and B

		PhenoDiet group A (n = 121)		PhenoDiet group B (n = 121)		*P*-value		
	n*	week 0	week 12	week 0	week 12	Group	Time	Group x Time
Glucose metabolism								
Disposition index (AU)	199	412 (369 - 460)	406 (365 - 451)	357 (321 - 398)	380 (343 - 423)	0.068	0.640	0.109
Fasting glucose (mmol/L)	199	5.3 (5.2 - 5.5)	5.3 (5.2 - 5.4)	5.5 (5.3 - 5.6)	5.3 (5.2 - 5.4)	0.179	0.146	0.238
Fasting insulin (pmol/L)	199	47.5 (44.0 - 51.4)	46.0 (42.4 - 49.9)	52.7 (48.9 - 56.9)	46.0 (42.4 - 49.9)	0.063	0.285	**0.019**
2-hr glucose (mmol/L)	199	6.1 (5.8 - 6.5)	6.2 (5.8 - 6.5)	6.5 (6.1 - 6.9)	6.1 (5.8 - 6.5)	0.123	0.561	**0.020**
2-hr insulin (pmol/L)	199	349.7 (308.3 - 396.3)	337.0 (297.9 - 381.1)	397.0 (350.8 - 449.8)	322.9 (285.1 - 365.6)	0.154	0.569	**0.023**
HOMA-IR (AU)	199	1.6 (1.5 - 1.8)	1.6 (1.4 - 1.7)	1.8 (1.7 - 2)	1.6 (1.4 - 1.7)	0.052	0.203	**0.017**
HOMA-β (AU)	199	76.5 (71.3 - 81.8)	76.2 (70.8 - 82)	79.8 (74.5 - 85.5)	73.9 (68.5 - 79.6)	0.301	0.931	0.079
Matsuda index (AU)	199	4.8 (4.4 – 5.3)	5.1 (4.6 – 5.6)	4.2 (3.9 – 4.6)	5.1 (4.6 – 5.5)	**0.032**	0.150	**0.004**
Insulinogenic index (AU)	199	32.2 (29.6 - 35)	30.4 (27.8 - 33.2)	32.3 (29.8 - 35.1)	28.8 (26.4 - 31.5)	0.957	0.072	0.234
MISI (AU)	191	0.123 (0.11 - 0.138)	0.130 (0.114 - 0.147)	0.116 (0.104 - 0.13)	0.151 (0.133 - 0.171)	0.424	0.583	**0.038**
HIRI (AU)	198	383 (348 - 421)	346 (311 - 385)	404 (367 - 444)	340 (305 - 378)	0.505	**0.021**	0.253
HbA1c (mmol/mol)	199	36.0 (35.2 - 36.7)	36.0 (35.4 - 36.7)	36.5 (35.7 - 37.2)	35.9 (35.2 - 36.6)	0.635	0.976	0.091
Anthropometrics								
Weight (kg)	221	86.9 (84.9 - 88.9)	85.2 (83.4 - 87.1)	87.9 (85.9 - 89.7)	85.5 (83.6 - 87.5)	0.408	**<0.001**	0.224
Waist circumference (cm)	221	101.2 (99.5 - 102.8)	99.1 (97.7 - 100.7)	102.4 (100.9 - 104)	100.5 (99.1 - 102.1)	0.187	**<0.001**	0.789
Waist-to-hip ratio	221	0.93 (0.92 - 0.94)	0.92 (0.91 - 0.94)	0.94 (0.93 - 0.95)	0.94 (0.92 - 0.95)	0.156	0.149	0.947
Body composition								
Body fat mass (%)	195	36.1 (35.2 - 37.1)	35.0 (34.0 - 36.1)	37.0 (36.1 - 37.9)	35.4 (34.4 - 36.4)	0.186	**<0.001**	0.078
Body fat mass (kg)	195	31.4 (30.1 - 32.8)	29.8 (28.4 - 31.3)	32.7 (31.3 - 34)	30.5 (29.1 - 31.9)	0.193	**<0.001**	0.058
Lean body mass (kg)	195	51.6 (50.6 - 52.7)	51.4 (50.4 - 52.5)	52.0 (51.1 - 53.1)	52.0 (50.9 - 53.1)	0.604	0.130	0.466
Android fat mass (kg)	195	3.2 (3 - 3.3)	3.0 (2.8 - 3.1)	3.3 (3.1 - 3.4)	3.0 (2.9 - 3.2)	0.399	**<0.001**	0.535
Gynoid fat mass (kg)	195	4.9 (4.6 - 5.1)	4.7 (4.4 - 4.9)	5.1 (4.9 - 5.4)	4.8 (4.6 – 5.0)	0.114	**<0.001**	**0.035**

Table 2. Continued

		PhenoDiet group A (n = 121)		PhenoDiet group B (n = 121)		*P*-value		
	n*	week 0	week 12	week 0	week 12	Group	Time	Group x Time
Android/gynoid ratio	195	1.21 (1.18 - 1.23)	1.19 (1.16 - 1.22)	1.19 (1.16 - 1.21)	1.18 (1.15 - 1.21)	0.261	**0.031**	0.498
VAT (L)[a]	70	5.4 (4.9 – 6.0)	5.0 (4.5 - 5.5)	5.3 (4.8 - 5.8)	5.0 (4.6 - 5.5)	0.808	**<0.001**	0.489
VAT (cm^2)[b]	88	158 (146 - 170)	145 (134 - 158)	176 (163 - 191)	162 (149 - 176)	**0.047**	**<0.001**	0.972
Cardiometabolic parameters								
Total cholesterol (mmol/L)	198	5.3 (5.1 - 5.5)	4.8 (4.7 - 5)	5.4 (5.2 - 5.6)	4.8 (4.6 - 5)	0.432	**<0.001**	0.078
HDL cholesterol (mmol/L)	198	1.3 (1.2 - 1.3)	1.2 (1.2 - 1.3)	1.3 (1.2 - 1.3)	1.2 (1.1 - 1.2)	0.266	**<0.001**	0.101
Total cholesterol:HDL ratio	198	4.2 (4.0 - 4.4)	4.0 (3.8 - 4.2)	4.4 (4.2 - 4.6)	4.2 (4.0 - 4.4)	0.146	**<0.001**	0.980
TAG (mmol/L)	196	1.3 (1.2 - 1.4)	1.2 (1.2 - 1.3)	1.5 (1.4 - 1.6)	1.3 (1.2 - 1.4)	**0.033**	0.103	**0.028**
FFA (mmol/L)	196	0.5 (0.4 - 0.5)	0.4 (0.4 - 0.5)	0.5 (0.4 - 0.5)	0.4 (0.4 - 0.5)	0.884	**0.013**	0.684
SBP (mmHg)	198	123.7 (121.1 - 126.2)	121.5 (119.1 - 123.9)	126.5 (123.9 - 129.1)	121.6 (119.1 - 124.2)	0.137	**0.033**	0.077
DBP (mmHg)	198	77.9 (76.2 - 79.7)	76.3 (74.6 – 78.0)	79.4 (77.7 - 81.1)	77.1 (75.4 - 78.7)	0.257	**0.013**	0.495
Inflammatory profile								
CRP (mg/L)	197	0.98 (0.81 - 1.17)	0.97 (0.78 - 1.19)	1.12 (0.94 - 1.34)	0.88 (0.71 - 1.08)	0.298	0.892	**0.034**

* n represent number of individuals of which data was available from both week 0 and week 12.

[a] At MUMC+, VAT was assessed using a whole-body MRI scan. b At WUR, VAT was assessed using single-slice MRI. Values are estimated marginal means with 95% confidence intervals, adjusted for age, sex and center.

P-values <0.05 are highlighted in bold.

CRP, C-reactive protein; DBP, diastolic blood pressure; FFA, free fatty acid; HDL, high density lipoprotein; HIRI, hepatic insulin resistance index; MISI, muscle insulin sensitivity index; SBP, systolic blood pressure; TAG, triacylglyceride; VAT, visceral adipose tissue.

The primary outcome disposition index was not significantly affected in either of the intervention groups

Glucose homeostasis and insulin sensitivity was assessed with a 7-point venous OGTT (75 gr of glucose) before and at the end of the intervention. The primary outcome was the disposition index, which is a composite measure of insulin sensitivity and insulin secretion. The disposition index was 412 (369 - 460) (estimated marginal mean with adjustment for age, sex, and center (95% CI)) before intervention and 406 (365 - 451) after intervention in PhenoDiet group A, and 357 (321 - 398) before intervention and 380 (343 - 423) after intervention in PhenoDiet group B. Differences between groups did not reach statistical significance (*P* for group x time = 0.109) (Table 2, Fig. 2A). Also, there was no change over time in either of the intervention groups (*P* time = 0.640).

Insulin sensitivity and glucose homeostasis improved to a greater extent in PhenoDiet group B

Fasting insulin, 2-hour glucose, 2-hour insulin, and HOMA-IR decreased, and MISI increased significantly in PhenoDiet group B, but not in PhenoDiet group A (all *P* for group x time < 0.05) (Fig. 2B-E). The Matsuda index, which reflects whole-body insulin sensitivity, also increased significantly in PhenoDiet group B (from 4.2 [3.9 - 4.6] to 5.1 [4.6 - 5.5]) compared to PhenoDiet group A (from 4.8 [4.4 – 5.3] to 5.1 [4.6 - 5.6]) (*P* for group x time = 0.004) (Fig. 2F). HIRI decreased significantly in both groups (*P* time = 0.021), with no difference between the groups (*P* for group x time = 0.25). HbA1c tended to decrease slightly in PhenoDiet group B compared to PhenoDiet group A (*P* for group x time = 0.091) (Table 2). Additional statistical adjustment for weight change did not affect these results (results not shown).

We additionally compared changes in glucose and insulin area under the curves (AUCs) in response to the OGTT between the two groups. The AUCs of postprandial glucose showed a larger reduction (*P* for group x time = 0.004) and a trend for larger reduction in postprandial insulin (*P* for group x time = 0.076) in PhenoDiet group B compared to PhenoDiet group A. (Fig. S2).

These greater improvements in PhenoDiet group B were observed in both individuals with the MIR and LIR phenotype

We performed *post-hoc* analyses with stratification for IR phenotype for the outcomes with significant group x time interaction (Fig. 3 and Table S7). Fasting insulin, HOMA-IR, Matsuda index, and MISI improved in both individuals with the MIR and individuals with the LIR phenotype in PhenoDiet group B, whereas these parameters did not improve in

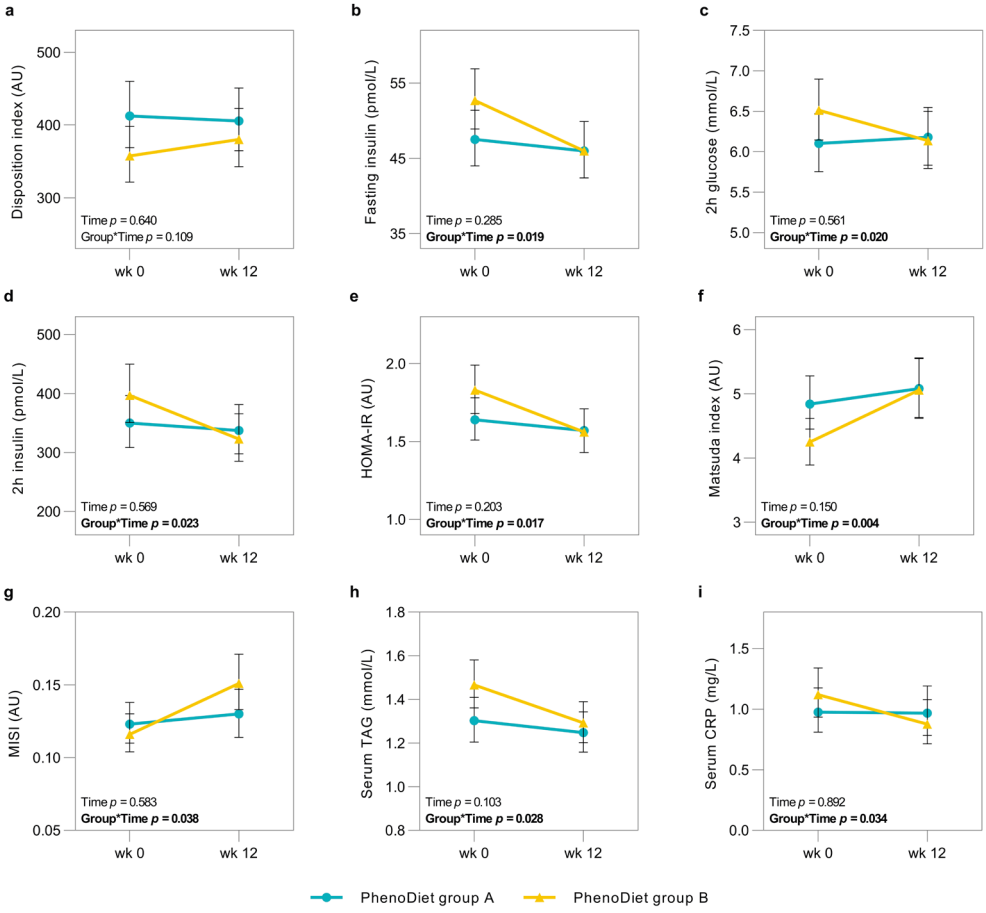

Fig. 2. Insulin sensitivity, glucose tolerance, fasting TAG and CRP improved to a greater extent in PhenoDiet group B compared to PhenoDiet group A. Individuals in PhenoDiet group B (n = 121) had more pronounced improvements in fasting insulin (B), 2-hour glucose (C), 2-hour insulin (D), HOMA-IR (E), Matsuda index (F), muscle sensitivity index (MISI) (F), serum triacylglycerol (TAG) (H), and plasma C-reactive protein (CRP) (i), but not disposition index (A) after 12 weeks of dietary intervention compared to PhenoDiet group A (n = 121). Data are presented as estimated marginal means with 95% confidence intervals, adjusted for age, sex and center. P-values <0.05 are highlighted in bold. Intervention effects were tested using a repeated measures linear mixed model.

individuals with either IR phenotype within PhenoDiet group A. Within PhenoDiet group B, 2-hour glucose and insulin decreased significantly in the MIR group following the LFHP diet, but the decreases did not reach significance in the LIR group on the HMUFA diet.

Glycemic variability was not affected in either of the groups

In addition to measuring glucose parameters in response to a laboratory challenge test, we assessed glycemic variability in daily-life settings for 6 days using continuous glucose monitoring (CGM). Mean glucose, glucose standard deviation (SD), glucose coefficient of variation (CV) %, % glucose time in range 3.9-7.8 mmol/L, and mean amplitude of glucose excursions (MAGE) were not affected in either of the groups (Table 3).

Minor weight loss and reduction in body fat and ectopic fat in both groups

Body weight decreased to a similar extent in both groups, with 2.0% and 2.7% in PhenoDiet group A and B, respectively (*P* for time <0.001; *P* for group x time = 0.22) (Table 2). We performed a dual X-ray absorptiometry (DXA) to assess body composition. The weight loss was caused by a reduction in body fat mass, which tended to be greater in PhenoDiet group B compared to PhenoDiet group A (*P* for group x time = 0.058). Both android and gynoid fat mass decreased in both groups (*P* for time <0.001), but the reduction in gynoid fat mass was slightly larger in PhenoDiet group B, compared to PhenoDiet group A (*P* for group x time = 0.035). Additionally, at MUMC+, visceral adipose tissue (VAT), liver fat, and muscle fat were assessed using a whole-body magnetic resonance imaging (MRI) scan and at WUR, VAT was assessed using single-slice MRI and liver fat was measured using proton magnetic resonance spectroscopy (1H-MRS). Visceral adipose tissue (VAT) decreased in both groups in both centers, without significant differences between groups (*P* for group x time = 0.49 [whole-body MRI]; 0.97 [single-slice MRI]) (Table 2). Liver fat and muscle fat decreased to a similar extent in both groups, with no significant differences between groups (*P* for group x time = 0.58 [liver fat measured by MRI], 0.15 [liver fat measured by MRS] and 0.73 [muscle fat], respectively) (Table 3).

Larger reduction in serum TAG in PhenoDiet group B and similar reductions in cholesterol, FFA, and blood pressure in both groups

Both groups showed a decrease in fasting serum total cholesterol and HDL cholesterol levels, with a tendency for a greater decrease in total cholesterol in PhenoDiet group B (*P* for time <0.001; *P* for group x time = 0.078) (Table 2). Fasting serum triacylglycerol (TAG) decreased in PhenoDiet group B, whereas it did not change in PhenoDiet group A (*P* for group x time = 0.028) (Fig. 2H). The lack of improvement in serum TAG in PhenoDiet group

Table 3. Secondary outcomes at baseline and after 12 weeks in PhenoDiet groups A and B

		PhenoDiet group A (n = 121)		PhenoDiet group B (n = 121)		*P*-value		
	n*	week 0	week 12	week 0	week 12	Group	Time	Group x Time
Glycemic variability								
Mean glucose (mmol/L)	211	6.0 (5.9 - 6.1)	6.0 (5.9 - 6.1)	6.2 (6.1 - 6.3)	6.1 (6 - 6.2)	0.031	0.545	0.178
SD glucose (mmol/L)	211	0.85 (0.80 - 0.91)	0.89 (0.83 - 0.94)	0.93 (0.88 - 0.99)	0.91 (0.86 - 0.97)	0.046	0.185	0.148
CV glucose (%)	211	14.2 (13.4 – 15.0)	14.8 (14.0 - 15.6)	15.1 (14.3 - 15.9)	15.0 (14.2 - 15.8)	0.115	0.134	0.227
Time in range 3.9-7.8 mmol/L (%)	211	93.6 (86.7 - 101.2)	93.3 (90.6 - 95.9)	84.3 (78 - 91)	89.6 (87.1 - 92.3)	0.055	0.887	0.102
MAGE (mmol/L)	211	2.1 (2 - 2.3)	2.2 (2.1 - 2.4)	2.4 (2.2 - 2.5)	2.3 (2.1 - 2.4)	0.045	0.438	0.159
Ectopic fat								
Liver fat (%) (MRI)a	69	5.2 (3.9 - 6.8)	3.4 (2.5 - 4.5)	6.1 (4.7 - 7.9)	4.2 (3.2 - 5.5)	0.367	<0.001	0.580
Liver fat (%) (1MRS)b	84	2.6 (2.0 - 3.5)	1.3 (1.0 - 1.7)	3.2 (2.4 - 4.4)	1.3 (0.9 - 1.7)	0.347	<0.001	0.154
Muscle fat (%)	70	7.7 (7.2 - 8.2)	7.6 (7.1 - 8.1)	7.4 (7.0 - 7.9)	7.3 (6.9 - 7.8)	0.427	0.036	0.728
Physical activity								
LPA (h/day)	187	5.1 (4.8 – 5.3)	4.8 (4.6 – 5.1)	5.1 (4.9 – 5.4)	5.0 (4.7 – 5.3)	0.942	0.030	0.233
MVPA (h/day)	187	1.2 (1.1 – 1.3)	1.2 (1.1 – 1.3)	1.2 (1.1 – 1.3)	1.2 (1.1 – 1.3)	0.562	0.241	0.297
Quality of life								
RAND-36 PCS	220	65.7 (64.2 - 67.3)	65.8 (64.3 - 67.4)	65.3 (63.8 - 66.9)	66.3 (64.6 - 67.9)	0.721	0.451	0.543
RAND-36 MCS	220	60.4 (58.9 - 61.9)	59.5 (58.2 - 60.9)	59.4 (58 - 60.9)	60.2 (58.8 - 61.6)	0.353	0.140	0.946
Sleep and fatigue								
Global PSQI score	220	5.0 (4.3 - 5.2)	5.1 (4.6 - 5.6)	4.7 (4.3 - 5.2)	5.2 (4.7 - 5.7)	0.700	0.189	0.534
Epworth Sleepiness Scale score	220	7.1 (6.4 - 7.7)	6.5 (5.8 - 7.1)	7.2 (6.6 - 7.9)	7.2 (6.4 - 7.9)	0.325	0.044	0.115
Chalder Fatigue score	220	11.7 (11.2 - 12.3)	11.4 (10.7 - 12.1)	11.7 (11.1 - 12.3)	11.1 (10.4 - 11.8)	0.892	0.576	0.090
Perceived well-being								
Perceived Stress Score (PSS-10)	220	8.8 (7.9 - 9.6)	8.2 (7.4 - 9)	8.6 (7.8 - 9.4)	9.4 (8.5 - 10.4)	0.333	0.592	0.003

* n represent number of individuals of which data was available from both week 0 and week 12. [a] At MUMC+, liver fat was assessed using a whole-body MRI scan. [b] At WUR, liver fat was measured using 1H-MRS. Values are estimated marginal means with 95% confidence intervals, adjusted for age, sex and center. *P*-values <0.05 are highlighted in bold. CV, coefficient of variation; LPA, light physical activity; MAGE, mean amplitude glucose excursion; MCS, mental component summary; MRI, magnetic resonance imaging; MRS, magnetic resonance spectroscopy; MVPA, moderate-to-vigorous physical activity; PCS, physical component summary; PSQI, Pittsburgh sleep quality index; SD, standard deviation.

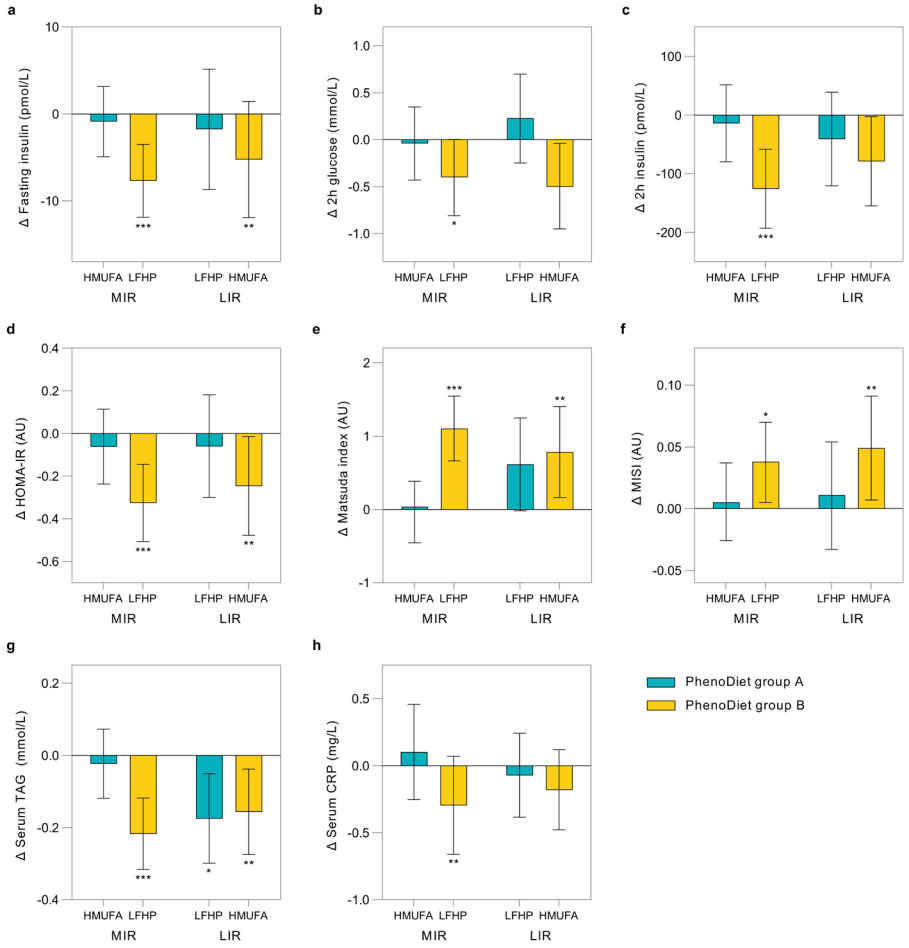

Fig. 3. The greater improvements in PhenoDiet group B were observed in both the MIR and LIR phenotype. Greater improvements in fasting insulin (A), 2-hour glucose (B), 2-hour insulin (C), HOMA-IR (D), Matsuda index (E), and MISI (F) were observed in PhenoDiet B (n = 121) in both individuals with MIR and LIR, whereas PhenoDiet A (n = 121) did not affect outcomes in either IR phenotype. Serum TAG (G) was reduced after 12 weeks in PhenoDiet group B in both individuals with MIR and LIR, and in PhenoDiet group A in LIR individuals only. Plasma CRP (H) was reduced in PhenoDiet group B in individuals with MIR and was not affected in the other groups. Data are presented as estimated marginal means with 95% confidence intervals, adjusted for age, sex and center. * P<0.05, ** P<0.01, *** P<0.001 for time effect, as tested with a repeated measures linear mixed-model, stratified for IR phenotype (*post-hoc* analysis).

A was mainly driven by a lack of improvement of individuals with the MIR phenotype on the HMUFA diet (Fig. 3G). Fasting free fatty acids (FFA) decreased in both groups to a similar extent (*P* for time = 0.013; *P* for group x time = 0.68) (Table 2). Both interventions significantly reduced SBP and DBP (Table 2). The reduction in SBP tended to be larger in PhenoDiet group B (*P* for group x time = 0.077).

Systemic inflammation marker CRP decreased in PhenoDiet group B only

Plasma CRP decreased significantly from 1.12 (0.94 - 1.34) to 0.88 (0.71 - 1.08) mg/L in PhenoDiet group B, whereas it did not change in PhenoDiet group A (from 0.98 [0.81 – 11.7] to 0.97 [0.78 – 119]) (*P* for group x time = 0.034) (Table 2; Fig. 2I). *Post-hoc* analysis revealed that plasma CRP only improved in individuals with the MIR phenotype in the LFHP diet but did not significantly improve in other combinations of diet and phenotype (Fig. 3H).

Similar reductions in postprandial glucose, insulin, TAG, and FFA upon a high-fat mixed meal in both groups

In addition to an OGTT, we also performed a liquid high-fat mixed-meal (HFMM) test to assess postprandial responses to a meal containing fat, carbohydrates, and protein. The AUCs for postprandial glucose, insulin, and FFA response decreased for both interventions (*P* for time all < 0.05) without differences between PhenoDiet groups A and B (Fig. S3 and S4). The postprandial increase in serum TAG decreased slightly in PhenoDiet group B compared to PhenoDiet group A, but this did not reach statistical significance (*P* for group x time = 0.11) (Fig. S4).

The interventions had mixed effects on perceived well-being

Next to physiological measures, we included questionnaires to assess perceived well-being. Health-related quality of life was not affected in either of the groups (Table 3). Of the questionnaires related to sleep and fatigue, only the Epworth Sleepiness Scale score significantly decreased in both groups, indicating a reduction in daytime sleepiness, but with no difference between the groups (*P* for time = 0.044; *P* for group x time = 0.12). The Chalder fatigue score tended to decrease in PhenoDiet group B only, indicating a reduction in self-reported fatigue (*P* for time = 0.58; *P* for group x time = 0.090). The Perceived Stress Score increased in PhenoDiet group B, indicating an increase in perceived stress compared to PhenoDiet group A (*P* for group x time = 0.003).

Light-intensity physical activity decreased slightly in both groups Physical activity was objectively measured throughout ~7 days in free-living conditions at the start and end of

the intervention period using a thigh-worn accelerometer. In both groups, light-intensity physical activity decreased from baseline to week 12, with no difference between the groups (*P* for time = 0.030; *P* for group x time = 0.23) (Table 3). Moderate-to-vigorous physical activity did not change in either of the groups.

DISCUSSION

In this study, we show for the first time that improvements in cardiometabolic health after modulation of dietary macronutrient composition are dependent on tissue-specific IR phenotype. We defined two PhenoDiet groups, with PhenoDiet group A including individuals with MIR following HMUFA diet and individuals with LIR following a LFHP diet, and PhenoDiet group B including individuals with LIR following a HMUFA diet and MIR following a LFHP diet. The present data demonstrate pronounced and clinically relevant improvements in insulin sensitivity, fasting plasma insulin and TAG concentrations, glucose tolerance, and CRP in PhenoDiet group B compared to PhenoDiet group A. These findings provide evidence for greater effectiveness of a precision nutrition strategy based on tissue-specific IR phenotypes over a 'one-size-fits-all' dietary approach within the general dietary guidelines in improving cardiometabolic health.

Here, we demonstrate for the first time in a prospective study that individuals with distinct tissue-specific IR phenotypes respond differentially to dietary macronutrient modification. Interestingly, especially peripheral, rather than hepatic, insulin sensitivity showed a distinct differential response between PhenoDiet groups A and B. The Matsuda index significantly improved by ~20% in PhenoDiet group B compared to ~5% in PhenoDiet group A. Besides, MISI, 2-hour glucose, and 2-hour insulin concentrations improved more in PhenoDiet group B, independent of IR phenotype, while no distinct responses between PhenoDiet groups A and B were observed for HIRI and fasting plasma glucose. The Matsuda index (29) and MISI (26) have previously been validated against the glucose disposal rate, as determined by the gold-standard two-step hyperinsulinemic-euglycemic clamp. Both indices represent primarily peripheral, or skeletal muscle, insulin sensitivity.

The underlying mechanisms for the more pronounced improvements in particularly peripheral insulin sensitivity and overall cardiometabolic health in individuals with the MIR phenotype on the LFHP diet and individuals with the LIR phenotype on the HMUFA diet remain to be elucidated. Interestingly, modification of microbial composition by either fecal transplantation from lean donors to men with the metabolic syndrome, or dietary

fiber intervention improved peripheral but not hepatic insulin sensitivity (30, 31). These data suggest that modulation of gut microbial composition may primarily affect peripheral insulin sensitivity, and this may thus be a putative underlying mechanism for the more pronounced effects on peripheral insulin sensitivity in individuals with the MIR phenotype on the LFHP diet and individuals with the LIR phenotype on the HMUFA diet. The high content of slowly fermentable fibers in the LHFP diet (such as β-glucan, the fiber that was provided in the present LFHP diet) may ferment more distally in the colon, whereby the produced short chain fatty acids (SCFA) may bypass the liver and elicit metabolic effects more peripherally (32). Additionally, high-fermented foods, including yoghurt and quark (largely provided within the LFHP diet), can increase microbial diversity and decrease inflammatory markers (33). Together, several components within the LFHP diet may have elicited improvements in peripheral insulin sensitivity and inflammation, possibly via modulation of the gut microbiota.

Despite these indications that microbial modulation may target peripheral insulin sensitivity specifically, a role of microbiota composition in hepatic metabolism, possibly depending on initial microbial composition as well as site of colonic fermentation, cannot be excluded (34). A diet rich in MUFA and thereby rich in polyphenols may also affect microbial composition and liver lipid metabolism (34-36). Besides that, we have previously shown that a meal high in PUFA or MUFA acutely decreased circulating VLDL-TAG levels (liver-derived TAG), increased the fractional synthetic rate of TAG in the skeletal muscle, and increased postprandial insulin sensitivity, compared to SFA (23). In line, in the present study the HMUFA diet reduced fasting TAG levels and tended to reduce postprandial TAG levels in individuals with LIR compared to MIR. These data suggest that the HMUFA diet may affect hepatic lipid metabolism, thereby possibly contributing to improved peripheral insulin sensitivity through inter-organ crosstalk.

The present findings are in line with a recent *post-hoc* analysis of the CORDIOPREV-DIAB study, which showed that individuals with distinct tissue-specific IR phenotypes benefit most from diets that differ in macronutrient composition (14). Based on the CORDIOPREV-DIAB study, we hypothesized that individuals with the MIR phenotype would benefit more from a HMUFA diet and individuals with the LIR phenotype more from a LFHP diet. We, however, observed a nonsignificant tendency for an improved disposition index and a more pronounced improvement in cardiometabolic health in individuals with the MIR phenotype on a LFHP diet and individuals with the LIR phenotype on a HMUFA diet (PhenoDiet group B as compared to PhenoDiet group A). These conflicting findings may relate to several factors, including differences in study populations (overall more healthy

population in the present PERSON study), in assessment of LIR, and in composition of diet interventions. These contrasting results illustrate the complexity of precision nutrition. Further advancement of the field of precision nutrition requires more well-designed, clinical trials with deep phenotyping to better understand the mechanisms that underlie inter-individual variation in response to diet. Such studies are needed to identify the most important factors that explain individual response to diet, as well as to validate precision nutrition-based strategies.

Interestingly, 76% of both individuals with MIR and LIR were considered normal glucose-tolerant at baseline. Nevertheless, based on elevated waist circumference, body fat percentage, and total cholesterol levels observed in the present study population, individuals with MIR or LIR may already be at increased risk for metabolic perturbations before the onset of disturbed glucose homeostasis as defined by established clinical cut-off values for IFG and IGT. Previous findings also show that tissue-specific IR phenotypes are related to disturbances in metabolome, lipidome, and inflammatory profiles (11-13). An important finding in the present study is that individuals in both study arms showed improvements in body composition, body fat distribution, ectopic fat, and several cardiometabolic parameters, regardless of intervention group (PhenoDiet group A or B) and without substantial weight loss (average weight loss: 2.3% or ~2 kg). To illustrate, liver fat decreased by more than 40% on average in the total population and total cholesterol levels decreased on average to values within the healthy range (<5.0 mmol/L). These results highlight the effectiveness and clinical relevance of a healthy diet in individuals with tissue-specific IR. Importantly, however, we demonstrate that health improvements can be remarkably enhanced when modulating dietary macronutrient composition based on tissue-specific IR phenotype.

We included questionnaires related to perceived well-being to explore the relationship between objective (clinical parameters) health, and subjective health and well-being. Although slight changes in fatigue and perceived stress were observed, the effects on subjective health and well-being were not consistent. These findings suggest that improvements in cardiometabolic health were not reflected in detectable improvements in perceived well-being.

A major strength of the present study is that it is the first to investigate the effects of modulating dietary macronutrient composition according to tissue-specific IR with a prospective, double-blind, randomized design in a large number of individuals. Another strength of the present study is the classification of individuals by using only one

measurement (7-point OGTT), paving the way for implementation of precision nutrition into clinical practice, although even more easily measurable biomarkers may be identified in future research. Finally, the dietary interventions were implemented by intensive dietary counselling and provision of key products. Dietary compliance was high, with substantial differences in reported MUFA, protein and fiber intake between the HMUFA and LFHP diet, while keeping carbohydrate and SFA intake similar between the diets. The macronutrient composition that we aimed for was largely achieved in both diets, although reported MUFA and fiber intakes were slightly lower than advised in the HMUFA and LFHP diet, respectively. This may be due to either lower actual intake or misreporting (37). Nevertheless, the two intervention diets clearly differed in key macronutrients, and both diets were a considerable modification to the participants' habitual diet.

In conclusion, we here demonstrate for the first time that clinically relevant improvements in cardiometabolic health after dietary macronutrient intervention are driven by IR phenotype, with the optimal macronutrient composition for each phenotype leading to a more pronounced improvement in cardiometabolic health, independent of weight loss. Our findings indicate that precision nutrition based on metabolic phenotype may be superior to a 'one-size-fits-all' diet based on general guidelines with respect to improving cardiometabolic health.

Limitations of the study

We acknowledge several limitations of this study. Firstly, more individuals with the MIR phenotype were included in the study compared to LIR (149 vs. 93). Due to equal distribution of phenotypes between PhenoDiet groups A and B, by design, more individuals followed the HMUFA diet in PhenoDiet group A and more LFHP in PhenoDiet group B. Still, *post-hoc* analyses revealed that the more pronounced improvements in PhenoDiet group B as compared to PhenoDiet group A were driven by improvements in both individuals with the MIR on the LFHP diet and individuals with the LIR phenotype on the HMUFA diet. Furthermore, it appeared that the individuals in PhenoDiet group A were by chance somewhat more insulin sensitive at baseline compared to PhenoDiet group B. Nevertheless, statistical adjustments for baseline differences were made, indicating that the conclusions of larger improvements observed in PhenoDiet group B cannot be explained by a more unfavorable metabolic profile at baseline. Tissue-specific IR was assessed with a 7-point OGTT. This method has been validated again the gold-standard hyperinsulinemic euglycemic clamp technique (25, 26). Nevertheless, contrary to the highly standardized hyperinsulinemic euglycemic clamp technique, OGTT-derived measurement of tissue-specific IR may partially be affected by biological processes

associated with the oral ingestion of glucose, including differences in gastrointestinal factors, such as the rate of glucose absorption by the gut and the related incretin response (38). Furthermore, glucose and insulin responses to an OGTT may be affected by an individual's body size, as the dose of ingested glucose is the same for all. In addition, all blood samples were taken from a venous forearm catheter. Therefore, it should be noted that the degree of forearm glucose uptake may have contributed to inter-individual variation in venous plasma glucose concentrations (39). Importantly however, we have shown that based on just one OGTT, regardless of whether we were truly able to distinguish LIR and MIR, we identified distinct metabolic phenotypes, which could be replicated in independent cohorts (11-13). and which in this prospective study responded differentially to dietary intervention. We hereby provide support for the efficacy of the clinical use of (7-point) OGTT-derived measures of metabolic heterogeneity. Finally, the present study is a proof-of-concept study, focused on specific IR phenotypes which are prevalent in ~30% of the overweight population. Future research has to demonstrate whether more metabolic and IR phenotypes that respond differentially to dietary macronutrient modulation can be defined.

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METHODS

Experimental model and subject details

The PERSON study (PERSonalized glucose Optimization through Nutritional intervention) was a two-center, randomized, double-blinded, 12-week dietary intervention study with a parallel design (Fig. 1). The rationale and methodology of the PERSON study have been described in detail previously (24). The study was conducted from May 2018 until November 2021 at Maastricht University Medical Center+ (MUMC+) and Wageningen University (WUR) in the Netherlands, in line with the principles of the Declaration of Helsinki. The protocol was approved by the Medical Ethical Committee of the MUMC+(NL63768.068.17) and registered at ClinicalTrials.gov (NCT03708419). All participants gave written informed consent.

Study participants

Participants were recruited via a volunteer database, flyers, and advertisements in local and online media. Inclusion criteria were: age 40–75 years, BMI 25–40 kg/m2, body weight stability for at least 3 months (no weight gain or loss >3 kg), and tissue-specific IR, characterized as predominant LIR or MIR, as assessed by a 7-point oral glucose tolerance test (OGTT) based on venous plasma glucose and insulin concentrations. Exclusion criteria included among others pre-diagnosis of type 2 diabetes mellitus (T2DM), diseases or use of medication that affect glucose and/or lipid metabolism, major gastrointestinal diseases, history of major abdominal surgery, uncontrolled hypertension, smoking, alcohol consumption >14 units/week, and >4 h/week moderate-to-vigorous physical activity (24).

Assessment of eligibility

Compliance with in- and exclusion criteria was assessed according to standard protocols during a screening visit as described previously (24). Data on demographics, medical history, family history of DM (≥1 first-degree relative with DM), and medication use were collected by a screening questionnaire. Education level was categorized into low (no education, primary education, lower or preparatory vocational education, lower general secondary education), medium (intermediate vocational education, higher general senior secondary education, or pre-university secondary education) and high (higher vocational education, university).

Tissue-specific IR was assessed based on the plasma glucose and insulin concentrations during a 7-point OGTT. Participants ingested 200 ml of a ready-to-use 75 g glucose solution (Novolab) within 5 min, and blood samples were collected from the

antecubital vein via an intravenous cannula under fasting conditions (t = 0 min) and after ingestion of the glucose drink (t = 15, 30, 45, 60, 90, and 120 min) for determination of plasma glucose and insulin concentrations. LIR and MIR were estimated using calculations for the hepatic insulin resistance index (HIRI) and muscle insulin sensitivity index (MISI) respectively, by Abdul-Ghani and colleagues (26). The MISI calculation has been optimized using the cubic spline method (25). HIRI and MISI were calculated as follows:

HIRI = glucose 0-30 [AUC in mmol/L x h] x insulin 0-30 [AUC in pmol/L x h]
MISI = (dGlucose/dt) / insulin [mean during OGTT in pmol/L]

In the calculation for MISI, dGlucose/dt is the rate of decay of plasma glucose concentration (mmol/L) during the OGTT, calculated as the slope of the least square fit to the decline in plasma glucose concentration from peak to nadir. Deviating glucose curves that were flagged by the calculator were visually inspected for MIR and LIR classification. Individuals were classified as "No MIR/LIR," "MIR," "LIR," or "combined MIR/LIR," using tertile cutoffs for MISI and HIRI. The lowest tertile of MISI represented individuals with MIR, while the highest tertile of HIRI represented individuals with LIR. The cutoffs for these tertiles were based on values of a selected study population of The Maastricht Study (27), which resembles the target population of the PERSON study. After inclusion of 163 participants, the median HIRI of the current study screening population was used for classification due to an apparent discrepancy in LIR prevalence between the two populations. Additional OGTT-derived indices and other outcomes were determined as described below and as previously reported (24). Eligible participants started the study within 3 months after screening.

Randomization

Eligible participants were randomly assigned to either PhenoDiet group A or PhenoDiet group B, which consisted of unique combinations of the MIR and LIR metabolic phenotypes and two distinct diets meeting the Dutch dietary guidelines (40). PhenoDiet group A included individuals with MIR following a high-monounsaturated fatty acids (HMUFA), and individuals with LIR following a low-fat, high-protein, and high-fiber (LFHP) diet. PhenoDiet group B included individuals with LIR and MIR on HMUFA and LFHP diets, respectively.

Random allocation to either PhenoDiet group A or B in 1:1 ratio was conducted by an independent researcher using center-specific minimization (41, 42), with randomization factors of 1.0 for the LIR/MIR phenotype, and 0.8 for age and sex, and a base probability

of 0.7 by means of biased-coin (43). Both researchers and participants were blinded to the participants' metabolic phenotype (LIR or MIR), and thus blinded to whether participants were allocated to PhenoDiet A or B.

METHOD DETAILS

Dietary intervention

The HMUFA diet had a targeted macronutrient composition of 38% of energy from fat (20% MUFA, 8% PUFA, 8% SFA), 48% of energy from carbohydrates (CHO) (30% polysaccharides; 3 g/MJ fiber), and 14% of energy from protein. The macronutrient composition of the LFHP diet was targeted at 28% of energy from fat (10% MUFA, 8% PUFA, 8% SFA), 48% of energy from CHO (30% polysaccharides; >4 g/MJ fiber), and 24% of energy from protein (Table S1). Energy from CHO was similar between diets. Key products that largely distinguished the two diets with regards to macronutrient composition were provided in pre-measured amounts. For the HMUFA diet, key products included olive oil, olives, olive tapenade, and low-fat margarine with olive oil. Key products for the LFHP diet included low-fat yogurt and quark, reduced-fat cheese, very low-fat spread, pumpkin seeds, baking margarine with olive oil, and a dietary fiber supplement (2 g β-glucan per 6 g, DSM Nutritional Products, Basel, Switzerland) providing 6–12 g of additional fiber per day. Participants were instructed to consume a certain amount of every provided product each day. Apart from the fiber supplement, all products were commercially available. Alcohol consumption was restricted to ≤ 1 glass/day, in agreement with the current Dutch dietary guidelines (40).

Participants were assigned to one of eight energy groups ranging from 6 to 13 MJ/d according to their estimated individual energy requirement, which was calculated by averaging self-reported energy intake from a food frequency questionnaire (FFQ) (44) with the product of the predicted BMR, as calculated with Schofield equations (45), and self-reported physical activity level.

Individual counselling sessions with a dietician or research nutritionist were scheduled weekly at the research facilities to monitor adherence to the diet, adverse events and body weight to assess weight stability. Additional support was provided via e-mail or phone if needed. In case of weight instability, the participant's energy group was adjusted to avoid further weight change. During the period of COVID-19 restrictions, all counseling sessions took place via phone or video call. The dietary intervention strategy has been described in more detail before (24).

Dietary compliance

During the 12-week intervention, dietary compliance was assessed by three unannounced 1-day food records on two non-consecutive weekdays and one weekend day using the mobile app "Traqq" (28). In addition, plasma fatty acid profile was measured by nuclear magnetic resonance spectroscopy as a biomarker for MUFA, PUFA and SFA consumption (46).

Habitual dietary intake

A validated 163-item semi-quantitative FFQ (44) was used to assess habitual dietary intake before the start of the dietary intervention period. Dietary misreporting was evaluated by Goldberg's method (47, 48), using the ratio of daily energy intake (EI) to estimated basal metabolic rate (BMR). Energy under- (EI/BMR < 0.87) and over reporters (EI/BMR > 2.75) were excluded from data analyses.

Measurements

In the week before start of the dietary intervention (baseline) and in the last week of the 12-week intervention (week 12), participants were extensively phenotyped during a characterization week. This week included three or four (depending on study center and participation in additional subgroup measurements) clinical test days including a broad spectrum of laboratory analyses and three at-home days for additional data collection in daily-life settings (24). On the clinical test days, participants were instructed to travel to the facility by car or public transport. The day prior to and during the characterization weeks, participants were requested to refrain from alcohol and vigorous physical activity.

7-point oral glucose tolerance test

A 7-point OGTT was performed according to the same procedures as during the screening visit. Participants consumed a standardized low-fat macaroni meal (30% of energy intake [en%] fat, 49 en% CHO, 21 en% protein; 1,560–2,460 kJ, depending on energy group) the evening before the OGTT, after which they remained fasted until the OGTT.

The primary outcome disposition index was calculated as: [Matsuda index * (AUC30 min insulin/AUC30 min glucose)], where AUC30 min is the area under the curve between baseline and 30 min of the OGTT for insulin (pmol/L) and glucose (mmol/L) as calculated using the trapezoidal method, respectively. The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as (fasting glucose [mmol/L] × fasting insulin [mU/L])/22.5 (49). HOMA of β-cell function (HOMA-β) was calculated as (20 × fasting

insulin [mU/L])/(fasting glucose [mmol/L] – 3.5). Matsuda index was defined as: [10,000 ÷ square root of [fasting plasma glucose (mg/dL) × fasting insulin (mU/L)] × [mean glucose (mg/dL) x mean insulin (mU/L)]], using glucose and insulin values of time points 0, 30, 60, 90, and 120 min (29). Criteria of the WHO (50) were used define glucose status: normal glucose tolerance (NGT), fasting glucose <6.1 mmol/L and 2-hour glucose <7.8 mmol/L; impaired fasting glucose (IFG), fasting glucose 6.1 – 6.9 mmol/L and 2-hour glucose <7.8 mmol/L; impaired glucose tolerance (IGT), fasting glucose <6.1 mmol/L and 2-hour glucose 7.8 – 11.0 mmol/L; combined IFG/IGT, fasting glucose 6.1 – 6.9 mmol/L and 2-hour glucose 7.8-11.0 mmol/L; T2DM, fasting glucose ≥7.0 mmol/L and/or 2-hour glucose ≥11.1 mmol/L.

High-fat mixed-meal challenge test

A high-fat mixed-meal challenge test was performed at least 4 days after the OGTT, to determine the effects of the diets on postprandial glucose and lipid metabolism after a high-fat challenge. Participants consumed the same standardized low-fat macaroni meal as before the OGTT, after which they fasted 12 hours overnight. The liquid HFMM (350 g containing 2.8 MJ, 49 g [64 en%] fat, 48 g [29 en%] CHO, 12 g [7 en%] protein) was prepared in the university kitchen using whipped cream ice cream, whipped cream, full-fat milk, and sugar. An intravenous cannula was inserted in the antecubital vein for blood sampling. At least 30 min following insertion of the catheter, a fasting blood sample was drawn (t = 0 min). Subsequently, participants were asked to consume the liquid HFMM within 5 min and postprandial blood samples were drawn at t = 30, 60, 90, 120, 180, and 240 min for determination of glucose, insulin, free fatty acids (FFA) and triacylglycerol (TAG). Total cholesterol and HDL cholesterol were determined in fasting serum.

Body composition, fat distribution and ectopic fat deposition

Measurements of body weight and waist and hip circumference were performed according to standardized measurements (24). Whole-body and regional fat mass, fat percentage, and lean body mass were assessed using dual-energy X-ray absorptiometry (DXA), while participants were fasted for ≥2 h (MUMC+, Discovery A, Hologic; WUR, Lunar Prodigy, GE Healthcare).

Fat distribution and ectopic fat deposition were assessed using magnetic resonance imaging (MRI) and/or magnetic resonance spectroscopy (MRS). At MUMC+, a whole-body scan was made after a ≥2 h fast with a 3T MRI scanner (3T MAGNETOM Prisma fit, Siemens Healthcare), using a radiofrequency transmit/receive body coil at Scannexus, Maastricht, the Netherlands. Analyses were performed using a computational modeling

method [AMRA Medical AB, Linköping, Sweden] for quantification of visceral adipose tissue (VAT), intrahepatic lipid content (IHL), and muscle fat infiltration (MFI) in the anterior thighs. At WUR, IHL and abdominal fat were assessed with proton magnetic resonance spectroscopy (1H-MRS) and MRI, respectively, on a 3T whole-body scanner (Siemens, Munich, Germany; Philips Healthcare, Best, the Netherlands from November 2020 onwards). MRI measurements were performed after a ≥2 h fast at hospital Gelderse Vallei, Ede, the Netherlands. Spectra for determination of IHL were obtained from a 30 × 30 × 20 mm voxel placed in the right lobe of the liver, avoiding blood vessels and bile ducts. Participants were instructed to hold their breath when spectra were acquired to reduce respiratory motion artifacts. Spectra were post-processed and analyzed using the AMARES algorithm in jMRUI software (51). VAT was quantified in single-slice axial T1-weighted spin echo transverse images at the inter-vertebral space L3-L4 using the image analysis software program c (version 5.0, Tomovision).

Continuous glucose monitoring

Participants wore a continuous glucose monitor (CGM) for 6 days during characterization weeks 1 and 2. The CGM device (iPro2 and Enlite Glucose Sensor; Medtronic, Tolochenaz, Switzerland) was worn lateral to the umbilicus and recorded subcutaneous interstitial glucose values every 5 minutes. Participants were asked to perform four daily capillary glucose self-measurements (SMBG) via Contour XT (Ascensia Diabetes Care, Mijdrecht, the Netherlands) while wearing the CGM device. The CGM data files were then calibrated retrospectively using the SMBG values in CareLink (Medtronic, Tolochenaz, Switzerland) according to manufacturer's instructions. To avoid insufficient calibration, sensor glucose readings outside the time interval of the first and last SMBG measurements were excluded from the analysis. Participants were blinded to the CGM recording, but not to the SMBG values. In addition, CGM data files with irregular measurement frequencies (i.e. other than 5 minute) were excluded from the analysis (n = 3). The iglu package (52) (version 3.3.0) in R (version 4.0.2) was used to calculate mean glucose, standard deviation (SD), coefficient of variation (CV), time in range (between 3.9 and 7.8 mmol/L; TIR) and mean amplitude of glycemic excursions (MAGE).

Blood pressure

Systolic and diastolic pressure were measured in triplicate on the non-dominant arm with an automated sphygmomanometer after a 5-minute rest. The first measurement was used to acclimatize the subject to the measurements, and therefore omitted from the data.

Physical activity monitoring

Physical activity was assessed with the activPAL3 micro triaxial accelerometer (PAL Technologies Ltd., Glasgow, UK). The monitor was worn continuously attached to the anterior thigh, in the middle between the knee and the greater trochanter for ~14 days during both the characterization weeks, of which ~7 days in free-living conditions. Parameters of physical activity were quantified with a modified version of a home-written script (53), using sleeping and waking times recorded by the participants as input. We distinguished light-intensity physical activity (LPA) and moderate-to-vigorous physical activity (MVPA). LPA includes standing and stepping times with Metabolic Equivalent of Task (MET) values <3 (53). MVPA includes activities with MET values ≥3. Both measures were determined in hours per day. In the present study, only LPA and MVPA during the free-living days were used because physical activity during the characterization weeks with university visits and measurements is not reflective of regular physical activity level.

Self-reported sleep, well-being, and physical (in-)activity

General perceived health was assessed by the Physical and Mental Component Summary (PCS and MCS) scores obtained from the RAND-36 (54). Perceived stress was assessed with the 10-item Perceived Stress Scale (PSS-10) (55). Physical and mental fatigue were assessed using the 14-item Chalder fatigue scale (56). Sleep quality was assessed with the 10-item Pittsburgh Sleep Quality Index (57). Daytime sleepiness was assessed with the 8-item Epworth Sleepiness scale (58). Self-reported habitual physical activity and sedentary behavior were assessed using the Baecke questionnaire (59).

Adjusted COVID-19 protocol

Due to strict Dutch COVID-19 restrictions from March to June 2020, post-intervention measurements of 22 individuals were performed according to an adjusted protocol. The protocol included CGM measurements, anthropometric measurements and questionnaires as described above. The participants performed the measurements at home under guidance of the researcher via video connection. All other measurements were not performed during this period. The dietary intervention part of the study was completed according to the original protocol. The COVID-19 protocol was approved by the Medical Ethical Committee of the MUMC+ and participants gave their written informed consent.

Biochemical analyses of blood samples and biobanking

Venous blood was collected in EDTA tubes (Becton Dickinson, Eysins, Switzerland), which were centrifuged at 1,200 g, 4°C for 10 min and plasma was aliquoted subsequently. Serum tubes were left at room temperature for at least 30 min to allow clotting after

sampling and centrifuged at 1,200 g, 20°C for 10 min before aliquoting of serum. All biological samples were snap-frozen in liquid nitrogen and stored at −80°C until analysis. Samples from both centers were analyzed at central laboratories. Plasma glucose, insulin, and FFA were measured on a Cobas Pentra C400 using ABX Pentra Glucose HK CP reagens (Horiba ABX Diagnostics, Montpellier, France), ELISA (Meso Scale Discovery, Gaithersburg, USA), and NEFA HR reagens (Wako chemicals, Neuss, Germany), respectively. Serum TAG, total cholesterol, and HDL cholesterol were measured on a Cobas Pentra C400 using ABX Pentra Triglycerides HK CP reagens, ABX Pentra Cholesterol CP reagens, and ABX Pentra HDL Direct, respectively. A fasting blood sample was drawn for determination of glycated hemoglobin (HbA1c) by the hospital laboratories of MUMC+ and Ziekenhuis Gelderse Vallei, Ede, the Netherlands. The inflammatory marker C-reactive protein (CRP) was measured in fasting plasma using a Luminex immunoassay performed by DSM Nutritional Products (Kaiseraugst, Switzerland).

QUANTIFICATION AND STATISTICAL ANALYSIS

Power and sample size

A total sample size of 202 was previously calculated to be required to detect a standardized effect size of 0.46 with a power of 90% (24). Due to practical issues related to the unforeseen COVID-19 pandemic, 199 individuals completed the measures related to the primary outcome the disposition index. Participants for whom data that was missing due to the adjusted COVID-19 protocol were not considered dropouts but were excluded from the analyses related to these missing data to limit interference with study outcomes.

Statistical analyses

The number of dropouts between the two intervention groups was not significantly different ($p = 0.11$), and baseline characteristics did not differ between dropouts and completers (all $p > 0.05$) (Table S3). An intention to treat (ITT) analysis, which assumes that data was missing at random, was performed using a mixed-model with repeated measures to test intervention effects on primary and secondary parameters comparing PhenoDiet groups A and B. The model included age, sex, and study center as covariates, and time (baseline and week 12) as repeated measure. *Post-hoc* analyses with stratification for IR phenotype were performed in case of a significant group x time interaction. Estimated marginal means with 95% confidence intervals adjusted for the covariates are reported. For OGTT and high-fat mixed-meal responses, the AUC was calculated using the trapezoid method. Baseline characteristics were compared between the MIR and LIR phenotype, and between the diet groups within MIR and LIR groups using independent samples T-test

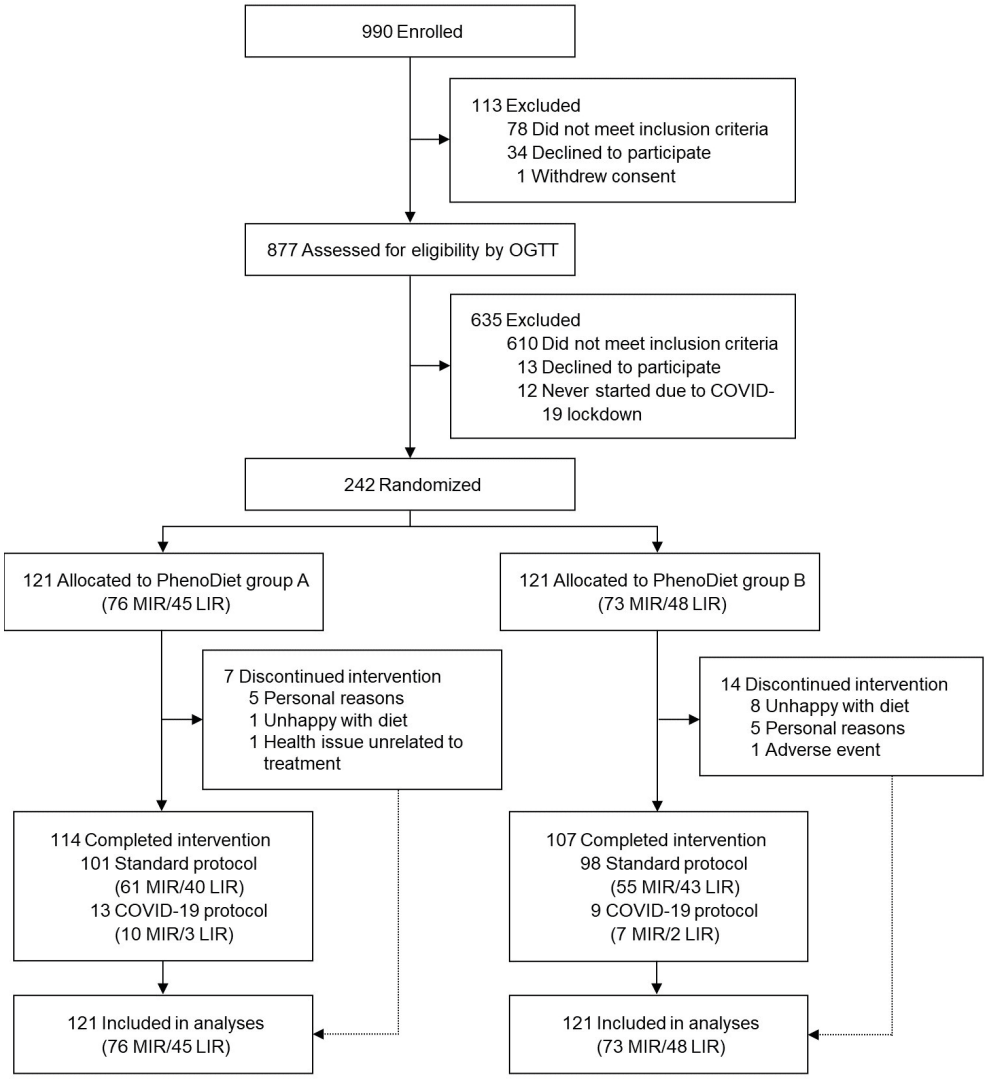
for numerical data (mean ± SD) and using Fisher's exact test for categorical data (%).

Model assumptions were tested by plotting residual and predicted values and by visually inspecting residual Q-Q plots, to test homogeneity of variances and normality of residuals, respectively. Skewed variables were log-transformed (log10) to improve normality. Two-tailed $p < 0.05$ was considered statistically significant. Analyses were performed using IBM® SPSS® Statistics software version 28.

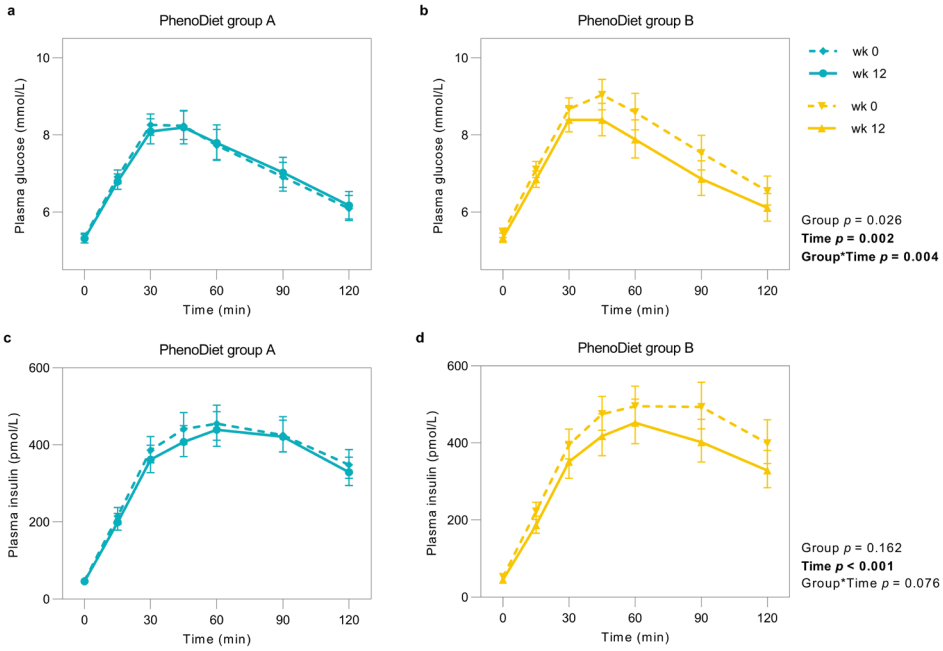
Additional resources

The trial was registered on: https://clinicaltrials.gov/ct2/show/NCT03708419, and the design paper was published previously: https://www.frontiersin.org/articles/10.3389/fnut.2021.694568.

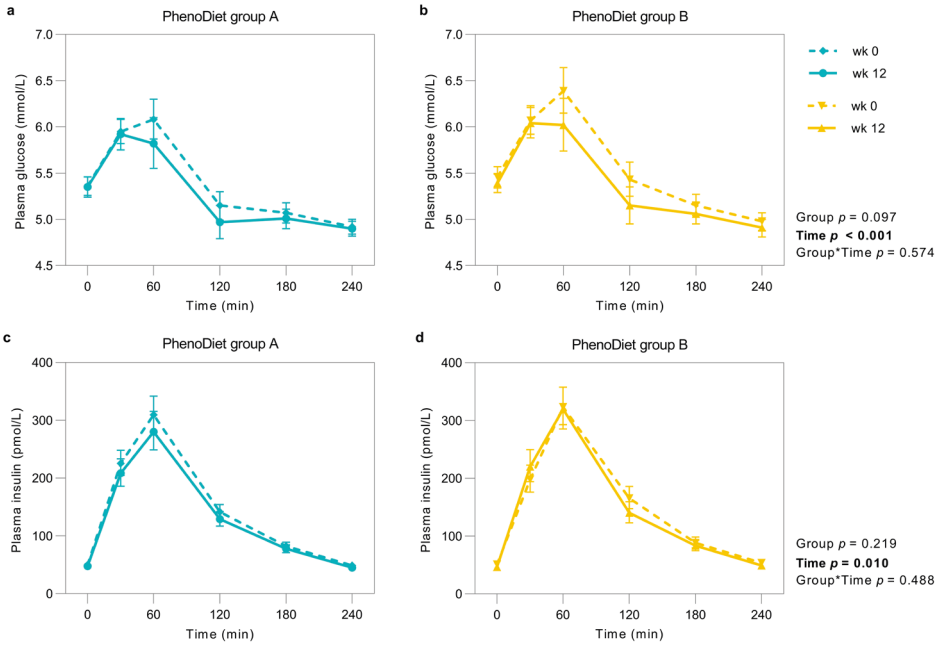
SUPPLEMENTARY MATERIAL



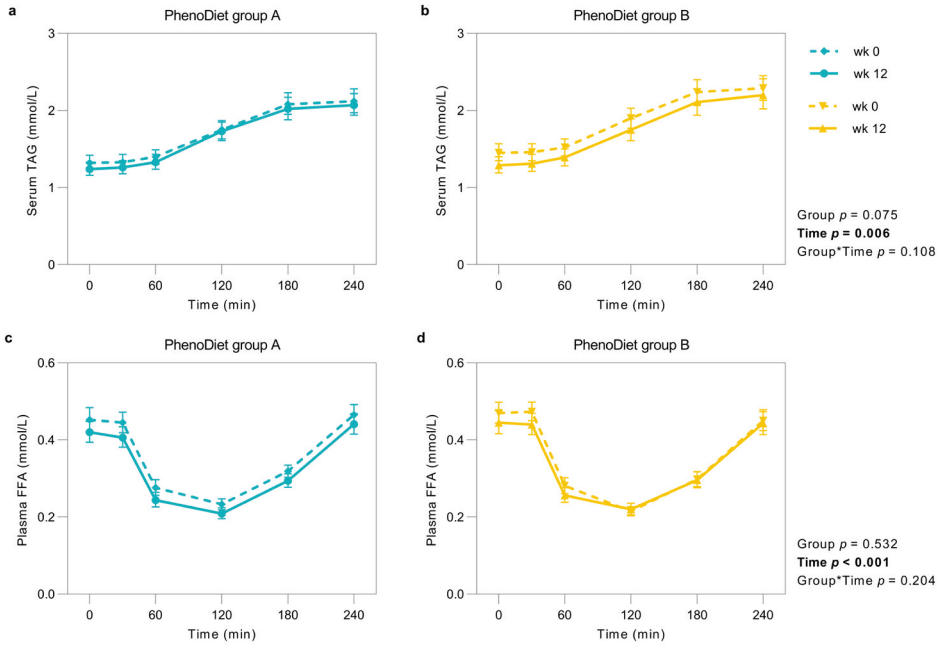
Supplementary Fig 1. Flowchart of participant enrolment and eligibility



Supplementary Fig 2. Plasma glucose and insulin concentrations in response to a 75g oral glucose tolerance test. a, Plasma glucose concentrations at week 0 and week 12 in PhenoDiet group A. b, Plasma glucose concentrations at week 0 and week 12 in PhenoDiet group B. c, Plasma insulin concentrations at week 0 and week 12 in PhenoDiet group A. d, Plasma insulin concentrations at week 0 and week 12 in PhenoDiet group B. Dashed lines indicate week 0, solid lines week 12. Data are presented as geometric means with 95% CI and differences in area under the curves (AUCs) between PhenoDiet group A and B were assessed using a linear mixed model with repeated measures with adjustments for age, sex, and center. The AUCs of both postprandial glucose and insulin decreased in both groups (P for time all < 0.05) with a larger reduction in postprandial glucose (P for group x time = 0.004) and a trend for larger reduction in postprandial insulin (P for group x time = 0.076) in PhenoDiet group B compared to PhenoDiet group A. *P*-values <0.05 are highlighted in bold.



Supplementary Fig. 3. Glucose and insulin responses to a liquid high-fat mixed meal. Plasma glucose concentrations in PhenoDiet group A (a) and B (b), and plasma insulin concentrations in PhenoDiet group A (c) and B (d) in response to a liquid high-fat mixed meal at week 0 and week 12. Dashed lines indicate week 0, solid lines week 12. Data are presented as geometric means with 95% CI. The differences in area under the curves (AUCs) between PhenoDiet group A and B were assessed using a mixed model with repeated measures with adjustments for age, sex, and center. The AUCs for both postprandial glucose and insulin decreased in both groups (*P* for time all < 0.05) without differences between PhenoDiet group A and B.



Supplementary Fig. 4. Postprandial triglyceride (TAG) and free fatty acids (FFA) responses to a liquid high- fat mixed meal. Serum TAG concentrations in PhenoDiet group A (a) and B (b), and plasma FFA concentrations in PhenoDiet group A (c) and B (d) in response to a liquid high-fat mixed meal at week 0 and week 12. Dashed lines indicate week 0, solid lines week 12. Data are presented as geometric means with 95% CI. The differences in area under the curves (AUCs) between PhenoDiet group A and B were assessed using a mixed model with repeated measures with adjustments for age, sex, and center. The AUCs for both postprandial FFA and TAG decreased in both groups (P for time all <0.05), with a trend for a slightly larger reduction in postprandial TAG in PhenoDiet group B compared to PhenoDiet group A, but this did not reach statistical significance (P for group x time = 0.108). *P*-values <0.05 are highlighted in bold. FFA, free fatty acids; TAG, triacylglycerol.

Supplementary Table 1. Targeted nutrient composition of the HMUFA and LFHP diet

	HMUFA	LFHP
Fat (en%)	38	28
Monounsaturated fat	20	10
Polyunsaturated fat	8	8
Saturated fat	8	8
Protein(en%)	14	24
Animal-based (% of total protein)	45	60
Plant-based (% of total protein)	55	40
Carbohydrates (en%)	42	42
Mono- and disaccharides	12	12
Polysaccharides	30	30
Fiber(g/MJ)	3	>4
Alcohol	<3	<3

en%, energy percentage of total energy intake; MJ, megajoule

Supplementary Table 2. Baseline characteristics with stratification for IR phenotype and diet intervention

	MIR phenotype			LIR phenotype			*p*-value	
	PhenoDiet group A	PhenoDiet group B	*p*-value	PhenoDiet group A	PhenoDiet group B	*p*-value		
	HMUFA diet	LFHP diet	HMUFA vs. LFHP	LFHP diet	HMUFA diet	HMUFA vs. LFHP	MIR vs. LIR	HMUFA vs. LFHP
Age, years	60 ± 8	61 ± 8	0.752	60 ± 7	60 ± 8	0.972	0.596	0.808
Women, n (%)	45 (59.2%)	48 (65.8%)	0.499	21 (46.7%)	27 (56.3%)	0.409	0.109	1.000
BMI, kg/m^2	29.7 ± 3.6	29.3 ± 3.0	0.691	30.1 ± 1.3	30.7 ± 3.8	0.259	**0.037**	0.277
Medication use, n (%)								
Antidepressants	2 (2.6%)	8 (11.0%)	0.053	3 (6.7%)	4 (8.3%)	1.000	0.801	0.212
Antihypertensives	18 (23.7%)	12 (16.4%)	0.311	9 (20.0%)	4 (8.3%)	0.138	0.300	1.000
Anti-inflammatory medication	11 (14.5%)	8 (11.0%)	0.626	3 (6.7%)	1 (2.1%)	0.351	**0.041**	1.000
Statins	6 (7.9%)	5 (6.8%)	1.000	3 (6.7%)	2 (4.2%)	0.671	0.606	1.000
Other	27 (35.5%)	24 (32.9%)	0.863	15 (33.3%)	13 (27.1%)	0.652	0.574	1.000
Family history of diabetes, n (%)	15 (19.7%)	19 (26.0%)	0.436	7 (15.6%)	13 (27.1%)	0.212	0.875	1.000
Glucose status, n (%)			0.231			0.712	0.131	0.256
NGT	61 (80.3%)	52 (71.2%)		33 (73.3%)	36 (75.0%)			
IFG	2 (2.6%)	0 (0%)		3 (6.7%)	4 (8.3%)			
IGT	7 (9.2%)	13 (17.8%)		5 (11.1%)	3 (6.3%)			
Combined IFG/IGT	3 (3.9%)	2 (2.7%)		0 (0%)	2 (4.2%)			
T2DM	3 (3.9%)	6 (8.2%)		2 (4.4%)	3 (6.3%)			
Habitual physical activity, Baecke score	8.3 ± 1.2	8.3 ± 1.1	0.714	8.5 ± 1.3	8.3 ± 1.3	0.390	0.436	0.401
Employment status, n (%)			**0.009**			0.482	0.262	0.064
Paid job	45 (60.0%)	29 (39.7%)		28 (62.2%)	26 (54.2%)			
Retired	25 (33.3%)	28 (38.4%)		9 (20.0%)	15 (31.3%)			
Other	5 (6.7%)	16 (21.9%)		8 (17.8%)	7 (14.6%)			
Education level, n (%)			**0.034**			**0.045**	**0.035**	**0.002**
Low	3 (3.9%)	12 (16.4%)		15 (33.3%)	6 (12.5%)			
Intermediate	35 (46.1%)	27 (37.0%)		12 (26.7%)	21 (43.8%)			
High	37 (48.7%)	33 (45.2%)		18 (40.0%)	21 (43.8%)			

Values are n (%) or mean ± SD. *P*-values <0.05 are highlighted in bold MIR, muscle insulin resistance; LIR, liver insulin resistance; BMI, body mass index; NGT, normal glucose tolerance; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; T2DM, type 2 diabetes mellitus.

Supplementary Table 3. Baseline characteristics of drop-outs compared to completers

	PhenoDiet group A		PhenoDiet group B	
	Study completers (n = 114)	Drop-outs (n = 114)	Study completers (n = 107)	Drop-outs (n = 14)
Age, years	60 ± 7	54 ± 13	61 ± 8.2	59 ± 8
Women, n (%)	60 (52.6%)	6 (85.7%)	67 (62.6%)	8 (57.1%)
BMI, kg/m^2	29.7 ± 3.4	32.0 ± 5.6	29.9 ± 3.4	30.2 ± 3.1
Medication use, n (%)				
Antidepressants	4 (3.5%)	1 (14.3%)	11 (10.3%)	1 (7.1%)
Antihypertensives	25 (21.9%)	2 (28.6%)	15 (14.0%)	1 (7.1%)
Anti-inflammatory medication	14 (12.3%)	0 (0%)	8 (7.5%)	1 (7.1%)
Statins	9 (7.9%)	0 (0%)	7 (6.5%)	0 (0%)
Other	40 (35.1%)	2 (28.6%)	33 (30.8%)	4 (28.6%)
Family history of diabetes, n (%)	19 (16.7%)	3 (42.9%)	29 (27.1%)	3 (21.4%)
Glucose status, n (%)				
NGT	89 (78.1%)	5 (100%)	78 (72.9%)	10 (71.4%)
IFG	5 (4.4%)	0 (0%)	4 (3.7%)	0 (0%)
IGT	12 (10.5%)	0 (0%)	15 (14.0%)	1 (7.1%)
Combined IFG/IGT	3 (2.6%)	0 (0%)	2 (1.9%)	2 (14.3%)
T2DM	5 (4.4%)	0 (0%)	8 (7.5%)	1 (7.1%)
Habitual physical activity, Baecke score	8.4 ± 1.2	8.3 ± 1.3	8.3 ± 1.2	8.4 ± 1.1
Employment status, n (%)				
Paid job	68 (59.6%)	5 (71.4%)	47 (43.9%)	8 (57.1%)
Retired	33 (28.9%)	1 (14.3%)	39 (36.4%)	4 (28.6%)
Other	12 (10.5%)	1 (14.3%)	21 (19.6%)	2 (14.3%)
Education level, n (%)				
Low	17 (14.9%)	1 (14.3%)	16 (15.1%)	2 (14.3%)
Intermediate	42 (36.8%)	5 (71.4%)	43 (40.6%)	5 (35.7%)
High	54 (47.4%)	1 (14.3%)	47 (44.3%)	7 (50.0%)

Values are n (%) or mean ± SD. MIR, muscle insulin resistance; LIR, liver insulin resistance; BMI, body mass index; NGT, normal glucose tolerance; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; T2D, type 2 diabetes.

Supplementary Table 4. Habitual dietary intake at baseline as assessed by FFQ

	PhenoDiet group A (n=104)	PhenoDiet group B (n=109)
Energy (MJ)	9.6 [7.8,10.9]	8.6 [7.4,10.6]
Fat (en%)	37.9 ± 5.9	37.5 ± 5.5
Monounsaturated fat	13.6 ± 2.8	13.4 ± 2.2
Polyunsaturated fat	7.3 ± 1.8	7.2 ± 1.8
Saturated fat	13.8 ± 2.5	13.8 ± 2.9
Protein (en%)	15.6 ± 2.0	15.6 ± 2.1
Animal-based (% of total protein)	58.9 ± 9.3	59.2 ± 8.2
Plant-based (% of total protein)	41.1 ± 9.3	40.9 ± 8.2
Carbohydrates (en%)	41.1 ± 5.9	41.9 ± 5.6
Mono-and disaccharides	19.0 ± 5.4	19.0 ± 5.2
Polysaccharides	22.1 ± 4.7	22.8 ± 4.5
Fiber(g/MJ)	2.6 ± 0.6	2.6 ± 0.6
Alcohol	2.2 [0.6,4.0]	1.5 [0.6,3.5]

Values are mean ± SD or median [IQR] if not normally distributed.
FFQ, food frequency questionnaire; MJ, megajoule; en%, energy percentage.

Supplementary Table 5. Advised macronutrient composition of the intervention diets and reported intake

	Advised		PhenoDiet group A		PhenoDiet group B		*p*-value
	HMUFA	LFHP	MIR–HMUFA (n=62)	LIR–LFHP (n=41)	LIR–HMUFA (n=42)	MIR-LFHP (n=61)	HMUFA vs. LFHP
Energy (MJ)	9.8 ± 1.6	9.5 ± 1.6	8.1 ± 1.4	8.6 ± 1.7	8.6 ± 2	8.1 ± 1.4	0.966
Fat (en%)	38.3 ± 0.4	27.5 ± 0.5	34.8 ± 5.1	27.1 ± 3.3	33.9 ± 5.4	28.3 ± 4.4	**<0.001**
Monounsaturated fat	19.8 ± 0.5	9.7 ± 0.1	16.5 ± 3.7	9.2 ± 1.6	16.0 ± 3.7	9.4 ± 1.7	**<0.001**
Polyunsaturated fat	8.1 ± 0.3	7.7 ± 0.2	7.4 ± 1.7	6.6 ± 1.5	7.2 ± 1.4	7.4 ± 1.8	0.365
Saturated fat	7.8 ± 0.2	7.3 ± 0.2	8.4 ± 1.3	8.7 ± 1.7	8.3 ± 1.1	8.8 ± 1.8	0.071
Protein(en%)	14.0 ± 0.2	23.5 ± 0.2	16.6 ± 2.2	24.5 ± 2.1	17.3 ± 2.5	22.9 ± 3.2	**<0.001**
Animal-based, % of total	45.1 ± 1.3	60.1 ± 0.6	50.9 ± 9.2	65.7 ± 5.4	52.2 ± 7.8	60.9 ± 8.9	**<0.001**
Plant-based, % of total	54.9 ± 1.3	39.9 ± 0.6	49.1 ± 9.2	34.4 ± 5.4	47.8 ± 7.8	39.1 ± 8.9	**<0.001**
Carbohydrates(en%)	41.4 ± 0.4	42.4 ± 0.5	43.8 ± 4.8	43.4 ± 4.4	44.0 ± 4.5	43.6 ± 4.2	0.538
Mono- and disaccharides	12.9 ± 0.5	13.4 ± 0.3	15.6 ± 3.6	16.4 ± 2.7	15.2 ± 2.5	15.6 ± 3	0.204
Polysaccharides	28.5 ± 0.8	29.0 ± 0.5	28.3 ± 4.4	27.0 ± 3.5	28.8 ± 3.6	28.0 ± 3.3	0.084
Fiber (g/MJ)	3.0 ± 0.1	4.4 ± 0.2	3.3 ± 0.5	4.0 ± 0.6	3.3 ± 0.5	4.0 ± 0.6	**<0.001**
Alcohol	3.3 ± 0.4	2.4 ± 0.3	1.5 ± 2.2	0.9 ± 1.7	1.5 ± 1.9	1.3 ± 2.2	0.166

Values are mean ± SD. Advised intake is based on the targeted macronutrient composition (Supplementary Table 1) according to the individual meal plans as described in more detail elsewhere (24). *P*-values <0.05 are highlighted in bold. MIR, muscle insulin resistance; LIR, liver insulin resistance; en%, energy percentage of total energy intake; MJ, megajoule.

Supplementary Table 7. *Post-hoc* analyses of intervention effects on secondary outcomes with stratification for IR phenotype that were significantly different between PhenoDiet groups A and B.

	MIR phenotype		**LIR phenotype**	
	PhenoDiet group A HMUFA diet	PhenoDiet group B LFHP diet	PhenoDiet group A LFHP diet	PhenoDiet group B HMUFA diet
Fasting insulin (pmol/L)				
Week 0	47.5 (42.9 - 52.6)	49.9 (45.1 - 55.3)	47.5 (41.8 - 53.9)	57.0 (50.7 - 64.2)
Week 12	47.0 (42.6 - 51.8)	43.7 (39.5 - 48.3)	44.4 (38.5 - 51.2)	49.4 (43.1 - 56.5)
p-value time	0.762	**< 0.001**	0.230	**0.008**
2-hr glucose (mmol/L)				
Week 0	6.5 (6.1 - 6.9)	6.8 (6.4 - 7.3)	5.5 (5.0 - 6.1)	6.1 (5.5 - 6.7)
Week 12	6.4 (6.0 - 6.9)	6.4 (6.0 - 6.9)	5.8 (5.3 - 6.4)	5.8 (5.3 - 6.3)
p-value time	0.714	**0.034**	0.193	0.123
2-hr insulin (pmol/L)				
Week 0	370.8 (316.7 - 434.2)	429.5 (366.9 - 502.8)	295.9 (233.3 - 375.2)	342.8 (274.6 - 427.8)
Week 12	362.7 (310.8 - 423.2)	343.3 (292.9 - 402.3)	285.0 (230.7 - 352.2)	291.1 (238.1 - 355.8)
p-value time	0.722	**< 0.001**	0.688	0.069
HOMA-IR (AU)				
Week 0	1.6 (1.4 - 1.8)	1.7 (1.5 - 1.9)	1.6 (1.4 - 1.9)	2.0 (1.8 - 2.3)
Week 12	1.6 (1.4 - 1.8)	1.5 (1.3 - 1.7)	1.5 (1.3 - 1.8)	1.7 (1.5 - 2.0)
p-value time	0.481	**< 0.001**	0.274	**0.003**
Matsuda index (AU)				
Week 0	5.0 (4.5 - 5.6)	4.5 (4.0 - 5.0)	4.6 (4.0 - 5.4)	3.9 (3.4 - 4.6)
Week 12	0.5.1 (4.5 - 5.7)	5.4 (4.8 - 6.1)	5.1 (4.4 - 6)	4.6 (4.0 - 5.3)
p-value time	0.697	**< 0.001**	0.099	**0.008**
MISI (AU)				
Week 0	0.114 (0.098 - 0.132)	0.119 (0.102 - 0.139)	0.146 (0.118 - 0.181)	0.112 (0.092 - 0.137)
Week 12	0.112 (0.094 - 0.133)	0.147 (0.123 - 0.176)	0.162 (0.136 - 0.194)	0.156 (0.131 - 0.185)
p-value time	0.887	**0.030**	0.388	**0.007**
Serum TAG (mmol/L)				
Week 0	1.2 (1.1 - 1.4)	1.5 (1.4 - 1.6)	1.4 (1.2 - 1.6)	1.4 (1.3 - 1.6)
Week 12	1.2 (1.1 - 1.4)	1.3 (1.2 - 1.4)	1.3 (1.1 - 1.4)	1.3 (1.1 - 1.4)
p-value time	0.894	**< 0.001**	**0.019**	**0.006**
Serum CRP (mg/L)				
Week 0	1.1 (0.9 - 1.4)	1.3 (1.0 - 1.6)	0.8 (0.6 - 1.1)	0.9 (0.7 - 1.2)
Week 12	1.2 (0.9 - 1.6)	0.9 (0.7 - 1.2)	0.7 (0.5 - 1.0)	0.8 (0.6 - 1.1)
p-value time	0.727	**0.005**	0.433	0.240

Values are estimated marginal means with 95% confidence intervals, adjusted for age, sex and center.
P-values <0.05 are highlighted in bold.
MIR, muscle insulin resistance; LIR, liver insulin resistance; HOMA-IR, homeostasis model assessment of insulin resistance; MISI, muscle insulin sensitivity index; TAG, triacylglyceride; CRP, C-reactive protein

Supplementary Table 5. Effects of 12-week HMUFA and LFHP diet on plasma FFA concentrations

	HMUFA		LFHP		*p*-value		
	Week 0	Week 12	Week 0	Week 12	Diet	Time	Diet x Time
TotalFA (mmol/L)	13.2 (12.7-13.6)	12.5 (12.1-12.9)	13.4 (12.9-13.8)	12.4 (11.9-12.8)	0.918	**<0.001**	0.117
MUFA (mmol/L)	3.4 (3.2-3.5)	3.3 (3.2-3.4)	3.5 (3.3-3.7)	3.2 (3.0-3.3)	0.977	**<0.001**	**0.001**
MUFA (%)	25.7 (25.3-26)	26.4 (26.0-26.8)	26.2 (25.8-26.6)	25.8 (25.4-26.2)	0.891	0.074	**<0.001**
PUFA (mmol/L)	5.4 (5.3-5.5)	5.1 (5.0-5.3)	5.4 (5.3-5.6)	5.1 (5.0-5.3)	0.861	**<0.001**	0.902
PUFA (%)	41.0 (40.6-41.5)	41.0 (40.6-41.5)	40.6 (40.1-41.1)	41.6 (41.1-42.0)	0.879	**<0.001**	**<0.001**
PUFA/MUFA ratio	1.60 (1.56-1.64)	1.56 (1.52-1.59)	1.55 (1.51-1.59)	1.61 (1.57-1.65)	0.888	0.467	**<0.001**
SFA (mmol/L)	4.4 (4.2-4.5)	4.0 (3.9-4.2)	4.4 (4.2-4.6)	4.0 (3.9-4.2)	0.927	**<0.001**	0.261
SFA (%)	33.1 (32.9-33.3)	32.4 (32.2-32.7)	33.0 (32.8-33.3)	32.5 (32.2-32.7)	0.990	**<0.001**	0.364

Values are estimated marginal means with 95% confidence intervals, adjusted for age, sex and center.

Chapter 7

Body composition and body fat distribution in tissue-specific insulin resistance and in response to a 12-week isocaloric dietary macronutrient intervention

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ABSTRACT

Background: Body composition and body fat distribution are important predictors of cardiometabolic diseases. The etiology of cardiometabolic diseases is heterogenous, and partly driven by inter-individual differences in tissue-specific insulin sensitivity.

Objectives: To investigate 1) the associations between body composition and whole-body, liver and muscle insulin sensitivity, and 2) changes in body composition and insulin sensitivity and their relationship after a 12-week isocaloric diet high in mono-unsaturated fatty acids (HMUFA) or a low-fat, high-protein, high-fiber (LFHP) diet.

Design: 93 individuals (53% women, BMI 25-40 kg/m^2, 40-75 years) participated in this randomized intervention study. At baseline and after 12 weeks of following the LFHP, or HMUFA diet, we performed a 7-point oral glucose tolerance test to assess whole-body, liver, and muscle insulin sensitivity, and whole-body magnetic resonance imaging to determine body composition and body fat distribution. Both diets are within the guidelines of healthy nutrition.

Results: At baseline, liver fat content was associated with worse liver insulin sensitivity ([95%CI]; 0.12 [0.01; 0.22]). Only in women, thigh muscle fat content was inversely related to muscle insulin sensitivity (-0.27 [-0.48; -0.05]). Visceral adipose tissue (VAT) was inversely associated with whole-body, liver, and muscle insulin sensitivity. Both diets decreased VAT, abdominal subcutaneous adipose tissue (aSAT), and liver fat, but not whole-body and tissue- specific insulin sensitivity with no differences between diets. Waist circumference, however, decreased more following the LFHP diet as compared to the HMUFA diet (-3.0 vs. -0.5 cm, respectively). After the LFHP but not HMUFA diet, improvements in body composition were positively associated with improvements in whole-body and liver insulin sensitivity.

Conclusions: Liver and muscle insulin sensitivity are distinctly associated with liver and muscle fat accumulation. Although both LFHP and HMUFA diets improved in body fat, VAT, aSAT, and liver fat, only LFHP-induced improvements in body composition are associated with improved insulin sensitivity.

INTRODUCTION

Obesity is accompanied by an increased risk for the development of chronic cardiometabolic diseases, including cardiovascular diseases (CVD), type 2 diabetes (T2D) and several types of cancer. However, individuals with obesity make a heterogeneous group where some develop cardiometabolic diseases while others remain relatively healthy (1, 2). Furthermore, the etiology towards obesity-associated cardiometabolic diseases is highly heterogenous. Insulin resistance for example can develop to a different extent in insulin-sensitive organs such as the liver and skeletal muscle within individuals with obesity, representing different etiologies towards T2D and cardiometabolic risk (3).

The body mass index (BMI) is a simple and inexpensive measurement that has been extensively used to identify obesity, but it is not a good indicator of cardiometabolic health at the individual level (4). Body composition and body fat distribution, which include the distribution of fat storage in different adipose tissue depots, skeletal muscle mass, and ectopic fat deposition, can help to explain the differences in cardiometabolic disease risk observed among individuals with overweight or obesity (1, 5-8). Indeed, excessive abdominal visceral and subcutaneous adipose tissue (VAT and SAT) (6) and low skeletal muscle mass (1, 7), as well as liver fat (6), are strongly associated with whole-body insulin resistance in humans. Body composition and body fat distribution may be important determinants of tissue-specific metabolic disturbances and may thus also be associated with tissue-specific insulin resistance (7, 9). Notably, clear sex differences in body composition and its relationship to cardiometabolic diseases have been reported, as extensively reviewed (10). However, whether body composition and body fat distribution can (partially) explain the distinct etiologies of the tissue-specific insulin resistant phenotypes in obesity, and whether this is different between men and women, is unclear.

Adopting a healthy diet is an important strategy for decreasing cardiometabolic disease risk, at least partially due to positive effects on body composition (11-13). Both quality and quantity of dietary protein, fat and carbohydrate seem to impact body composition, body fat distribution, and ectopic fat deposition (9, 12), as well as affect insulin sensitivity and glucose control (14). We have recently shown that two isocaloric diets within guidelines of healthy nutrition - a low- fat, high-protein, high-fiber diet (LFHP) and a high mono-unsaturated fatty acid diet (HMUFA) - can both elicit pronounced improvements in body composition and several cardiometabolic parameters (15). Nevertheless, it is not yet clear what the effect of these two isocaloric healthy diets differing in macronutrient composition are on body composition and body fat distribution, measured with state-of-the-art

methodology, including characterization of VAT and SAT, ectopic fat deposition, and skeletal muscle volume and whether these improvements are related to improvements in (whole-body and tissue-specific) insulin sensitivity.

The present study aimed to investigate the relationship between body composition, body fat distribution, ectopic fat deposition, and muscle volume with whole-body and tissue-specific insulin sensitivity. Furthermore, we investigated the impact of a 12-week dietary intervention with either an isocaloric LFHP or a HMUFA diet, both within the context of the Dutch dietary guidelines for healthy nutrition, on changes in body composition and insulin sensitivity, as well as the relationship between improvements in body composition and improvements in insulin sensitivity. The findings may provide leads of dietary intervention strategies that better target cardiometabolic risk factors in obesity.

METHODS

Study design and population

The present analysis is part of the larger two-center (Maastricht University Medical Center+ (MUMC+) and Wageningen University (WUR), both the Netherlands) double-blind, randomized trial, the PERSonalized Glucose Optimization Through Nutritional Intervention (PERSON) study. The rationale and methodology of the PERSON study have been described previously (16). In short, 242 men and women (age 40 – 75 years) with overweight or obesity (BMI 25 – 40 kg/m^2) with either predominant liver or muscle insulin resistance participated in a 12-week isocaloric dietary intervention investigating the effect modulation of dietary macronutrient composition according to tissue-specific insulin resistance phenotypes on measures of glucose homeostasis and quality of life. The presence of tissue-specific insulin resistance was based the glucose and insulin responses from a 7-point oral glucose tolerance test (OGTT) at screening, from which the hepatic insulin resistance index (HIRI) and the muscle insulin sensitivity index (MISI) were calculated (16). Tertile cut-offs for MISI and HIRI from a previous study (the Maastricht Study (17)) were used to identify individuals with predominant muscle or liver insulin resistance. Before and after the intervention, extensive measurements in laboratory and daily life were performed. Exclusion criteria for participation included amongst others: not weight stable (>3 kg weight change in last 3 months), smoking, alcohol abuse (>14 glasses/week), pre-diagnosis of diabetes, major cardiovascular disease, major gastrointestinal disease or surgery, and dietary restrictions interfering with the dietary study protocol. For the present analysis, 93 individuals from the PERSON study that underwent a whole-body magnetic resonance imaging (MRI) scan at MUMC+ were included in the analysis.

The study was approved by The Medical Ethics Committee of MUMC+ (NL63768.068.17) and registered at ClinicalTrials.gov (identifier: NCT03708419). All participants provided written informed consent. The study was carried out in accordance with the principles of the Declaration of Helsinki.

Dietary intervention

Participants were allocated to follow an isocaloric diet either high in mono-unsaturated fatty acids (HMUFA) or low in fat and high in protein and fiber (LFHP) for 12 weeks. The HMUFA diet had a targeted macronutrient composition of 38% of energy from fat (20 en% mono- unsaturated, 8ven% poly-unsaturated, and 8 en% saturated fatty acids), 48% of energy from carbohydrates (30 en% polysaccharides, 3 g/MJ fiber), and 14% of energy from protein. For the LFHP diet, the targeted macronutrient composition was 28% of energy from fat (10 en% mono-unsaturated, 8 en% poly-unsaturated, and 8 en% saturated fatty acids), 48% of energy from carbohydrates (30 en% polysaccharides, >4 g/MJ fiber), and 24% of energy from protein. Participants received verbal and written dietary instructions at the start of the intervention and received weekly dietary counseling and were provided with key food products. Body weight was monitored every week to ensure participants remained relatively weight stable throughout the intervention. Dietary adherence was assessed with three unannounced one-day food records during the intervention. An extensive description of the details of the dietary intervention are described in detail elsewhere (16).

Anthropometrics and body composition

Body weight (kg) and height (cm), and waist and hip circumference (cm) were measured to the closest 0.1 unit in duplicate and in underwear. A subgroup (n=93) of the total PERSON study population (MUMC+ participants) underwent a whole body 3T MRI scan (3T MAGNETOM Prisma fit, Siemens Healthcare). Analyses were performed using computational modeling (method by AMRA Medical AB, Linköping, Sweden (18)). Fat ratio: (%, total abdominal adipose tissue / (total abdominal adipose tissue + total thigh muscle volume)*100), VAT volume (L), abdominal subcutaneous adipose (aSAT) volume (L), thigh muscle fat (%), liver fat: (%), abdominal adipose tissue (AT) index (L/m^2, (VAT+aSAT)/height2), weight-to- muscle ratio (kg/L, body weight/total muscle volume), thigh muscle volume (L), and thigh muscle volume Z-score (adjusted for sex and body size (height, body weight, BMI) invariant) were quantified. Participants underwent a dual-energy X-ray absorptiometry (DXA) (MUMC+, Discovery A, Hologic; WUR, Lunar Prodigy, GE Healthcare) to determine body fat %.

Whole-body and tissue-specific insulin sensitivity

Whole-body and tissue-specific (liver and muscle) insulin sensitivity were estimated based on a 7-point OGTT at baseline and after 12 weeks of intervention. Participants ingested a 200ml, 75gr glucose solution (Novolab), after an overnight fast. Blood draws were performed from the antecubital vein at t = 0, 15, 30, 45, 60, 90, and 120 minutes and plasma glucose and insulin were determined. From these glucose and insulin values, we calculated the Matsuda index for whole-body insulin sensitivity, HIRI for liver insulin sensitivity, and MISI for muscle insulin sensitivity. The Matsuda index was calculated by: 10.000 ÷ square root of (fasting plasma glucose (mmol/L) x fasting insulin (pmol/L) x (mean glucose (mmol/L) x mean insulin (pmol/L)) using 5 timepoints of the OGTT (excluding t = 15 and 45). The calculations of the HIRI and MISI have been described previously (16). Briefly, HIRI was calculated as the area under the curve from both glucose and insulin in the first 30 minutes of the OGTT while the MISI was calculated by the slope of the peak of the OGTT to the nadir divided by mean insulin concentrations throughout the OGTT. The Matsuda index (19), HIRI, and MISI (20) have been validated against the golden standard hyperinsulinemic clamp technique.

Statistical analysis

Participant characteristics are presented as mean ± standard deviation (SD) and an independent t-test was performed to assess differences between sexes at baseline.

Cross-sectional relationship between body composition and body fat distribution with (whole-body and tissue-specific) insulin sensitivity:

Linear regression analysis was performed to assess crude associations (model 1), with adjustments for age and sex (model 2), additionally adjusted for body weight (model 3), and additionally adjusted for MISI in the model with HIRI and vice versa (model 4). Variables that are expressed relative to body size (for example body fat %) were not adjusted for body weight in model 3 and 4. Effect modification by sex was assessed by addition of an interaction term. In case of significant interaction, results are presented separately for women and men. Data are reported as (95% confidence interval (CI)).

The effects of both diets on changes in (whole-body and tissue-specific) insulin sensitivity, body composition, and body fat distribution:

Repeated mixed-model analysis was performed including time (pre- and post-intervention) as repeated measure, while adjusting for age and sex. To examine whether the two diets differentially affected these parameters, an interaction term between diet*time was added. Data are reported as Estimated Marginal Mean (EMM) (95% CI).

The associations between diet-induced changes in body composition and changes in (whole-body and tissue-specific) insulin sensitivity:

Linear regression analysis was performed including model 1 (with adjustment for diet) is reported, as well as model 2 (additionally adjusted for age and sex), model 3 (additionally adjusted for weight change (D weight)), and model 4 (additionally adjusted for MISI or HIRI in the model with HIRI and MISI as dependent variable, respectively). Interaction terms were included to test for effect modification of diet and sex, and stratified analysis are reported in case of significance. Data are reported as β (95% CI).

Data were transformed with the natural logarithm in case of not-normally distributed residuals. Statistical significance was defined as $P < 0.05$. Analyses were performed using the IBM SPSS Statistics software (version 25).

RESULTS

Participant characteristics

Complete data of 49 women and 44 men was available for the present analysis. As indicated in Table 1, women were slightly younger compared to men (58.3 ± 8.9 vs. 62.0 ± 7.5 years, respectively) ($P = 0.034$). BMI was similar between sexes, on average 31.7 ± 3.8 and 30.8 ± 2.9 kg/m^2, for women and men, respectively ($P = 0.231$). Parameters of glucose homeostasis were comparable between sexes. On average, individuals were normal glucose tolerant according to clinical cut-off values for fasting and 2-hr glucose values (21). Large differences between most parameters of body composition were observed between sexes. Women showed greater total body fat %, higher abdominal AT index, and lower VAT volume and muscle fat % compared to men (all $P < 0.001$). Liver fat % and muscle volume Z-score (normalized for sex and body size) were similar between sexes (Table 1).

Associations between body composition and whole-body and tissue-specific insulin sensitivity

Body weight was significantly associated with the Matsuda index and HIRI, but not with MISI (Table 2). The Matsuda index, HIRI, and MISI showed distinct associations with parameters of body composition. Only for the association between MISI and muscle fat, a significant sex interaction was present, and data are reported separately for women and men. Specifically, higher muscle fat (%) was associated with lower MISI (i.e., with lower muscle insulin sensitivity) in women (-0.27 (-0.48; -0.05),$P = 0.016$), but not in men. Muscle fat (%) was not related to the Matsuda index or HIRI. Higher liver fat was associated

Table 1. Baseline participant characteristics, stratified by sex.

	Women (n=49)	Men (n=44)	*P*-value
General characteristics			
Age (years)	58.3 ± 8.9	62.0 ± 7.5	**0.034**
Premenopausal (n, %)	8 (16%)	n/a	
BMI (kg/m^2)	31.7 ± 3.8	30.8 ± 2.9	0.231
Body weight (kg)	86.9 ± 11.5	97.4 ± 11.9	**<0.001**
Waist circumference (cm)	98.2 ± 9.5	107.7 ± 8.2	**<0.001**
Waist-to-hip (ratio)	0.86 ± 0.06	1.02 ± 0.06	**<0.001**
Total cholesterol (mmol/L)	5.52 ± 0.92	4.86 ± 0.78	**<0.001**
Systolic blood pressure (mmHg)	123 ± 12	133 ± 12	**<0.001**
Diastolic blood pressure (mmHg)	84 ± 8	85 ± 9	0.700
Glucose parameters			
HbA1c (mmol/L)	52.1 [37.2; 66.4]	56.7 [41.1; 67.9]	0.879
Fasting glucose (mmol/L)	5.5 ± 0.7	5.7 ± 0.8	0.187
2-hr glucose (mmol/L)	6.5 [5.3; 7.3]	6.4 [5.1; 8.5]	0.979
Fasting insulin (pmol/L)	52.1 [37.2; 66.4]	56.7 [41.1; 67.9]	0.310
HOMA-IR (A.U.)	1.80 [1.18; 2.49]	2.02 [1.37; 2.55]	0.221
Matsuda index (A.U.)	11.2 [7.3; 17.3]	10.3 [7.0; 13.9]	0.310
HIRI (A.U.)	418 [308; 588]	423 [311; 599]	0.945
MISI (A.U.)	0.129 [0.093; 0.199]	0.134 [0.088; 0.209]	0.911
Body composition			
Body fat (%)	43.5 [40.5; 46.0]	30.9 [27.5; 32.6]	**<0.001**
Fat ratio (%)	63.6 ± 6.2	52.3 ± 6.7	**<0.001**
VAT (L)	4.4 ± 1.4	7.3 ± 1.9	**<0.001**
aSAT (L)	12.7 [9.9; 14.6]	7.9 [6.4; 9.6]	**<0.001**
Muscle fat (%)	8.3 [7.4; 9.8]	6.7 [5.6; 7.7]	**<0.001**
Liver fat (%)	4.6 [3.1; 13.6]	5.3 [3.1; 12.2]	0.721
Abdominal AT index (L/m^2)	6.2 ± 1.4	5.0 ± 1.3	**<0.001**
Weight-to-muscle (ratio)	9.4 [8.4; 10.3]	7.1 [6.5; 7.5]	**<0.001**
Muscle volume (L)	9.3 [8.4; 10.3]	13.9 [12.6; 14.6]	**<0.001**
Muscle volume (Z-score)	0.100 ± 1.148	0.220 ± 0.921	0.584

Data are presented as mean ± standard deviation or median [inter quartile range] in case of non-normally distributed values. An independent sample t-test (normally distributed variables) or a Mann-Whitney U test (non- normally distributed variables) was performed to assess differences between sexes. Significant *P*-values (<0.05) are highlighted in bold. aSAT, abdominal subcutaneous adipose tissue; HIRI, hepatic insulin resistance index; HOMA-IR, homeostatic model assessment for insulin resistance; MISI, muscle insulin sensitivity index; VAT, visceral adipose tissue

with lower whole-body, liver, and muscle insulin sensitivity (i.e. Matsuda index, HIRI and MISI, respectively), but the significant association between liver fat and MISI disappeared after adjustment for HIRI. All measures of insulin sensitivity were independently associated with the abdominal AT index, VAT, and waist circumference, although the latter two did not reach statistical significance in model 4 (adjustment for age, sex, body weight, HIRI) for MISI ($P = 0.073$ and $P = 0.063$, respectively). Fat ratio (%) was only associated with the Matsuda index (-0.25 (-0.38; -0.13), $P < 0.001$), but not with MISI or HIRI.

Diet-induced changes in body composition and whole-body and tissue-specific insulin sensitivity

Body weight decreased to a similar extent in both diets, from 90.3 (95% CI, 87.5; 93.3) to 88.4 (85.6; 91.3) kg following HMUFA diet and from 91.3 (88.0; 94.8) to 89.3 (86.0; 92.8) kg following the LFHP diet (time $P < 0.001$, diet x time $P = 0.824$) (Table 3). Body fat, fat ratio, VAT, aSAT, liver fat, the abdominal AT index, and muscle volume also decreased following both diets (all time $P < 0.05$), without significant differences between diets. Waist circumference decreased from 103.8 (101.1; 106.7) to 100.8 (98.2; 103.5) cm following the LFHP diet, while it remained similar (from 100.8 (98.5; 103.1) to 100.3 (98.0; 102.6) cm) following the HMUFA diet (diet x time $P = 0.006$). Trends for greater decrease in muscle fat (diet x time $P = 0.071$), and lower muscle volume (z-score) decrease (diet x time $P = 0.071$) were observed following the LFHP compared to HMUFA diet. The Matsuda index, HIRI, and MISI were not significantly affected by either the HMUFA or LFHP diet (Table 3).

Associations between diet-induced changes in body composition and changes in whole-body and tissue-specific insulin sensitivity

The associations between changes in body composition and changes in (whole-body and tissue-specific) insulin sensitivity upon the dietary intervention are reported in Table 4. Several interactions with diet and with sex were present. In case of these interactions, data are reported for women and men or for both diets separately. In the fully adjusted model, the change in body weight was positively associated with change in HIRI (0.14 (0.05; 0.23), $P = 0.002$) following both diets. Furthermore, the decrease in VAT was associated with a decrease in HIRI, but only following the LFHP diet (0.27 (0.02; 0.52), $P = 0.039$). No associations between change in body weight and VAT were observed with the Matsuda index or with MISI in the fully adjusted models. The change in body fat was associated with the change in MISI only in men in all models (fully adjusted model: -0.11 (-0.21; -0.01), $P = 0.028$). The change in fat ratio following the LFHP diet was negatively associated with the change in Matsuda index, whilst the change in muscle volume was

Table 2. Associations between tissue-specific and whole-body insulin sensitivity and body composition.

		Model 1 Crude		Model 2 Adj. for age and sex		Model 3 Adj. for age, sex, body weight*		Model 4 Adj. for age, sex, body weight*, MISI/HIRI	
		β (95%CI)	*P*-value	β (95%CI)	*P*-value	β (95%CI)	*P*-value	β (95%CI)	*P*-value
HIRI	Body weight (kg)	0.17 (0.07; 0.27)	**0.001**	0.21 (0.10; 0.32)	**<0.001**			0.21 (0.09; 0.33)	**0.001**
	Waist circumference (cm)	0.24 (0.15; 0.34)	**<0.001**	0.25 (0.15; 0.34)	**<0.001**	0.27 (0.10; 0.43)	**0.002**	0.22 (0.05; 0.39)	**0.014**
	Body fat (%)	0.00 (-0.11; 0.11)	0.970	0.03 (-0.18; 0.23)	0.795			0.00 (-0.19; 0.20)	0.974
	Fat ratio (%)	0.07 (-0.04; 0.17)	0.230	0.16 (-0.01; 0.33)	0.064			0.10 (-0.04; 0.24)	0.157
	VAT (L)	0.16 (0.06; 0.27)	**0.002**	0.27 (0.14; 0.40)	**<0.001**	0.18 (0.04; 0.33)	**0.016**	0.16 (0.01; 0.31)	**0.042**
	aSAT (L)	0.06 (-0.08; 0.20)	0.374	0.14 (0.00; 0.28)	0.051	-0.13 (-0.33; 0.07)	0.193	-0.14 (-0.33; 0.06)	0.159
	Muscle fat (%)	0.03 (-0.08; 0.14)	0.599	0.04 (-0.09; 0.16)	0.577	-0.06 (-0.18; 0.07)	0.346	-0.06 (-0.18; 0.06)	0.350
	Liver fat (%)	0.16 (0.05; 0.26)	**0.003**	0.16 (0.05; 0.26)	**0.003**	0.12 (0.01; 0.22)	**0.028**	0.12 (0.01; 0.22)	**0.029**
	Abdominal AT index (L/m²)	0.12 (0.01; 0.22)	**0.032**	0.15 (0.04; 0.27)	**0.012**			0.13 (0.01; 0.25)	**0.028**
	Weight-to-muscle (ratio)	0.04 (-0.07; 0.14)	0.500	0.10 (-0.07; 0.26)	0.243			0.07 (-0.09; 0.22)	0.401
	Muscle volume (L)	0.06 (-0.05; 0.17)	0.256	0.21 (0.01; 0.41)	**0.038**	0.05 (-0.17; 0.27)	0.643	0.08 (-0.13; 0.29)	0.471
	Muscle volume (Z-score)	-0.07 (-0.26; 0.11)	0.420	-0.07 (-0.22; 0.07)	0.312			-0.04 (-0.18; 0.10)	0.536
MISI	Body weight (kg)	-0.01 (-0.14; 0.12)	0.930	-0.02 (-0.18; 0.14)	0.818			0.07 (-0.09; 0.22)	0.402
	Waist circumference (cm)	-0.10 (-0.23; 0.03)	0.118	-0.14 (-0.29; 0.01)	0.062	-0.30 (-0.52; -0.08)	**0.009**	-0.21 (-0.44; 0.01)	0.063
	Body fat (%)	-0.02 (-0.16; 0.11)	0.725	-0.07 (-0.31; 0.18)	0.591			-0.05 (-0.28; 0.19)	0.700
	Fat ratio (%)	-0.08 (-0.21; 0.06)	0.260	-0.12 (-0.30; 0.05)	0.160			-0.08 (-0.25; 0.09)	0.351
	VAT (L)	-0.10 (-0.23; 0.03)	0.115	-0.18 (-0.35; -0.01)	**0.034**	-0.23 (-0.43; -0.04)	**0.018**	-0.17 (-0.37; 0.02)	0.073
	aSAT (L)	-0.03 (-0.16; 0.11)	0.690	-0.03 (-0.22; 0.13)	0.610	-0.08 (-0.34; 0.19)	0.560	-0.13 (-0.38; 0.12)	0.292
	Muscle fat (%) Women	-0.18 (-0.38; 0.02)	0.070	-0.20 (-0.42; 0.02)	0.067	-0.19 (-0.42; 0.05)	0.115	-0.27 (-0.48; -0.05)	**0.016**
	Muscle fat (%) Men	0.13 (-0.08; 0.34)	0.228	0.34 (-0.09; 0.34)	0.234	0.11 (-0.13; 0.35)	0.343	0.10 (-0.13; 0.33)	0.396
	Liver fat (%)	-0.14 (-0.27; -0.01)	**0.040**	-0.14 (-0.27; -0.01)	**0.042**	-0.15 (-0.28; -0.01)	**0.041**	-0.09 (-0.23; 0.05)	0.210
	Abdominal AT index (L/m²)	0.04 (-0.22; 0.04)	0.166	0.17 (-0.26; 0.03)	0.132			-0.19 (-0.38; 0.00)	**0.045**
	Weight-to-muscle (ratio)	-0.06 (-0.19; 0.07)	0.348	0.35 (-0.32; 0.07)	0.204	0.04 (-0.09; 0.17)	0.498	-0.09 (-0.28; 0.10)	0.342
	Muscle volume (L)	0.50 (-0.12; 0.38)	0.294	0.18 (-0.10; 0.46)	0.205	0.18 (-0.10; 0.46)	0.205	0.19 (-0.08; 0.45)	0.159
	Muscle volume (Z-score)	0.12 (-0.03; 0.26)	0.113	0.11 (-0.05; 0.24)	0.210			0.06 (-0.07; 0.19)	0.340

Table 2. Continued

		Model 1 Crude		Model 2 Adj. for age and sex		Model 3 Adj. for age, sex, body weight*		Model 4 Adj. for age, sex, body weight*, MISI/HIRI	
		β (95%CI)	*P*-value	β (95%CI)	*P*-value	β (95%CI)	*P*-value	β (95%CI)	*P*-value
Matsuda index	Body weight (kg)	-0.12 (-0.22; -0.02)	**0.023**	-0.17 (-0.29; -0.06)	**0.004**				
	Waist circumference (cm)	-0.23 (-0.33; -0.14)	**<0.001**	-0.27 (-0.38; -0.17)	**<0.001**	-0.33 (-0.49; -0.18)	**<0.001**		
	Body fat (%)	0.01 (-0.10; 0.11)	0.905	-0.18 (-0.37; 0.01)	0.057				
	Fat ratio (%)	-0.10 (-0.20; 0.00)	0.054	-0.25 (-0.38; -0.13)	**<0.001**				
	VAT (L)	-0.26 (-0.34; -0.17)	**<0.001**	-0.36 (-0.47; -0.25)	**<0.001**	-0.36 (-0.48; -0.23)	**<0.001**		
	aSAT (L)	-0.03 (-0.14; 0.07)	0.522	-0.16 (-0.28; -0.03)	**0.019**	-0.02 (-0.21; 0.18)	0.862		
	Muscle fat (%)	-0.08 (-0.18; 0.03)	0.135	-0.11 (-0.23; 0.00)	0.058	-0.05 (-0.17; 0.07)	0.382		
	Liver fat (%)	-0.26 (-0.35; -0.17)	**<0.001**	-0.26 (-0.35; -0.17)	**<0.001**	-0.24 (-0.33; -0.14)	**<0.001**		
	Abdominal AT index (L/m^2)	-0.16 (-0.26; -0.06)	**0.002**	-0.24 (-0.35; -0.14)	**<0.001**				
	Weight-to-muscle (ratio)	-0.05 (-0.16; 0.05)	0.317	-0.21 (-0.36; -0.07)	**0.005**				
	Muscle volume (L)	-0.03 (-0.13; 0.08)	0.611	0.02 (-0.18; 0.21)	0.847	0.19 (-0.01; 0.4)	0.065		

* Variables that are expressed relative to body size (body fat, fat ratio, abdominal AT index, weight-to-muscle ratio, muscle volume Z-score) were not adjusted for body weight in model 3 and 4.
Data are reported as β (95% confidence interval (CI)). In case of significant sex interaction, data are reported separately for women and men. Significant *P*-values (<0.05) are highlighted in bold. Adj, adjusted; aSAT, abdominal subcutaneous adipose tissue; AT, adipose tissue; HIRI, hepatic insulin resistance index; MISI, muscle insulin sensitivity index; VAT, visceral adipose tissue.

Table 3. Changes in parameters of body composition and insulin sensitivity following the HMUFA and LFHP diet.

	HMUFA diet		LFHP diet		*P*-value		
	Week 0	Week 12	Week 0	Week 12	Time	Diet	Diet x time
Glucose parameters							
Matsuda index (A.U.)	11.2 (9.8; 12.8)	12.0 (10.5; 13.8)	10.1 (8.6; 11.8)	10.7 (9.1; 12.5)	0.160	0.304	0.895
HIRI (A.U.)	432.9 (375.4; 499.2)	424.4 (358.2; 503.2)	426.0 (360.7; 503.7)	382.3 (313.2; 466.4)	0.777	0.886	0.416
MISI (A.U.)	0.126 (0.106; 0.149)	0.138 (0.116; 0.164)	0.135 (0.111; 0.166)	0.139 (0.114; 0.169)	0.330	0.578	0.635
Body composition							
Body weight (kg)	90.3 (87.5; 93.3)	88.4 (85.6; 91.3)	91.3 (88.0; 94.8)	89.3 (86.0; 92.8)	**<0.001**	0.650	0.824
Waist circumference (cm)	100.8 (98.5; 103.1)	100.3 (98.0; 102.6)	103.8 (101.1; 106.7)	100.8 (98.2; 103.5)	0.422	0.101	**0.006**
Body fat (%)	36.1 (35.0; 37.2)	35.2 (34.0; 36.6)	37.3 (36.0; 38.6)	36.4 (34.9; 38.0)	**0.002**	0.177	0.900
Fat ratio (%)	57.6 (55.8; 59.3)	56.6 (54.8; 58.4)	59.5 (57.5; 61.5)	58.1 (56.1; 60.2)	**<0.001**	0.155	0.212
VAT (L)	5.19 (4.76; 5.65)	4.90 (4.51; 5.33)	5.53 (5.01; 6.11)	5.13 (4.65; 5.65)	**<0.001**	0.332	0.279
aSAT (L)	9.54 (8.84; 10.3)	9.09 (8.40; 9.84)	10.37 (9.48; 11.34)	9.79 (8.93; 10.74)	**<0.001**	0.165	0.476
Muscle fat (%)	7.48 (7.08; 7.89)	7.44 (7.05; 7.85)	7.54 (7.08; 8.03)	7.39 (6.94; 7.86)	0.383	0.847	0.071
Liver fat (%)	5.12 (4.08; 6.44)	3.33 (2.62; 4.24)	7.26 (5.53; 9.54)	5.02 (3.77; 6.69)	**<0.001**	0.055	0.515
Abdominal AT index (L/m^2)	5.25 (4.92; 5.61)	4.98 (4.65; 5.34)	5.62 (5.20; 6.07)	5.27 (4.86; 5.7)	**<0.001**	0.196	0.433
Weight-to-muscle (ratio)	8.05 (7.78; 8.33)	7.99 (7.72; 8.26)	8.26 (7.93; 8.60)	8.16 (7.85; 8.49)	**0.012**	0.343	0.515
Muscle volume (L)	11.21 (10.82; 11.61)	11.08 (10.7; 11.47)	11.06 (10.61; 11.52)	10.98 (10.54; 11.43)	**<0.001**	0.629	0.343
Muscle volume (Z-score)	0.227 (-0.058; 0.511)	0.172 (-0.108; 0.451)	0.027 (-0.304; 0.357)	0.048 (-0.276; 0.37)	0.051	0.366	0.078

Data are presented as Estimated Marginal Mean (EMM) adjusted for age, sex, and study center as analyzed with a mixed model with repeated measure. Significant *p*-values (<0.05) are highlighted in bold. aSAT, abdominal subcutaneous adipose tissue; AT, adipose tissue; HMUFA, high mono-unsaturated fatty acids; LFHP, low-fat high-protein; VAT, visceral adipose tissue.

positively associated with the change in Matsuda index, independent of age, sex, and change in body weight. Associations with fat ratio and muscle volume were not observed in relation to the tissue-specific insulin resistance indices HIRI and MISI.

DISCUSSION

In this study, we investigated the relationship between body composition and body fat distribution and whole-body and tissue-specific insulin sensitivity in men and women with overweight and obesity using state-of-the-art whole-body MRI technology. Additionally, we investigated diet-induced changes in body composition and the relationship with changes in (tissue-specific) insulin sensitivity after a 12-week isocaloric dietary intervention. We show that impaired liver and muscle insulin sensitivity are associated with distinct body composition profiles. Interestingly, liver fat was associated with impaired liver insulin sensitivity, and in women, an association between muscle fat and impaired muscle insulin sensitivity was found. Furthermore, both the LFHP and HMUFA diet resulted in improvements in body composition in the presence of only minor weight loss (~2 kg). The LFHP diet resulted in a greater reduction in waist circumferences as compared to the HMUFA diet. The diet-induced changes in whole- body and tissue-specific insulin sensitivity in the current analyses were in line with changes found in the complete study population of the PERSON study (15) but did not reach statistical significance, which seems related to the smaller sample size in the present study. Despite this, the LFHP-induced reduction in VAT was associated with improved liver insulin sensitivity. Furthermore, the decrease in fat ratio and lower decrease in muscle volume were associated with improvements in whole-body insulin sensitivity following the LFHP but not HMUFA diet.

We observed that women had higher total body fat and muscle fat at baseline compared to men, but with similar insulin sensitivity, which is in line with previous studies (10, 22, 23). This observation may reflect the higher fat storage capacity of women in peripheral tissues including the gluteofemoral region but also in the skeletal muscle without developing adverse effects on insulin sensitivity. In line, when newly diagnosed with type 2 diabetes, the BMI of women has shown to be almost 2 kg/m^2 higher despite similar levels of HbA1c (24). Interestingly, an increase in muscle fat was linked to worse insulin sensitivity in women, but not in men. The underlying mechanisms remain unclear but may related to the fact that women need to attain higher levels of muscle fat to develop insulin resistance, but once women develop prediabetes or T2D, impaired glucose metabolism is more strongly associated with cardiometabolic risk factors compared to men, as reported previously (25). Notably, most women in the present study were in the postmenopausal

Table 4. Associations between the diet-induced changes in tissue-specific insulin resistance and changes in body composition.

			Model 1 Adj. for diet		Model 2 Adj. for diet, age and sex		Model 3 Adj. for diet, age, sex, diet, Δ body weight		Model 4 Adj. for diet, age, sex, diet, Δ body weightv and Δ MISI/HIRI	
			β (95%CI)	*P*-value	β (95%CI)	*P*-value	β (95%CI)	*P*-value	β (95%CI)	*P*-value
Δ HIRI	Δ Body weight (kg)		0.16 (0.06; 0.23)	**0.001**	0.15 (0.06; 0.23)	**<0.001**			0.14 (0.05; 0.23)	**0.002**
	Δ Waist circumference (cm)		0.05 (-0.03; 0.13)	0.241	0.05 (-0.04; 0.13)	0.275	0.00 (-0.08; 0.08)	0.926	0.00 (-0.08; 0.08)	0.985
	Δ Body fat (%)		0.03 (-0.04; 0.11)	0.401	0.03 (-0.05; 0.11)	0.499	-0.01 (-0.09; 0.06)	0.732	-0.02 (-0.10; 0.06)	0.652
	Δ Fat ratio (%)		0.09 (0.02; 0.15)	**0.007**	0.09 (0.02; 0.15)	**0.008**	0.03 (-0.05; 0.11)	0.493	0.03 (-0.05; 0.11)	0.478
	Δ VAT (L)	HMUFA	0.04 (-0.02; 0.11)	0.202	0.05 (-0.02; 0.12)	0.153	0.01 (-0.09; 0.10)	0.873	0.01 (-0.09; 0.11)	0.818
		LFHP	0.27 (0.13; 0.42)	**<0.001**	0.27 (0.10; 0.43)	**0.002**	0.23 (0.02; 0.45)	**0.037**	0.27 (0.02; 0.52)	**0.039**
	Δ aSAT (L)		0.12 (0.04; 0.19)	**0.002**	0.13 (0.05; 0.21)	**0.002**	0.05 (-0.09; 0.19)	0.494	0.06 (-0.09; 0.21)	0.402
	Δ Muscle fat (%)		0.02 (-0.06; 0.10)	0.656	0.02 (-0.07; 0.10)	0.706	-0.05 (-0.13; 0.04)	0.275	-0.04 (-0.13; 0.04)	0.304
	Δ Liver fat (%)		0.05 (-0.03; 0.13)	0.245	0.05 (-0.03; 0.13)	0.220	0.00 (-0.08; 0.08)	0.967	0.00 (-0.09; 0.08)	0.926
	Δ Abdominal AT index (L/m²)		0.12 (0.05; 0.19)	**0.002**	0.13 (0.05; 0.20)	**<0.001**	0.05 (-0.08; 0.18)	0.462	0.06 (-0.08; 0.20)	0.413
	Δ Weight-to-muscle (ratio)		0.05 (-0.03; 0.13)	0.181	0.06 (-0.03; 0.14)	0.179	-0.01 (-0.10; 0.08)	0.808	-0.01 (-0.10; 0.08)	0.788
	Δ Muscle volume (L)		0.07 (0.00; 0.15)	0.063	0.08 (0.00; 0.16)	0.051	0.01 (-0.08; 0.10)	0.817	0.02 (-0.08; 0.11)	0.738
	Δ Muscle volume (Z-score)		0.00 (-0.08; 0.08)	0.924	0.01 (-0.08; 0.09)	0.870	-0.01 (-0.08; 0.07)	0.886	0.00 (-0.08; 0.08)	0.983
Δ MISI	Δ Body weight (kg)		-0.05 (-0.12; 0.01)	0.108	-0.05 (-0.12; 0.02)	0.137			-0.04 (-0.12; 0.03)	0.244
	Δ Waist circumference (cm)		-0.05 (-0.11; 0.00)	0.060	-0.06 (-0.11; 0.00)	0.053	-0.05 (-0.11; 0.01)	0.123	-0.05 (-0.11; 0.02)	0.131
	Δ Body fat (%)	Women	0.02 (-0.05; 0.08)	0.637	0.00 (-0.07; 0.06)	0.891	0.01 (-0.06; 0.09)	0.715	0.01 (-0.07; 0.09)	0.806
		Men	0.04 (-0.11; 0.20)	0.564	-0.11 (-0.21; -0.02)	**0.020**	-0.11 (-0.20; -0.01)	**0.027**	-0.11 (-0.21; -0.01)	**0.028**
	Δ Fat ratio (%)		-0.03 (-0.08; 0.02)	0.251	0.36 (-0.08; 0.02)	0.290	-0.01 (-0.08; 0.05)	0.695	-0.01 (-0.08; 0.05)	0.717
	Δ VAT (L)		-0.03 (-0.09; 0.02)	0.248	-0.04 (-0.10; 0.02)	0.192	-0.02 (-0.11; 0.06)	0.568	-0.02 (-0.11; 0.06)	0.614
	Δ aSAT (L)		-0.01 (-0.07; 0.05)	0.741	0.00 (-0.06; 0.06)	0.937	0.09 (-0.02; 0.19)	0.112	0.09 (-0.02; 0.20)	0.114
	Δ Muscle fat (%)		0.00 (-0.06; 0.06)	0.987	0.00 (-0.06; 0.05)	0.884	0.01 (-0.05; 0.08)	0.723	0.01 (-0.05; 0.08)	0.704
	Δ Liver fat (%)		-0.06 (-0.11; 0.00)	**0.039**	-0.06 (-0.11; 0.00)	0.050	0.05 (-0.11; 0.01)	0.109	-0.05 (-0.11; 0.01)	0.126
	Δ Abdominal AT index (L/m²)		-0.03 (-0.09; 0.03)	0.354	-0.02 (-0.08; 0.04)	0.449	0.02 (-0.09; 0.13)	0.700	0.02 (-0.09; 0.13)	0.684
	Δ Weight-to-muscle (ratio)		-0.03 (-0.09; 0.02)	0.235	-0.03 (-0.09; 0.03)	0.303	-0.02 (-0.08; 0.05)	0.589	-0.02 (-0.08; 0.05)	0.633
	Δ Muscle volume (L)		0.01 (-0.04; 0.07)	0.655	0.01 (-0.05; 0.07)	0.671	0.04 (-0.02; 0.11)	0.198	0.04 (-0.03; 0.11)	0.206
	Δ Muscle volume (Z-score)		0.04 (-0.02; 0.10)	0.178	0.04 (-0.02; 0.10)	0.179	0.04 (-0.01; 0.10)	0.138	0.04 (-0.02; 0.10)	0.147

Table 4. Continued

		Model 1 Adj. for diet		Model 2 Adj. for diet, age and sex		Model 3 Adj. for diet, age, sex, diet, Δ body weight		Model 4 Adj. for diet, age, sex, diet, Δ body weight and Δ MISI/HIRI	
		β (95%CI)	*P*-value	β (95%CI)	*P*-value	β (95%CI)	*P*-value	β (95%CI)	*P*-value
Δ Matsuda index	Δ Body weight (kg)	-0.13 (-0.28; 0.01)	0.066	-0.11 (-0.25; 0.03)	0.107				
	Δ Waist circumference (cm)	-0.10 (-0.23; 0.03)	0.130	-0.12 (-0.24; 0.01)	0.063	-0.10 (-0.23; 0.04)	0.153		
	Δ Body fat (%)	-0.07 (-0.20; 0.05)	0.244	-0.11 (-0.23; 0.01)	0.071	-0.09 (-0.22; 0.04)	0.163		
	Δ Fat ratio (%) HMUFA	-0.09 (-0.18; 0.00)	0.052	-0.06 (-0.15; 0.03)	0.157	0.00 (-0.11; 0.12)	0.937		
	Δ Fat ratio (%) LFHP	-0.21 (-0.43; 0.00)	0.054	-0.25 (-0.48; -0.03)	**0.029**	-0.30 (-0.57; -0.04)	**0.025**		
	Δ VAT (L)	-0.12 (-0.24; 0.01)	0.064	-0.14 (-0.26; -0.02)	**0.021**	-0.13 (-0.3; 0.03)	0.117		
	Δ aSAT (L)	-0.15 (-0.27; -0.02)	**0.022**	-0.12 (-0.25; 0.01)	0.060	-0.11 (-0.33; 0.12)	0.364		
	Δ Muscle fat (%)	-0.08 (-0.21; 0.05)	0.238	-0.11 (-0.24; 0.01)	0.074	-0.08 (-0.22; 0.05)	0.219		
	Δ Liver fat (%)	-0.13 (-0.25; 0.00)	**0.044**	-0.11 (-0.23; 0.01)	0.067	-0.09 (-0.22; 0.05)	0.199		
	Δ Abdominal AT index (L/m^2)	-0.16 (-0.28; -0.04)	**0.011**	-0.14 (-0.26; -0.02)	0.023	-0.18 (-0.39; 0.04)	0.112		
	Δ Weight-to-muscle (ratio)	-0.12 (-0.25; 0.00)	0.054	-0.10 (-0.22; 0.02)	0.107	-0.07 (-0.21; 0.07)	0.346		
	Δ Muscle volume (L) HMUFA	-0.10 (-0.22; 0.01)	0.082	-0.08 (-0.19; 0.04)	0.187	-0.01 (-0.14; 0.12)	0.888		
	Δ Muscle volume (L) LFHP	0.18 (-0.07; 0.43)	0.147	0.18 (-0.07; 0.42)	0.145	0.28 (0.01; 0.56)	**0.045**		
	Δ Muscle volume (Z-score)	0.05 (-0.08; 0.18)	0.447	0.06 (-0.07; 0.18)	0.361	0.07 (-0.06; 0.19)	0.277		

Data are reported as β (95% confidence interval (CI)).
Indicates the change from week 0 till 12, calculated as value week 12 minus the value of week 0. In case of significant diet or sex interaction, data are reported separately for HMUFA and LFHP or women and men, respectively. Significant *P*-values (<0.05) are highlighted in bold.
Adj, adjusted; aSAT, abdominal subcutaneous adipose tissue; AT, adipose tissue; HIRI, hepatic insulin resistance index; HMUFA, high mono-unsaturated fatty acids; LFHP, low-fat high-protein; MISI, muscle insulin sensitivity index; VAT, visceral adipose tissue.

state which is associated with decreased estrogen and increased testosterone levels compared to premenopausal women. This, in turn, may impact the association between body composition and metabolic health (22, 26). It can be speculated that above a certain threshold women may lose their reserve capacity to handle a higher muscle fat storage after menopause. Sexual dimorphism in relation to body composition and cardiometabolic health is well established (1, 27, 28), and confirmed in the present study, but the underlying mechanisms for the present findings remain to be elucidated.

Furthermore, we found that waist circumference and the abdominal AT index were inversely associated with whole-body, as well as tissue-specific insulin sensitivity. Waist circumference is often used as an indicator of abdominal adipose tissue mass (29). Adipose tissue within the abdominal region, including both aSAT and VAT, has been linked to an adverse cardiometabolic health profile in previous studies (30, 31). The measurement of waist circumference in the present study thus was a good indicator for overall abdominal-obesity related cardiometabolic risk (i.e. insulin resistance). Nevertheless, determining waist circumference does not allow for discrimination between abdominal SAT and VAT (32). With more detailed characterization of body composition, as done in the present study, we were able to demonstrate that the associations between abdominal adiposity and whole-body and tissue-specific insulin resistance appeared to be mainly driven by VAT volume, rather than aSAT volume. In line with this, VAT has been previously reported to be linked to an even more adverse metabolic, inflammatory, and dyslipidemic phenotype compared to aSAT (30, 33). It should be noted however that VAT may be a result of spillover of other AT depots, such as the aSAT, resulting from dysfunctional adipose tissue which fails to appropriately expand (1, 34).

Besides cross-sectional associations, we found improvements in body composition following either a 12-week LFHP or HMUFA diet, in the presence of minor weight loss (~2 kg). Interestingly, the LFHP diet demonstrated more pronounced improvements in waist circumference, a tendency towards a higher reduction in muscle fat, and lower reduction in muscle volume (Z-score). Previous studies also reported beneficial effects of a low-fat diet on body composition (35, 36). For example, the LIPGENE study investigated the effect of diets differing in dietary fat quantity and quality on metabolic health in individuals with the metabolic syndrome during a 12-wk period, showing that two isocaloric low-fat diets (28 en% fat) resulted in greater reduction in body weight compared to isocaloric higher-fat diets (38 en% fat) (35). In another study, body weight and waist circumference decreased more following a low-fat (28 en%) versus a Mediterranean diet rich in MUFA's without energy restrictions for 2 years (36). The higher dietary fiber content (37, 38) and

higher protein content (39) in the LFHP diet in the present study may also have contributed to the more beneficial effects on body composition. Despite the lack of significant improvements in insulin sensitivity in this subgroup that participated in the PERSON study, we found that LFHP diet-induced reductions in VAT were associated with improvement in HIRI. Additionally, the LFHP-induced decrease in fat ratio and lower reduction of muscle volume were associated with improvements in whole-body insulin sensitivity, whereas this was not seen following the HMUFA diet. The present study is one of the first studies to show that LFHP-, but not HMUFA-induced improvements in body composition are associated with improvements in whole-body and liver insulin sensitivity.

One of the strengths of the present study is the very well-detailed analysis of body composition and body fat distribution. Body composition can be measured with high accuracy and precision with a whole-body MRI, and this technique may even be considered the gold standard to measure body fat distribution (40). Nevertheless, this study also has some limitations. The association between body composition and insulin sensitivity might be different for women in the premenopausal compared to postmenopausal state. Due to the relatively small number of premenopausal women in the present analysis (n=8), we were not able to confirm this. Exclusion of premenopausal women (data not shown), however, did not alter the conclusions of the present study. Future studies are warranted to investigate the relationship between body composition and insulin sensitivity in premenopausal women and age-matched men. Furthermore, we did not observe significant improvements in the Matsuda index, HIRI, and MISI following dietary intervention on a group level, as a result of limited power in this subset, since improvements were observed in the complete PERSON study population (15). Despite this, we were still able to identify significant associations between changes in body composition and changes in insulin sensitivity.

In summary, tissue-specific insulin resistant phenotypes are associated with distinct body composition profiles. Specifically, body weight and liver fat were associated with liver insulin sensitivity while muscle fat was associated with muscle insulin sensitivity in women but not in men. VAT was independently associated with both whole-body and tissue specific insulin sensitivity. Distinct phenotypes of body composition have previously been identified in individuals with similar BMI, possibly representing different etiologies towards T2D or CVD (5, 18, 41). Our findings support the presence of distinct metabolic profiles depending on the tissue site of insulin resistance (either in the liver or muscle), which is in line with previous research which identified distinct lipidome, metabolome, and inflammatory profiles in individuals with liver versus muscle insulin resistance (42-44). Furthermore, we

observed that body composition and body fat distribution improved following isocaloric 12-week healthy diets, in the presence of only minor weight loss. Although both the LFHP and HMUFA diet elicited beneficial effects on body composition and body fat distribution, the LFHP appeared most effective in reducing waist circumference. Additionally, only LFHP-induced improvements in body composition were associated with improved (whole-body and liver) insulin sensitivity. Findings of this study give more insight into the heterogeneity of the etiology towards cardiometabolic diseases and can have implications for the development of more targeted dietary intervention strategies to improve cardiometabolic health.

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Comment on Espinosa De Ycaza et al.: Adipose tissue inflammation is not related to adipose insulin resistance in humans

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Adipose tissue (AT) dysfunction in obesity is associated with insulin resistance and an increased risk of cardiometabolic diseases (1). We have read the article by Espinosa De Ycaza and colleagues (2) with great interest. The authors conclude that AT senescence and inflammation are not causally linked to AT insulin resistance (as determined by the insulin concentration that suppresses AT lipolysis by 50% (IC50)) and suggest that fat cell size (FCS) is a stronger predictor of AT insulin resistance. The team of investigators based their conclusions on cross-sectional analyses in people with normal weight and obesity (n=86) and a subgroup of people with obesity (n=25) that was studied before and after a 6-month weight loss intervention.

We believe, however, that the present findings are preliminary in nature. First, it is well established that adipocyte hypertrophy is associated with AT inflammation and insulin resistance (3). The lack of a significant association between AT inflammation (assessed by AT macrophage (ATM) content and AT cytokine expression) and AT insulin sensitivity after adjustment for FCS does not necessarily imply that AT inflammation does not induce insulin resistance. In fact, AT inflammation could mediate the association between FCS and AT insulin resistance. Notably, previous observations indicated that ATM content was higher in abdominal subcutaneous and omental AT in people with obesity with metabolic impairments compared to those without metabolic perturbations (matched for age, sex and BMI), which was not explained by differences in adipocyte size (4). Secondly, more detailed examination of the AT inflammatory phenotype (i.e. immunophenotyping and transcriptional profiling of adipocytes and immune cells) would have provided better insight into the association between FCS, inflammation, and AT insulin resistance. Finally, the authors did not find significant associations between ATMs and AT insulin resistance after weight loss. We feel it would have been more informative if the authors had examined weight-loss induced changes in ATM, FCS, AT senescence and pro-inflammatory gene expression and alterations in AT insulin sensitivity. Given the dynamic immune response to weight loss, the duration of the weight-stable period (2 weeks) following weight loss might have been too short to exclude that weight-loss induced cellular stress and AT remodeling, which is accompanied by an inflammatory response (5), has influenced these associations. Moreover, because of sexual dimorphism in adipose tissue function and insulin sensitivity (3), and the fact that changes in body composition were different between men and women following weight loss, we were surprised that the correlations assessed following weight loss were not corrected for sex.

Overall, the work of Espinosa De Ycaza and colleagues (2) contribute to our current understanding of the relationship between abdominal subcutaneous and femoral FSC,

AT inflammation and AT insulin resistance. However, these findings require confirmation in future studies. Because of inter-individual differences in the etiology of obesity-related metabolic complications, the importance of FCS, AT inflammation and AT senescence in tissue-specific insulin resistance warrants further investigation, taking age, sex, body composition, obesity duration and weight cycling into account.

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Chapter 9

General discussion

Overweight and obesity is accompanied by increased risk for development of cardiometabolic diseases including cardiovascular disease and type 2 diabetes (T2D) (1). Obesity is a complex and multifactorial disease and better insight in the underlying metabolic disturbances is essential to develop effective (dietary) intervention strategies. However, it is increasingly evident that 'one size does not fit all' when it comes to lifestyle or dietary approaches to improve health. It is well acknowledged that about 40-50% of participants do not respond or adhere to general dietary interventions, wherein response partly relates back to specific metabolic phenotypes (2-7). As a result, population-level dietary strategies may have a suboptimal effect in a part of the population, and thus more targeted or personalized dietary approaches are warranted. Targeting diets according to a metabolic phenotype may improve the efficacy of dietary interventions (8). Nevertheless, effective precision nutrition strategies require a better understanding of these metabolic phenotypes.

Distinct phenotypes can be identified in the etiology towards cardiometabolic diseases, characterized by distinct general (including age and sex), anthropometric, and/or metabolic characteristics (including inflammation and glucose homeostasis) (9-11). Previous studies have demonstrated that individuals with LIR and MIR have distinct differences in metabolome and lipidome profiles and tissue metabolism and may also show a differential response to diet (5, 12). An important aim of this thesis is to better understand differences in insulin resistant phenotypes, in particular the distinction between individuals with more pronounced liver or muscle insulin resistance (LIR and MIR). Additionally, the efficacy of adapting dietary macronutrient composition (within the context of a healthy diet) towards these metabolic phenotypes to enhance improvements in cardiometabolic health was investigated.

For this thesis, primarily data from the PERSonalized Glucose Optimization Through Nutritional intervention (PERSON) study was used (Chapter 4, 5, 6, and 7). The PERSON study investigated the efficacy of modulation of dietary macronutrient composition according to tissue-specific insulin resistant phenotypes (LIR and MIR) on parameters related to glucose homeostasis, body composition, inflammation, insulin resistance, and cardiometabolic disease risk. Furthermore, Chapter 2 is a review article to obtain insight into ectopic fat deposition, specifically in relation to (tissue-specific) insulin sensitivity, and how ectopic fat may be affected by macronutrient quality and quantity. Furthermore, data from the Diet, Obesity, and Genes (DiOGenes) study was used to assess sex-specific differences in diet-induced weight loss and associated metabolic alterations (Chapter 3). In Chapter 8, we commented on the interpretations and study design of a recently published

article that examined the relation between adipose tissue inflammation and adipose tissue insulin resistance (13). Finally, in this General Discussion, the main findings of the individual thesis chapters will be discussed and integrated, finalized by suggestions for future research.

Distinct metabolic phenotypes towards cardiometabolic diseases

Heterogeneity in etiology of obesity-related disease exists already before its manifestation (9, 11). As such, individuals at risk for these diseases can be identified in an early stage. These different risk profiles, including metabolic phenotypes, may also determine the response to dietary intervention (5, 11). Nevertheless, numerous factors may determine an individuals' risk toward obesity-related diseases. Therefore, identification of clinically relevant factors that largely determine metabolic health outcomes are warranted. Aside from more complex measures of heterogeneity in tissue metabolism such as tissue-specific insulin resistance, which will be discussed later, classification of individuals by simple measures may provide easy and effective distinction in metabolic health risk. In the present thesis, we therefore identified sex as a relevant factor in relation to metabolic health outcomes and in response to dietary intervention.

Sexual dimorphism in metabolic health and in response to intervention

In Chapter 3, we compared the metabolic response to a low-calorie diet aimed at weight loss between men and women. At baseline, we observed clear cardiometabolic differences between sexes. More specifically, women had among other factors a lower blood pressure, lower plasma triacylglycerol (TAG), total cholesterol levels and glucose levels, and greater whole-body and liver insulin sensitivity compared to men. Furthermore, data from Chapter 5 and 6, in the PERSON study population, revealed that women were more often classified as MIR, while men were more often LIR. In line, in the DiOGenes study, we observed that men were more insulin resistant in the liver compared to women (Chapter 3). Finally, we reported lower visceral adipose tissue (VAT), higher abdominal subcutaneous adipose tissue (aSAT), and higher muscle fat, despite similar BMI, in Chapter 7.

Interestingly, we also found distinct sex-related responses following a weight loss period (Chapter 3). Per kilogram of body weight that was lost as a result of dietary intervention (~800 kcal/day for 8 weeks), men improved more in cardiometabolic parameters compared to women. However, men regained more weight, and were less able to maintain the cardiometabolic improvements after 6 months of follow-up, compared to women. We hypothesized these differences to be attributed to differential loss and regain of different adipose tissue depots. Men generally have a higher metabolically disadvantageous VAT

mass, as we also observed in Chapter 7. The loss and subsequent regain in VAT mass may thereby result in greater metabolic alterations compared to the loss or regain of adipose tissue mass in other fat depots, such as in the SAT in the gluteofemoral region, which is often seen to a greater extent in women compared to men (14).

These metabolic differences between men and women impact the risk towards cardiometabolic disease (15). Before menopause, women appear relatively protected against metabolic perturbations compared to men, despite higher adiposity (16, 17). For example, for a given BMI, women are relatively protected against lipid-induced insulin resistance (18, 19). These differences can at least partly be ascribed to elevated estrogen concentrations and generally higher concentrations of the adipokines adiponectin and leptin in premenopausal women compared to men of similar age, which may be protective against a pro-inflammatory phenotype and other metabolic complications associated with obesity (20, 21). Nevertheless, the metabolic advantage in pre-menopausal women compared to men largely disappears after menopause (22). Notably, once women develop prediabetes or T2D, more detrimental changes in cardiometabolic risk factors seem to develop compared to men (23). The latter appears to be in accordance with the association between higher muscle fat and worse muscle insulin sensitivity, in women, but not in men that we reported in Chapter 7.

Women gain approximately 2.5 kg in total body weight in 3 years during the menopausal transition, with is accompanied by an increase in fat storage in the abdominal region (22). Hence, the risk for the development of hypertension, T2D, cardiovascular disease and other diseases increases significantly during and following menopause (24). It should be noted that the majority of the women in the DiOGenes study (Chapter 3) were in the premenopausal state, while most women in the PERSON study (Chapter 4-7) were in the postmenopausal state. Although we did not adjust for menopausal state in the statistical analyses related to the PERSON study, we observed similar percentages (~11%) of women in the premenopausal state in both intervention groups (data not reported in thesis). We thus expect the impact of the menopausal state to be similar in both intervention groups. Regardless, the findings in this thesis clearly show sex differences in cardiometabolic health markers, both in the pre- and post-menopausal state, compared to men of similar age. It is tempting to postulate that these differences may at least partially be a result of differences in body composition and body fat distribution such as reported in Chapter 7. This knowledge may have important consequences for sex-specific prevention and treatment strategies. However, research on sex differences related to metabolic health currently remains understudied.

Tissue-specific insulin resistant phenotypes

Methodologies for the assessment of distinct insulin resistant phenotypes

Quantification of insulin resistance and insulin secretion is an important aspect in the metabolic characterization of individuals at risk of developing cardiometabolic diseases. Different methods and indices are available, which each come with their own advantages and disadvantages (25). The choice of method may depend on the type of information that is required (such as either whole-body or tissue-specific insulin resistance), the number of study participants, and the available resources (budget, time, facilities) (26).

The two-step hyperinsulinemic euglycemic clamp technique allows for determination of tissue- specific insulin resistance and is considered the gold standard technique for measuring insulin action per se (27), but is rather invasive, costly, and time consuming. The details of this method are described in more detail in Chapter 4. In short, insulin is infused at two set rates (10 and 40 mU/min/m^2). D-[6.6-$^2H^2$] glucose tracer is co-infused at constant rate, while glucose is co- infused at variable rate to maintain normoglycemia (~5.0 mmol/L). The low insulin infusion step is used to calculate the relative suppression of endogenous glucose production (EGP), as a measure of liver insulin sensitivity and relative suppression of free fatty acids (FFA's) in the circulation as a measure of adipose tissue insulin sensitivity. The high insulin infusion step is used to calculate peripheral glucose uptake, or rate of disappearance (Rd), as a measure of muscle insulin sensitivity. The clamp is a very standardized and controlled assessment of (tissue-specific) insulin resistance.

Surrogate markers of insulin sensitivity are often derived from fasting glucose and insulin values, or after ingestion of a standardized glucose drink (OGTT) and may more easily be implemented in clinical practice as well as in large clinical trials. In Chapter 3 to 7, quantification of whole-body and tissue-specific insulin sensitivity was done by using OGTT- derived measures. Whole-body insulin sensitivity can amongst others be quantified with the HOMA-IR (28) and Matsuda index (29), while tissue-specific insulin resistance can be quantified with the hepatic insulin resistance index (HIRI), muscle insulin sensitivity index (MISI), and the adipose tissue insulin resistance index (ATIRI) (30, 31). HIRI is assessed by quantifying the area under the curve for both glucose and insulin in the first half hour of an OGTT, which reflects the suppression of endogenous (primarily hepatic) glucose production. MISI is assessed by measuring the decline in plasma glucose following the glucose peak, relative to mean insulin concentrations, which reflects glucose uptake in peripheral tissues, mainly by the skeletal muscle. ATIRI is assessed in fasting blood and is the product of fasting insulin and fasting FFA's, reflecting the insulin-mediated

suppression of adipose tissue lipolysis. HIRI, MISI, and ATIRI have been validated against the two-step hyperinsulinemic euglycemic clamp technique and show reasonably good correlations of 0.64 (30), 0.58 (31), and 0.90 (32), respectively.

Nevertheless, clamp-derived and OGTT-derived assessment of insulin sensitivity do show discrepancies. The hyperinsulinemic-euglycemic clamp is a highly standardized, gold- standard method to assess insulin action perse. This method elicits a sub-physiological state of sustained elevated insulin concentrations for several hours, while the glucose infusion rate is adapted to maintain euglycemia at 5.0 mmol/L. Importantly, however, OGTT-derived measures of insulin sensitivity may represent a more physiological condition. More specifically, the methods differ in mode of glucose delivery, being either oral (OGTT), or intravenously (two- step hyperinsulinemic euglycemic clamp). As such, the glucose and insulin responses during an OGTT may be affected by differences in gastrointestinal factors, including the rate of glucose absorption by the gut and the related incretin response (33). The latter describes the release of hormones such as GLP-1 and PYY by the gut into the circulation in response to the ingestion of food, which in its turn stimulates insulin secretion. This results in higher plasma insulin concentrations with oral as compared to intravenous glucose administration using the same glucose load (33). Thus, OGTT-related measures may better reflect human physiology in daily life. We (Chapters 4 to 7) and others (34-36) have shown that based on just one OGTT, distinct metabolic phenotypes can be identified, thereby providing support for the efficacy of the clinical use of (7-point) OGTT-derived measures of tissue-specific insulin sensitivity.

Metabolic heterogeneity of tissue-specific insulin resistant phenotypes

Insulin resistance is a heterogeneous condition and can develop simultaneously or separately in insulin-sensitive tissues such as adipose tissue, skeletal muscle, and the liver. In Chapter 4 we demonstrate that isolated LIR and MIR are present in about 30% of the population with overweight and obesity. Importantly, insulin resistance can be present already well before the development of hyperglycemia and cardiometabolic complications but may also develop later and coincide with disturbances in glucose homeostasis, including impaired fasting glucose (IFG; fasting plasma glucose 26.1 mmol/L) and IGT (2-hr plasma glucose 27.8 mmol/L) (37). IGT may be mainly characterized by peripheral insulin resistance, and thus primarily represents the MIR phenotype, whilst IFG seems primarily characterized by LIR (38). The extent to which insulin resistance is present in different tissues and associated disturbances in glucose homeostasis may determine an individuals' risk of developing cardiometabolic disease. Indeed, recent reports have indicated that individuals with more pronounced LIR have a distinct metabolome (36),

lipidome (35, 39), adipose tissue transcriptome (34), and systemic inflammatory profile (34) compared to individuals with more pronounced MIR. The findings of the present thesis (Chapters 4 to 7) further strengthen the concept of LIR and MIR phenotypes as distinct phenotypes.

Dysfunctional adipose tissue in obesity is characterized by enlarged adipocytes and a pro- inflammatory phenotype, which has been associated with the development of insulin resistance and other cardiometabolic complications. In Chapter 5, we found distinct associations between whole-blood immune cell populations as measured with flow cytometry and inflammatory adipose tissue gene expression in MIR and LIR phenotypes. More specifically, LIR, but not MIR, was associated with several blood immune cell populations in the PERSON study. We were able to replicate the association between monocytes, specifically classical monocytes, with worse liver insulin sensitivity in The Maastricht Study (n=273). Additionally, we observed aSAT gene expression of the inflammatory marker IL-6 and the immune cell marker CD14 to be specifically related to MIR, but not LIR. These findings are in line with previous research reporting that individuals with MIR showed higher expression of aSAT inflammatory genes, compared to individuals without IR, while this was not observed in LIR (34). Furthermore, in Chapter 6, we found that circulating CRP levels were higher in MIR compared to LIR. Together, these findings suggest that MIR and LIR phenotypes are related to distinct inflammatory profiles.

Furthermore, body composition and body fat distribution are important predictors of cardiometabolic disease risk. However, the relationship between body composition and tissue- specific insulin sensitivity has not been investigated previously. In Chapter 7, we showed distinct associations of HIRI and MISI with body composition and body fat distribution using state-of-the-art whole-body MRI methodology. Specifically, HIRI was associated with body weight and liver fat percentage, while MISI was inversely associated with muscle fat percentage (only in women). Remarkably, despite the inverse association between muscle fat and MISI in women in the PERSON study, we found that muscle insulin sensitivity was similar between sexes, despite higher muscle fat storage in women compared to men. This suggest that women may be capable of storing more fat in the muscle without adverse effects. This appears in line with the generally higher fat storage capacity in peripheral tissues in particular gluteo-femoral adipose tissue, and with the fact the women have an about 2 kg/m^2 higher BMI when they develop T2D compared to men (40). Nevertheless, once women develop prediabetes or T2D, this coincides with more a disadvantageous cardiometabolic risk profile compared to men, as reported previously (23). Possibly, after a certain threshold of skeletal muscle lipid storage has been reached,

women might show a catch-up in cardiometabolic impairments compared to men. This may affect the association between body composition and cardiometabolic health, as discussed in Chapter 7. Furthermore, while MISI was similar between men and women in the PERSON study (Chapter 7), as also observed in the DiOGenes study (Chapter 3), women were more often classified as isolated MIR compared to men. In contrast, men were more often classified as combined LIR+MIR (Chapter 4). The latter may possibly be a result of the higher liver fat and higher VAT mass in men compared to women, predisposing men to develop LIR at an earlier stage, reflecting difference in pathways towards cardiometabolic diseases.

These cross-sectional analyses of body composition and body fat distribution with (tissue- specific insulin sensitivity do not provide insights into the cause-effect relationship of ectopic fat and insulin resistance, and a bi-directional association has been suggested in literature (41- 45), as also discussed in Chapter 2. Importantly, some measures of body composition were associated with both HIRI and MISI, such as VAT and waist circumference, suggesting that although LIR and MIR are distinct phenotypes, there seems to be overlap in their risk profile. This partly overlapping risk profile may also explain why the LIR and MIR phenotypes may not only develop separately but may also develop simultaneously. Indeed, about 28% of the overweight and obese population was classified as combined MIR/LIR, as reported in Chapter 4.

The underlying pathophysiology for the development of distinct MIR and LIR phenotypes remains speculative. A potential explanation relates to the differential blood supply to the skeletal muscle and liver. The skeletal muscle is more affected by the systemic circulation, while the liver is more strongly affected by the portal vein (46, 47). As a result, the muscle may be more affected by SAT, while the liver may be more affected by tissues in the abdominal region, such as the VAT and gut. Another explanation for the differential development of MIR and LIR relates to the gut microbiome. A recent study comparing the gut microbiota composition of individuals with normal glucose tolerance (NGT), IFG, and IGT, revealed that gut microbiota of people with IGT differs from that of individuals with NGT, while no differences were observed when comparing IFG and NGT groups (48). As described above, IGT may be mainly determined by peripheral insulin resistance, and may thus primarily represent the MIR phenotype, whilst IFG seems predominantly characterized by LIR (38). Furthermore, modification of microbial composition by either fecal transplantation from lean donors to men with the metabolic syndrome (49), or by dietary fiber intervention (50), improved peripheral (mainly muscle), but not liver, insulin sensitivity. Furthermore, individuals may have a genetic predisposition to develop insulin resistance,

which interacts with the presence of overweight or obesity. There are many genetic variants present that may impact tissue-specific metabolism, and thus may impact liver or muscle insulin sensitivity to a different extent (51), although research in this field is limited. Furthermore, sex may impact the development of MIR and LIR, as discussed above, where MIR possibly plays a more pronounced role in the development towards cardiometabolic diseases in women. Finally, differences in lifestyle- related factors, including diet and physical exercise, may impact the development of insulin resistance in the liver and skeletal muscle. However, the exact mechanisms involved in the etiology of the LIR and MIR phenotypes remain to be elucidated.

Taken together, the presence of distinct metabolic phenotypes in relation to cardiometabolic disease risk is evident. Tissue-specific insulin resistant phenotypes can already be observed in the overweight or obese state, also before the development of cardiometabolic complications, providing a window of opportunity for (dietary) prevention strategies.

Precision nutrition strategies to improve cardiometabolic health

Currently, dietary recommendations are often developed for the general population, with some exceptions of recommendations for specific nutrients or supplements for at risk groups (e.g. older individuals or pregnant women) (52). This one-size-fits all approach is successful in lowering cardiometabolic disease risk at the population level but may fail to elicit beneficial effects in all. The metabolic heterogeneity in the development of cardiometabolic diseases may partly underly the response to dietary intervention, implying that adapting the diet according to metabolic phenotypes may improve dietary intervention success. It is thus evident that there is a need for precision nutrition in order to optimize health outcomes.

Dietary intervention success may depend on general characteristics, such as age and sex. Chapter 3 provides evidence for differences in dietary intervention response by subdivision of individuals based on sex. Also, age has previously been identified as a negative predictor of intervention success, specifically in relation to weight loss (53). Women, and older adults, may thus benefit less from a low-calorie diet. Low-calorie diets may thus be more effective in younger men, provided there is good supervision throughout the weight maintenance period. Furthermore, heterogeneity in dietary intervention response may at least partly be explained by underlying differences in metabolic phenotype (2, 3, 6, 54-59). To illustrate, individuals with insulin resistance and metabolic syndrome were more susceptible to a health effect from the substitution of saturated fatty acids in

the diet by either unsaturated fatty acids, or complex carbohydrates compared to individuals without insulin resistance (6). Additionally, in a *post-hoc* analysis of three large randomized clinical trials, elevated fasting glucose before weight loss predicted greater weight loss, and greater weight maintenance was found following a diet with a low glycemic load (2). Clearly, these studies show an interaction between metabolic phenotype and dietary macronutrient composition in the response to dietary intervention.

Also, the presence of insulin resistance in either the liver or skeletal muscle determine may the outcome to a dietary intervention. An important study in this context is the CORDIOPREV- DIAB study (5). A *post-hoc* analysis of this study revealed that individuals with predominant LIR benefited most from a diet low in fat and high in complex carbohydrates while individuals with MIR benefitted most from a more Mediterranean diet, high in mono-unsaturated fatty acids, with respect to improvements in the Disposition index (combined score of insulin sensitivity and insulin secretion). Moreover, multiple intervention studies reported beneficial effect of proteins, fats, and dietary fibers on either liver (60-64) or muscle metabolism (39, 65). Furthermore, several studies revealed that the LIR and MIR phenotypes show distinct metabolic differences, amongst others related to metabolome (36), lipidome (35, 39), adipose tissue transcriptome (34), and systemic inflammatory profile (34). The present findings extend this by demonstrating significant differences in circulating immune cells and adipose tissue inflammatory gene expression (Chapter 5), as well as differences in body composition and body fat distribution (Chapter 7) between individuals with MIR and LIR.

Taken together, tissue-specific insulin resistant phenotypes are distinct phenotypes that may respond differentially to diets differing in macronutrient quality and quantity. Targeting these phenotypes by means of dietary macronutrient manipulation may thus provide a novel strategy to further enhance the efficacy of dietary interventions in improving cardiometabolic health. However, evidence supporting the latter comes from retrospective analysis, and prospective studies were thus far lacking. We have therefore initiated the PERSON study to assess prospectively whether tissue-specific insulin resistant phenotypes would benefit more from healthy diets differing in macronutrient composition (Chapter 4 to 7).

Precision nutrition by targeting tissue specific insulin resistance – the PERSON study
In the PERSON study we investigated two healthy isocaloric diets, a high-monounsaturated fatty acid (HMUFA) and a low-fat, high-protein, and high-fiber (LFHP) diet of which the study design is described in Chapter 4. Protein and fat quantity and quality differed

between both diets. While the diets were similar in amount of carbohydrates, the LFHP diet was higher in complex carbohydrates (fibers). Both diets were low in mono- and disaccharides (12 en%) and low in saturated fatty acids (8 en%). Importantly, both diets were within the Dutch guidelines of healthy nutrition. Lifestyle interventions that comply to these guidelines, including reduced intake of saturated fat, increase intake of unsaturated fatty acids, increased fiber intake, and reduced intake of simple carbohydrates, have been shown to reduce T2D incidence by more than 50%, also in the presence of minor weight loss (66-69). In the PERSON study, we compared two PhenoDiet groups. The PhenoDiet group A included individuals with MIR following a HMUFA diet, and individuals with LIR following a LFHP diet. The PhenoDiet group B included individuals with the opposite phenotype and diet combinations, namely LIR following a HMUFA diet and MIR following a LFHP diet. Based on *post-hoc* analysis, mechanistic data, progressing insights in the scientific field, and the literature search in Chapter 2, we hypothesized that MIR individuals on a HMUFA diet, and LIR individuals on a LFHP diet (PhenoDiet group A) would improve most in the Disposition index, compared to MIR individuals on a LFHP and LIR individuals on a HMUFA diet (PhenoDiet group B).

Chapter 6 and 7 describe the outcomes to the PERSON study intervention. We did not observe significant differences in the primary outcome, the Disposition index, between the two PhenoDiet groups (Chapter 6). Notably, however, we did show that individuals in the PhenoDiet group B, rather than PhenoDiet group A, improved significantly more in insulin sensitivity and other cardiometabolic parameters, independent of slight changes in body weight. Diet-induced improvements in the Matsuda index (whole-body insulin sensitivity) were pronounced, with on average ~20% improvement in the PhenoDiet group B, versus ~5% increase in the PhenoDiet group A. Notably, improvements in whole-body and muscle insulin sensitivity, circulating TAG and CRP were observed in both the LIR and MIR individuals within the PhenoDiet group B but not PhenoDiet group A, except for a similar improvement in TAG in people with LIR in both PhenoDiet groups. These data suggest that these beneficial health effects were driven by improvements in both tissue-specific insulin resistant phenotypes. We hereby confirm that an isocaloric modulation of dietary macronutrient composition targeting liver and skeletal muscle insulin resistance can significantly improve glucose metabolism and cardiometabolic risk factors (including plasma TAG and CRP) in about one third of the individuals with overweight and obesity (which was the prevalence of tissue-specific insulin resistance), independent of weight loss. These findings may prove direct leads for prevention strategies and health care professionals, since it opens the possibility to advice more optimal diets based on an OGTT or a derived simplified measure.

Nevertheless, the primary outcomes of the PERSON study were contrary to our hypothesis, indicating that developing precision nutrition strategies based on metabolic phenotypes is complex, and detailed mechanistic studies are warranted. Outcomes to dietary interventions depend on many factors, including the health status and overall characteristics of the study population, the exact composition of the diet, dietary adherence, the mode of delivery of the dietary intervention, and other external factors (e.g., geographic region, climate, and culture). These factors might also explain the contradictory findings of the PERSON study (Chapter 6) compared to observations from the CORDIOPREV-DIAB study (5). Furthermore, the day-to- day variation in OGTT responses on which the LIR and MIR classification was based, as well as the fact that classification of individuals was performed at screening, both possibly resulted in misclassification of some individuals at the start of the intervention.

Despite the complexities involved in developing precision nutrition strategies, we were able to confirm the proof-of-concept of precision nutrition. To better understand underlying mechanisms of tissue-specific insulin resistant phenotypes and their metabolic response to the PERSON study dietary intervention, a two-step hyperinsulinemic euglycemic clamp was performed in a subgroup of the PERSON study population in Maastricht, using stable isotope methodology. In Chapter 6, we observed greater improvements in MISI in the PhenoDiet group B compared to A and an improvement in HIRI in both groups, without significant differences between PhenoDiet groups. Strikingly, no significant changes in peripheral (insulin- mediated glucose disposal) or liver insulin sensitivity (insulin-mediated EGP suppression), as assessed by the clamp, were found in either of the intervention groups (Table 1). As discussed previously, an important difference between clamp- and OGTT-derived measures of insulin sensitivity is the bypass of the gastrointestinal track when performing a hyperinsulinemic- euglycemic clamp. Although the underlying mechanisms for the differential effect of the LFHP and HMUFA diet in the LIR and MIR phenotype remains to be elucidated, these findings suggest that the differential response in PhenoDiet group A and B may at least partially involve gastrointestinal factors. More specifically, diet-induced alterations in gut microbiota composition and functionality in individuals with LIR and MIR may underlie the differential responses, as also described in Chapter 6. Other explanations for the differential effect of the LFHP and HMUFA in the LIR and MIR phenotype may relate to a diet- and phenotype-specific effects on inflammatory processes, hepatic lipid metabolism, and inter-organ crosstalk, as discussed in more detail in Chapter 6. Clearly, more studies are warranted to obtain insight in the underlying mechanisms.

Table 1. Clamp-derived measures of tissue-specific insulin sensitivity at baseline and after 12 weeks in PhenoDiet group A and B

		PhenoDiet group A (n=28)	PhenoDiet group B (n=30)	*P*-value Group	Time	Group x Time
Insulin-mediated EGP suppression (%)	Week 0	60.3 (54.3 – 66.4)	59.8 (54.1 – 65.5)	0.903	0.238	0.893
	Week 12	63.4 (58.6 – 68.2)	62.4 (57.8 – 67.0)			
Insulin-mediated Rd (umol/kg/min)	Week 0	30.6 (26.9 – 34.4)	31.6 (28.1 – 35.1)	0.708	0.173	0.725
	Week 12	32.7 (28.5 – 36.9)	34.4 (30.4 – 38.3)			

Values are estimated marginal means with 95% confidence intervals, adjusted for age, sex and center. EGP, endogenous glucose production; Rd, rate of disappearance

Although individuals in PhenoDiet group B benefitted on average from the dietary intervention, not surprisingly, some variation between individuals in dietary intervention response was still present. In future studies on precision nutrition, classification of individuals may be further optimized. Besides tissue-specific insulin resistant phenotypes, other clinically relevant parameters may contribute to the heterogeneity in dietary intervention response, including β-cell functioning, body composition, microbiome profile, and metabolome profile (54, 58, 59, 70- 73). This knowledge may hint towards the identification of additional metabolic subgroups in the population, using prespecified clinically relevant variables. Targeting these metabolic subgroups would possibly allow for even more successful precision nutrition strategies. Nevertheless, already in this sub-classification of two phenotypes, the distinct LIR and the MIR phenotype, as done in the PERSON study, we were able to significantly optimize the effect of a dietary macronutrient intervention on cardiometabolic health.

Besides the hypothesis driven classification approach as applied in the PERSON study, a data- driven machine learning approach has been investigated in precision nutrition research. In this approach, a great number of variables, such as data related to the gut microbiota, blood parameters, questionnaires, anthropometrics, and others, can be integrated by machine- learning algorithms that may predict intervention outcomes. Algorithms that predict individual glucose and TAG responses have been developed previously (58, 59, 74), and appear to predict postprandial and intervention response relatively well. Nevertheless, this data-driven, rather than hypothesis-based approach, limits the possibility to identify the underlying mechanisms of the phenotypic complexities, which may hamper replication of the findings in different populations. Together, implementation of machine-learning algorithms into evidence- based dietary guidelines

and prevention strategies may thus be challenging.

Taken together, the findings in this thesis outcomes support the proof-of-concept of precision nutrition by showing that it is possible to optimize the effect of dietary interventions based on an individual's metabolic phenotype, independent of changes in body weight. The PERSON study applies a precision nutrition strategy by classifying individuals using pre-specified variables that are hypothesized to be of importance for dietary intervention response (i.e., tissue-specific insulin resistance). This strategy allows for a better understanding of underlying mechanisms of the dietary responses, providing a solid basis for the development of dietary recommendations targeted to metabolic subgroups within the population. And thus, hypothesis-based classification for precision nutrition appears to have a high potential for implementation into clinical practice. The future perspectives of precision nutrition strategies are discussed in more detail in the final section of this General Discussion.

General health benefits of isocaloric healthy diets

Irrespective of metabolic phenotype, both the HMUFA and LFHP diet resulted in clinically relevant improvements in cardiometabolic risk profile (total cholesterol and blood pressure amongst others) and parameters of body composition, in the presence of only minor weight loss (Chapter 6 and 7). These findings highlight the relevance of adopting a healthy diet in the prevention of cardiometabolic diseases, also when weight loss is not aimed for or cannot be achieved. Interestingly, however, we identified the LFHP diet to perform better in relation to several clinical parameters and body composition compared to the HMUFA diet. As such, the LFHP diet elicited greater improvements in fasting and postprandial insulin, the Matsuda index, TAG, and CRP, compared to the HMUFA diet (Chapter 6), as well as waist circumference, a slightly greater decrease in muscle fat (%), and a lower decrease in muscle volume compared to the HMUFA diet (Chapter 7). The improvements in body composition seem to be dependent on diet but were independent of baseline tissue-specific insulin resistant phenotype or sex. To illustrate, body weight decreased with ~2% with corresponding changes in liver fat and VAT of ~33% and ~8%, respectively, in the total population. This was comparable in LIR and MIR individuals (Chapter 6) and similar in men and women (Chapter 7). Furthermore, improvements in insulin sensitivity were only associated with improvements in body composition following the LFHP, but not HMUFA diet. Together, these results indicate that despite different associations of liver and muscle insulin sensitivity with parameters of body composition at baseline (Chapter 7), diet-induced body composition changes are not different in the LIR and MIR phenotypes but do rather depend on the type of diet. This is in line with previous work, as discussed in Chapter 2. Importantly, as aforementioned, our findings

clearly demonstrate that improvements in glucose homeostasis, insulin resistance, and other cardiometabolic parameters can be remarkably enhanced when modulating dietary macronutrient composition according to tissue-specific insulin resistant phenotypes.

MAIN FINDINGS

The studies presented in this thesis provide a better understanding of the distinct metabolic phenotypes towards cardiometabolic diseases, which can have implications for precision nutrition strategies. The main conclusions of the present thesis are summarized below.

Based on a *post-hoc* analysis, mechanistic data, progressing insights in the scientific field, and the literature search in Chapter 2, we hypothesized that tissue-specific insulin resistant phenotypes would respond differentially to diet and that the beneficial effects of a healthy diet could be further improved when dietary macronutrient composition is modulated according to tissue-specific insulin resistant phenotype. To test this hypothesis, we developed the prospective randomized PERSON study as described in Chapter 4. In Chapter 6 we clearly identified differential responses to dietary intervention depending on baseline tissue-specific insulin resistant phenotype. More specifically, individuals with LIR benefitted most from the HMUFA diet, and with MIR from the LFHP diet, with respect to improvements in insulin sensitivity, glucose homeostasis, serum TAG, and CRP, and independent of weight loss. We hereby confirmed that isocaloric dietary macronutrient modulation within context of guidelines healthy nutrition based on baseline insulin resistant phenotype leads to a clinically relevant pronounced further improvement in cardiometabolic health, hereby providing the proof-of- concept of a subgroup-based precision nutrition (Chapter 6).

Based on previous research we know that tissue-specific insulin resistant phenotypes are linked to distinct metabolome (36), lipidome (35, 39), adipose tissue transcriptome (34), and systemic inflammatory profile (34). In the present thesis we provide further understanding of these metabolic phenotypes. In Chapter 5 we found that higher relative presence of circulating immune cell populations, specifically (classical) monocytes, were associated with impaired liver but not muscle insulin sensitivity. Furthermore, aSAT gene expression of IL-6 and CD14, and circulating CRP concentrations, were associated with worse muscle but not liver insulin sensitivity. In the context of obesity-associated inflammation, we discussed in Chapter 8 that the conclusion by Espinosa De Ycaza and colleagues (13) that adipose tissue inflammation is not related to adipose tissue insulin

resistance may be preliminary and required further well- controlled studies. Furthermore, we describe in Chapter 7 that tissue-specific insulin resistant phenotypes are also distinctly related to body composition. Specifically, body weight and liver fat were associated with reduced liver insulin sensitivity, and muscle fat was associated with impaired muscle insulin sensitivity in women, but not in men. These findings are in line with findings of Chapter 2, which highlights the relevance of ectopic fat in relation to (tissue- specific) insulin resistance.

With data from the PERSON study as described in Chapter 6 and 7 we show that two isocaloric diets (LFHP and HMUFA) which are according to the guidelines of healthy nutrition, and irrespective of metabolic phenotype, are effective in improving cardiometabolic risk factors, including body composition. In Chapter 6 we observed improvements in amongst other total cholesterol and blood pressure, and in Chapter 7, we observed improvements in body composition, body fat distribution, and ectopic fat in following both the LFHP and HMUFA diet, with only minor weight loss (~2%). Adopting a healthy diet thus should always be considered, even if weight loss cannot be achieved. However, the LFHP diet appeared to be slightly more beneficial compared to the HMUFA diet with respect to improvements in cardiometabolic parameters and body composition. Additionally, the LFHP diet resulted in greater decrease in waist circumference compared to the HMUFA diet, LFHP-induced reduction in VAT was associated with improved liver insulin sensitivity, and LFHP-induced reduction in fat ratio and maintenance of muscle volume was associated with improvements in whole-body insulin sensitivity, while this was not observed following a HMUFA diet (Chapter 7).

Finally, from literature it is well known that men and women show clear differences in cardiometabolic risk factors and body composition, which we also observed in Chapter 3 and 7. In Chapter 3, we showed that men and women respond differentially to a low-calorie diet, in which men lost more body weight, and improved more in cardiometabolic health per kilograms of weight loss compared to women, but were less able to maintain the improvements after 6 months of follow-up. Sex should thus be considered in the development of weight loss strategies.

Future perspectives

In the present thesis we obtained more insight into distinct metabolic phenotypes, in particular tissue-specific insulin resistant phenotypes. Furthermore, we demonstrated that the effect of a healthy diet on cardiometabolic health can be further improved by modulation of dietary macronutrient composition within the dietary guidelines according

to an individual's tissue- specific insulin resistant phenotype, thus providing the proof-of-concept of subgroup-based precision nutrition in a prospective study. Based on findings of the PERSON study, we can improve cardiometabolic health in about one third of the overweight and obese population, which may have implications for clinical practice and prevention strategies or contribute to the development of subgroup-specific dietary recommendations and guidelines. Nevertheless, some questions remain unanswered and require further investigation. Several future perspectives are discussed below.

» An important determinant for successful implementation of precision nutrition strategies into clinical practice will be the availability of easy, accessible, and cheap methods to classify individuals. As a result, the precision nutrition strategy may be implemented on a larger scale and would allow re-evaluation of an individual's metabolic phenotype after some time, as precision nutrition may be a longitudinal and dynamic process. The 7-point OGTT is a relatively easy and cheap method to assess tissue-specific insulin sensitivity but may already be relatively time- and resource consuming to integrate into clinical practice. Results from the PERSON study indicate that based on just one OGTT, dietary intervention success can be successfully predicted, and metabolic heterogeneity between subgroups can be identified. However, often in clinical practice, only fasting blood samples are collected. A simplified version of the OGTT, for example by collecting at least one postprandial blood sample, could be an important first step in the identification of at-risk individuals for the development of cardiometabolic diseases, who could potentially benefit from more personalized dietary recommendations.

» The primary focus of the present thesis was on tissue-specific insulin resistant phenotypes as target for precision nutrition strategies. Although parameters related to glucose metabolism play an important role in nutrient partitioning and are therefore important determinants of outcomes to dietary interventions, it may not explain all the variation that is present in dietary intervention response. At present it is not known how many distinct diet-sensitive phenotypes can be identified in an overweight population at risk for T2D. Future studies in the field of precision nutrition could thus be aimed at a more detailed classification or at other metabolic subgroups, that include other prespecified clinically relevant risk factors, including, but not limited to, -cell functioning, body composition, microbiome profile, lipidome profile, metabolome profile, age, sex, and ethnicity. Subgroups could be identified by

means of a spectral clustering approach, developing for example five of six relevant clusters, or metabolic phenotypes (75). The clinical usefulness of these metabolic phenotypes in the prevention or treatment of cardiometabolic diseases should be examined in randomized clinical trials or field studies.

» Besides baseline metabolic phenotype, dietary adherence is an important contributor to dietary intervention success, and may depend on factors including dietary preferences, motivation, psychological well-being, dietary knowledge, self-efficacy, and dietary behaviors (76, 77). Dietary counseling (including behavioral treatment strategies), web-based technologies, and wearables that provide direct feedback (e.g., continuous glucose monitors (CGM's) providing direct information on glucose concentrations), may offer possibilities to improve sustained dietary behavioral change, adherence, and engagement, and should be evaluated in future studies. Furthermore, validation in daily life settings is crucial to understand the potential success of a dietary intervention. To this regard, studies may for example include online coaching instead of weekly dietary visits, more focus on dietary preferences and dietary feasibility (e.g., meals that can easily be consumed outside a home-setting), and the performance of less invasive measurements that can also be performed at home (such as with CGM's).

» We collected immune cells of both whole blood as well as from aSAT samples. Nevertheless, circulating immune cells may not solely reflect aSAT inflammation, but may also reflect inflammation in other tissues, such as the liver and VAT. To obtain more insight in the relationship between immune cells and tissue-specific insulin sensitivity, biopsies of these tissues could be performed for the identification of immune cells. Besides, ectopic fat within the liver and skeletal muscle was quantified in the PERSON study. However, it is well established that not only lipid quantity, but also quality, as well as the accumulation of bioactive lipid metabolites are linked to metabolic perturbations. Future studies could include the collection of tissue biopsies and/or perform a magnetic resonance spectroscopy scan for the assessment of tissue-specific lipid quality.

» Finally, we found clear differences between men and women in body composition, and in response to a diet-induced weight loss. Furthermore, MIR was more prevalent in women compared to men, suggesting a different

etiology towards cardiometabolic diseases between sexes. These findings highlight the relevance of the development of sex-specific dietary recommendations, which are currently largely lacking. Especially, the relationship between diet and cardiometabolic diseases in women, compared to men, is currently understudied. And more specifically, the effect of the pre- and postmenopausal state in relation to tissue-specific insulin resistance and related metabolic disturbances remains to be elucidated. Inclusion of a (larger) group of premenopausal women in clinical trials related to precision nutrition to develop sex- and age-specific dietary recommendations is therefore warranted.

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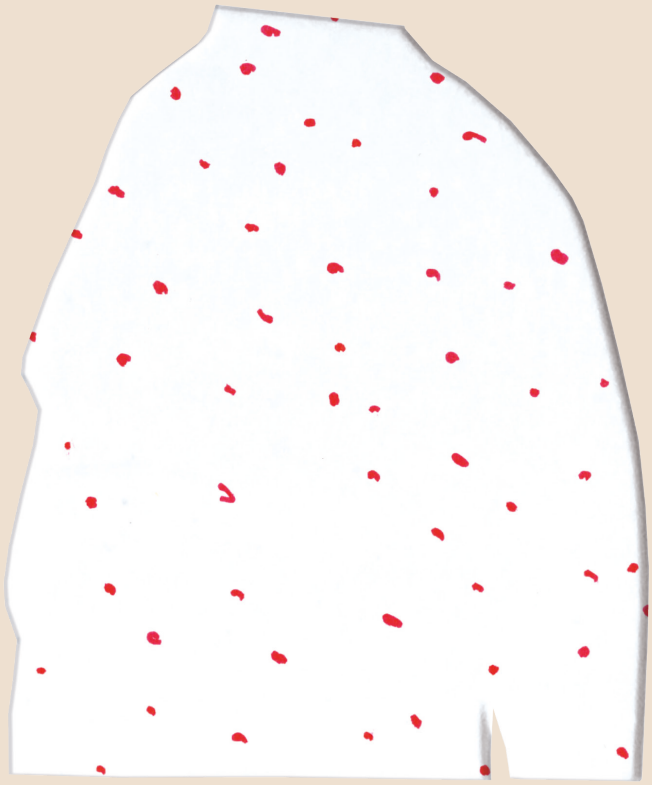
Appendix



IMPACT

In this section, the potential societal, scientific, and commercial impact of the research findings as described in this thesis will be discussed.

Societal impact of obesity research and precision nutrition

Obesity is a complex chronic disease and is related to the development of cardiometabolic diseases such as cardiovascular diseases (CVD) and type 2 diabetes (T2D). Obesity significantly impacts the average life expectancy and the quality of years alive. 40-year-old men and women with obesity have an about 6- and 7-year lower life expectancy, respectively, compared to men and women of the same age with normal body weight (1). Furthermore, individuals with obesity have on average an additional loss of one in ten disease-free years (2). Individuals with obesity are 25% more likely to be admitted to the hospital and health care cost are about 25% higher compared to someone with healthy body weight (3-5). Together, obesity poses a major burden on an individual's health as well as the healthcare system.

Many advances have been made in the prevention and treatment of obesity and obesity-related diseases, including the development and implementation of lifestyle and pharmacological interventions. An effective way to prevent CVD and T2M is via dietary intervention, focusing on weight loss, or improving health in general. However, it is increasingly evident that 'one size does not fit all' when it comes to dietary approaches to improve health. Dietary advice is often given at population-level based on general nutritional guidelines that rely on the group mean, while it is well acknowledged that 40-50% of participants does not respond or adhere to general dietary interventions. Interestingly, response relates back to specific metabolic phenotypes, as evidenced in studies like DiOGenes (6-8), LIPGENE (9), and CORDIOPREV-DIAB (10). Thus, there is an urgent need for more tailored personalized nutrition, or precision nutrition, to improve the efficacy of dietary interventions in people living with overweight and obesity.

In the prospective randomized PERSON study described in this thesis, we provide evidence that we can optimize the cardiometabolic health benefits of diet when the diet is modulated according to metabolic phenotype. We found that individuals with either liver (LIR) or muscle insulin resistance (MIR) showed more pronounced improvements in cardiometabolic risk factors following a different healthy diet, which seemed independent of the minor weight loss that occurred (~2 kg). A diet low in fat and high in protein and fiber (LFHP) appeared most beneficial for individuals with MIR, while a diet higher in healthy

(mono-unsaturated) fatty acids (HMUFA) was more beneficial for individuals with LIR, with respect to improvements in cardiometabolic health. These findings provide support for precision nutrition strategies over a general 'one-size-fits-all' dietary approach and may provide direct leads for translation into precision-based dietary guidelines to contribute to more successful prevention or treatment of obesity-related diseases. Importantly, individuals may be more motivated to follow dietary recommendations that are targeted for their individual phenotype, as expected health improvements are larger compared to general dietary advice.

Furthermore, in the present thesis we have identified two effective healthy diets within the guidelines for healthy nutrition, the isocaloric LFHP and HMUFA diet, in improving cardiometabolic risk factors. Importantly, irrespectively of baseline metabolic phenotype, both diets were effective in improving cardiometabolic risk factors. As such, liver fat decreased by more that 40% on average in the total population. Additionally, total cholesterol concentrations, which were slightly elevated in the total population at baseline according to clinical cut-off values (mean: 5.4 mmol/L), decreased following the dietary intervention, reaching concentrations within the healthy range (<5.0 mmol/L). These results highlight that adopting a healthy diet will result in cardiometabolic health benefits, also in the presence of minor weight loss. Importantly however, we demonstrate that health-related improvements can be remarkably enhanced when modulating dietary macronutrient composition according to tissue- specific insulin resistant phenotypes.

Scientific and commercial impact – towards precision nutrition

The research field for the prevention or treatment of obesity and obesity-related diseases shows a paradigm shift from general to more personalized interventions. Although more research has become available in the last decennia on phenotypic differences between (groups of) individuals that determines health status, there is a knowledge gap on the identification of phenotypic characteristics that largely determine dietary intervention response.

The PERSON study is the first prospective randomized trials investigating the effect of precision-based nutrition targeting tissue-specific insulin resistance phenotypes. The confirmed concept of precision nutrition provides leads for future research. Other metabolic (sub)phenotypes may be identified in future research to minimalize heterogeneity in dietary intervention response within a subgroup, thereby developing even more effective targeted dietary strategies. Furthermore, strategies to implement precision nutrition into daily life may be investigated. For example, future studies on precision nutrition may be focused on

the inclusion of web-based technologies that include direct feedback (possibly via wearables such as continuous glucose monitors) and online coaching, as well as the focusing on dietary adherence and preferences. The scientific insights of the present work together with future studies on precision nutrition may ultimately contribute to more personalized dietary recommendations to optimize cardiometabolic health improvements in the population.

Different researchers and companies have shown interest in the findings of the PERSON study. Furthermore, plans for future collaboration and research projects based on our findings are being developed at the moment. The research in this thesis is partly funded by industrial partners (food companies) that could use the present findings as leads for future product development. Commercial companies could benefit from the research by translating these findings into products that could support precision nutrition strategies or food products that specifically target metabolic subgroups in the population. Furthermore, companies may develop products that provide information for real-time personalized feedback, such as continuous glucose monitors linked to smartphone apps.

Dissemination

Based on the exciting outcomes of the PERSON study, we have drawn a dissemination and communication plan. We will disseminate our findings through social media and through press releases to both scientific and popular press from local and (inter)national news networks. Furthermore, a symposium on precision nutrition will be organized to disseminate the findings related to the PERSON study, thus attracting health care professionals and researchers in the field of metabolic health and precision nutrition. The results presented in this thesis are, or will be, published in international peer-reviewed scientific journals. The articles will be published with open access, to enable researchers and health care professionals from all over the world to read them. Furthermore, parts of the data presented in this thesis have been, and will be, presented at (inter)national conferences related to nutrition, obesity, and T2D. Furthermore, the information will be further shared via social media channels, including the website of our research group (www.nimolab.nl), Facebook, and LinkedIn. This attention may reach the general population, health care professionals (including dieticians, doctors, and lifestyle coaches), scientists, and other people within our own (inter)national network. Finally, specifically for the participants of the PERSON study, we have communicated the present outcomes, including information on (the changes in) their individual health parameters (e.g., parameters of glucose homeostasis, blood lipid profile, body composition, etc.).

SUMMARY

Obesity is a chronic disease and increases the risk for the development of other chronic noncommunicable diseases, including cardiovascular disease (CVD) and type 2 diabetes (T2D). Many lifestyle interventions have been developed to prevent and treat obesity and obesity-related metabolic diseases. However, long-term adherence to dietary interventions is often poor and there is a large variability in response, and thus, it is increasingly evident that 'one size does not fit all' when it comes to dietary approaches to improve health. To improve the effectiveness of nutritional intervention, there is an urgent need for more personalized approaches such as precision nutrition strategies. Importantly, dietary intervention success often relates back to specific underlying metabolic phenotypes related to general (including age and sex), anthropometric, and/or metabolic characteristics (including inflammation and glucose homeostasis). The present thesis primarily focusses on distinct metabolic phenotypes, more specifically individuals with either more pronounced liver or muscle insulin resistance (LIR and MIR). Previous studies demonstrated that individuals with LIR and MIR have a distinct lipidome, metabolome, and adipose tissue transcriptome profile, which may (partly) underly the differential response to dietary intervention. Clearly, this may have implications for precision nutrition strategies. Therefore, the aim of the present thesis was to further characterize these LIR and MIR phenotypes in individuals with overweight and obesity. Additionally, the efficacy of adapting dietary macronutrient composition (within the context of a healthy diet) towards these tissue-specific metabolic phenotypes to enhance improvements in cardiometabolic health was investigated.

In Chapter 4 we described the study design of the two-center randomized PERSonalized Glucose Optimization Through Nutritional intervention (PERSON) study, which was designed to gain more insight into tissue-specific insulin resistant phenotypes, and to test whether these phenotypes would benefit from two healthy diets differing in macronutrient composition. Individuals with overweight and obesity (n=242) aged between 40 and 75 years, with predominant LIR or MIR, were included in the study. The study participants followed one of two isocaloric diets for 12 weeks: a high-monounsaturated fatty acid (HMUFA) or a low-fat, high-protein, and high-fiber (LFHP) diet. Both diets are in line with Dutch dietary guidelines and have been implicated in the prevention of cardiometabolic diseases, also in the presence of only minor weight loss. We predefined two intervention groups, namely PhenoDiet group A which included individuals with MIR following a HMUFA diet, and individuals with LIR following a LFHP diet, and PhenoDiet group B which included individuals with LIR following a HMUFA diet and MIR following a LFHP diet. We

hypothesized the diet and phenotype combinations in PhenoDiet group A to be most beneficial with respect to improvements in the disposition index (combined score of insulin secretion and insulin sensitivity) and other cardiometabolic parameters.

In Chapter 6 and 7 we described the intervention outcomes of the PERSON study. We clearly demonstrated that we could optimize the effect of a healthy diet when modulating dietary macronutrient composition according to tissue-specific insulin resistant phenotypes (Chapter 6). We did not observe significant differences in the primary outcome, the disposition index, between the two PhenoDiet groups. Strikingly, however, we found that individuals in the PhenoDiet group B rather than PhenoDiet group A, improved significantly more in in insulin sensitivity, glucose homeostasis, serum TAG, and CRP compared to individuals in PhenoDiet group A, independent of modest weight loss (~2.5 kg). To illustrate, whole-body insulin sensitivity improved with ~20% in PhenoDiet group B, compared to only ~4% in the PhenoDiet group A. In other words, individuals with LIR benefitted most from the HMUFA diet, and individuals with MIR from the LFHP diet, with respect to improvements in cardiometabolic risk factors. The findings of this prospective study provide the proof-of-concept for precision nutrition based on metabolic phenotypes to further improve the effect of a dietary intervention on cardiometabolic health.

Importantly, we found that both the LFHP and HMUFA diets, irrespective of tissue-specific insulin resistant phenotype, were effective in improving cardiometabolic risk factors, including body composition. In Chapter 6 we observed improvements in amongst other total cholesterol and blood pressure, and in Chapter 7 we observed improvements in body composition, body fat distribution, and ectopic fat in following both the LFHP and HMUFA diet, in the presence of only minor weight loss (~2.5 kg). Nevertheless, the LFHP diet appeared to be slightly more beneficial compared to the HMUFA diet with respect to improvements in waist circumference (Chapter 7). Moreover, the LFHP diet-induced improvements in body composition were associated with improved whole-body and liver insulin sensitivity, while this was not observed following the HMUFA diet (Chapter 7).

In Chapter 7 we furthermore demonstrated that body composition and body fat distribution were distinctly associated with liver and muscle insulin sensitivity. Body weight and liver fat were associated with impaired liver insulin sensitivity while muscle fat was associated with impaired muscle insulin sensitivity in women, but not in men. These findings are in line with the literature search in Chapter 2, were we highlight the relevance of ectopic fat in relation to (tissue-specific) insulin resistance. In Chapter 7 we furthermore found that women have a higher total fat percentage, higher muscle fat, and more

abdominal adipose tissue, but less visceral adipose tissue (VAT) compared to men, while whole-body and tissue-specific insulin sensitivity were comparable. Interestingly, as muscle fat was higher in women, and muscle fat was inversely associated with muscle insulin sensitivity in women only, it can be speculated that women might show a catch-up in cardiometabolic impairments after a certain threshold of skeletal muscle lipid storage has been reached compared to men. In Chapter 3, we further investigated sex-differences in relation to cardiometabolic health and demonstrated that sex impacts the response to dietary intervention. Men and women responded differentially to a low-calorie diet within the context of the Diet, Obesity, and Genes (DiOGenes) study. Men lost more body weight and improved more in cardiometabolic health compared to women, while women were better in maintaining the improvements during follow-up on ad libitum diets varying in protein content and glycemic index. These observations are possibly a result of a sex-specific loss of adipose tissue depots, where men possibly lose and subsequently regain more of the metabolically disadvantageous VAT depot.

In Chapter 5, we performed a cross-sectional analysis within the PERSON study and The Maastricht Study, a large population-based cohort (n=273), to investigate the relationship between circulating and abdominal subcutaneous adipose tissue (aSAT) immune cells and inflammatory markers, and tissue-specific insulin sensitivity. We found that a higher relative abundance of circulating immune cell populations, specifically (classical) monocytes, were associated with impaired liver but not muscle insulin sensitivity. Furthermore, we demonstrated that aSAT gene expression of the pro-inflammatory adipokine IL-6 and the immune cell marker CD14 was associated with impaired muscle, but not liver, insulin sensitivity. Related to the importance of adipose tissue inflammation in insulin sensitivity, in Chapter 8, we commented on the article by Espinosa De Ycaza and colleagues that was recently published in Diabetes, in which the relationship between adipose tissue inflammation and adipose tissue insulin resistance was investigated.

In conclusion, the results from the work described in this thesis show that distinct metabolic phenotypes towards the development of cardiometabolic diseases can be identified. Within the PERSON study, we specifically investigated the (intervention response in) tissue-specific insulin resistant phenotypes, LIR and MIR. We demonstrated that these phenotypes are distinct in relation to circulating immune cells, aSAT inflammatory gene expression, body composition, body fat distribution, and ectopic fat deposition. Furthermore, we provide evidence, for the first time, that we can optimize the effect of a healthy diet on cardiometabolic health when the dietary macronutrient

composition is modulated according to the (tissue- specific insulin resistant) metabolic phenotype. With this, we provide the proof-of-concept that precision nutrition may enhance the cardiometabolic improvements following dietary intervention, which may have important implications in the prevention of cardiometabolic diseases.

SAMENVATTING

Obesitas is een chronische ziekte en verhoogd het risico op de ontwikkeling van andere chronische cardio-metabole ziekten, waaronder hart- en vaatziekten en type 2 diabetes (T2D). Er zijn veel leefstijlinterventies ontwikkeld om obesitas en obesitas-gerelateerde ziekten te voorkomen en te behandelen. Echter is het voor mensen vaak lastig om een gezond voedingspatroon op de lange termijn vol te blijven houden, en daarnaast is er een grote variatie te zien in de effectiviteit van voeding tussen individuen. Om die redenen wordt het steeds duidelijker dat 'one size fits all' niet geldt als het gaat om het verbeteren van de gezondheid door middel van gezonde voeding. Om de effectiviteit van voedingsinterventies te verbeteren, is er dringend behoefte aan meer gepersonaliseerde benaderingen. Het succes van voedingsinterventies wordt gedeeltelijk verklaard door verschillen in specifieke onderliggende fenotypes tussen mensen dat gerelateerd is aan algemene kenmerken (inclusief leeftijd en geslacht), antropometrische kenmerken (inclusief lichaamssamenstelling), en/of stofwisselingskenmerken (inclusief ontstekingswaardes en de suikerhuishouding). Dit proefschrift richt zich voornamelijk op verschillende weefselspecifieke insuline resistente fenotypes, en meer specifiek op individuen met een meer uitgesproken lever- of spierinsulineresistentie (LIR en MIR). Eerdere studies hebben aangetoond dat individuen met LIR en MIR een duidelijk verschil in lipidoom-, metaboloom- en vetweefseltranscriptoomprofiel laten zien, dat mogelijk (gedeeltelijk) ten grondslag ligt aan de verschillen in response op een voedingsinterventie. Het doel van dit proefschrift was om deze LIR- en MIR-fenotypes verder te karakteriseren bij personen met overgewicht en obesitas. Daarnaast hebben we onderzocht wat het effect van het aanpassen van de samenstelling van macronutriënten in de voeding (in de context van een gezond dieet) aan deze twee fenotypes op de cardio-metabole gezondheid was.

In Hoofdstuk 4 beschreven we de onderzoeksopzet van de gerandomiseerde PERsonalized Glucose Optimization Through Nutritional intervention (PERSON) studie, die was opgezet om meer inzicht te krijgen in weefselspecifieke insulineresistente fenotypes, en om te testen of deze fenotypes baat zouden hebben bij een ander gezond voedingspatroon, die verschillend zijn in de samenstelling van macronutriënten. Personen met overgewicht en obesitas (n=242), tussen 40 en 75 jaar, met meer uitgesproken LIR of MIR, werden in het onderzoek geïncludeerd. De studiedeelnemers volgden gedurende 12 weken een van de twee isocalorische diëten: een hoog enkelvoudig onverzadigd vetzuren (HEOV) of een laag vet, hoog eiwit, en vezelrijk (LVHE) voedingspatroon. Beide voedingspatronen zijn in lijn met de Nederlandse voedingsrichtlijnen en worden aanbevolen in de preventie van obesitas-gerelateerde ziekten, ook wanneer dit samengaat met slechts

gering gewichtsverlies. We hebben vooraf twee interventiegroepen gedefinieerd, namelijk PhenoDiet groep A met personen met MIR die het HEOV voedingspatroon volgden, en personen met LIR die het LVHE voedingspatroon volgden, en PhenoDiet groep B met personen met LIR die het HEOV voedingspatroon volgden en MIR die het LVHE voedingspatroon volgden. We veronderstelden dat de combinaties van voedingspatroon en fenotype in PhenoDiet groep A het meest gunstig waren met betrekking tot verbeteringen in de dispositie-index (gecombineerde score van insulinesecretie en insulinegevoeligheid) en andere cardio-metabole parameters.

In Hoofdstuk 6 en 7 hebben we de interventie-uitkomsten van de PERSON studie beschreven. We hebben duidelijk aangetoond dat we het effect van een gezond voedingspatroon konden optimaliseren door de samenstelling van macronutriënten in de voeding aan te passen naar de weefselspecifieke insulineresistente fenotypes, LIR en MIR (Hoofdstuk 6). We hebben geen significante verschillen waargenomen in de primaire uitkomstmaat, de dispositie index, tussen de twee PhenoDiet groepen. Opvallend was echter dat we ontdekten dat individuen in de PhenoDiet groep B in plaats van in de PhenoDiet groep A, significant meer verbeterden in insulinegevoeligheid, suikerhuishouding, bloedtriglyceriden, en de ontstekingswaarde CRP, in vergelijking met individuen in PhenoDiet groep A, onafhankelijk van het kleine gewichtsverlies (~2,5 kilo) in beide groepen. Ter illustratie, de insulinegevoeligheid van het hele lichaam verbeterde met ~20% in PhenoDiet groep B, vergeleken met slechts ~4% in de PhenoDiet groep A. Met andere woorden, individuen met LIR hadden het meest baat bij het HEOV voedingspatroon, terwijl individuen met MIR het meest baat hadden bij het LVHE voedingspatroon, met betrekking tot verbeteringen in cardio-metabole risicofactoren. De bevindingen van dit onderzoek leveren het proof-of-concept voor meer gepersonaliseerde voeding op basis van metabole fenotypes om het effect van een voedingsinterventie op de cardio-metabole gezondheid verder te verbeteren.

Belangrijk is dat we vonden dat zowel het LVHE als het HEOV voedingspatroon, ongeacht het weefselspecifieke insulineresistente fenotype, effectief waren in het verbeteren van cardio-metabole risicofactoren. Zo zagen we in Hoofdstuk 6 verbeteringen in onder andere cholesterol en bloeddruk, en in Hoofdstuk 7 zagen we verbeteringen in lichaamssamenstelling, lichaamsvetverdeling, en ectopisch vet bij het volgen van zowel het LVHE als het HEOV voedingspatroon. Toch bleek het LVHE voedingspatroon iets gunstiger te zijn dan het HEOV voedingspatroon met betrekking tot verbeteringen in middelomtrek (Hoofdstuk 7). Bovendien waren de door het LVHE voedingspatroon geïnduceerde verbeteringen in lichaamssamenstelling geassocieerd met een verbeterde

insulinegevoeligheid van het hele lichaam en van de lever, terwijl dit niet werd waargenomen na het HEOV voedingspatroon (Hoofdstuk 7).

In Hoofdstuk 7 hebben we verder aangetoond dat lichaamssamenstelling en lichaamsvetverdeling duidelijk geassocieerd waren met lever- en spierinsulinegevoeligheid. Lichaamsgewicht en levervet was geassocieerd met een verminderde insulinegevoeligheid in de lever, terwijl spiervet bij vrouwen, maar niet bij mannen, was geassocieerd met een verminderde insulinegevoeligheid in de spier. Deze bevindingen zijn in lijn met het literatuuronderzoek in Hoofdstuk 2, waar we de relevantie van ectopisch vet in relatie tot (weefselspecifieke) insulineresistentie belichten. In Hoofdstuk 7 vonden we verder dat vrouwen een hoger totaal vetpercentage, hoger spiervet en meer buikvetweefsel hebben, maar minder visceraal vetweefsel in vergelijking met mannen, terwijl de insulinegevoeligheid van het gehele lichaam en van de lever en de spier vergelijkbaar waren. Interessant is dat, aangezien spiervet hoger was bij vrouwen en spiervet geassocieerd was met mindere spierinsulinegevoeligheid alleen bij vrouwen, kan worden gespeculeerd dat vrouwen een inhaalslag in cardio-metabole risicofactoren kunnen vertonen nadat een bepaalde drempel voor vetopslag in de spier is bereikt, ten opzichte van mannen. In Hoofdstuk 3 hebben we sekseverschillen in relatie tot cardio-metabole gezondheid verder onderzocht en aangetoond dat sekse de respons op dieetinterventie beïnvloedt. Mannen en vrouwen reageerden verschillend op een caloriearm dieet (800 kcal per dag) in de Diet, Obesity, and Genes (DiOGenes) studie. Mannen verloren meer lichaamsgewicht en verbeterden meer in cardio-metabole gezondheid in vergelijking met vrouwen, terwijl vrouwen de verbeteringen beter volhielden tijdens de periode na gewichtsverlies tijdens het volgen van een ad libitum voedingspatronen variërend in eiwitgehalte en glycemische index. Deze waarnemingen zijn mogelijk een gevolg van een sekse-specifiek verlies van vetweefseldepots, waarbij mannen mogelijk meer van het metabool nadelige visceraal vetdepot verliezen en vervolgens weer bijkomen.

In Hoofdstuk 5 hebben we een cross-sectionele analyse uitgevoerd binnen de PERSON studie en De Maastricht Studie, een groot populatie-gebaseerd cohort (n=273), om de relatie tussen immuun cellen in de circulatie en in het abdominaal onderhuids vetweefsel en inflammatoire markers en weefselspecifieke insulinegevoeligheid te onderzoeken. We vonden dat een hogere relatieve hoeveelheid van circulerende immuuncellen, met name (klassieke) monocyten, geassocieerd was met een verminderde lever-, maar niet met spier-insulinegevoeligheid. Verder toonden we aan dat vetweefsel genexpressie van de pro-inflammatoire IL-6 en de immuuncelmarker CD14 geassocieerd was met verminderde spier-, maar niet leverinsulinegevoeligheid. In verband met het belang van

vetweefselontsteking bij insulinegevoeligheid, hebben we in Hoofdstuk 8 commentaar gegeven op het artikel van Espinosa De Ycaza en collega's dat onlangs is gepubliceerd in Diabetes, waarin de relatie tussen vetweefselontsteking en vetweefselinsulineresistentie werd onderzocht.

Concluderend, de resultaten van dit proefschrift laten zien dat verschillende metabole fenotypes in de ontwikkeling van cardio-metabole ziekten kunnen worden geïdentificeerd. Binnen de PERSON-studie hebben we specifiek de (interventierespons in) weefsel-specifieke insulineresistente fenotypes, LIR en MIR, onderzocht. We hebben aangetoond dat deze fenotypes verschillend zijn in relatie tot circulerende immuuncellen, inflammatoire genexpressie van het onderhuids vetweefsel, lichaamssamenstelling, lichaamsvetverdeling, en ectopische vetophoping. Bovendien leveren we voor het eerst bewijs dat we het effect van een gezond dieet op de cardio-metabole gezondheid kunnen optimaliseren wanneer de samenstelling van de macronutriënten in de voeding wordt aangepast aan het (weefselspecifieke insulineresistente) metabole fenotype. Hiermee leveren we het proof-of-concept dat meer gepersonaliseerde voeding de cardio-metabole verbeteringen na dieetinterventie kan optimaliseren, dat belangrijke implicaties kan hebben bij de preventie van cardio-metabole ziekten.

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LIST OF SCIENTIFIC PUBLICATIONS AND PRESENTATIONS

Scientific publications

Published or accepted for publication

2022 Trouwborst, I.*; Gijbels A.*; Jardon K.M.*; Siebelink E.; Hul G.; Wanders L.; Erdos B.; Péter S.; Singh-Povel C.M.; de Vogel-van den Bosch J.; Adriaens M.E.; Arts I.C.W.; Thijssen D.H.J.; Feskens E.J.M.; Goossens G.H.; Afman L.A.; Blaak E.E.. Cardiometabolic health improvements upon dietary intervention are driven by tissue-specific insulin resistance phenotype: a precision nutrition trial. Accepted for publication in Cell Metabolism. *Shared first authorship.

2022 Trouwborst, I.; Goossens, G.H. Comment On Espinosa De Ycaza et al. Adipose Tissue Inflammation Is Not Related to Adipose Insulin Resistance in Humans. Diabetes. 2022, 71, 381–393, doi:10.2337/db21-1141

2021 Trouwborst, I.*; Gijbels A.*; Jardon K.M.; Hul G.B.; Siebelink E.; Bowser S.M.; Yildiz D.; Wanders L.; Erdos B.; Thijssen D.H.J.; Feskens E.J.M; Goossens G.H.; Afman L.A.; Blaak E.E. The PERSonalized glucose Optimization through Nutritional intervention (PERSON) study: rationale, design and preliminary screening results. Front. Nutr. 2021, 8, doi: 10.3389/fnut.2021.694568. *Shared first authorship

2021 Trouwborst, I.; Goossens, G.H.; Astrup, A.; Saris, W.H.M.; Blaak, E.E. Sexual Dimorphism in Body Weight Loss, Improvements in Cardiometabolic Risk Factors and Maintenance of Beneficial Effects 6 Months after a Low-Calorie Diet: Results from the Randomized Controlled DiOGenes Trial. Nutrients. 2021, 13, 1588, doi:10.3390/nu13051588

2019 Tieland, M.; Trouwborst, I.; Verreijen, A.; Memelink, R.; Weijs P.J.M. Sarcopenic Obesity in the Elderly. Book: Sarcopenia. Edition 1st Edition. First Published 2019. Imprint CRC Press. eBook ISBN 9780429155260

2018 Trouwborst, I.; Bowser, S.M.; Goossens, G.H.; Blaak. E.E. Ectopic fat accumulation in distinct insulin resistant phenotypes; targets for personalized nutritional interventions. Front. Nutr. 2018, 5, 77, doi:10.3389/fnut.2018.00077

2018 Trouwborst I.; Verreijen A.; Memelink R.; Massanet P.; Boirie Y.; Weijs P.; Tieland

M.. Exercise and nutrition strategies to counteract Sarcopenic obesity. Nutrients. 2018, 10.5, 605. doi:10.3390/nu10050605

2018 Tieland, M.; Trouwborst, I.; Clark B.C. Skeletal muscle performance and ageing. J. Cachexia Sarcopenia Muscle. 2018, 9, 3–19, doi:10.1002/jcsm.12238

Under review, submitted, or in preparation

2022 Trouwborst I.*; Wouters K.*; Jocken J.W.; Jardon K.M.; Gijbels A.; Dagnelie P.C.; van Greevenbroek M.M.J.; van der Kallen C.J.; Stehouwer C.D.A.; Schalkwijk C.G.; Afman L.A.; Blaak E.E.; Goossens G.H. Systemic and abdominal subcutaneous adipose tissue immune cells in tissue-specific insulin resistance in overweight and obesity. Submitted to Obesity. *Shared first authorship.

2022 Trouwborst I.; Jardon K.M.; Gijbels A.; Hul G.; Feskens E.J.M.; Afman L.A.; Linge J.; Goossens G.H.; Blaak E.E. Body composition and body fat distribution in tissue-specific insulin resistance and in response to a 12-week isocaloric dietary macronutrient intervention. To be submitted

2022 Gijbels A.; Trouwborst I.; Jardon K.M.; Feskens E.J.M; Goossens G.H.; Blaak E.E.; Afman L.A.. Hepatic insulin resistance and muscle insulin resistance are characterized by distinct postprandial metabolite profiles. In preparation

2022 Jardon K.M.; Umanets A.; Trouwborst I.; Gijbels A.; Hul G.; Feskens E.J.M.; Venema K.; Afman L.A.; Goossens G.H.; Blaak E.E.. The PERSON study: Effects of a 12-week metabolically targeted dietary intervention on the gut microbial profile in overweight and obesity. In preparation

2022 Gijbels A.; Erdos B.; Jardon K.M.; Trouwborst I.; Feskens E.J.M; Goossens G.H.; Adriaens M.E.; Arts I.C.W.; Blaak E.E.; Afman L.A.. Effects of a 12-week metabolically targeted dietary intervention on fasting and postprandial metabolome in overweight and obese adults: a secondary analysis of the PERSON study. In preparation

2022 Jardon K.M.; Umanets A.; Venema K.; Gijbels A.; Trouwborst I; Hul G.; Afman L.A.; Goossens G.H.; Blaak E.E.. Gut microbiome profiling in tissue-specific insulin resistance: A cross-sectional analysis of the PERSON study. In preparation

2022 Wanders, L.; Gijbels A.; Bakker E.A.; Trouwborst I.; Jardon K.M.; Manusama K.C.; Hul G.B.; Feskens E.J.M.; Afman L.A.; Blaak E.E.; Hopman M.T.E.; Goossens G.H.; Thijssen D.H.J. Physical activity and sedentary behavior show distinct associations with tissue-specific insulin sensitivity in adults with overweight. In revision at Acta Physiologica

2022 Sloun B.; Goossens G.H.; Erdõs B.; O'Donovan S.D.; Gijbels A.; Trouwborst I.; Jardon K.M.; Blaak E.E.; Lydia Afman L.A.; van Riel N.A.W.; Arts I.C.W. Predicting the postprandial glucose response in tissue-specific insulin resistant phenotypes: a computational modeling approach. Submitted

Scientific presentations

Oral presentations

Nutritional Science Days 2022. Precision nutrition by modulating dietary macronutrients according to tissue-specific insulin resistance phenotypes improves cardiometabolic health.

DTMC 2022. Systemic and abdominal subcutaneous adipose tissue immune cells in tissue-specific insulin resistance in overweight and obesity.

European Congress on Obesity - Zoom Forward, 2022. Immune cells and inflammation in relation to tissue-specific insulin resistance.

Nutritional Science Days, 2021. Sex-specific metabolic differences following weight loss and after weight maintenance: the DiOGenes study.

NUTRIM division 1 symposium, 2021. Personalized nutrition to target inter-individual differences in tissue-specific insulin resistance.

European Congress on Obesity, 2020. Sex-specific metabolic differences following weight loss and after weight maintenance: the DiOGenes study.

Annual Dutch Diabetes Research Meeting, 2020. Sex-specific metabolic differences following weight loss and after weight maintenance: the DiOGenes study

Young Investigators of the Dutch Association for Diabetes Research meeting, 2019. The PERSON-study: PERSonalized glucose Optimization through Nutritional intervention.

Poster presentations

Winter school on Obesity, European Association for the Study of Obesity (EASO), 2022. Systemic and abdominal subcutaneous adipose tissue immune cells are associated with hepatic- but not with muscle insulin resistance in people with overweight and obesity.

MUMC+ science day, 2021. Personalized nutrition to target inter-individual differences in tissue-specific insulin resistance; study design and preliminary screening results.

NUTRIM symposium, 2019. Personalized nutrition to target inter-individual differences in tissue-specific insulin resistance.

Personalized Nutrition Congress, 2019. The PERSON-study: PERSonalized glucose Optimization through Nutritional intervention: study design and rationale.

NUTRIM symposium, 2019. Sex-specific differences in weight loss and insulin resistance in overweight adults following an 8-week low calorie diet and 6 months of weight maintenance.

NUTRIM symposium, 2018. The PERSON-study: PERSonalized glucose Optimization through Nutritional intervention: study design and rationale.

Inez Trouwborst was born on the 31st of December in 1993, in Nijmegen. She attended secondary school in Nijmegen at the Montessori College where she received her Atheneum degree in 2011. In that same year, she started her Bachelor of Science in Nutrition and Health at Wageningen University. After obtaining her diploma in 2014, she continued her studies at Wageningen University with the Master Nutrition and Health, specialized in Nutritional Physiology and Health Status. During her master she performed an internship at the University of Applied Sciences in Amsterdam, investigating the effect of protein intake on the preservation of muscle mass in elderly with type 2 diabetes. After graduating from her Master of Science in 2017, she started her PhD project at the department of Human Biology at Maastricht University, under supervision of Prof. dr. Ellen Blaak and dr. Gijs Goossens. Her research focused on the effect of targeted, more personalized, nutrition to prevent and treat obesity-related diseases in an *in vivo* clinical setting, where she specifically focused on the distinct metabolic phenotypes towards cardiometabolic diseases. Inez presented her work at several national and international conferences. In 2022, she received the Foppe ten Hoor award for her work and CV as a young nutritional scientist. Inez will continue her scientific career as a research associate in Metabolic Health at Nestlé Institute of Health Science in Lausanne, Switzerland.