

Tissue-specific insulin resistance in human obesity

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Tissue-specific insulin resistance in human obesity

A physiological approach to lipid metabolism



School of Nutrition and
Translational Research
in Metabolism



NASO

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Tissue-specific insulin resistance in human obesity

A physiological approach to lipid metabolism

PROEFSCHRIFT

Ter verkrijging van de graad doctor aan de Universiteit Maastricht,
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The background of the page is a light gray gradient. On the left side, there is a vertical strip of abstract, semi-transparent white and light gray patterns. These patterns include various geometric shapes like squares and hexagons, as well as molecular-like structures with nodes (circles) and connecting lines. Some of these structures resemble chemical frameworks or network diagrams. The overall aesthetic is clean, modern, and scientific.

Chapter 1

General Introduction



Obesity and insulin resistance

Obesity prevalence

The prevalence of obesity has risen to epidemic proportions over the last decades and affects people of all ages and socioeconomic groups (1). In 2014, more than 39% of the world adult population was overweight (BMI > 25 kg/m²) and about 13% was obese (BMI > 30 kg/m²) (2). In 2015, in the Netherlands, over 50% of the individuals over 20 years of age were overweight or obese (3).

The increasing prevalence of obesity is a major health concern. In 2010, overweight and/or obesity was estimated to cause 3.4 million deaths worldwide (4). Additionally, obesity increases the risk for developing chronic diseases such as type 2 diabetes mellitus (5), cardiovascular diseases (6), mental disorders (7) and several cancer types (8). In the upcoming years, the expectation is that obesity-related complications will continue to increase and will have large public health and socioeconomic consequences.

Obesity and cardiometabolic health

A disrupted energy homeostasis, due to a dysbalance between energy intake and energy expenditure, is often accompanied by the development of obesity and its related metabolic complications. A positive energy balance, which is a long-term surplus of energy intake from carbohydrates, lipids and/or proteins over energy expenditure, leads to energy storage as fat in the body and to weight gain (9). An inactive lifestyle as well as calorie-dense food consumption are main causes for body weight changes, next to genetic predisposition and other environmental and biological factors (10). Impaired energy homeostasis and obesity are strongly associated with insulin resistance, development of type 2 diabetes and cardiometabolic complications (11).

Obesity is linked to the development of insulin resistance, which is defined as an impaired sensitivity to insulin of its main metabolic target organs e.g. adipose tissue, skeletal muscle, liver and pancreas. Insulin resistance is a major contributor to hyperglycemia and hyperlipidemia, which are all risk factors towards the development of type 2 diabetes and cardiovascular diseases. Notably, the 'World Health Organization (WHO) Global report on diabetes 2016' reports a doubled prevalence of diabetes since 1980, rising from 4.7% to 8.5% in the worldwide adult population. Moreover, this report estimates the total burden of deaths from high blood glucose near 3.7 million cases (12), including 2.2 million deaths caused by

higher-than-optimal blood glucose concentrations and related cardiovascular disease complications (12). In addition, the WHO estimated that 17.7 million people died from cardiovascular diseases in 2015, representing 31% of all global deaths (13). Strikingly, insulin resistance increases the risk for cardiovascular diseases even in the absence of hyperglycemia (14,15). Thus, understanding the etiology of insulin resistance is a key step in the prevention of cardiometabolic diseases.

Insulin resistance and tissue-specificity

In humans, intermediate states of impaired glucose metabolism (prediabetes) can be identified by measuring plasma glucose and insulin concentrations in the fasting state and after an oral glucose tolerance test (OGTT). Insulin resistance together with increased β -cell failure results in an increased blood glucose concentrations in the non-diabetic range. These prediabetes states are classified as impaired fasting glucose (IFG) or impaired glucose tolerance (IGT) (16). Individuals with IFG, defined by fasting glucose > 5.6 mmol/L, mainly show hepatic insulin resistance and a normal or slightly lower whole-body insulin sensitivity. In contrast, individuals with IGT, defined by a 2 h glucose concentration > 7.8 mmol/L, have a normal to slightly reduced hepatic insulin resistance and show moderate to severe reduced peripheral (skeletal muscle) insulin sensitivity. However, the pathophysiological mechanisms do overlap substantially between IFG and IGT as both states are characterized by hyperglycemia. In addition, individuals who have combined IFG and IGT show both hepatic and peripheral (skeletal muscle) insulin resistance (17).

These prediabetes states represent distinct pathophysiological pathways towards cardiometabolic diseases. Up to now, there is evidence that interventions that increase insulin sensitivity are organ specific, at least for a part of their effects. For instance, physical activity was shown to mainly affect muscle insulin sensitivity (18), while metformin treatment might improve hepatic insulin resistance (19). In addition, a diet low in fat and high in complex carbohydrates may be beneficial for prediabetic individuals with hepatic insulin resistance, while the Mediterranean diet may improve muscle insulin sensitivity (20).

Rodent studies may provide important links to a better understanding of human substrate metabolism in relation to insulin resistance. In mice, genetic manipulation of the insulin receptor signaling pathways in different organs had major effects on the mice phenotypes and metabolic functioning of these organs (21). For instance, mice with muscle-specific insulin receptor knockout (MIRKO) had profound insulin resistance in muscle, but glucose tolerance

was normal due to insulin-independent glucose uptake in muscle (22). This comes along with increased glucose uptake in adipose tissue (22). In mice with liver-specific insulin receptor deletion (LIRKO), insulin suppression of hepatic glucose output was completely abolished, and hyperlipidemia and progressive liver dysfunction was observed (23). Notably, the MIRKO mouse demonstrated a development of adaptive mechanisms in adipose tissue to compensate for muscle insulin resistance to maintain glucose homeostasis while the LIRKO mouse was unable to respond to hyperinsulinemia (21). So far, these rodent studies have helped us revealing distinct metabolic profiles when insulin receptor signaling pathways are affected in a tissue-specific manner. However, human studies focusing on tissue-specific insulin resistance phenotypes are limited so far.

Interorgan cross-talk in obesity and insulin resistance

As illustrated above, insulin resistance can develop simultaneously in multiple organs and the insulin resistance severity may vary between different organs. Insulin tightly regulates the distribution and utilization of the energy-rich carbohydrates and fatty acids. Therefore the metabolic insulin sensitive organs such as adipose tissue, liver and skeletal muscle play an important role in human energy metabolism. Dysfunction of these organs cause or contribute to the development of (tissue-specific) insulin resistance and cardiometabolic diseases. Figure 1 shows a schematic overview of organ crosstalk in obese insulin resistant conditions.

Adipose tissue plays a central role in energy metabolism and is closely related to the adverse health effects associated with obesity. The two major functions of the adipose tissue related to lipid metabolism are: i) lipid storage by the uptake of meal-derived fatty acids from the circulation and store them as triacylglycerol (TAG) in the postprandial phase and ii) to supply non-adipose tissue with energy in the form of non-esterified fatty acids (NEFAs) in conditions of increased energy demand (e.g. fasting and exercise) (9). Under conditions of chronic positive energy balance, the adipocytes become enlarged and the adipose tissue lipid buffering capacity is often exceeded. This results in lipid overflow into the circulation and subsequently to non-adipose tissues such as liver, skeletal muscle and pancreas (24). If lipid supply exceeds the fat oxidative capacity in these non-adipose tissues, ectopic fat accumulation may occur. This ectopic fat accumulation is associated with the development of insulin resistance.

In the liver, an increased lipid supply from the adipose tissue may stimulate the endogenous formation of TAG under obese insulin resistant conditions. The lipid overflow

leads to elevated secretion of very low-density lipoprotein (VLDL)-TAG into the circulation (25). Together with a higher hepatic glucose production and reduced insulin clearance, this results in increased systemic glucose and insulin concentrations. Subsequently, these processes might affect the lipid overflow to other tissues (26).

The skeletal muscle is recognized as a key organ in peripheral insulin sensitivity as it accounts for 70-80% of the total glucose disposal under postprandial conditions (27). Often skeletal muscle insulin resistance refers to an impaired insulin regulation of glucose homeostasis. Notably, lipid metabolism is important as well. An increased lipid supply from adipose tissue and liver results in an increased uptake of systemic endogenous and dietary lipids into the skeletal muscle. Furthermore, a reduced capacity to oxidize fatty acids is often observed in obese insulin resistant conditions, which might be due to mitochondrial dysfunction (28). These factors together may contribute to the increased accumulation of intramuscular lipids (e.g. TAG, diacylglycerol (DAG)) and lipid intermediates (e.g. ceramides and acylcarnitines), leading to lipotoxicity and cellular dysfunction (29). Subsequently, these processes might disrupt the insulin signaling and insulin-mediated glucose uptake, resulting in lipid-induced insulin resistance (30).

In the pancreas, ectopic fat deposition might result in lipotoxicity and decreased glucose-stimulated insulin secretion by the beta-cell, thereby accelerating hyperglycemia and the development of insulin resistance in other tissues. This process may require a long time as this is illustrated by the observation that the obese insulin resistant state is often associated with higher plasma insulin concentrations (i.e. compensatory hyperinsulinemia) rather than decreased insulin levels (24).

Finally, the gastrointestinal tract plays a major part in organ crosstalk and lipid metabolism as well. The primary task of the gastrointestinal tract is to digest and absorb macronutrients, including lipids. For instance, dietary lipids are packaged into lipoprotein particles called chylomicrons, which will be transported via the lymph nodes towards peripheral tissues and the liver. Additionally, the gut is an endocrine organ regulating the secretion of satiety hormones and incretins, thereby affecting energy and substrate metabolism of the host (31). Importantly in the last decade, the residents of the intestine, the gut microbiota, have been recognized as an active metabolic organ. They produce short-chain fatty acids, bile acids and inflammatory factors with significant impact on host energy/substrate metabolism and insulin sensitivity (32). Up to now, there is accumulating evidence that obesity-associated alterations in the gut microbiota can also affect host lipid and

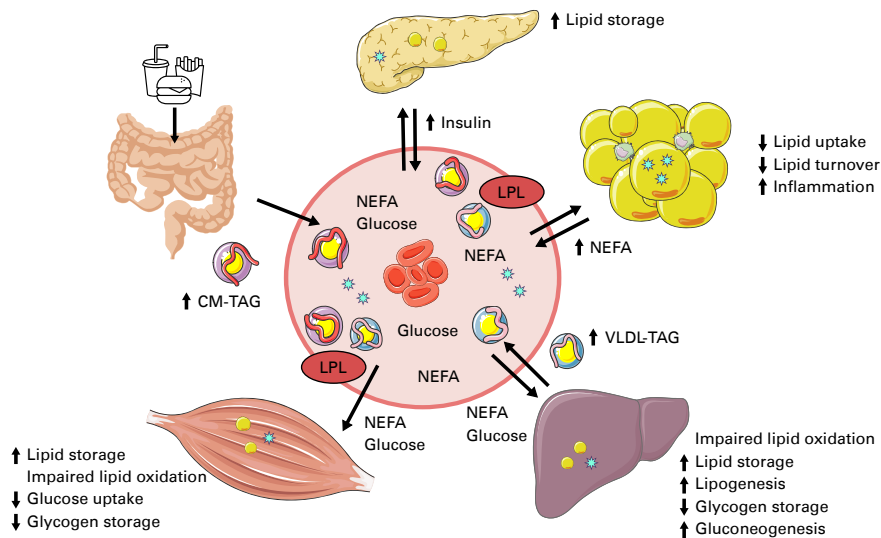


Figure 1. Schematic overview of inter-organ crosstalk in obesity-induced insulin resistance

During long-term positive energy balance (i.e. when energy intake exceeds energy expenditure), systemic lipid overload contributes to ectopic (non-adipose) lipid storage. Loss of the adipose tissue buffer capacity may increase circulating NEFA and TAG. Fatty acids are taken up by peripheral tissues like skeletal muscle, liver and pancreas. An impaired lipid oxidation in these tissues may direct these lipids towards storage, possibly leading to the development of insulin resistance. LPL, located at the luminal side of the capillary endothelium, plays a central role in the clearance of TAG-rich plasma lipoprotein particles in skeletal muscle and adipose tissue. In addition, inflammatory cytokines may be secreted from adipose tissue and they may decrease local and whole-body insulin sensitivity. LPL: lipoprotein lipase, NEFA: non-esterified fatty acid, CM-TAG: chylomicron triacylglycerol (●), VLDL-TAG: very-low density lipoprotein triacylglycerol (●), ectopic lipid storage (●), inflammatory cytokines (★).

glucose metabolism in adipose tissue, liver and skeletal muscle. However most mechanistic evidence is based on animal studies so far (32,33).

To better understand the complex inter-organ crosstalk in insulin resistance, the next paragraphs will discuss in more detail the role of adipose tissue (dys)function as well as the role of liver and skeletal muscle in lipid metabolism and insulin resistance.

Adipose tissue function and dysfunction

The development of obesity is accompanied by a substantial increase in adipose tissue mass. However, increased adipose tissue mass *per se* might not be the most important contributor to the development of obesity-related metabolic disorders. Adipose tissue function and distribution of body fat may also play a prominent role in cardiometabolic health (34). The adipose tissue organ is composed of different discrete anatomical depots, all with different size and metabolic function (35,36). Adipose tissue accumulation in the upper body (abdominal region) is associated with increased cardiometabolic and mortality risk, while lower body (gluteofemoral region) fat accumulation is considered to be protective against obesity-related disorders (35). Moreover, with respect to the upper-body adipose tissue depots, enlarged visceral adipose tissue confers increased risk for cardiometabolic risk factors compared to subcutaneous adipose tissue. This could possibly be due to functional differences such as a pro-inflammatory phenotype (37).

Traditionally, the only function that was ascribed to adipose tissue was that of insulator and energy storing tissue. However, nowadays it is commonly accepted that adipose tissue is a dynamic endocrine organ. It is involved in the regulation of various homeostatic processes including lipid storage and mobilization which is also called lipid buffering capacity. The adipose tissue continuously switches from being a net storage organ in times of a positive energy balance to a net release organ in times of a negative energy balance. In addition, adipose tissue is involved in regulation of appetite and satiety, and secretion of cytokines and adipokines related to the development of local and systemic low-grade inflammation (9,38-40).

In the next paragraphs, I will discuss the relationship between adipose tissue lipid buffering capacity and insulin resistance in more detail. Additionally, I will highlight the role of adipose tissue differentiation capacity and adipose tissue inflammation in obesity-induced insulin resistance.

Adipose tissue lipid uptake and storage

In times of a positive energy balance, i.e. the fed state, the adipose tissue buffers and stores dietary fatty acids in the form of TAGs (41). Lipoprotein lipase (LPL), located at the luminal side of the capillary endothelium, plays a central role in the clearance of TAG-rich plasma lipoproteins particles (42). It is the rate-limiting enzyme in extracellular lipolysis as it catalyzes the hydrolysis of endogenous VLDL-TAG and exogenous chylomicron-TAG into glycerol

and NEFAs (43). The activity of LPL is under tight nutritional and hormonal control in a tissue-specific manner. In humans, adipose LPL activity is markedly elevated in the fasted state compared with the fed state (44). While adipose tissue LPL activity oscillates, LPL mRNA and protein mass differ only slightly (42). This suggests that the activity of LPL is primarily regulated at the post-translational level. Indeed, in adipose tissue, the postprandial rise in insulin is a major activator of LPL activity (43,45).

Recently, angiopoietin-like protein 4 (ANGPTL4) has emerged as an important inhibitor of LPL activity in the fasted state (46). But the role of ANGPTL4 in human adipose tissue LPL activity is not well understood and current data are mainly derived from animal studies. In rodent adipose tissue studies, increased ANGPTL4 concentrations inhibit adipose tissue LPL activity via transient binding to LPL and conversion to inactive monomers under fasting conditions (47). Moreover, it has been shown that ANGPTL4 promotes intracellular degradation of LPL in murine adipocytes in *in vitro* studies (48). It has also been reported in rodent studies that increased ANGPTL4 expression leads to a reduced plasma TAG clearance by adipose tissue (49) and this inhibiting effect was blunted in ANGPTL4 knockout mice (49). It is known that ANGPTL4 is expressed in human adipocytes (50) and it has been shown that during long-term fasting (50) and following acute physical activity (50-52) adipose tissue expression and plasma ANGPTL4 concentrations are increased in humans. Moreover, insulin has been shown to reduce circulating ANGPTL4, as revealed by hyperinsulinemic-euglycemic clamp studies in humans (53-55). Nevertheless, it remains to be elucidated whether systemic and/or adipose tissue ANGPTL4 levels are also involved in regulating adipose tissue LPL activity in humans and whether this is impaired under insulin resistant conditions. Besides insulin and ANGPTL4, LPL activity is also regulated by apolipoproteins (APO-C1, -2, -3, -5, APO-E), Gastric inhibitory polypeptide (GIP), Glycosylphosphatidylinositol anchored high density lipoprotein binding protein 1 (GPIHBP1) and other ANGPTLs (-3 and -8), which are reviewed in detail elsewhere (42,56).

LPL activity is not well understood in humans under obese insulin resistant conditions. Basal LPL activity has been reported to be delayed but preserved in obese individuals when compared to lean healthy individuals (57). During hyperinsulinemia, it has been shown that fatty acid spillover from LPL-mediated TAG hydrolysis across adipose tissue was less suppressed in obese individuals (57,58). It was recently demonstrated that men with type 2 diabetes have a similar ability to store VLDL-TAG in adipose tissue compared to non-diabetic men, despite greater circulating postprandial VLDL-TAG concentrations (59).

Moreover, a delayed insulin-mediated stimulation of adipose tissue LPL activity was found in obesity, insulin resistance and type 2 diabetes (60-64). This resulted in an impaired clearance of chylomicron-derived TAG. In obese individuals, a decreased clearance of VLDL-TAG by adipose tissue was observed after an overnight fast, whilst in the postprandial state chylomicron-TAG clearance was reduced (65). Subsequently, VLDL-TAG seemed to compete with chylomicrons for LPL-mediated clearance (60,65). In line, the latter study demonstrated that the relative quantity of meal lipids stored in adipose tissue after the intake of subsequent meals was significantly reduced in abdominally obese versus lean individuals (65). Together, these data suggest a less efficient removal of dietary lipids by adipose tissue under obese insulin resistant conditions. The impaired removal of dietary lipids in combination with increased liver VLDL production (26,66) may contribute to increased lipid spillover and ectopic fat deposition. To summarize, in obese insulin resistant conditions, elevated TAG concentrations are observed. Both an increased liver VLDL production (26,66) as well a reduced clearance of circulating VLDL-TAG and chylomicron-TAG by adipose tissue have been shown to play a significant role in this phenomenon.

In addition to an impaired LPL action in obese and insulin-resistant conditions, disturbances in the uptake of the liberated NEFAs may contribute to an impaired total lipid uptake by adipose tissue. A significant proportion of the fatty acids that are hydrolyzed from TAG by intravascular LPL lipolysis are not taken up by the adipose tissue and they will 'spill over' into the plasma NEFA pool (67). Under normal physiological conditions, LPL-derived NEFAs are taken up by passive diffusion or active fatty acid transporters, like fatty acid translocase (CD36), plasma membrane bound fatty acid binding protein (FABPpm) and a family of fatty acid transport proteins (FATP1-6) (68). The process of fatty acids trapping into the adipocytes is dependent on the concentration gradient across the adipocyte membrane and plasma. The concentration gradient is regulated by enzymes involved in fatty acid re-esterification and intracellular lipolysis (41,69). In the adipocytes, the NEFAs will be mostly directed towards re-esterification and storage of TAG, thereby lowering the NEFA concentration intracellularly.

Adipose tissue lipolysis

In times of a negative energy balance, e.g. during fasting or increased physical activity, the adipose tissue releases fatty acids in the form of NEFAs for oxidation in other organs. This process is called intracellular lipolysis (70). It involves the hydrolysis of stored neutral TAGs into glycerol and NEFAs via the action of three consecutive lipases: adipose tissue TAG lipase

(ATGL), hormone-sensitive lipase (HSL) and monoacylglyceride lipase (MGL) (71,72). The catecholamines, adrenalin and noradrenalin, and the natriuretic peptides, atrial natriuretic peptide (ANP) and the brain natriuretic peptide (BNP), act as pro-lipolytic hormones, whereas insulin is the most potent anti-lipolytic hormone in human adipose tissue (70).

In obesity, whole-body lipolysis under fasting conditions (also called basal lipolysis) may be increased because of the increased total adipose tissue mass. Indeed, an increased total NEFA flux is often observed during fasting in obese insulin resistant conditions. In contrast, the rate of NEFA release per kg fat mass is considerably decreased in obesity (73). In line, this reduced lipolysis per unit fat mass during fasting and catecholamine stimulation has been shown to be associated with lower adipose tissue ATGL and HSL expression and activity in obese insulin resistant individuals (74,75). Furthermore, insulin-mediated suppression of fatty acid release has been shown to be blunted in obesity, impaired glucose tolerant and type 2 diabetes individuals (62,76). Nevertheless, the differences in circulating NEFA concentrations between obese and lean participants are generally modest and appear not to be proportionally increased to the size of adipose tissue mass (77). In summary, obesity and insulin resistance are characterized by an increased basal and a blunted catecholamine and natriuretic peptide-stimulated lipolysis in subcutaneous adipocytes.

Adipose tissue differentiation capacity

During periods of a chronic positive energy balance, adipocytes become overloaded with TAG and the adipose tissue will expand to accomodate extra TAG storage. This process is firstly determined by an increase in adipocyte size (hypertrophy) and subsequently by an increased adipocyte number (hyperplasia) through a process called adipogenesis (78). In general, the total adipocyte number remains stable during adulthood although cells are renewed during life. It has been estimated that there is a yearly ~10% adipocyte turnover (79). During the ~10 year lifespan of human adipocytes, the TAG content is renewed six times on average (80).

Hypertrophic adipocytes have been more strongly associated with insulin resistance and its metabolic complications than hyperplastic adipose tissue (81-83). Moreover, adipocyte hypertrophy has been shown to be a key phenotypic characteristic of non-obese and obese type 2 diabetes patients (84), while adipocyte number was lower compared to BMI-matched obese persons without metabolic complications (85-87). Finally, a reduced lipid turnover, due to mitochondrial dysfunction, in adipocytes was also associated with obesity (88) and hypertrophic dysfunctional adipose tissue (89). Together these data a diminished adipocyte

differentiation capacity, which might result in a reduced TAG storing capacity in obese insulin resistant individuals. Subsequently, this may contribute to a limited lipid buffering capacity.

Adipose tissue inflammation

Adipose tissue contains, besides adipocytes, a wide variety of stromal vascular cells, including endothelial cells, fibroblasts, preadipocytes and immune cells. The latter have been increasingly implicated in the development of insulin resistance (90). Next to being a lipid storage organ, adipose tissue is also an secretory organ (40) that actively secretes adipokines, including cytokines, chemokines and hormones. These factors can act locally (autocrine and/or paracrine) or systemically as they can be secreted into the circulation (figure 1).

Obese adipose tissue is characterized by secretion of pro-inflammatory cytokines (40), but the trigger for adipose tissue low-grade inflammation is poorly understood. Low-grade inflammation could originate from a rapid expansion of adipose tissue. This could provide intrinsic signals to stimulate an inflammatory response, thereby accommodating tissue remodeling (91). These intrinsic signals may include adipocyte death, hypoxia and mechanotransduction stress arising from interactions between the cell and the extracellular matrix. Alternatively, gut-derived substances such as dietary components or metabolites might stimulate an inflammatory response in adipose tissue (92).

In rodents, an expanded adipose tissue mass is associated with a switch towards a more pro-inflammatory phenotype (93). This is accompanied by an increased production of pro-inflammatory cytokines such as tumor necrosis factor alpha (TNF- α), interleukin-6 (IL-6) and monocyte chemoattractant protein-1 (MCP-1) (39). For instance, mice studies show that increased MCP-1 expression attracts macrophages to the adipose tissue, which might polarize from anti-inflammatory M2 to pro-inflammatory cytokine producing M1 macrophages (94,95). This in turn might create an environment for attraction of other immune cells (96) and might accelerate local inflammation (93). However, the role of pro-inflammatory macrophages in human adipose tissue is still under intense debate (97).

Local adipose tissue inflammation interacts also with adipose tissue lipid metabolism. Through release of inflammatory factors, immune cells can affect adipose tissue lipid buffering capacity and lipolysis (98). For instance, *in vitro* studies show that IL-6 and TNF- α stimulate adipocyte lipolysis (99-101), and might thereby contribute to systemic fatty acid release. Moreover, these cytokines can inhibit adipocyte differentiation (102,103), further limiting

adipose tissue buffering capacity. This deteriorates adipose tissue function and accelerates local and whole-body insulin resistance.

In addition, adipose tissue inflammation might result in systemic low-grade inflammation and may exert detrimental effects on insulin signaling in peripheral tissues, including skeletal muscle and liver. For example, *in vitro* evidence shows that adipose tissue derived TNF- α impairs GLUT-4 translocation and glucose uptake in human myotubes, although findings are inconsistent (104,105). Moreover, it has been demonstrated that the adipokine adiponectin increases skeletal muscle and liver fat oxidation *in vitro* (106,107). Therefore, decreased adiponectin concentrations, as observed in obese conditions, may impact fat oxidation, and as such, affect lipid accumulation and insulin sensitivity in both skeletal muscle and liver.

Hepatic lipid metabolism

The liver plays an important role in whole-body lipid homeostasis and a pathophysiological link between hepatic lipid metabolism and insulin resistance has been known for decades (26). Hepatic insulin resistance is related to accumulation of lipid metabolites in the liver, which may be caused by multiple factors (108). This phenomenon can be explained by an increased NEFA supply from the expanded visceral adipose tissue (the ‘portal hypothesis’) or an increased intake of dietary fat. In addition, hepatic lipid accumulation may be explained by a reduction in postprandial lipid clearance, an increased uptake of lipoprotein remnants and an increased spillover of NEFA from subcutaneous adipose tissue in the circulation (109,110). Furthermore, hepatic mitochondrial dysfunction and an increased *de novo* lipogenesis are associated with elevated hepatic lipid accumulation as well (111,112). These processes are reviewed in detail elsewhere (24,26).

As mentioned before, insulin resistance is also linked with an increased secretion of hepatic VLDL-TAG. One role of VLDL is to transport excess energy out of the liver, predominantly in the form of TAG, which in turn elevates plasma TAG levels. VLDL assembly and secretion is a substrate-dependent process that is highly regulated by the availability of fatty acids (25). The fatty acids that are used for VLDL synthesis originate from several endogenous sources. They are mainly coming from the circulating NEFA pool, lipoprotein remnants that are cleared by the liver, intracellular lipids and *de novo* lipogenesis (113). The total flux of lipids to the liver is an important determinant of hepatic VLDL production and secretion, which can be suppressed by insulin (114,115). Hepatic insulin

resistance may increase circulating VLDL-TAG concentrations due to diminished insulin-mediated suppression of VLDL production and secretion (26). In addition, as VLDL- and chylomicron-TAG compete for the same clearance by LPL in peripheral tissues (116), increased VLDL-TAG in the fasting state may result in delayed clearance of TAG-rich lipoprotein particles in the postprandial state due to prolonged elevated VLDL-TAG concentrations.

Skeletal muscle function and dysfunction

As skeletal muscle is responsible for 70-80% of insulin-stimulated whole-body glucose disposal, it plays an important role in the pathogenesis of whole-body insulin resistance (27). Disturbances in skeletal muscle lipid metabolism have been suggested to contribute to the development of muscle insulin resistance and type 2 diabetes (30).

An increased lipid supply from adipose tissue and liver may result in an increased muscle uptake of systemic lipids, but this depends on the source and type of fatty acids. An increased lipid supply together with a reduced capacity to adapt fat oxidation accordingly might contribute to the accumulation of detrimental bioactive lipid intermediates, such as DAG and ceramides (29). This in turn may induce lipotoxicity. In recent years, it has become clear that there is a complex interplay between lipid supply, muscle lipid turnover and subcellular localization and composition of bioactive lipid metabolites. All these factors are involved in the development of a reduced insulin-mediated glucose uptake. They may interfere with the glucose transporter 4 (GLUT-4) translocation to the skeletal muscle membrane (117).

In the next paragraphs, skeletal muscle lipid metabolism will be described in more detail. Lipid uptake, muscle LPL activity, lipid synthesis, lipolysis, lipid oxidation and lipotoxicity will be reviewed. In addition, it will be discussed how a disturbed lipid metabolism may contribute to the development of insulin resistance.

Skeletal muscle lipid uptake

Under fasting conditions, skeletal muscle takes up fatty acids derived from the plasma NEFA pool as well as those liberated after LPL-mediated lipolysis of VLDL-TAG in the capillary bed of skeletal muscle. However, the quantitative contribution of the latter process compared to total NEFA uptake in fasting conditions is not clear. A slightly but significantly higher uptake of plasma-derived NEFA during fasting has been shown in skeletal muscle of obese versus lean (118) and insulin-resistant versus control individuals (63). This increased uptake

was observed despite comparable plasma NEFA concentrations. In contrast, two other human *in vivo* studies showed no difference in skeletal muscle fatty acid uptake (119,120). The first study compared an insulin resistant group with a control group, while the second compared impaired glucose tolerant individuals with normal glucose tolerant individuals. Despite the somewhat contradicting results, these data may point towards more pronounced disturbances in TAG metabolism rather than NEFA under obese insulin resistant conditions.

In the postprandial state, both VLDL-TAG and chylomicron-TAG are present in the circulation and these particles compete for LPL-mediated hydrolysis. It has been shown that chylomicron-TAG is the preferred substrate for LPL, but it competes with VLDL-TAG for extracellular lipolysis (116). VLDL-TAG makes a large contribution to circulating TAG, even in the postprandial phase (121). Compared to adipose tissue, it has been suggested that uptake of TAG-derived fatty acids after LPL hydrolysis is more efficient in skeletal muscle (122). Moreover, substantial fatty acid spillover also occurs in skeletal muscle, although it has been suggested that the extent of fatty acid spillover is less in skeletal muscle than in adipose tissue (123). These fatty acids merge with the plasma NEFA pool to compete for cellular uptake (124).

The contribution of TAG-derived fatty acids to skeletal muscle lipid uptake may be at least equally important as plasma NEFA (122,124). Elevated plasma TAG concentrations have been associated with whole-body insulin resistance. As discussed above, this might be due to increased liver VLDL production (26,66) and/or impaired clearance of circulating TAG by adipose tissue in the postprandial state (60-64). Previously, our group has demonstrated higher postprandial plasma TAG concentrations and an increased net TAG extraction across forearm muscle in individuals with IFG compared to IGT (125). Furthermore, despite a similar TAG supply, we showed higher postprandial VLDL-TAG extraction by skeletal muscle in insulin resistant men compared to BMI and age matched controls with the metabolic syndrome (126). However, another study including overweight men with insulin resistance could not confirm an increased postprandial muscle TAG extraction, despite elevated TAG concentrations (63). Recently, it was shown that muscle VLDL-TAG extraction was significantly greater in men with type 2 diabetes compared with nondiabetic men in the fasting state (127). Although the evidence is not conclusive, these studies indicate an impaired skeletal muscle TAG metabolism under insulin resistant conditions.

Skeletal muscle LPL activity

The expression and activation of muscle LPL plays a major role in skeletal muscle TAG extraction. Mice studies showed that skeletal muscle-specific deletion of LPL reduces lipid storage and increases insulin signaling in skeletal muscle (128). Additionally, muscle-specific LPL overexpression induced muscle insulin resistance, while skeletal muscle LPL knockdown showed the reverse effect (129). Although human data is limited, most studies indicate that fasting and exercise raises total LPL activity in human skeletal muscle (130,131), but results are not consistent (44). Moreover, insulin has been shown to stimulate LPL activity in adipose tissue, but does not appear to activate LPL in skeletal muscle (132). It has been hypothesized that these divergent tissue-specific LPL responses to insulin would serve to direct TAG-derived fatty acids away from muscle and towards the adipose tissue for storage (132).

In recent years, ANGPTL4 has emerged as an important inhibitor of skeletal muscle LPL activity at the post-translational level (46). In line with adipose tissue, mRNA expression of ANGPTL4 in human skeletal muscle is markedly increased during fasting (51). This is likely mediated via elevated plasma NEFA concentrations (50,133) and this may offset an increase in LPL mRNA/protein concentrations. This could lead to a decreased functional LPL activity (42). However, the role of skeletal muscle ANGPTL4 in muscle LPL activity and insulin resistance in humans remains to be elucidated.

The fatty acids that are liberated via LPL-mediated lipolysis as well as these from the plasma NEFA pool can be taken up into the skeletal muscle via passive diffusion and by protein-mediated transport, such as fatty acid translocase CD36, FABPm and FATP1-6 (68,134). Of these fatty acid transporters, CD36 is the best characterized. In humans, it was shown that CD36 protein expression may be acutely upregulated by insulin (135) and this upregulation may be more pronounced in insulin resistant conditions (136). Moreover, increased rates of fatty acid transport have been associated with increased localization of CD36 at the plasma membrane in individuals with type 2 diabetes, while total CD36 protein expression was not changed (137). This suggest that a permanent redistribution of CD36 to the plasma membrane occurred, which might play an essential role in the increased skeletal muscle uptake.

Skeletal muscle lipid synthesis

Once fatty acids have entered the myocyte, they bind to the cytoplasmic FABPc for transport through the cell (138). The enzyme long chain fatty acyl-CoA synthetase (LC-FACS) will

catalyze their binding to acetyl CoA. Subsequently, they can either be directed towards storage in lipid droplets or they can be directed towards the mitochondria to be used as energy source.

Intramyocellular storage of lipids is part of lipid turnover and is regulated by several processes. This includes enzymes catalyzing the esterification of fatty acyl-CoA into TAG and lipolytic enzymes. Initiation of TAG synthesis requires coupling of fatty acids to a glycerol backbone. The subsequent steps are dependent on the rate limiting enzymes glycerol-3-phosphatases (GPAT1-4) and diglyceride acyltransferase (DGAT1-2) (29). The latter enzyme has a dual role as it promotes TAG storage, but also decreases its precursor DAG which has been shown to have an inhibitory effect on insulin signaling in several studies (139). Although, there is limited evidence from human studies for a role of DGAT in insulin resistance (29), a short-term overexpression of DGAT1 in rat skeletal muscle showed beneficial effects on insulin sensitivity, despite increased TAG and DAG concentrations (140). Up to now, knowledge on muscle lipid turnover measured *in vivo* in humans in relation to insulin resistance is limited. In insulin resistant smokers compared with less insulin resistant non-smokers, muscle TAG concentrations and its fractional synthetic rate were not altered (141). Furthermore, a reduced fractional synthetic rate of muscle TAG was found in obese pre-diabetic individuals as compared with normal glucose tolerant individuals after exercise. This was found together with higher TAG concentrations, a reduced oxidative capacity and an impaired peripheral insulin action (142). Notably, these disturbances in muscle TAG metabolism were not found in women, indicating sex-related differences in muscle fatty acid handling (143). In addition, it was shown that IGT individuals (either isolated or in combination with IFG) had a reduced saturation and fractional synthesis of the DAG and TAG pool after a high saturated fatty acid mixed-meal (125). In combination with a reduced expression of oxidative genes, these data indicate that the insulin resistant muscle is characterized by disturbances in lipid turnover, particularly when exposed to a meal high in saturated fatty acids.

Lipid synthesis in skeletal muscle is also controlled by fatty acid desaturation which is tightly controlled by the enzyme $\Delta 9$ -desaturase or steroyl-CoA desaturase 1 (SCD1). It desaturates saturated fatty acids into mono unsaturated fatty acids, more specifically stearate (C18:0) and palmitate (C16:0) into the less toxic oleate (C18:1 n -9) and palmitoleate (C16:1 n -7) (144). It has been shown that unsaturated fatty acids are the preferred substrates for TAG synthesis (145). In rodent myotubes, overexpression of *SCD1* resulted in a significant increase in fatty acid esterification into TAG, with decreases in fatty acid oxidation and accumulation of lipid metabolites such as ceramides. This suggests that SCD1 protects

muscle cells from fatty acid induced insulin resistance by reducing ceramide and DAG accumulation, at least in rodents (146). However, an increased SCD1 mRNA expression has been observed in the skeletal muscle of extremely obese humans with severe muscle insulin resistance (147). Therefore, it remains an open question whether SCD1 plays a central role in the pathogenesis of insulin resistance.

Skeletal muscle lipolysis

Lipid turnover in skeletal muscle is not only determined by TAG synthesis but also by TAG lipolysis and subsequent fatty acid oxidation. As in adipose tissue, skeletal muscle lipolysis is mainly regulated by insulin and the consecutive action of ATGL and HSL (74,148). An *in vivo* microdialysis study with obese individuals has shown a blunted catecholamine-induced lipolysis in skeletal muscle (149). Moreover, obese insulin resistant men have lower HSL and higher ATGL protein content in skeletal muscle as well as a reduced HSL mRNA expression, phosphorylation and activity (150). This marked difference in muscle lipase content in obese insulin-resistant men was accompanied by a lower DAG hydrolase activity, resulting in a 60% lower ratio of DAG to TAG hydrolase activity (151). This suggests an incomplete muscle TAG hydrolysis. Of interest, this dysbalance in lipase activity was not accompanied by increased total DAG accumulation but rather an increase in saturated DAG lipid species in the muscle membrane and increased protein kinase C (PKC) activation in men with type 2 diabetes versus normal glucose tolerant men (151,152). These data together point towards a disturbed skeletal muscle lipolysis in obese insulin resistant conditions.

Fatty acid oxidation in skeletal muscle

In normal physiology, the aim of skeletal muscle lipid turnover is to provide intracellular fatty acids for oxidation when needed. If fatty acids are destined for oxidation, the fatty acyl-CoA is transported into mitochondria via the action of carnitine palmitoyl transferase I (CPTI) (153). This enzyme is expressed on the outer mitochondrial membrane. Next to CPTI, CD36 is expressed on mitochondrial membranes and it has been hypothesized to influence mitochondrial fatty acid transport and oxidation (154). Once inside mitochondria, fatty acyl-CoA enters β -oxidation, a process which generates acetyl-CoA for the tricarboxylic acid cycle. There, NADH and FADH₂ are formed and transferred to the electron transport chain, where ultimately the intracellular energy transfer molecule adenosine triphosphate (ATP) is produced. This process is called oxidative phosphorylation.

An impaired capacity to increase fat oxidation upon increased fatty acid availability and to switch between fat and glucose oxidation during insulin-mediated conditions has been referred to as metabolic inflexibility (28,155). Under fasting conditions, fatty acid oxidation measured across skeletal muscle has been shown to be reduced in obese individuals with type 2 diabetes (156,157) as well as the postprandial suppression of skeletal muscle fat oxidation (120). This apparent inflexibility also included a high reliance of obese individuals on glucose oxidation in the fasting state, but this could not be further increased during insulin infusion (120). As the absolute rate of lipid oxidation in the obese muscle was not able to change between fasting and postprandial conditions, this forms the basis for the 'metabolic inflexibility' model of skeletal muscle insulin resistance (24,158).

Mitochondrial dysfunction might be one of the key factors that is responsible for the intracellular accumulation of lipids and the inhibition of insulin signaling in skeletal muscle (28). This hypothesis is based on links between insulin resistance and decreased *ex vivo* mitochondrial respiration, enzyme activity, fatty acid oxidation (159) and mitochondrial size (160). Several studies found low skeletal muscle oxidative capacity in insulin resistant individuals (161,162), but results are not consistent (163). Currently, in the scientific community there is a large debate ongoing whether the association between mitochondrial dysfunction and skeletal muscle insulin resistance is a causal relationship or rather a consequence of lipotoxicity (164).

Lipotoxicity: lipid accumulation in skeletal muscle

Multiple studies have reported a negative association between intramyocellular lipid (IMCL) content and insulin sensitivity in sedentary lean, obese and type 2 diabetes individuals (165,166). However, this association is not present in endurance trained athletes who also have high levels of IMCL, but also are very insulin sensitive (167). This paradox lead to the hypothesis that not IMCL per se, but rather lipid intermediates, like long-chain fatty acyl-CoAs (LCFA-CoAs), DAG and ceramides, and their localization and fatty acid composition are related to insulin resistance. They may interfere in the insulin signaling pathway and negatively affect mitochondrial function (29).

LCFA-CoAs are the metabolically activated form of intracellular fatty acids and it commits the fatty acids to either lipid synthesis or fatty acid oxidation in the mitochondria. Several data support a link between LCFA-CoAs and insulin resistance. In rodents, studies utilizing lipid infusion (168) and high-fat diets (169) have demonstrated increased skeletal

muscle LCFA-CoAs in conjunction with insulin resistance. In humans, weight loss reduces skeletal muscle LCFA-CoA content in obesity, concomitant with improvements in insulin sensitivity (170). Although associations with insulin resistance have been described, so far no mechanism by which LCFA-CoA causes insulin resistance have been identified. It is possible that LCFA-CoA acts as a precursor for DAG or ceramide synthesis, resulting in insulin resistance (171).

In skeletal muscle, increased DAG concentrations are associated with insulin resistance (29). DAG is an intermediate in TAG synthesis and breakdown and it is an important intracellular second messenger. Induction of insulin resistance in healthy lean individuals by lipid infusion resulted in increased DAG levels in skeletal muscle (172), while weight loss or exercise interventions decreased intramuscular DAG concentrations and improved insulin sensitivity (173,174). Nevertheless, the role of DAG in this process is controversial (171). It has also been proposed that it is not total DAG content per se but rather specific DAG-subspecies (175,176) or DAG intracellular localization (152,177) that drives insulin resistance in humans.

Ceramides, which belong to the sphingolipid class, are frequently associated with insulin resistance as well. Accumulation of ceramides can impair insulin signaling resulting in an inhibition of GLUT-4 translocation (178). Indeed, elevated skeletal muscle ceramide levels have been observed in obese insulin-resistant individuals (179-182), but not all studies show this association (183,184). Like DAG, it has recently been postulated that specific ceramide-subspecies might play a more central role in driving insulin resistance rather than total ceramide content (185,186). Moreover, diet composition plays a role in skeletal muscle lipid composition as well. For instance, it has been shown that increased levels of the saturated fatty acid palmitate can drive ceramide synthesis (187). Nevertheless, it remains an open question whether ceramides play a central role in the pathogenesis of insulin resistance.

Outline of the thesis

Research in this thesis focused on multiple aspects of lipid metabolism in relation to whole-body and tissue-specific insulin resistance.

As indicated in this introduction, the obese insulin resistant state is characterized by lipid overflow. This is mainly driven by an impaired lipid buffering capacity, and an impaired capacity to increase muscle fat oxidation upon increased supply. However, little knowledge is

available on the contribution of dietary versus endogenous fatty acids to lipid overflow, their extraction and uptake by skeletal muscle as well fractional synthetic rate, content and composition of the skeletal muscle lipid pools in insulin resistance. In addition, previous studies have used a relatively small sample size due to methodological difficulties as well as the high costs associated with using stable isotope tracers. Therefore, in **chapter 2** we studied the contribution of endogenous and dietary fatty acid sources to skeletal muscle uptake and storage in a study of overweight or obese participants ($n = 74$) with a wide range of insulin resistance. We used a combination of differential stable isotope labeling of endogenously ($[^2\text{H}_2]$ -palmitate) and meal-derived fatty acids ($[\text{U-}^{13}\text{C}]$ -palmitate tracers) in combination with arterio-venous tracee and tracer concentration measurements across forearm muscle and biochemical analysis in muscle biopsies.

Chylomicron- and VLDL-TAG are hydrolysed in the process of intravascular lipolysis by LPL. Insulin is an important regulator of LPL activity, but a considerable part of the variation in LPL activity may also be explained by other factors. The LPL inhibitor ANGPTL4 is an interesting candidate and might be involved in the altered skeletal muscle TAG extraction previously observed in insulin resistant conditions. Hence, in **chapter 3**, the role of plasma ANGPTL4 in skeletal muscle lipid metabolism in overweight or obese insulin resistant individuals was studied. We investigated the relationship between plasma ANGPTL4 concentration and *in vivo* skeletal muscle LPL activity, but we also addressed the impact of dietary fat quality on plasma ANGPTL4 concentrations and examined whether human forearm muscle secretes ANGPTL4.

In addition to skeletal muscle, the role of plasma ANGPTL4 in relation to *in vivo* adipose tissue fatty acid metabolism in humans is currently unknown. Limited information is available on the role of ANGPTL4 in the impaired adipose tissue TAG extraction as observed in obese individuals in the postprandial state and whether weight loss affects *in vivo* adipose tissue LPL activity and ANGPTL4 in parallel. In **chapter 4**, the aim was to investigate fasting and postprandial plasma ANGPTL4 concentrations and their relationship with *in vivo* adipose tissue LPL activity and intracellular lipolysis in overweight and obese humans before and after diet-induced weight loss.

Although the development of type 2 diabetes and cardiometabolic risk is tightly coupled to insulin resistance, there are indications that insulin resistance may not develop simultaneously in different organs. For instance, in prediabetes, the IFG phenotype may be merely characterized by hepatic insulin resistance, whilst the IGT phenotype has more

pronounced peripheral (muscle) insulin resistance. To obtain more evidence for intervention strategies targeting different tissue-related or prediabetic phenotypes, more knowledge on the distinct phenotypes is required. In **chapter 5**, we studied cross-sectional associations of tissue-specific insulin resistance with plasma lipidome profiles. We identified 140 lipid species which were measured by liquid chromatography-mass spectrometry. Tissue-specific insulin resistance was estimated based on a 0-120 min oral glucose tolerance test with 5 time-points. This was studied in a relatively large group of overweight or obese individuals within the context of the European multicenter DiOGenes dietary intervention study. Furthermore, in **chapter 6**, in the same cohort, we investigated abdominal subcutaneous adipose tissue transcriptome by means of RNA sequencing in relation to tissue-specific insulin resistance.

Finally, in **chapter 7** the main findings from the studies described in this thesis are integrated and discussed in a broader perspective and implications for future research are provided.

References

1. Ng M, Fleming T, Robinson M, Thomson B, Graetz N, Margono C, et al. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980–2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet*. 2014;384:766–81.
2. World Health Organization. Fact sheet 311: obesity and overweight. 2015. <http://www.who.int/mediacentre/factsheets/fs311/en/>; 2016.
3. GGD'en G. CBS en RIVM. Gezondheidsmonitor GGD'en; 2016.
4. Lim SS, Vos T, Flaxman AD, Danaei G, Shibuya K, Adair-Rohani H, et al. A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet*. 2012;380:2224–60.
5. Kahn SE, Hull RL, Utzschneider KM. Mechanisms linking obesity to insulin resistance and type 2 diabetes. *Nature*. 2006;444:840–6.
6. Emerging Risk Factors Collaboration, Wormser D, Kaptoge S, Di Angelantonio E, Wood AM, Pennells L, et al. Separate and combined associations of body-mass index and abdominal adiposity with cardiovascular disease: collaborative analysis of 58 prospective studies. *Lancet*. 2011;377:1085–95.
7. Preiss K, Brennan L, Clarke D. A systematic review of variables associated with the relationship between obesity and depression. *Obes Rev*. 2013;14:906–18.
8. Renehan AG, Zwahlen M, Egger M. Adiposity and cancer risk: new mechanistic insights from epidemiology. *Nat Rev Cancer*. 2015;15:484–98.
9. Goossens GH. The role of adipose tissue dysfunction in the pathogenesis of obesity-related insulin resistance. *Physiol Behav*. 2008;94:206–18.
10. Apovian CM. Obesity: definition, comorbidities, causes, and burden. *Am J Manag Care*. 2016;22:s176–85.
11. Samuel VT, Petersen KF, Shulman GI. Lipid-induced insulin resistance: unravelling the mechanism. *Lancet*. 2010;375:2267–77.
12. World Health Organization. Global report on diabetes. 2016.
13. World Health Organization. Factsheet nr 317: Cardiovascular diseases (CVDs). 2017. <http://www.who.int/mediacentre/factsheets/fs317/en/>.
14. DeFronzo RA. Insulin resistance, lipotoxicity, type 2 diabetes and atherosclerosis: the missing links. The Claude Bernard Lecture 2009. *Diabetologia*. 2010;53:1270–87.
15. Bornfeldt KE, Tabas I. Insulin Resistance, Hyperglycemia, and Atherosclerosis. *Cell Metab*. 2011;14:575–85.
16. Stefan N, Fritsche A, Schick F, Häring H-U. Phenotypes of prediabetes and stratification of cardiometabolic risk. *Lancet Diabetes Endocrinol*. 2016;4:789–98.
17. Faerch K, Hulmán A, Solomon TPJ. Heterogeneity of Pre-diabetes and Type 2 Diabetes: Implications for Prediction, Prevention and Treatment Responsiveness. *Curr Diabetes Rev*. 2016;12:30–41.
18. Bird SR, Hawley JA. Update on the effects of physical activity on insulin sensitivity in humans. *BMJ Open Sport Exerc Med*. 2016;2:e000143.

19. Zheng J, Woo S-L, Hu X, Botchlett R, Chen L, Huo Y, et al. Metformin and metabolic diseases: a focus on hepatic aspects. *Front Med.* 2015;9:173–86.
20. Blanco-Rojo R, Alcalá-Díaz JF, Wopereis S, Pérez-Martínez P, Quintana-Navarro GM, Marin C, et al. The insulin resistance phenotype (muscle or liver) interacts with the type of diet to determine changes in disposition index after 2 years of intervention: the CORDIOPREV-DIAB randomised clinical trial. *Diabetologia.* 2015.
21. Rask-Madsen C, Kahn CR. Tissue-specific insulin signaling, metabolic syndrome, and cardiovascular disease. *Arterioscler Thromb Vasc Biol.* 2012;32:2052–9.
22. Brüning JC, Michael MD, Winnay JN, Hayashi T, Hörsch D, Accili D, et al. A muscle-specific insulin receptor knockout exhibits features of the metabolic syndrome of NIDDM without altering glucose tolerance. *Mol Cell.* 1998;2:559–69.
23. Biddinger SB, Hernandez-Ono A, Rask-Madsen C, Haas JT, Alemán JO, Suzuki R, et al. Hepatic insulin resistance is sufficient to produce dyslipidemia and susceptibility to atherosclerosis. *Cell Metab.* 2008;7:125–34.
24. Stinkens R, Goossens GH, Jocken JWE, Blaak EE. Targeting fatty acid metabolism to improve glucose metabolism. *Obes Rev.* 2015;16:715–57.
25. Nielsen S, Karpe F. Determinants of VLDL-triglycerides production. *Curr Opin Lipidol.* 2012;23:321–6.
26. Choi SH, Ginsberg HN. Increased very low density lipoprotein (VLDL) secretion, hepatic steatosis, and insulin resistance. *Trends Endocrinol Metab.* 2011;22:353–63.
27. DeFronzo RA, Jacot E, Jequier E, Maeder E, Wahren J, Felber JP. The effect of insulin on the disposal of intravenous glucose. Results from indirect calorimetry and hepatic and femoral venous catheterization. *Diabetes.* 1981;30:1000–7.
28. Affourtit C. Mitochondrial involvement in skeletal muscle insulin resistance: A case of imbalanced bioenergetics. *Biochim Biophys Acta.* 2016;1857:1678–93.
29. Bosma M, Kersten S, Hesselink MKC, Schrauwen P. Re-evaluating lipotoxic triggers in skeletal muscle: relating intramyocellular lipid metabolism to insulin sensitivity. *Prog Lipid Res.* 2012;51:36–49.
30. Shulman GI. Ectopic Fat in Insulin Resistance, Dyslipidemia, and Cardiometabolic Disease. *N Engl J Med.* 2014;371:1131–41.
31. Mishra AK, Dubey V, Ghosh AR. Obesity: An overview of possible role(s) of gut hormones, lipid sensing and gut microbiota. *Metab Clin Exp.* 2016;65:48–65.
32. Canfora EE, Jocken JW, Blaak EE. Short-chain fatty acids in control of body weight and insulin sensitivity. *Nat Rev Endocrinol.* 2015;11:577–91.
33. Reijnders D, Goossens GH, Hermes GDA, Neis EPJG, van der Beek CM, Most J, et al. Effects of Gut Microbiota Manipulation by Antibiotics on Host Metabolism in Obese Humans: A Randomized Double-Blind Placebo-Controlled Trial. *Cell Metab.* 2016;24:63–74.
34. Goossens GH. The Metabolic Phenotype in Obesity: Fat Mass, Body Fat Distribution, and Adipose Tissue Function. *Obes Facts.* 2017;10:207–15.
35. Karpe F, Pinnick KE. Biology of upper-body and lower-body adipose tissue--link to whole-body phenotypes. *Nat Rev Endocrinol.* 2015;11:90–100.
36. Tchkonja T, Thomou T, Zhu Y, Karagiannides I, Pothoulakis C, Jensen MD, et al. Mechanisms and Metabolic Implications of Regional Differences among Fat Depots. *Cell Metab.* 2013;17:644–56.

37. Ibrahim MM. Subcutaneous and visceral adipose tissue: structural and functional differences. *Obes Rev.* 2010;11:11–8.
38. Klöting N, Blüher M. Adipocyte dysfunction, inflammation and metabolic syndrome. *Rev Endocr Metab Disord.* 2014;15:277–87.
39. Ouchi N, Parker JL, Lugus JJ, Walsh K. Adipokines in inflammation and metabolic disease. *Nat Rev Immunol.* 2011;11:85–97.
40. Leal V de O, Mafra D. Adipokines in obesity. *Clin Chim Acta.* 2013;419:87–94.
41. Frayn K. Adipose tissue as a buffer for daily lipid flux. *Diabetologia.* 2002;45:1201–10.
42. Kersten S. Physiological regulation of lipoprotein lipase. *Biochim Biophys Acta.* 2014;1841:919–33.
43. Goldberg IJ, Eckel RH, Abumrad NA. Regulation of fatty acid uptake into tissues: lipoprotein lipase- and CD36-mediated pathways. *J Lipid Res.* 2009;50 Suppl:S86–90.
44. Yost TJ, Jensen DR, Haugen BR, Eckel RH. Effect of dietary macronutrient composition on tissue-specific lipoprotein lipase activity and insulin action in normal-weight subjects. *Am J Clin Nutr.* 1998;68:296–302.
45. Brunzell JD, Schwartz RS, Eckel RH, Goldberg AP. Insulin and adipose tissue lipoprotein lipase activity in humans. *Int J Obes.* 1981;5:685–94.
46. Dijk W, Kersten S. Regulation of lipoprotein lipase by Angptl4. *Trends Endocrinol Metab.* 2014;25:146–55.
47. Sukonina V, Lookene A, Olivecrona T, Olivecrona G. Angiopoietin-like protein 4 converts lipoprotein lipase to inactive monomers and modulates lipase activity in adipose tissue. *Proc Natl Acad Sci USA.* 2006;103:17450–5.
48. Dijk W, Beigneux AP, Larsson M, Bensadoun A, Young SG, Kersten S. Angiopoietin-like 4 promotes intracellular degradation of lipoprotein lipase in adipocytes. *J Lipid Res.* 2016;57:1670–83.
49. Kroupa O, Vorrjs E, Stienstra R, Mattijssen F, Nilsson SK, Sukonina V, et al. Linking nutritional regulation of Angptl4, Gpihbp1, and Lmf1 to lipoprotein lipase activity in rodent adipose tissue. *BMC Physiol.* 2012;12:1–1.
50. Kersten S, Lichtenstein L, Steenbergen E, Mudde K, Hendriks HFJ, Hesselink MK, et al. Caloric restriction and exercise increase plasma ANGPTL4 levels in humans via elevated free fatty acids. *Arterioscler Thromb Vasc Biol.* 2009;29:969–74.
51. Catoire M, Alex S, Paraskevopoulos N, Mattijssen F, Evers-van Gogh I, Schaart G, et al. Fatty acid-inducible ANGPTL4 governs lipid metabolic response to exercise. *Proc Natl Acad Sci USA.* 2014;111:E1043–52.
52. Norheim F, Hjorth M, Langleite TM, Lee S, Holen T, Bindesbøll C, et al. Regulation of angiopoietin-like protein 4 production during and after exercise. *Physiol Rep.* 2014;2.
53. Brands M, Sauerwein HP, Ackermans MT, Kersten S, Serlie MJ. Omega-3 long-chain fatty acids strongly induce angiopoietin-like 4 in humans. *J Lipid Res.* 2013;54:615–21.
54. Ruge T, Sukonina V, Kroupa O, Makoveichuk E, Lundgren M, Svensson MK, et al. Effects of hyperinsulinemia on lipoprotein lipase, angiopoietin-like protein 4, and glycosylphosphatidylinositol-anchored high-density lipoprotein binding protein 1 in subjects with and without type 2 diabetes mellitus. *Metab Clin Exp.* 2012;61:652–60.
55. van Raalte DH, Brands M, Serlie MJ, Mudde K, Stienstra R, Sauerwein HP, et al. Angiopoietin-like

- protein 4 is differentially regulated by glucocorticoids and insulin in vitro and in vivo in healthy humans. *Exp Clin Endocrinol Diabetes*. 2012;120:598–603.
56. Dijk W, Kersten S. Regulation of lipid metabolism by angiopoietin-like proteins. *Curr Opin Lipidol*. 2016;27:249–56.
 57. Sadur CN, Yost TJ, Eckel RH. Insulin responsiveness of adipose tissue lipoprotein lipase is delayed but preserved in obesity. *J Clin Endocrinol Metab*. 1984;59:1176–82.
 58. Riemens SC, Sluiter WJ, Dullaart RP. Enhanced escape of non-esterified fatty acids from tissue uptake: its role in impaired insulin-induced lowering of total rate of appearance in obesity and Type II diabetes mellitus. *Diabetologia*. 2000;43:416–26.
 59. Søndergaard E, Johansen RF, Jensen MD, Nielsen S. Postprandial VLDL-TG metabolism in type 2 diabetes. *Metab Clin Exp*. 2017;75:25–35.
 60. Potts JL, Coppack SW, Fisher RM, Humphreys SM, Gibbons GF, Frayn KN. Impaired postprandial clearance of triacylglycerol-rich lipoproteins in adipose tissue in obese subjects. *Am J Physiol*. 1995;268:E588–94.
 61. Panarotto D, Rémillard P, Bouffard L, Maheux P. Insulin resistance affects the regulation of lipoprotein lipase in the postprandial period and in an adipose tissue-specific manner. *Eur J Clin Invest*. 2002;32:84–92.
 62. Coppack SW, Evans RD, Fisher RM, Frayn KN, Gibbons GF, Humphreys SM, et al. Adipose tissue metabolism in obesity: lipase action in vivo before and after a mixed meal. *Metab Clin Exp*. 1992;41:264–72.
 63. Bickerton AST, Roberts R, Fielding BA, Tornqvist H, Blaak EE, Wagenmakers AJM, et al. Adipose tissue fatty acid metabolism in insulin-resistant men. *Diabetologia*. 2008;51:1466–74.
 64. Annuzzi G, Giacco R, Patti L, Di Marino L, De Natale C, Costabile G, et al. Postprandial chylomicrons and adipose tissue lipoprotein lipase are altered in type 2 diabetes independently of obesity and whole-body insulin resistance. *Nutr Metab Cardiovasc Dis*. 2008;18:531–8.
 65. McQuaid SE, Hodson L, Neville MJ, Dennis AL, Cheeseman J, Humphreys SM, et al. Downregulation of Adipose Tissue Fatty Acid Trafficking in Obesity: A Driver for Ectopic Fat Deposition? *Diabetes*. 2010;60:47–55.
 66. Sparks JD, Sparks CE, Adeli K. Selective hepatic insulin resistance, VLDL overproduction, and hypertriglyceridemia. *Arterioscler Thromb Vasc Biol*. 2012;32:2104–12.
 67. Frayn KN, Coppack SW, Fielding BA, Humphreys SM. Coordinated Regulation of Hormone-Sensitive Lipase and Lipoprotein-Lipase in Human Adipose-Tissue in-Vivo - Implications for the Control of Fat Storage and Fat Mobilization. *Adv Enzyme Regul*. 1995;35:163–78.
 68. Glatz JFC, Luiken JJFP, Bonen A. Membrane fatty acid transporters as regulators of lipid metabolism: implications for metabolic disease. *Physiol Rev*. 2010;90:367–417.
 69. Frayn KN, Shadid S, Hamrani R, Humphreys SM, Clark ML, Fielding BA, et al. Regulation of fatty acid movement in human adipose tissue in the postabsorptive-to-postprandial transition. *Am J Physiol*. 1994;266:E308–17.
 70. Morigny P, Houssier M, Mouisel E, Langin D. Adipocyte lipolysis and insulin resistance. *Biochimie*. 2016;125:259–66.
 71. Zimmermann R, Strauss JG, Haemmerle G, Schoiswohl G, Birner-Gruenberger R, Riederer M, et al. Fat mobilization in adipose tissue is promoted by adipose triglyceride lipase. *Science*. 2004;306:1383–6.

72. Fredrikson G, Tornqvist H, Belfrage P. Hormone-sensitive lipase and monoacylglycerol lipase are both required for complete degradation of adipocyte triacylglycerol. *Biochim Biophys Acta*. 1986;876:288–93.
73. Mittendorfer B, Magkos F, Fabbrini E, Mohammed BS, Klein S. Relationship between body fat mass and free fatty acid kinetics in men and women. *Obesity (Silver Spring)*. 2009;17:1872–7.
74. Jocken JWE, Langin D, Smit E, Saris WHM, Valle C, Hul GB, et al. Adipose triglyceride lipase and hormone-sensitive lipase protein expression is decreased in the obese insulin-resistant state. *J Clin Endocrinol Metab*. 2007;92:2292–9.
75. Langin D, Dicker A, Tavernier G, Hoffstedt J, Mairal A, Rydén M, et al. Adipocyte lipases and defect of lipolysis in human obesity. *Diabetes*. 2005;54:3190–7.
76. Laws A, Hoen HM, Selby JV, Saad MF, Haffner SM, Howard BV. Differences in insulin suppression of free fatty acid levels by gender and glucose tolerance status. Relation to plasma triglyceride and apolipoprotein B concentrations. *Insulin Resistance Atherosclerosis Study (IRAS) Investigators. Arterioscler Thromb Vasc Biol*. 1997;17:64–71.
77. Karpe F, Dickmann JR, Frayn KN. Fatty acids, obesity, and insulin resistance: time for a reevaluation. *Diabetes*. 2011.
78. Arner P, Spalding KL. Fat cell turnover in humans. *Biochim Biophys Acta*. 2010;396:101–4.
79. Spalding KL, Arner E, Westermark PO, Bernard S, Buchholz BA, Bergmann O, et al. Dynamics of fat cell turnover in humans. *Nature*. 2008;453:783–7.
80. Arner P, Bernard S, Salehpour M, Possnert G, Liebl J, Steier P, et al. Dynamics of human adipose lipid turnover in health and metabolic disease. *Nature*. 2011;478:110–3.
81. Weyer C, Foley JE, Bogardus C, Tataranni PA, Pratley RE. Enlarged subcutaneous abdominal adipocyte size, but not obesity itself, predicts type II diabetes independent of insulin resistance. *Diabetologia*. 2000;43:1498–506.
82. Eriksson JW, Smith U, Waagstein F, Wysocki M, Jansson PA. Glucose turnover and adipose tissue lipolysis are insulin-resistant in healthy relatives of type 2 diabetes patients: is cellular insulin resistance a secondary phenomenon? *Diabetes*. 1999;48:1572–8.
83. Moreno-Indias I, Tinahones FJ. Impaired adipose tissue expandability and lipogenic capacities as ones of the main causes of metabolic disorders. *J Diabetes Res*. 2015;2015:970375–12.
84. Acosta JR, Douagi I, Andersson DP, Bäckdahl J, Rydén M, Arner P, et al. Increased fat cell size: a major phenotype of subcutaneous white adipose tissue in non-obese individuals with type 2 diabetes. *Diabetologia*. 2016;59:560–70.
85. Pasarica M, Xie H, Hymel D, Bray G, Greenway F, Ravussin E, et al. Lower total adipocyte number but no evidence for small adipocyte depletion in patients with type 2 diabetes. *Diabetes Care*. 2009;32:900–2.
86. Rydén M, Andersson DP, Bergström IB, Arner P. Adipose tissue and metabolic alterations: regional differences in fat cell size and number matter, but differently: a cross-sectional study. *J Clin Endocrinol Metab*. 2014;99:E1870–6.
87. van Tienen FHJ, van der Kallen CJH, Lindsey PJ, Wanders RJ, Van Greevenbroek MM, Smeets HJM. Preadipocytes of type 2 diabetes subjects display an intrinsic gene expression profile of decreased differentiation capacity. *Int J Obes (Lond)*. 2011;35:1154–64.
88. Rydén M, Andersson DP, Bernard S, Spalding K, Arner P. Adipocyte triglyceride turnover and lipolysis in lean and overweight subjects. *J Lipid Res*. 2013;54:2909–13.

89. Arner E, Westermark PO, Spalding KL, Britton T, Rydén M, Frisén J, et al. Adipocyte turnover: relevance to human adipose tissue morphology. *Diabetes*. 2010;59:105–9.
90. Rosen ED, Spiegelman BM. What We Talk About When We Talk About Fat. *Cell*. 2014;156:20–44.
91. Reilly SM, Saltiel AR. Adapting to obesity with adipose tissue inflammation. *Nat Rev Endocrinol*. 2017;13:633–43.
92. Blüher M. Adipose tissue inflammation: a cause or consequence of obesity-related insulin resistance? *Clin Sci*. 2016;130:1603–14.
93. Asghar A, Sheikh N. Role of immune cells in obesity induced low grade inflammation and insulin resistance. *Cell Immunol*. 2017;315:18–26.
94. Thomas D, Apovian C. Macrophage functions in lean and obese adipose tissue. *Metab Clin Exp*. 2017;72:120–43.
95. Castoldi A, Naffah de Souza C, Câmara NOS, Moraes-Vieira PM. The Macrophage Switch in Obesity Development. *Front Immunol*. 2015;6:637.
96. Haase J, Weyer U, Immig K, Klötting N, Blüher M, Eilers J, et al. Local proliferation of macrophages in adipose tissue during obesity-induced inflammation. *Diabetologia*. 2014;57:562–71.
97. Schipper HS, Prakken B, Kalkhoven E, Boes M. Adipose tissue-resident immune cells: key players in immunometabolism. *Trends Endocrinol Metab*. 2012;23:407–15.
98. Mathis D. Immunological goings-on in visceral adipose tissue. *Cell Metab*. 2013;17:851–9.
99. Souza SC, Palmer HJ, Kang YH, Yamamoto MT, Muliro KV, Eric Paulson K, et al. TNF- α induction of lipolysis is mediated through activation of the extracellular signal related kinase pathway in 3T3-L1 adipocytes. *J Cell Biochem*. 2003;89:1077–86.
100. Zhang HH, Halbleib M, Ahmad F, Manganiello VC, Greenberg AS. Tumor necrosis factor- α stimulates lipolysis in differentiated human adipocytes through activation of extracellular signal-related kinase and elevation of intracellular cAMP. *Diabetes*. 2002;51:2929–35.
101. van Hall G, Steensberg A, Sacchetti M, Fischer C, Keller C, Schjerling P, et al. Interleukin-6 stimulates lipolysis and fat oxidation in humans. *J Clin Endocrinol Metab*. 2003;88:3005–10.
102. Sopasakis VR, Sandqvist M, Gustafson B, Hammarstedt A, Schmelz M, Yang X, et al. High local concentrations and effects on differentiation implicate interleukin-6 as a paracrine regulator. *Obes Res*. 2004;12:454–60.
103. Petruschke T, Hauner H. Tumor necrosis factor- α prevents the differentiation of human adipocyte precursor cells and causes delipidation of newly developed fat cells. *J Clin Endocrinol Metab*. 1993;76:742–7.
104. Dyck DJ, Heigenhauer GJF, Bruce CR. The role of adipokines as regulators of skeletal muscle fatty acid metabolism and insulin sensitivity. *Acta Physiol*. 2005;1–12.
105. Sell H, Eckel J, Dietze-Schroeder D. Pathways leading to muscle insulin resistance--the muscle--fat connection. *Arch Physiol Biochem*. 2006;112:105–13.
106. Lumeng CN, Saltiel AR. Inflammatory links between obesity and metabolic disease. *J Clin Invest*. 2011;121:2111–7.
107. Kwon H, Pessin JE. Adipokines Mediate Inflammation and Insulin Resistance. *Front Endocrinol (Lausanne)*. 2013;4:1–13.

108. Meex RCR, Watt MJ. Hepatokines: linking nonalcoholic fatty liver disease and insulin resistance. *Nat Rev Endocrinol.* 2017;:1–12.
109. Kabir M, Catalano KJ, Ananthnarayan S, Kim SP, Van Citters GW, Dea MK, et al. Molecular evidence supporting the portal theory: a causative link between visceral adiposity and hepatic insulin resistance. *Am J Physiol Endocrinol Metab.* 2005;288:E454–61.
110. Jensen MD. Role of body fat distribution and the metabolic complications of obesity. *J Clin Endocrinol Metab.* 2008;93:S57–63.
111. Vial G, Dubouchaud H, Leverve XM. Liver mitochondria and insulin resistance. *Acta Biochim Pol.* 2010;57:389–92.
112. Donnelly KL, Smith CI, Schwarzenberg SJ, Jessurun J, Boldt MD, Parks EJ. Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease. *J Clin Invest.* 2005;115:1343–51.
113. Barrows BR, Parks EJ. Contributions of different fatty acid sources to very low-density lipoprotein-triacylglycerol in the fasted and fed states. *J Clin Endocrinol Metab.* 2006;91:1446–52.
114. Sparks JD, Sparks CE, Adeli K. Selective hepatic insulin resistance, VLDL overproduction, and hypertriglyceridemia. *Arterioscler Thromb Vasc Biol.* 2012;32:2104–12.
115. Titchenell PM, Lazar MA, Birnbaum MJ. Unraveling the Regulation of Hepatic Metabolism by Insulin. *Trends Endocrinol Metab.* 2017;28:497–505.
116. Bickerton AST, Roberts R, Fielding BA, Hodson L, Blaak EE, Wagenmakers AJM, et al. Preferential uptake of dietary Fatty acids in adipose tissue and muscle in the postprandial period. *Diabetes.* 2007;56:168–76.
117. Jaldin-Fincati JR, Pavarotti M, Frendo-Cumbo S, Bilan PJ, Klip A. Update on GLUT4 Vesicle Traffic: A Cornerstone of Insulin Action. *Trends Endocrinol Metab.* 2017;28:597–611.
118. Jocken JWE, Goossens GH, van Hees AMJ, Frayn KN, van Baak M, Stegen J, et al. Effect of beta-adrenergic stimulation on whole-body and abdominal subcutaneous adipose tissue lipolysis in lean and obese men. *Diabetologia.* 2008;51:320–7.
119. Moors CCM, van der Zijl NJ, Diamant M, Blaak EE, Goossens GH. Impaired insulin sensitivity is accompanied by disturbances in skeletal muscle fatty acid handling in subjects with impaired glucose metabolism. *Int J Obes (Lond).* 2012;36:709–17.
120. Corpeleijn E, Mensink M, Kooi ME, Roekaerts PMHJ, Saris WHM, Blaak EE. Impaired skeletal muscle substrate oxidation in glucose-intolerant men improves after weight loss. *Obesity (Silver Spring).* 2008;16:1025–32.
121. Cohn JS, Johnson EJ, Millar JS, Cohn SD, Milne RW, Marcel YL, et al. Contribution of apoB-48 and apoB-100 triglyceride-rich lipoproteins (TRL) to postprandial increases in the plasma concentration of TRL triglycerides and retinyl esters. *J Lipid Res.* 1993;34:2033–40.
122. Evans K, Burdge GC, Wootton SA, Clark ML, Frayn KN. Regulation of dietary fatty acid entrapment in subcutaneous adipose tissue and skeletal muscle. *Diabetes.* 2002;51:2684–90.
123. Miles JM, Park YS, Walewicz D, Russell-Lopez C, Windsor S, Isley WL, et al. Systemic and forearm triglyceride metabolism: fate of lipoprotein lipase-generated glycerol and free fatty acids. *Diabetes.* 2004;53:521–7.
124. Teusink B, Voshol PJ, Dahlmans VEH, Rensen PCN, Pijl H, Romijn JA, et al. Contribution of Fatty Acids Released From Lipolysis of Plasma Triglycerides to Total Plasma Fatty Acid Flux and Tissue-Specific Fatty Acid Uptake. *Diabetes.* 2003;52:614–20.

125. Goossens G, Moors C, Jocken J, van der Zijl N, Jans A, Konings E, et al. Altered Skeletal Muscle Fatty Acid Handling in Subjects with Impaired Glucose Tolerance as Compared to Impaired Fasting Glucose. *Nutrients*. 2016;8:164–15.
126. van Hees AMJ, Jans A, Hul GB, Roche HM, Saris WHM, Blaak EE. Skeletal muscle fatty acid handling in insulin resistant men. *Obesity*. 2011;19:1350–9.
127. Andersen IR, Søndergaard E, Sørensen LP, Nellemann B, Gormsen LC, Jensen MD, et al. Increased VLDL-TG fatty acid storage in skeletal muscle in men with type 2 diabetes. *J Clin Endocrinol Metab*. 2016;:jc20162979.
128. Wang H, Knaub LA, Jensen DR, Young Jung D, Hong E-G, Ko H-J, et al. Skeletal muscle-specific deletion of lipoprotein lipase enhances insulin signaling in skeletal muscle but causes insulin resistance in liver and other tissues. *Diabetes*. 2009;58:116–24.
129. Kim JK, Fillmore JJ, Chen Y, Yu C, Moore IK, Pypaert M, et al. Tissue-specific overexpression of lipoprotein lipase causes tissue-specific insulin resistance. *Proc Natl Acad Sci USA*. 2001;98:7522–7.
130. Sugden MC, Holness MJ, Howard RM. Changes in lipoprotein lipase activities in adipose tissue, heart and skeletal muscle during continuous or interrupted feeding. *Biochem J*. 1993;292:113–9.
131. Greiwe JS, Holloszy JO, Semenkovich CF. Exercise induces lipoprotein lipase and GLUT-4 protein in muscle independent of adrenergic-receptor signaling. *J Appl Physiol*. 2000;89:176–81.
132. Farese RV, Yost TJ, Eckel RH. Tissue-Specific Regulation of Lipoprotein-Lipase Activity by Insulin Glucose in Normal-Weight Humans. *Metab Clin Exp*. 1991;40:214–6.
133. Staiger H, Haas C, Machann J, Werner R, Weisser M, Schick F, et al. Muscle-derived angiopoietin-like protein 4 is induced by fatty acids via peroxisome proliferator-activated receptor (PPAR)-delta and is of metabolic relevance in humans. *Diabetes*. 2009;58:579–89.
134. Bonen A, Chabowski A, Luiken JJFP, Glatz JFC. Is membrane transport of FFA mediated by lipid, protein, or both? Mechanisms and regulation of protein-mediated cellular fatty acid uptake: molecular, biochemical, and physiological evidence. *Physiol*. 2007;22:15–29.
135. Bonen A, Benton CR, Campbell SE, Chabowski A, Clarke DC, Han X-X, et al. Plasmalemmal fatty acid transport is regulated in heart and skeletal muscle by contraction, insulin and leptin, and in obesity and diabetes. *Acta Physiol Scand*. 2003;178:347–56.
136. Corpeleijn E, Pelsers MMAL, Soenen S, Mensink M, Bouwman FG, Kooi ME, et al. Insulin acutely upregulates protein expression of the fatty acid transporter CD36 in human skeletal muscle in vivo. *J Physiol Pharmacol*. 2008;59:77–83.
137. Bonen A, Parolin ML, Steinberg GR, Calles-Escandon J, Tandon NN, Glatz JFC, et al. Triacylglycerol accumulation in human obesity and type 2 diabetes is associated with increased rates of skeletal muscle fatty acid transport and increased sarcolemmal FAT/CD36. *FASEB J*. 2004;18:1144–6.
138. Glatz JFC, Luiken JJFP, van Bilsen M, van der Vusse GJ. Cellular lipid binding proteins as facilitators and regulators of lipid metabolism. *Mol Cell Biochem*. 2002;239:3–7.
139. Timmers S, Schrauwen P, de Vogel J. Muscular diacylglycerol metabolism and insulin resistance. *Physiol Behav*. 2008;94:242–51.
140. Timmers S, de Vogel-van den Bosch J, Hesslink MKC, van Beurden D, Schaart G, Ferraz MJ, et al. Paradoxical increase in TAG and DAG content parallel the insulin sensitizing effect of unilateral DGAT1 overexpression in rat skeletal muscle. *PLoS ONE*. 2011;6:e14503.
141. Bergman BC, Perreault L, Hunerdosse DM, Koehler MC, Samek AM, Eckel RH. Intramuscular lipid metabolism in the insulin resistance of smoking. *Diabetes*. 2009;58:2220–7.

142. Perreault L, Bergman BC, Hunerdosse DM, Playdon MC, Eckel RH. Inflexibility in Intramuscular Triglyceride Fractional Synthesis Distinguishes Prediabetes From Obesity in Humans. *Obesity*. 2009;18:1524–31.
143. Perreault L, Bergman BC, Hunerdosse DM, Eckel RH. Altered Intramuscular Lipid Metabolism Relates to Diminished Insulin Action in Men, but Not Women, in Progression to Diabetes. *Obesity*. 2009;18:2093–100.
144. Hodson L, Fielding BA. Stearoyl-CoA desaturase: rogue or innocent bystander? *Prog Lipid Res*. 2013;52:15–42.
145. Coleman R. Enzymes of triacylglycerol synthesis and their regulation. *Prog Lipid Res*. 2004;43:134–76.
146. Pinnamaneni SK, Southgate RJ, Febbraio MA, Watt MJ. Stearoyl CoA desaturase 1 is elevated in obesity but protects against fatty acid-induced skeletal muscle insulin resistance in vitro. *Diabetologia*. 2006;49:3027–37.
147. Hulver MW, Berggren JR, Carper MJ, Miyazaki M, Ntambi JM, Hoffman EP, et al. Elevated stearoyl-CoA desaturase-1 expression in skeletal muscle contributes to abnormal fatty acid partitioning in obese humans. *Cell Metab*. 2005;2:251–61.
148. Jacob S, Hauer B, Becker R, Artzner S, Grauer P, Loblein K, et al. Lipolysis in skeletal muscle is rapidly regulated by low physiological doses of insulin. *Diabetologia*. 1999;42:1171–4.
149. Blaak EE, Schiffrers SL, Saris WH, Mensink M, Kooi ME. Impaired beta-adrenergically mediated lipolysis in skeletal muscle of obese subjects. *Diabetologia*. 2004;47:1462–8.
150. Jocken JWE, Moro C, Goossens GH, Hansen D, Mairal A, Hesselink MKC, et al. Skeletal Muscle Lipase Content and Activity in Obesity and Type 2 Diabetes. *J Clin Endocrinol Metab*. 2010;95:5449–53.
151. Jocken JWE, Roepstorff C, Goossens GH, van der Baan P, van Baak M, Saris WHM, et al. Hormone-sensitive lipase serine phosphorylation and glycerol exchange across skeletal muscle in lean and obese subjects: effect of beta-adrenergic stimulation. *Diabetes*. 2008;57:1834–41.
152. Jocken JWE, Goossens GH, Boon H, Mason RR, Essers Y, Havekes B, et al. Insulin-mediated suppression of lipolysis in adipose tissue and skeletal muscle of obese type 2 diabetic men and men with normal glucose tolerance. *Diabetologia*. 2013;56:2255–65.
153. Rasmussen BB, Holmbäck UC, Volpi E, Morio-Liondore B, Paddon-Jones D, Wolfe RR. Malonyl coenzyme A and the regulation of functional carnitine palmitoyltransferase-1 activity and fat oxidation in human skeletal muscle. *J Clin Invest*. 2002;110:1687–93.
154. Holloway GP, Bezaire V, Heigenhauser GJF, Tandon NN, Glatz JFC, Luiken JJFP, et al. Mitochondrial long chain fatty acid oxidation, fatty acid translocase/CD36 content and carnitine palmitoyltransferase I activity in human skeletal muscle during aerobic exercise. *J Physiol*. 2006;571:201–10.
155. Corpeleijn E, Saris WHM, Blaak EE. Metabolic flexibility in the development of insulin resistance and type 2 diabetes: effects of lifestyle. *Obes Rev*. 2009;10:178–93.
156. Blaak EE, Wagenmakers AJ, Glatz JF, Wolffenbuttel BH, Kemerink GJ, Langenberg CJ, et al. Plasma FFA utilization and fatty acid-binding protein content are diminished in type 2 diabetic muscle. *Am J Physiol Endocrinol Metab*. 2000;279:E146–54.
157. Kelley DE. Skeletal muscle fat oxidation: timing and flexibility are everything. *J Clin Invest*. 2005;115:1699–702.

158. Goodpaster BH, Sparks LM. Metabolic Flexibility in Health and Disease. *Cell Metab.* 2017;25:1027–36.
159. Kelley DE, Goodpaster B, Wing RR, Simoneau JA. Skeletal muscle fatty acid metabolism in association with insulin resistance, obesity, and weight loss. *Am J Physiol.* 1999;277:E1130–41.
160. Kelley DE, He J, Menshikova EV, Ritov VB. Dysfunction of Mitochondria in Human Skeletal Muscle in Type 2 Diabetes. *Diabetes.* 2002;51:2944–50.
161. Schrauwen-Hinderling VB, Kooi ME, Hesselink MKC, Jeneson JAL, Backes WH, van Echteld CJA, et al. Impaired in vivo mitochondrial function but similar intramyocellular lipid content in patients with type 2 diabetes mellitus and BMI-matched control subjects. *Diabetologia.* 2006;50:113–20.
162. Phielix E, Schrauwen-Hinderling VB, Mensink M, Lenaers E, Meex R, Hoeks J, et al. Lower intrinsic ADP-stimulated mitochondrial respiration underlies in vivo mitochondrial dysfunction in muscle of male type 2 diabetic patients. *Diabetes.* 2008;57:2943–9.
163. Boushel R, Gnaiger E, Schjerling P, Skovbro M, Kraunsøe R, Dela F. Patients with type 2 diabetes have normal mitochondrial function in skeletal muscle. *Diabetologia.* 2007;50:790–6.
164. Schrauwen P, Schrauwen-Hinderling V, Hoeks J, Hesselink MKC. Mitochondrial dysfunction and lipotoxicity. *Biochim Biophys Acta.* 2010;1801:266–71.
165. Krssak M, Falk Petersen K, Dresner A, DiPietro L, Vogel SM, Rothman DL, et al. Intramyocellular lipid concentrations are correlated with insulin sensitivity in humans: a 1H NMR spectroscopy study. *Diabetologia.* 1999;42:113–6.
166. Pan DA, Lillioja S, Kriketos AD, Milner MR, Baur LA, Bogardus C, et al. Skeletal muscle triglyceride levels are inversely related to insulin action. *Diabetes.* 1997;46:983–8.
167. Goodpaster BH, He J, Watkins S, Kelley DE. Skeletal muscle lipid content and insulin resistance: evidence for a paradox in endurance-trained athletes. *J Clin Endocrinol Metab.* 2001;86:5755–61.
168. Chalkley SM, Hettiarachchi M, Chisholm DJ, Kraegen EW. Five-hour fatty acid elevation increases muscle lipids and impairs glycogen synthesis in the rat. *Metab Clin Exp.* 1998;47:1121–6.
169. Ellis BA, Poynten A, Lowy AJ, Furler SM, Chisholm DJ, Kraegen EW, et al. Long-chain acyl-CoA esters as indicators of lipid metabolism and insulin sensitivity in rat and human muscle. *Am J Physiol Endocrinol Metab.* 2000;279:E554–60.
170. Houmard JA, Tanner CJ, Yu C, Cunningham PG, Pories WJ, MacDonald KG, et al. Effect of weight loss on insulin sensitivity and intramuscular long-chain fatty acyl-CoAs in morbidly obese subjects. *Diabetes.* 2002;51:2959–63.
171. Coen PM, Goodpaster BH. Role of intramyocellular lipids in human health. *Trends Endocrinol Metab.* 2012;23:391–8.
172. Itani SI, Ruderman NB, Schmieder F, Boden G. Lipid-induced insulin resistance in human muscle is associated with changes in diacylglycerol, protein kinase C, and IkappaB-alpha. *Diabetes.* 2002;51:2005–11.
173. Dube JJ, Amati F, Toledo FGS, Stefanovic-Racic M, Rossi A, Coen P, et al. Effects of weight loss and exercise on insulin resistance, and intramyocellular triacylglycerol, diacylglycerol and ceramide. *Diabetologia.* 2011;54:1147–56.
174. Dubé JJ, Amati F, Stefanovic-Racic M, Toledo FGS, Sauers SE, Goodpaster BH. Exercise-induced alterations in intramyocellular lipids and insulin resistance: the athlete's paradox revisited. *Am J Physiol Endocrinol Metab.* 2008;294:E882–8.

175. Amati F, Dubé JJ, Alvarez-Carnero E, Edreira MM, Chomentowski P, Coen PM, et al. Skeletal muscle triglycerides, diacylglycerols, and ceramides in insulin resistance: another paradox in endurance-trained athletes? *Diabetes*. 2011;60:2588–97.
176. Bergman BC, Perreault L, Hunerdosse DM, Koehler MC, Samek AM, Eckel RH. Increased intramuscular lipid synthesis and low saturation relate to insulin sensitivity in endurance-trained athletes. *J Appl Physiol*. 2010;108:1134–41.
177. Bergman BC, Hunerdosse DM, Kerege A, Playdon MC, Perreault L. Localisation and composition of skeletal muscle diacylglycerol predicts insulin resistance in humans. *Diabetologia*. 2012;55:1140–50.
178. Hassan RH. Defect of insulin signal in peripheral tissues: Important role of ceramide. *WJD*. 2014;5:244–15.
179. Moro C, Galgani JE, Luu L, Pasarica M, Mairal A, Bajpeyi S, et al. Influence of gender, obesity, and muscle lipase activity on intramyocellular lipids in sedentary individuals. *J Clin Endocrinol Metab*. 2009;94:3440–7.
180. Strackowski M, Kowalska I, Baranowski M, Nikolajuk A, Oziomek E, Zabielski P, et al. Increased skeletal muscle ceramide level in men at risk of developing type 2 diabetes. *Diabetologia*. 2007;50:2366–73.
181. Thrush AB, Brindley DN, Chabowski A, Heigenhauser GJ, Dyck DJ. Skeletal muscle lipogenic protein expression is not different between lean and obese individuals: a potential factor in ceramide accumulation. *J Clin Endocrinol Metab*. 2009;94:5053–61.
182. Adams JM, Pratipanawatr T, Berria R, Wang E, DeFronzo RA, Sullards MC, et al. Ceramide Content Is Increased in Skeletal Muscle From Obese Insulin-Resistant Humans. *Diabetes*. 2004;53:25–31.
183. Serlie MJ, Meijer AJ, Groener JE, Duran M, Endert E, Fliers E, et al. Short-term manipulation of plasma free fatty acids does not change skeletal muscle concentrations of ceramide and glucosylceramide in lean and overweight subjects. *J Clin Endocrinol Metab*. 2007;92:1524–9.
184. Skovbro M, Baranowski M, Skov-Jensen C, Flint A, Dela F, Gorski J, et al. Human skeletal muscle ceramide content is not a major factor in muscle insulin sensitivity. *Diabetologia*. 2008;51:1253–60.
185. Ritter O, Jelenik T, Roden M. Lipid-mediated muscle insulin resistance: different fat, different pathways? *J Mol Med*. 2015;93:831–43.
186. Kitessa S, Abeywardena M. Lipid-Induced Insulin Resistance in Skeletal Muscle: The Chase for the Culprit Goes from Total Intramuscular Fat to Lipid Intermediates, and Finally to Species of Lipid Intermediates. *Nutrients*. 2016;8:466–14.
187. Chaurasia B, Summers SA. Ceramides - Lipotoxic Inducers of Metabolic Disorders. *Trends Endocrinol Metab*. 2015;26:538–50.



Chapter 2

Altered skeletal muscle fatty acid handling is associated with the degree of insulin resistance in overweight and obese humans

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Abstract

Introduction/ hypothesis Disturbances in skeletal muscle fatty acid (FA) handling may contribute to the development and progression of whole-body insulin resistance (IR). In this study, we compared fasting and postprandial skeletal muscle FA handling in individuals with varying degrees of IR.

Methods Seventy-four overweight/obese participants (62 men) were divided into two groups based on the HOMA-IR median (3.35). Fasting and postprandial skeletal muscle FA handling were determined by combining the forearm muscle balance technique with stable isotopes. [$^2\text{H}_2$]-palmitate was infused intravenously to label VLDL-triacylglycerol (VLDL-TAG) and NEFA in the circulation, whereas [$\text{U-}^{13}\text{C}$]-palmitate was incorporated in a high-saturated FA mixed-meal labelling chylomicron-TAG. Skeletal muscle biopsies were taken to assess intramuscular lipid content, fractional synthetic rate (FSR) and the transcriptional regulation of FA metabolism.

Results Postprandial forearm muscle VLDL-TAG extraction was elevated in the high-IR *vs* the mild-IR group ($\text{AUC}_{0-4\text{h}}$: 0.57 ± 0.32 *vs* -0.43 ± 0.38 $\text{nmol [100 mL tissue]}^{-1} \text{ min}^{-1}$, respectively, $p = 0.045$). Although no differences in skeletal muscle TAG, diacylglycerol, NEFA content and FSR were present between groups, the high-IR group showed increased saturation of the intramuscular NEFA pool ($p = 0.039$). This was accompanied by lower muscle *GPAT1* (also known as *GPAM*) expression ($p = 0.050$).

Conclusions/interpretation Participants with high-IR demonstrated increased postprandial skeletal muscle VLDL-TAG extraction and higher saturation of the intramuscular NEFA pool *vs* individuals with mild-IR. These data support the involvement of disturbances in skeletal muscle FA handling in the progression of whole-body IR.

Introduction

Systemic lipid overflow, which is driven by adipose tissue dysfunction and impaired skeletal muscle lipid handling, is associated with insulin resistance (IR) (1). Increased circulating triacylglycerol (TAG) and NEFA concentrations are often found in IR because of impaired adipose tissue lipid handling (2-4). This results in an increased lipid supply to other non-adipose tissues, such as liver and skeletal muscle. Due to an impaired capacity to oxidise fatty acids (FAs) (5,6), these lipids may accumulate and interfere with insulin signalling in the liver and skeletal muscle (7,8). Over the last decade, it has become clear that the amount of lipids per se does not determine IR. Rather, a complex interplay between FA supply, FA type, muscle lipid turnover, subcellular localisation and composition of specific bioactive lipid metabolites seems to determine skeletal muscle IR (1,8,9).

The contribution of dietary fat (chylomicron-TAG) and endogenous fat (NEFA and VLDL-TAG) to skeletal muscle FA handling is not well understood. Elevated plasma NEFA concentrations may result from both expanded fat mass (2) and reduced peripheral clearance (5,10). Despite a reduced lipolysis per unit fat mass because of hyperinsulinaemia (11,12), the total amount of NEFA released from adipose tissue in the postprandial state seems to be elevated in the obese insulin resistant state (13). Furthermore, in situations with plasma insulin concentrations comparable to insulin levels achieved during a hyperinsulinaemic-euglycaemic clamp, the spillover from FAs derived from lipoprotein lipase (LPL)-mediated TAG hydrolysis in adipose tissue has been shown to be less suppressed in obese patients with type 2 diabetes than in non-obese healthy controls (14). This increase in adipose tissue NEFA output might lead to increased hepatic VLDL-TAG production and elevated plasma TAG concentrations (15,16). Bickerton *et al.* (17) demonstrated that dietary FAs were preferentially taken up in adipose tissue and skeletal muscle in the postprandial state in healthy lean humans, even though VLDL particles were abundantly present after the meal (11).

Up to now, most studies investigating combined VLDL- and chylomicron-TAG metabolism have been performed in healthy, lean humans (17,18). Bickerton *et al.* (11) have shown elevated postprandial plasma VLDL- and chylomicron-TAG concentrations in overweight men with IR (11). Moreover, we have recently demonstrated that an increased postprandial VLDL-TAG extraction was associated with IR in men with the metabolic syndrome (19). However, studies involving the combined assessment of human skeletal muscle VLDL- and chylomicron-TAG metabolism, and intramuscular lipid species are limited. In addition, previous studies have used a relatively small sample size due to

methodological difficulties as well as the high costs associated with these measurements. Therefore, extensive human *in vivo* data on skeletal muscle FA handling in IR is currently lacking. The aim of this study was to investigate fasting and postprandial skeletal muscle FA handling in a large study cohort of overweight or obese participants with a wide range of IR. A dual stable isotope tracer technique using labelled palmitate in combination with measurements of differences in arteriovenous concentrations across forearm muscle and forearm blood flow was used in this study, as previously validated (17). This enabled us to differentiate between the metabolic fate of dietary and endogenous FA. In addition, skeletal muscle biopsies were taken to investigate skeletal muscle lipid metabolites, their fractional synthetic rates (FSRs) and the transcriptional regulation of FA metabolism.

Methods

Study participants

Seventy-four participants (62 men and 12 women) with the metabolic syndrome or impaired glucose metabolism were obtained from the Maastricht biobank. These participants (described elsewhere in more detail (19–21)) underwent a high-saturated FA (SFA) mixed-meal test. Participants were divided into two groups based on the median of HOMA-IR (3.35); participants below the median of HOMA-IR formed the ‘mild-IR’ group ($n = 37$) and participants above the median formed the ‘high-IR’ group ($n = 37$). The local Medical Ethical Committee of Maastricht University Medical Center* approved the study protocols. All participants gave their written informed consent before participation.

High-fat mixed-meal test

Participants were studied after an overnight fast and were asked to refrain from strenuous exercise and drinking alcohol for 24 h before the study day. In addition, they were asked to avoid food products naturally enriched with ^{13}C for 7 days before the study day. Forearm muscle metabolism was studied using arteriovenous concentration differences combined with measurements of forearm blood flow. Three catheters were inserted before the start of the experiment. One catheter was placed retrogradely into a superficial dorsal vein of a hand heated in a hot-box (60°C) to obtain an arterialised blood sample. In the same arm another catheter was placed in an antecubital vein for the infusion of the $[\text{}^2\text{H}_2]$ -palmitate tracer. A third catheter was placed retrogradely in a deep antecubital vein of the contralateral forearm to sample venous blood draining the forearm muscle. After taking an arterialised and

deep-venous background sample at 90 min before meal ingestion, a continuous intravenously infusion of the stable isotope tracer, [$^2\text{H}_2$]-palmitate (97% enrichment; Cambridge Isotope Laboratories, Andover, MA, USA) complexed to albumin was started ($0.035 \mu\text{mol} [\text{kg body weight}]^{-1} \text{min}^{-1}$). Baseline blood sampling was started after 1 h of tracer infusion to allow for isotopic equilibration to occur. Blood samples were taken simultaneously from the dorsal hand vein and the deep muscle vein at three time points during fasting. Samples were also taken at six time points postprandially after consumption of a high-SFA mixed-meal (at '0 min') containing 200 mg [$\text{U-}^{13}\text{C}$]-palmitate (98% enrichment; Cambridge Isotope Laboratories). The liquid meal provided 2.6 MJ energy, consisting of 61 energy % (E%) fat (35.5 E%, SFA; 18.8 E%, monounsaturated FA [MUFA]; 1.7 E% polyunsaturated FA [PUFA]), 33 E% carbohydrates and 6.3 E% protein. Analysis of forearm blood flow before blood sampling and details of other biochemical analyses have been described previously (19).

Skeletal muscle biopsies

Skeletal muscle biopsies were obtained from the vastus lateralis muscle after local anaesthesia of the skin and fascia using the Bergström method with suction (22). Muscle biopsies were taken during fasting and at the end of the postprandial period (240 min). Muscle biopsies were lyophilised and dissected free of extramyocellular lipid, blood and connective tissue under a microscope. Details of lipid extraction and quantification have been described previously (19). Skeletal muscle expression of genes related to transcription factors, oxidative metabolism, lipid synthesis and lipolysis were analysed. Gene expression was normalised relative to the geometric mean of the internal reference genes (β -actin, β -2-microglobulin and/or ribosomal protein L13a). Details of accession numbers, RNA primer sequences and RT-PCR analysis of these genes have been described previously (19,20). Since limited muscle biopsy samples were available, not all genes have been measured in all individuals.

Calculations

Net fluxes of metabolites (labelled and unlabelled) across the forearm were calculated by multiplying the arteriovenous concentration difference by forearm plasma flow. Plasma flow was measured using plethysmography and calculated by multiplying forearm blood flow with $[(1 - \text{haematocrit (\%vol.)}) / 100]$. A positive flux indicates net uptake across forearm muscle, whereas a negative flux indicates net release. Labelled NEFA and TAG concentrations were calculated as the product of tracer:tracee ratio (TTR) of [$^2\text{H}_2$]-palmitate and [$\text{U-}^{13}\text{C}$]-palmitate and the concentration of palmitate in NEFA and TAG, as reported previously (19).

The degree of saturation of skeletal muscle TAG, diacylglycerol (DAG), phospholipid (PL) and NEFA (%) was calculated by dividing the sum of unsaturated FAs by the total amount of FAs in a fraction multiplied by 100. The FSR of skeletal muscle NEFA, TAG, DAG and PL was calculated using skeletal muscle NEFA as the precursor pool for lipid synthesis. The increase in TTR of [U- ^{13}C] from fasting to 4 h postprandial measures was divided by the enrichment of skeletal muscle NEFA and expressed as per cent per hour (%/h). Postprandial areas under the curve ($\text{AUC}_{0-4\text{h}}$) of metabolites were calculated using the trapezium rule and in this study data are presented as $\text{AUC}_{0-4\text{h}} / \text{min}$.

Statistics

In this study, all data are expressed as means \pm SEM. Participant characteristics and differences in skeletal muscle lipid handling during fasting and postprandial conditions of the mild-IR and high-IR group were compared using independent samples t tests. A linear regression was performed with HOMA-IR and BMI and sex as co-variates. Variables were \log_e -transformed if the assumption of normality was not met. The data were analysed using SPSS for Mac version 22.0 (SPSS, Chicago, IL, USA) and statistical significance was set at $p < 0.05$.

Results

Study population

Participant characteristics are summarised in Table 1. Age, waist:hip ratio and blood pressure were comparable between groups, while BMI was significantly higher in the high-IR group ($p < 0.002$). By design, mean HOMA-IR was different between the two groups (mild-IR *vs* high-IR: 2.5 ± 0.1 *vs* 4.7 ± 0.3 , respectively, $p < 0.001$).

Table 1. Characteristics of participants

Characteristic	Mild-IR (n = 37)	High-IR (n = 37)	Total group (n = 74)	Range (n = 74)	<i>p</i> value ^a
Male (n)/Female (n)	33 / 4	29 / 8	62 / 12		
Age (y)	58.0 ± 1.4	59.0 ± 1.1	58.5 ± 0.9	36 – 70	0.582
Body weight (kg)	89.8 ± 1.9	95.1 ± 2.1	92.5 ± 1.4	64.0 – 115.0	0.064
BMI (kg/m ²)	29.2 ± 0.5	31.7 ± 0.6	30.5 ± 0.4	22.7 – 39.5	0.002
Waist:hip ratio	1.02 ± 0.01	1.03 ± 0.01	1.02 ± 0.01	0.89 – 1.17	0.494
Systolic blood pressure (mmHg)	135 ± 2	136 ± 2	135 ± 2	105 – 174	0.636
Diastolic blood pressure (mmHg)	84 ± 2	85 ± 1	85 ± 1	68 – 110	0.944
Fasting plasma glucose (mmol/L)	5.4 ± 0.1	5.7 ± 0.1	5.5 ± 0.1	4.6 – 6.7	0.002
Fasting plasma insulin (pmol/L)	72.3 ± 2.4	130.2 ± 6.6	101.2 ± 4.9	50 – 263.3	< 0.001
Fasting plasma NEFA (μmol/L)	561 ± 22	598 ± 26	580 ± 17	296 – 1002	0.287
Fasting plasma TAG (μmol/L)	1320 ± 103	1246 ± 85	1283 ± 66	415 – 3232	0.582
HOMA-IR	2.5 ± 0.1	4.7 ± 0.3	3.6 ± 0.2	1.68 – 11.3	< 0.001

Values are means ± SEM

^a*p* value for difference between mild-IR and high-IR group, Student's *t* test for unpaired samples

Arterialised metabolites, forearm muscle metabolism and forearm blood flow

Fasting arterialised plasma glucose (Figure 1A) and insulin (Figure 1B) concentrations were significantly higher in the high-IR group than in the mild-IR group ($p = 0.002$ and $p < 0.001$, respectively) and remained higher throughout the postprandial period ($p = 0.035$ and $p < 0.001$, respectively). Net glucose uptake across forearm muscle was similar in the high-IR and mild-IR groups under fasting conditions, but was significantly lower in the high-IR group than in the mild-IR group after meal ingestion ($\text{AUC}_{0-4\text{h}} 0.59 \pm 0.04$ vs $0.76 \pm 0.07 \mu\text{mol [100 ml tissue]}^{-1} \text{ min}^{-1}$, respectively, $p = 0.034$), indicating a lower postprandial insulin sensitivity in the high-IR group (see Table 2 and Figure 1C). Arterialised fasting plasma glycerol concentrations ($p = 0.006$) and lactate concentrations were significantly higher ($p = 0.006$) in the high-IR group than in the mild-IR group (Table 2). Forearm blood flow and glycerol release were similar between the two IR groups, both during fasting and postprandial conditions. Moreover, fasting ($p = 0.002$) and postprandial ($p = 0.048$) lactate release was positively associated with IR (Table 2).

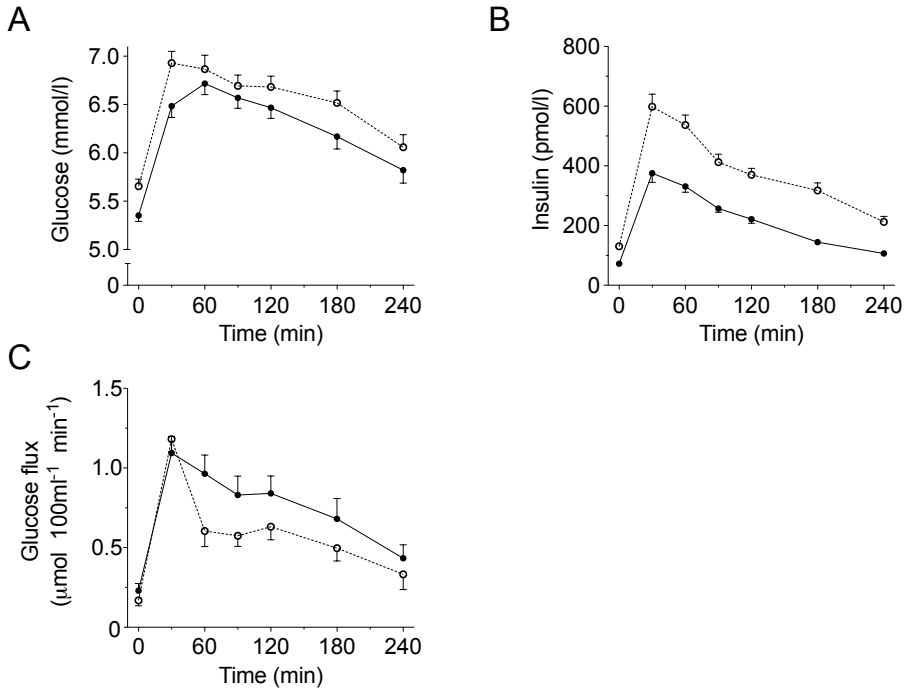


Figure 1. Arterialised plasma glucose (A) and insulin (B) concentrations and glucose flux (C) during fasting (0 min) and after consumption of a high-SFA meal. Black circles, mild-IR group; white circles, high-IR group. Student's *t* test for unpaired samples showed significant effect of group on fasting and postprandial plasma glucose ($p = 0.002$ and AUC_{0-4h} : $p = 0.035$, respectively), fasting and postprandial plasma insulin ($p < 0.001$ and AUC_{0-4h} : $p < 0.001$, respectively) and postprandial glucose flux (AUC_{0-4h} : $p = 0.034$). Values are presented as mean \pm SEM

Whole-body and forearm muscle NEFA metabolism

Fasting arterialised NEFA concentrations were similar in the high-IR and mild-IR groups (Figure 2A). After the high-SFA meal ingestion, arterialised NEFA concentrations decreased to the same extent in both groups and returned to near-baseline values at the end of the postprandial period, with no significant differences observed between the groups. [2H_2]-palmitate was infused intravenously and was mixed with the plasma NEFA pool. The TTR reached steady state during fasting measurements (Figure 2C,D). Consistent with these findings, the rate of appearance of NEFA (R_{NEFA}) decreased after the meal (Figure 2B), which is an indication of suppression of whole-body lipolysis. A reduction in postprandial

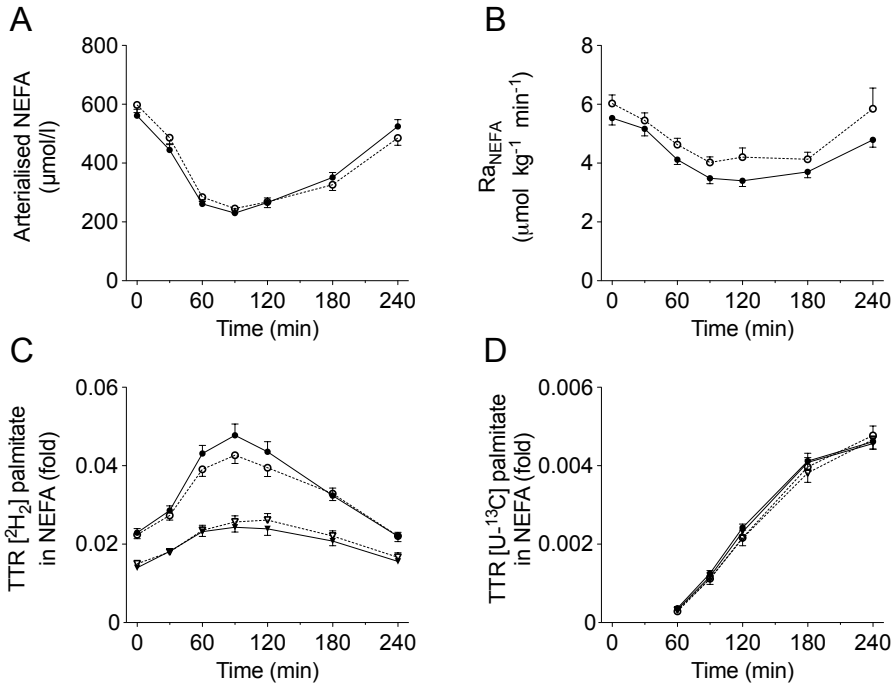


Figure 2. Postprandial whole-body NEFA metabolism. Arterialised plasma NEFA concentrations (A), R_{NEFA} (B), and the TTR of [$^2\text{H}_2$]-palmitate (C) and [$\text{U-}^{13}\text{C}$]-palmitate (D) in the plasma NEFA fraction during fasting (0 min) and after consumption of a high-SFA meal. Black symbols, mild-IR group; white symbols, high-IR group; in (C) and (D) circles, arterialised plasma concentrations and triangles, forearm venous plasma concentrations. Values are presented as mean \pm SEM

suppression of the R_{NEFA} was observed in the high-IR group compared with the mild-IR group, although the difference did not reach statistical significance ($p = 0.079$, Figure 2B). There were no differences in arterialised concentrations of [$^2\text{H}_2$]-palmitate and [$\text{U-}^{13}\text{C}$]-palmitate in NEFA between groups (data not shown).

The TTR of [$^2\text{H}_2$]-palmitate in NEFA was higher in arterialised *vs* deep-venous plasma at all time points in both groups. This reflects dilution of the $^2\text{H}_2$ tracer in the plasma NEFA pool across forearm muscle. The TTR of [$\text{U-}^{13}\text{C}$]-palmitate in NEFA (resulting from spillover of FA derived from chylomicron-TAG hydrolysis) was not different in arterialised *vs* deep-venous plasma at all time points in both groups (Figure 2C,D).

Table 2. Fasting and postprandial lipid metabolism

Variable	Mild-IR	High-IR	<i>p</i> value ^a	SE β	<i>p</i> value ^b
Forearm blood flow (mL [100 mL tissue] ⁻¹ min ⁻¹)					
Fasting	2.5 \pm 0.2	2.5 \pm 0.2	0.896	0.054	0.682
Postprandial	2.6 \pm 0.2	2.8 \pm 0.2	0.476	0.118	0.369
Glycerol (μ mol/L)					
Fasting	84.6 \pm 3.9	118.5 \pm 11.1	0.006	0.200	0.090
Postprandial	64.9 \pm 3.6	92.9 \pm 10.6	0.016	0.278	0.025
Lactate (μ mol/L)					
Fasting	0.62 \pm 0.04	0.79 \pm 0.05	0.006	0.438	< 0.001
Postprandial	0.98 \pm 0.05	1.10 \pm 0.05	0.081	0.224	0.087
Net flux across forearm muscle					
Glucose (μ mol [100 mL tissue] ⁻¹ min ⁻¹)					
Fasting	0.23 \pm 0.05	0.17 \pm 0.03	0.274	-0.083	0.524
Postprandial	0.76 \pm 0.07	0.59 \pm 0.04	0.034	-0.147	0.226
Glycerol (nmol [100 mL tissue] ⁻¹ min ⁻¹)					
Fasting	-28.3 \pm 5.5	-27.0 \pm 7.7	0.883	0.224	0.092
Postprandial	-20.4 \pm 4.5	-25.2 \pm 6.5	0.541	0.081	0.543
Lactate (nmol [100 mL tissue] ⁻¹ min ⁻¹)					
Fasting	-0.18 \pm 0.02	-0.12 \pm 0.03	0.123	0.384	0.002
Postprandial	0.01 \pm 0.02	0.04 \pm 0.03	0.472	0.249	0.048
NEFA (nmol [100 mL tissue] ⁻¹ min ⁻¹)					
Fasting	-6.9 \pm 20.4	26.6 \pm 24.6	0.300	0.165	0.210
Postprandial	-0.8 \pm 9.5	8.7 \pm 13.4	0.565	0.112	0.405
[² H ₂]-palmitate NEFA (nmol [100 mL tissue] ⁻¹ min ⁻¹)					
Fasting	1.69 \pm 0.12	1.58 \pm 0.14	0.539	0.017	0.902
Postprandial	1.50 \pm 0.09	1.53 \pm 0.14	0.846	0.171	0.208
TAG (nmol [100 mL tissue] ⁻¹ min ⁻¹)					
Fasting	10.2 \pm 13.2	45.5 \pm 12.1	0.052	0.094	0.466
Postprandial	53.9 \pm 17.2	51.9 \pm 28.4	0.954	-0.105	0.422

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Table 2. Fasting and postprandial lipid metabolism (*continued*)

Variable	Mild-IR	High-IR	<i>p</i> value ^a	SE β	<i>p</i> value ^b
<i>(Net flux across forearm muscle)</i>					
[² H ₂]-palmitate TAG (nmol [100 mL tissue] ⁻¹ min ⁻¹)					
Fasting	-0.24 ± 0.16	0.56 ± 0.41	0.069	0.241	0.076
Postprandial	-0.43 ± 0.38	0.57 ± 0.32	0.045	0.294	0.037
[U- ¹³ C]-palmitate TAG (nmol [100 mL tissue] ⁻¹ min ⁻¹)					
Fasting ^c					
Postprandial	0.86 ± 0.16	0.66 ± 0.16	0.353	-0.046	0.736

Values are means ± SEM. Postprandial values are calculated from AUC_{0-4h}. A positive flux indicates net uptake across forearm muscle, whereas a negative flux indicates net release. [²H₂]-palmitate NEFA fasting and ^a*p* value for difference between mild-IR and high-IR group, Student's *t* test for unpaired samples. ^b*p* value for multiple linear regression. ^c[U-¹³C]-palmitate was given with a meal, hence fasting data is not available. [²H₂]-palmitate TAG fasting, *n* = 34; [²H₂]-palmitate NEFA postprandial and [U-¹³C]-palmitate TAG postprandial, *n* = 33; [²H₂]-palmitate TAG postprandial (mild-IR), *n* = 29; [²H₂]-palmitate TAG postprandial (high-IR), *n* = 32. SE β , standardised β coefficient for total group for HOMA-IR, adjusted for sex and BMI

Fasting and postprandial net extraction of plasma NEFA across forearm muscle did not differ between groups. Furthermore, there was consistent uptake of [²H₂]-palmitate across forearm muscle during the study period and this was similar in both groups (Table 2).

Whole-body and forearm muscle TAG metabolism

Comparable arterialed TAG concentrations between the high-IR and mild-IR groups were observed during fasting and postprandial conditions (Figure 3A). The [²H₂]-palmitate tracer was measurable in plasma TAG from the first baseline sample onwards, reflecting incorporation of the intravenously infused tracer into VLDL-TAG (Figure 3B). The [U-¹³C]-palmitate tracer, which was given with the meal, appeared in plasma TAG from 60 min after meal ingestion, representing chylomicron-TAG in the circulation (Figure 3B). During the postprandial period both labelled TAG fractions increased without significant differences between groups.

Fasting net extraction of [²H₂]-palmitate TAG across forearm muscle was higher in the high-IR group than in the mild-IR group, although the difference did not reach statistical significance (*p* = 0.069). Postprandial net extraction of [²H₂]-palmitate TAG was significantly elevated in the high-IR group *vs* the mild-IR group (*p* = 0.045) (Figure 3C). In line with this,

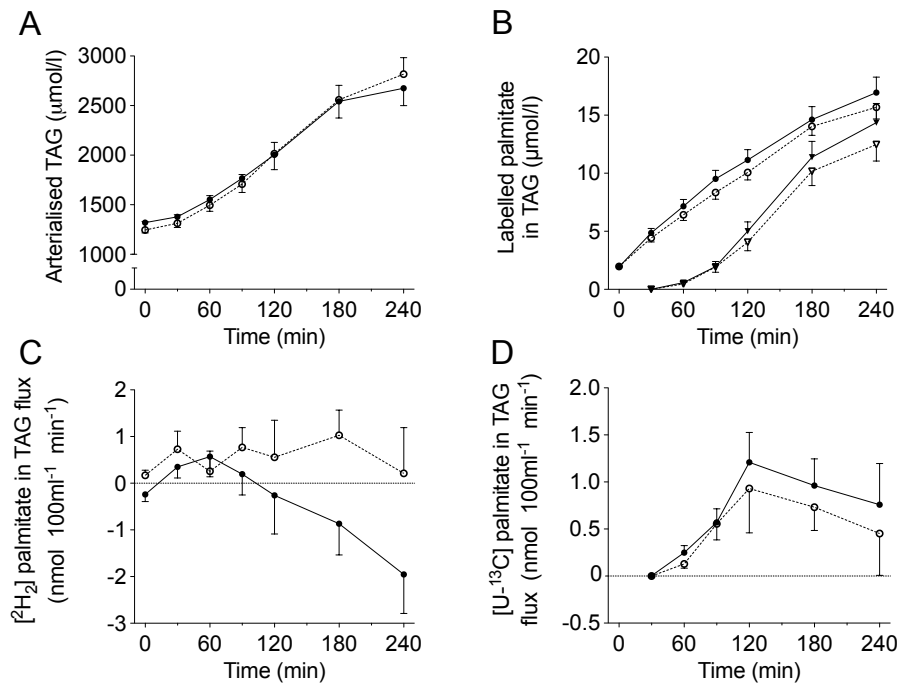


Figure 3. Postprandial whole-body and forearm muscle TAG metabolism. Arterialised plasma TAG concentrations (A), $[^2\text{H}_2]$ - and $[\text{U-}^{13}\text{C}]$ -palmitate concentrations in the plasma TAG fraction (B), and the net flux of $[^2\text{H}_2]$ -palmitate TAG (C) and $[\text{U-}^{13}\text{C}]$ -palmitate TAG (D) across forearm muscle during fasting (0 min) and after consumption of a high-SFA meal. A positive flux indicates net uptake across forearm muscle, whereas a negative flux indicates net release. Black symbols, mild-IR group; white symbols, high-IR group; in (B) circles, $[^2\text{H}_2]$ -palmitate TAG and triangles, $[\text{U-}^{13}\text{C}]$ -palmitate TAG. Student's *t* test for unpaired samples showed a significant effect of group on postprandial net flux of $[^2\text{H}_2]$ -palmitate TAG ($\text{AUC}_{0-4\text{h}}$; $p = 0.045$). Values are presented as mean \pm SEM

we found a significant linear association between postprandial net extraction of $[^2\text{H}_2]$ -palmitate TAG and HOMA-IR, even after adjustment for BMI and sex (standardised $\beta = 0.294$; $p = 0.037$; Table 2). The net extraction of $[\text{U-}^{13}\text{C}]$ -palmitate TAG across forearm muscle could be detected from 60 min onwards and did not differ significantly between groups (Table 2, Figure 3D).

Intramuscular lipid metabolism

Skeletal muscle TAG, DAG and NEFA content were comparable between high-IR and mild-IR groups (Table 3). The PL content was lower in the high-IR group than in the mild-IR group, although the difference did not reach statistical significance (60.5 ± 3.2 vs 70.2 ± 3.6 $\mu\text{mol} / [\text{g dry weight}]$, respectively, $p = 0.055$). However, there was no significant linear association between PL and measures of IR (Table 3). Intramuscular lipid composition was different between groups, with a significantly higher degree of saturation in the muscle NEFA pool in participants with high-IR compared with those with mild-IR ($51.8 \pm 2.8\%$ vs $43.9 \pm 2.5\%$, respectively, $p = 0.039$), but there was no significant linear association with IR (Table 3). This difference was mainly explained by higher percentages of myristic acid (C14:0; $3.5 \pm 0.7\%$ vs $2.1 \pm 0.4\%$, $p = 0.078$), pentadecyclic acid (C15:0; $4.5 \pm 1.5\%$ vs $1.2 \pm 0.3\%$, $p = 0.038$) and tricosylic acid (C23:0; $3.0 \pm 1.1\%$ vs $0.8 \pm 0.4\%$, $p = 0.064$) in the high-IR group vs mild-IR group (Figure 4A). Furthermore, the percentage of PUFA in the NEFA pool was lower in the high-IR group than in the mild-IR group ($13.0 \pm 1.1\%$ vs $17.0 \pm 1.4\%$, $p = 0.034$; Table 3). In addition, lipid composition in the intramuscular DAG pool differed between high-IR and mild-IR groups (Table 3): in the high-IR group, the percentage PUFA was significantly lower ($p = 0.022$). Linear regression showed a non-significant negative association between the percentage of PUFA in the DAG pool and HOMA-IR. Furthermore, a higher percentage of MUFA was observed in the DAG pool of the high-IR group compared with the mild-IR group ($p = 0.052$; Table 3). Although the total saturation of the lipid content in the DAG pool did not differ between groups (Table 3), the percentage of palmitate (C16:0) in the DAG pool of the high-IR group was significantly higher compared with that in the mild-IR group ($22.1 \pm 0.7\%$ vs $24.6 \pm 0.8\%$, respectively, $p = 0.024$; Figure 4B).

The FSR of skeletal muscle TAG, DAG and PL was comparable between groups (Table 3), indicating that a similar proportion of palmitate from the intramuscular NEFA pool was directed towards storage after the high-SFA meal.

Fasting skeletal muscle mRNA expression of genes encoding proteins involved in oxidative metabolism, lipid synthesis and lipolysis is shown in Table 4. Skeletal muscle gene expression of *GPAT1* (also known as *GPAM*), a gene involved in lipid synthesis, was significantly lower ($p = 0.050$) in the high-IR group than in the mild-IR group, even when adjusted for sex and BMI using linear regression analysis (standardised $\beta = -0.666$; $p = 0.002$). Furthermore, the expression level of *NDUFB5*, encoding a subunit of complex I in the electron transport chain, tended to be lower in the high-IR vs the mild-IR group ($p = 0.062$),

Table 3. Skeletal muscle lipid content and composition during fasting and the FSR of the muscle lipid pools after a high-SFA meal

Lipid	Mild-IR (n = 35)	High-IR (n = 28)	<i>p</i> value ^a	SE β	<i>p</i> value ^b
NEFA					
Total ($\mu\text{mol}/[\text{g dry weight}]$)	5.9 \pm 0.8	4.9 \pm 0.8	0.360	-0.058	0.642
% SFA	43.9 \pm 2.5	51.8 \pm 2.8	0.039	0.219	0.112
% MUFA	39.1 \pm 1.9	35.2 \pm 2.3	0.183	-0.183	0.162
% PUFA	17.0 \pm 1.4	13.0 \pm 1.1	0.034	-0.162	0.266
FSR (%/h)	0.38 \pm 0.05	0.36 \pm 0.07	0.747	-0.069	0.619
DAG					
Total ($\mu\text{mol}/[\text{g dry weight}]$)	10.8 \pm 2.8	6.2 \pm 0.7	0.151	-0.195	0.160
% SFA	37.8 \pm 1.9	36.6 \pm 0.8	0.594	-0.093	0.535
% MUFA	44.2 \pm 1.6	47.6 \pm 0.6	0.052	0.255	0.086
% PUFA	18.0 \pm 0.8	15.7 \pm 0.5	0.022	-0.271	0.059
FSR (%/h)	0.33 \pm 0.05	0.29 \pm 0.05	0.538	-0.058	0.692
TAG					
Total ($\mu\text{mol}/[\text{g dry weight}]$)	182.6 \pm 24.3	232.1 \pm 42.5	0.301	0.126	0.365
% SFA	36.7 \pm 0.9	35.8 \pm 0.9	0.477	-0.157	0.273
% MUFA	47.9 \pm 1.0	50.1 \pm 0.7	0.076	0.046	0.749
% PUFA	15.3 \pm 0.8	14.1 \pm 1.0	0.325	0.112	0.444
FSR (%/h)	0.28 \pm 0.05	0.21 \pm 0.04	0.325	-0.186	0.209
PL					
Total ($\mu\text{mol}/[\text{g dry weight}]$)	70.2 \pm 3.6	60.5 \pm 3.2	0.055	-0.155	0.260
% SFA	41.3 \pm 0.8	40.4 \pm 0.6	0.364	-0.046	0.754
% MUFA	11.3 \pm 0.3	11.4 \pm 0.5	0.903	0.017	0.909
% PUFA	47.4 \pm 1.0	48.2 \pm 0.7	0.496	0.030	0.838
FSR (%/h)	0.10 \pm 0.01	0.09 \pm 0.01	0.486	-0.006	0.966

Values are means \pm SEM. ^a*p* value for difference between mild-IR and high-IR group, Student's *t* test for unpaired samples. ^b*p* value for multiple linear regression. SE β , standardised β coefficient for total group for HOMA-IR, adjusted for sex and BMI

whilst the expression of the intracellular lipase gene *ATGL* (also known as *PNPLA2*) tended to be higher in the high-IR group (*p* = 0.052).

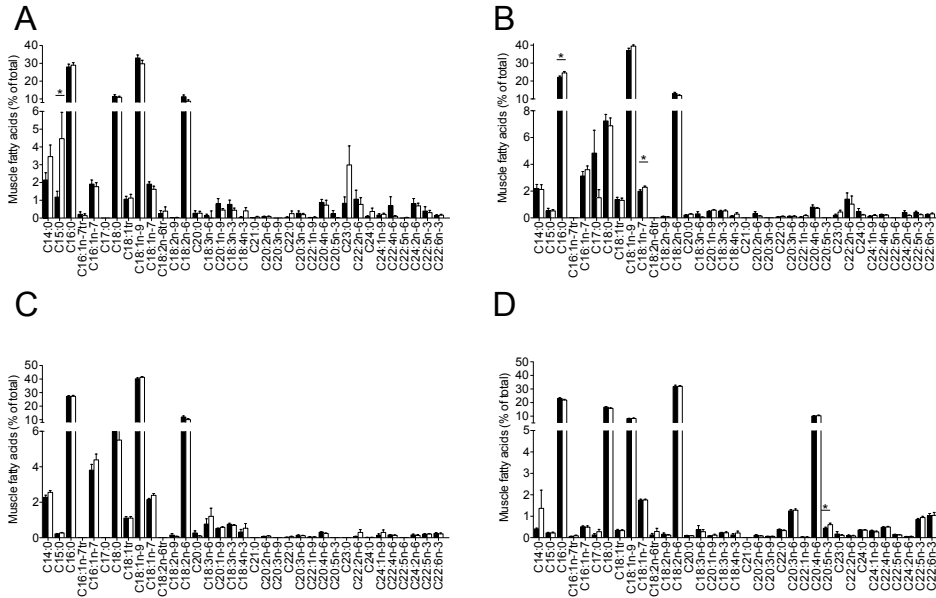


Figure 4. Intramuscular NEFA (A), DAG (B), TAG (C) and PL (D) composition under fasting conditions. Black bars, mild-IR group; white bars, high-IR group. * $p < 0.05$, mild-IR *vs* high-IR group, Student's *t* test for unpaired samples. Values are presented as mean \pm SEM

Discussion

The present study demonstrated that postprandial forearm muscle VLDL-TAG extraction was elevated in individuals with high-IR compared with mild-IR. This elevation in VLDL-TAG extraction was accompanied by increased saturation of the intramuscular NEFA pool. Both effects were independent of BMI. These data support the notion of an important role for disturbances in skeletal muscle FA handling in the progression of whole-body IR.

Disturbances in skeletal muscle FA handling have been implicated in the aetiology of IR and type 2 diabetes (1,23). Our group has recently demonstrated that higher postprandial plasma TAG concentrations and increased net TAG extraction across forearm muscle were accompanied by decreased postprandial insulin sensitivity in participants with impaired glucose metabolism compared with normal glucose tolerance (24). Furthermore, we showed a higher postprandial VLDL-TAG extraction by skeletal muscle in men with IR compared

Table 4. Fasting skeletal muscle gene expression

Gene	Mild-IR	High-IR	<i>p</i> value ^a	SE β	<i>p</i> value ^b
Oxidative metabolism	n = 32	n = 30			
<i>mCPT1B</i>	1.70 \pm 0.14	1.74 \pm 0.13	0.821	0.006	0.986
<i>PGC1α</i> (<i>PPARGC1A</i>)	0.51 \pm 0.05	0.46 \pm 0.04	0.428	0.027	0.847
<i>ACC2</i>	1.52 \pm 0.14	1.59 \pm 0.16	0.741	0.020	0.891
<i>SDHB</i>	2.40 \pm 0.21	1.97 \pm 0.22	0.153	-0.058	0.683
<i>NDUFB5</i>	2.67 \pm 0.17	2.14 \pm 0.23	0.062	-0.243	0.076
Transcription factors	n = 28	n = 23			
<i>PPARα</i> (<i>PPARA</i>)	1.63 \pm 0.16	1.56 \pm 0.11	0.743	0.034	0.808
<i>PPARδ</i> (<i>PPARD</i>)	0.36 \pm 0.04	0.48 \pm 0.07	0.114	0.175	0.259
<i>SREBP1c</i> (<i>SREBF1</i>)	0.92 \pm 0.10	1.12 \pm 0.14	0.238	0.028	0.840
<i>SREBP2</i> (<i>SREBF2</i>)	1.43 \pm 0.15	1.86 \pm 0.23	0.127	0.266	0.216
<i>ChREBP</i> (<i>CREBBP</i>)	1.02 \pm 0.19	0.99 \pm 0.15	0.906	-0.040	0.859
TAG synthesis	n = 10	n = 15			
<i>GPAT1</i> (<i>GPAM</i>)	2.55 \pm 0.23	1.81 \pm 0.25	0.050	-0.666	0.002
<i>DGAT1</i>	1.89 \pm 0.19	1.77 \pm 0.20	0.667	0.079	0.683
<i>DGAT2</i>	0.31 \pm 0.05	0.25 \pm 0.06	0.509	-0.103	0.671
TAG lipolysis	n = 15	n = 8			
<i>LPL</i>	0.50 \pm 0.08	0.62 \pm 0.11	0.342	0.018	0.927
<i>ATGL</i> (<i>PNPLA2</i>)	1.11 \pm 0.17	2.52 \pm 0.60	0.052	0.522	0.007
<i>HSL</i> (<i>LIPE</i>)	0.44 \pm 0.19	0.74 \pm 0.21	0.329	0.130	0.515

Values are means \pm SEM. ^a*p* value for difference between mild-IR and high-IR group, Student's *t* test for unpaired samples. ^b*p* value for multiple linear regression. *mCPT1b*, muscle *CPT1B*; SE β , standardised β coefficient for total group for HOMA-IR, adjusted for sex and BMI

with controls with the metabolic syndrome, matched for age and BMI, despite a similar TAG supply (19). More recently, we demonstrated that increased muscle VLDL-TAG extraction and reduced lipid turnover of SFA, rather than DAG content, accompany the more pronounced IR observed in humans with impaired glucose tolerance (IGT) compared with impaired fasting glucose (IFG) (20).

The present data extend our previous observations regarding FA handling by showing that postprandial forearm muscle VLDL-TAG extraction was elevated in participants with

high-IR when compared with those with mild-IR. We included participants who encompassed the entire spectrum of insulin sensitivity, from insulin-sensitive to very-insulin-resistant states (HOMA-IR, 1.7–11.3) and with a wide range of adiposity (BMI, 22.7–39.5 kg/m²). This allowed us to differentiate between the effect of obesity and IR per se, and our present findings confirm those of previous studies that have suggested the existence of a relationship between impaired skeletal muscle FA handling and IR (1,8,25). Moreover, our data imply that IR is primarily responsible for increased postprandial forearm muscle VLDL-TAG extraction, since we observed a significant linear association between VLDL-TAG extraction and IR. A potential mechanism for increased skeletal muscle VLDL-TAG extraction in participants with high-IR may involve differential apolipoprotein composition of the VLDL particles (16). For example, higher plasma apoCII:apoCIII ratios have been shown in diabetic individuals, compared with a control group (26). This variation in lipid composition might lead to higher a susceptibility for lipid degradation by in vivo skeletal muscle LPL (27). Of note, we did not perform measurements in the late postprandial phase. It has been shown that dietary FAs appear in VLDL-TAG from 2–3 h after meal ingestion, making it difficult to separate chylomicron- and VLDL-TAG in the late postprandial phase using the current dual isotope approach (17,28,29). Therefore, we cannot exclude the possibility that the increased TAG extraction observed in high-IR participants may also extend to chylomicron-TAG. In this context, an impaired inhibitory effect of insulin on skeletal muscle LPL action in the high-IR group (30), or impaired FA uptake via membrane-associated carrier proteins like CD36 (31) might possibly explain the observed differences.

In addition to an increased skeletal muscle lipid uptake, we observed differences in the intramyocellular FA partitioning between groups. Reduced re-esterification of NEFA into TAG may expose muscle to excess NEFA concentrations and bioactive lipid metabolites that may interfere with insulin signalling (23). In line with this, a reduced incorporation of NEFA into TAG in primary myotubes from obese individuals with type 2 diabetes has previously been shown (32), indicating that the ability to incorporate FAs into TAG is an intrinsic feature of human muscle cells that is reduced in individuals with type 2 diabetes. Moreover, *in vitro* work has shown that muscle cells incubated with palmitate incorporated more FA towards the DAG pool, while the unsaturated FAs were diverted towards storage in the TAG pool (33). In the present study, an increased percentage of palmitate in the DAG pool in the participants with high-IR was observed. Together with the reduced expression of *GPAT1*, which is involved in the first step in TAG synthesis in muscle, this might indicate a retention of SFA in the NEFA pool and may explain the lower percentage of PUFA in the DAG pool

in the individuals with high-IR *vs* mild-IR. More recently, we reported a reduced FSR of palmitate into intramuscular TAG and DAG in individuals with combined IGT and IFG compared with participants with isolated IFG (20). This has also been shown in obese humans with impaired glucose metabolism (34). However, in the present study we did not observe significant differences between the mild-IR and high-IR groups in the FSR of palmitate into intramuscular TAG and DAG. Nevertheless, these findings suggest that the postprandial incorporation of FAs in TAG or DAG is not affected by the degree of IR per se.

Strikingly, in this study the increased saturation in the NEFA pool was mainly confined to specific SFAs, namely myristic acid (C14:0), pentadecylic acid (C15:0) and tricosylic acid (C23:0). Recently, plasma odd-chain SFAs were shown to be inversely associated with type 2 diabetes and coronary heart disease incidence in large epidemiological studies (35). Interestingly, dairy products are known to be the most important source for C15:0 (36). This would certainly suggest a contribution of dietary fat to the increased saturation of the NEFA pool, since it has been shown that FA composition of skeletal muscle reflects dietary FA composition (37). However, data related to odd-chain FA metabolism in skeletal muscle is currently lacking and more research is needed to unravel the role of odd-chain FAs in intramyocellular lipid turnover and skeletal muscle IR. Moreover, it is important to note that the increased content of SFA in the high-IR group might be linked to an increased ceramide content and higher saturation in the long-chain fatty acyl-CoA. It has been shown that increased levels of the SFA palmitate drive ceramide synthesis (38). However, we did not measure skeletal muscle ceramide levels or expression of related genes in the present study. Finally, previous studies have shown that lower percentages of PUFA in the plasma membrane are associated with IR (39,40). However, in this study the percentages of total PUFAs in the muscle PL pool were comparable between groups. It is important to note that we have not specifically measured muscle membrane PL content but rather total muscle PL content. Since most PLs are located mainly in the plasma membrane, the reduced total PL content might reflect a lower absolute amount of PUFA in the muscle membrane fraction and might therefore contribute to the worsening of IR in individuals with high-IR.

A limitation of the present study is that we did not perform a hyperinsulinaemic-euglycaemic clamp test to assess insulin sensitivity. Rather, we divided groups based on a surrogate marker of whole-body insulin sensitivity, namely HOMA-IR. Nevertheless, previous studies have shown strong correlations between HOMA-IR and peripheral insulin sensitivity, as measured by the gold-standard hyperinsulinaemic-euglycaemic clamp (41). Importantly, in this study individuals in the high-IR group had a significantly lower

postprandial net glucose uptake across forearm muscle as compared with the individuals with mild-IR, despite having significantly higher postprandial plasma insulin concentrations. Since skeletal muscle accounts for approximately 80% of insulin-mediated glucose uptake in humans (42), these data clearly indicate a more pronounced skeletal muscle IR in the individuals with high-IR as compared with those with mild-IR.

In conclusion, increased skeletal muscle VLDL-TAG extraction in the postprandial state and higher saturation of the intramuscular NEFA pool are associated with more pronounced IR. These data support an important role for disturbances in skeletal muscle FA handling in the progression of whole-body IR.

References

1. Stinkens R, Goossens GH, Jocken JWE, Blaak EE. Targeting fatty acid metabolism to improve glucose metabolism. *Obes Rev.* 2015;16:715–57.
2. Goossens GH. The role of adipose tissue dysfunction in the pathogenesis of obesity-related insulin resistance. *Physiol Behav.* 2008;94:206–18.
3. Ebbert J, Jensen M. Fat Depots, Free Fatty Acids, and Dyslipidemia. *Nutrients.* 2013;5:498–508.
4. Mittendorfer B. Origins of metabolic complications in obesity: adipose tissue and free fatty acid trafficking. *Curr Opin Clin Nutr Metab Care.* 2011;14:535–41.
5. Mensink M, Blaak EE, van Baak MA, Wagenmakers AJ, Saris WH. Plasma free Fatty Acid uptake and oxidation are already diminished in subjects at high risk for developing type 2 diabetes. *Diabetes.* 2001;50:2548–54.
6. Dubé JJ, Coen PM, DiStefano G, Chacon AC, Helbling NL, Desimone ME, et al. Effects of acute lipid overload on skeletal muscle insulin resistance, metabolic flexibility, and mitochondrial performance. *Am J Physiol Endocrinol Metab.* 2014;307:E1117–24.
7. Schrauwen P, Schrauwen-Hinderling V, Hoeks J, Hesselink MKC. Mitochondrial dysfunction and lipotoxicity. *Biochim Biophys Acta.* 2010;1801:266–71.
8. Coen PM, Goodpaster BH. Role of intramyocellular lipids in human health. *Trends Endocrinol Metab.* 2012;23:391–8.
9. Samuel VT, Shulman GI. Mechanisms for Insulin Resistance: Common Threads and Missing Links. *Cell.* 2012;148:852–71.
10. Blaak EE, Wagenmakers AJ, Glatz JF, Wolffenbuttel BH, Kemerink GJ, Langenberg CJ, et al. Plasma FFA utilization and fatty acid-binding protein content are diminished in type 2 diabetic muscle. *Am J Physiol Endocrinol Metab.* 2000;279:E146–54.
11. Bickerton AST, Roberts R, Fielding BA, Tornqvist H, Blaak EE, Wagenmakers AJM, et al. Adipose tissue fatty acid metabolism in insulin-resistant men. *Diabetologia.* 2008;51:1466–74.
12. Jocken JWE, Goossens GH, van Hees AMJ, Frayn KN, van Baak M, Stegen J, et al. Effect of beta-adrenergic stimulation on whole-body and abdominal subcutaneous adipose tissue lipolysis in lean and obese men. *Diabetologia.* 2008;51:320–7.
13. Hodson L, Bickerton AST, McQuaid SE, Roberts R, Karpe F, Frayn KN, et al. The contribution of splanchnic fat to VLDL triglyceride is greater in insulin-resistant than insulin-sensitive men and women: studies in the postprandial state. *Diabetes.* 2007;56:2433–41.
14. Riemens SC, Sluiter WJ, Dullaart RP. Enhanced escape of non-esterified fatty acids from tissue uptake: its role in impaired insulin-induced lowering of total rate of appearance in obesity and Type II diabetes mellitus. *Diabetologia.* 2000;43:416–26.
15. Nielsen S, Karpe F. Determinants of VLDL-triglycerides production. *Curr Opin Lipidol.* 2012;23:321–6.
16. Choi SH, Ginsberg HN. Increased very low density lipoprotein (VLDL) secretion, hepatic steatosis, and insulin resistance. *Trends Endocrinol Metab.* 2011;22:353–63.
17. Bickerton AST, Roberts R, Fielding BA, Hodson L, Blaak EE, Wagenmakers AJM, et al. Preferential uptake of dietary Fatty acids in adipose tissue and muscle in the postprandial period. *Diabetes.* 2007;56:168–76.

18. Ruge T, Hodson L, Cheeseman J, Dennis AL, Fielding BA, Humphreys SM, et al. Fasted to Fed Trafficking of Fatty Acids in Human Adipose Tissue Reveals a Novel Regulatory Step for Enhanced Fat Storage. *J Clin Endocrinol Metab*. 2009;94:1781–8.
19. van Hees AMJ, Jans A, Hul GB, Roche HM, Saris WHM, Blaak EE. Skeletal muscle fatty acid handling in insulin resistant men. *Obesity*. 2011;19:1350–9.
20. Goossens G, Moors C, Jocken J, van der Zijl N, Jans A, Konings E, et al. Altered Skeletal Muscle Fatty Acid Handling in Subjects with Impaired Glucose Tolerance as Compared to Impaired Fasting Glucose. *Nutrients*. 2016;8:164–15.
21. Jans A, Konings E, Goossens GH, Bouwman FG, Moors CC, Boekschoten MV, et al. PUFAs acutely affect triacylglycerol-derived skeletal muscle fatty acid uptake and increase postprandial insulin sensitivity. *Am J Clin Nutr*. 2012;95:825–36.
22. Bergström J, Hermansen L, Hultman E, Saltin B. Diet, muscle glycogen and physical performance. *Acta Physiol Scand*. 1967;71:140–50.
23. Bosma M, Kersten S, Hesselink MKC, Schrauwen P. Re-evaluating lipotoxic triggers in skeletal muscle: relating intramyocellular lipid metabolism to insulin sensitivity. *Prog Lipid Res*. 2012;51:36–49.
24. Moors CCM, van der Zijl NJ, Diamant M, Blaak EE, Goossens GH. Impaired insulin sensitivity is accompanied by disturbances in skeletal muscle fatty acid handling in subjects with impaired glucose metabolism. *Int J Obes (Lond)*. 2012;36:709–17.
25. Samuel VT, Petersen KF, Shulman GI. Lipid-induced insulin resistance: unravelling the mechanism. *Lancet*. 2010;375:2267–77.
26. Hiukka A, Fruchart-Najib J, Leinonen E, Hilden H, Fruchart J-C, Taskinen M-R. Alterations of lipids and apolipoprotein CIII in very low density lipoprotein subpecies in type 2 diabetes. *Diabetologia*. 2005;48:1207–15.
27. Kersten S. Physiological regulation of lipoprotein lipase. *Biochim Biophys Acta*. 2014;1841:919–33.
28. Heath RB, Karpe F, Milne RW, Burdge GC, Wootton SA, Frayn KN. Selective partitioning of dietary fatty acids into the VLDL TG pool in the early postprandial period. *J Lipid Res*. 2003;44:2065–72.
29. Heath RB, Karpe F, Milne RW, Burdge GC, Wootton SA, Frayn KN. Dietary fatty acids make a rapid and substantial contribution to VLDL-triacylglycerol in the fed state. *Am J Physiol Endocrinol Metab*. 2007;292:E732–9.
30. Yost TJ, Jensen DR, Haugen BR, Eckel RH. Effect of dietary macronutrient composition on tissue-specific lipoprotein lipase activity and insulin action in normal-weight subjects. *Am J Clin Nutr*. 1998;68:296–302.
31. Goldberg IJ, Eckel RH, Abumrad NA. Regulation of fatty acid uptake into tissues: lipoprotein lipase- and CD36-mediated pathways. *J Lipid Res*. 2009;50 Suppl:S86–90.
32. Sparks LM, Bosma M, Brouwers B, van de Weijer T, Bilet L, Schaart G, et al. Reduced Incorporation of Fatty Acids Into Triacylglycerol in Myotubes From Obese Individuals With Type 2 Diabetes. *Diabetes*. 2014;63:1583–93.
33. Montell E, Turini M, Marotta M, Roberts M, Noé V, Ciudad CJ, et al. DAG accumulation from saturated fatty acids desensitizes insulin stimulation of glucose uptake in muscle cells. *Am J Physiol Endocrinol Metab*. 2001;280:E229–37.
34. Perreault L, Bergman BC, Hunerdosse DM, Playdon MC, Eckel RH. Inflexibility in Intramuscular Triglyceride Fractional Synthesis Distinguishes Prediabetes From Obesity in Humans. *Obesity*. 2009;18:1524–31.

35. Forouhi NG, Koulman A, Sharp SJ, Imamura F. Differences in the prospective association between individual plasma phospholipid saturated fatty acids and incident type 2 diabetes: the EPIC-InterAct case-cohort study. *Lancet Diabetes Endocrinol.* 2014;2:810–8.
36. Jenkins B, West J, Koulman A. A Review of Odd-Chain Fatty Acid Metabolism and the Role of Pentadecanoic Acid (C15:0) and Heptadecanoic Acid (C17:0) in Health and Disease. *Molecules.* 2015;20:2425–44.
37. Andersson A, Nälsén C, Tengblad S, Vessby B. Fatty acid composition of skeletal muscle reflects dietary fat composition in humans. *Am J Clin Nutr.* 2002;76:1222–9.
38. Chaurasia B, Summers SA. Ceramides - Lipotoxic Inducers of Metabolic Disorders. *Trends Endocrinol Metab.* 2015;26:538–50.
39. Borkman M, Storlien LH, Pan DA, Jenkins AB, Chisholm DJ, Campbell LV. The relation between insulin sensitivity and the fatty-acid composition of skeletal-muscle phospholipids. *N Engl J Med.* 1993;328:238–44.
40. Baur LA, O'Connor J, Pan DA, Kriketos AD, Storlien LH. The fatty acid composition of skeletal muscle membrane phospholipid: its relationship with the type of feeding and plasma glucose levels in young children. *Metabolism.* 1998;47:106–12.
41. Bonora E, Targher G, Alberiche M, Bonadonna RC, Saggiani F, Zenere MB, et al. Homeostasis model assessment closely mirrors the glucose clamp technique in the assessment of insulin sensitivity: studies in subjects with various degrees of glucose tolerance and insulin sensitivity. *Diabetes Care.* 2000;23:57–63.
42. DeFronzo RA, Jacot E, Jequier E, Maeder E, Wahren J, Felber JP. The effect of insulin on the disposal of intravenous glucose. Results from indirect calorimetry and hepatic and femoral venous catheterization. *Diabetes.* 1981;30:1000–7.





Chapter 3

Angiopoietin-like protein 4 and postprandial skeletal muscle lipid metabolism in overweight and obese prediabetics

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Abstract

Context: Angiopoietin-like protein 4 (ANGPTL4) decreases plasma triacylglycerol (TAG) clearance by inhibiting lipoprotein lipase (LPL) and may contribute to impairments in lipid metabolism under compromised metabolic conditions.

Objectives: To investigate the effects of a high-saturated fatty acid (SFA) mixed meal on plasma ANGPTL4 concentrations in relation to *in vivo* muscle LPL activity, to study the effects of dietary fat quality, and to examine skeletal muscle ANGPTL4 release.

Design, Participants, Setting, and Interventions: We used a dual stable-isotope tracer technique in combination with measurements of arteriovenous concentration differences across forearm muscle to investigate muscle ANGPTL4 secretion and fatty acid handling under fasting conditions and after a high-SFA mixed meal in 73 overweight and obese humans at the Metabolic Research Unit of Maastricht University. The effect of dietary fat quality manipulation on plasma ANGPTL4 was investigated in 10 obese insulin-resistant participants.

Results: The high-SFA meal decreased circulating ANGPTL4 concentrations (fasting, 5.2 ng/ml *vs* 4 hours postprandial, 4.0 ng/mL; $p < 0.001$). Furthermore, skeletal muscle ANGPTL4 secretion into the circulation was observed (AUC_{0-4h} , $p = 0.048$). However, no association was observed between plasma ANGPTL4 and skeletal muscle very low-density lipoprotein or dietary (chylomicron) TAG extraction (AUC_{0-4h} , $p = 0.372$ and $p = 0.139$, respectively). In contrast to a high-SFA or high-monounsaturated fat meal, plasma ANGPTL4 remained unchanged after a high-polyunsaturated fat meal.

Conclusions: ANGPTL4 is secreted by human forearm muscle in postprandial conditions after a high-SFA meal. Plasma ANGPTL4 concentrations were not associated with *in vivo* skeletal muscle LPL activity after a high-SFA meal. Dietary fat quality affects plasma ANGPTL4, but it remains to be elucidated whether this influences short-term skeletal muscle lipid handling.

Introduction

Systemic lipid overflow, driven by adipose tissue dysfunction and an impaired skeletal muscle lipid handling, may contribute to insulin resistance (1). Lipoprotein lipase (LPL) plays a central role in the clearance of triacylglycerol (TAG)-rich plasma lipoproteins (2). LPL is located at the capillary endothelium, where it catalyzes the hydrolysis of endogenous very low-density lipoprotein (VLDL)-TAG and exogenous chylomicron-TAG to glycerol and free fatty acids (FFAs) (3). The resulting FFAs are then taken up by the tissues and will be directed toward oxidation or storage or will be spilled over into the circulation (4,5). The activity of LPL is under tight nutritional and hormonal control. For instance, insulin is a major activator of LPL activity (6). Furthermore, angiopoietinlike protein 4 (ANGPTL4) has emerged as an important inhibitor of LPL activity, likely by inducing dissociation of catalytically active LPL dimers into inactive LPL monomers (7-9).

The regulation of ANGPTL4 under fasting and postprandial conditions in humans and its contribution to human tissue lipid handling is not well understood. Insulin reduces circulating ANGPTL4, as revealed by hyperinsulinemic-euglycemic clamps in humans (10-13). Interestingly, concomitant intravenous infusion of a lipid emulsion high in polyunsaturated fatty acids (PUFAs) blunted this insulin-mediated ANGPTL4 decrease. This suggests that dietary fat quality might be an important regulator of plasma ANGPTL4 (10). Furthermore, *in vitro* studies using human myotubes and adipocytes have shown that ANGPTL4 expression and secretion are stimulated by dietary fatty acid quality in a peroxisomal proliferator activator receptor-dependent manner (12,14-17). Interestingly, we have reported that dietary fat quality affects *in vivo* skeletal muscle fatty acid handling with an increased TAG extraction in obese insulin-resistant humans (18). However, it remains to be elucidated whether ANGPTL4 is involved in interindividual differences in skeletal muscle lipid metabolism.

ANGPTL4 is expressed and secreted by numerous cell types, and its expression is dependent on nutritional status (19). Human plasma ANGPTL4 concentrations are relatively stable, although plasma ANGPTL4 increases after long-term fasting (12,20) and chronic caloric restriction (12), as well as following physical activity (12,16,21). These conditions are characterized by elevated plasma FFA concentrations. However, the origin of circulating ANGPTL4 is currently unknown. Based on human ANGPTL4 mRNA expression and ANGPTL4 overexpression studies in mice, it has been postulated that the liver is the main contributor to systemic ANGPTL4 concentrations (12,22). In addition, adipose tissue and

intestine might also be important contributors to circulating ANGPTL4 concentrations (23,24). Interestingly, it has recently been shown that acute exercise induced gene and protein expression of ANGPTL4 in skeletal muscle and increased plasma ANGPTL4 concentrations (16,21). These data suggest that skeletal muscle might also contribute to plasma ANGPTL4 (16,21). However, data on *in vivo* ANGPTL4 secretion by skeletal muscle in humans are still lacking.

The aims of the present study were to investigate the determinants and postprandial regulation of plasma ANGPTL4 in overweight and obese prediabetic humans after the intake of a high saturated fatty acid (SFA) meal. Secondly, the relationship between plasma ANGPTL4 concentration and *in vivo* skeletal muscle LPL activity was studied. Moreover, we addressed the impact of dietary fat quality on plasma ANGPTL4 concentrations and examined whether human forearm muscle secretes ANGPTL4 under fasting conditions and after the intake of a high-SFA meal.

Subjects and methods

Study participants

In 150 participants (138 men and 12 women), fasting plasma ANGPTL4 concentrations were measured. Participants' characteristics are summarized in Table 1. From this cohort, 73 participants (61 men and 12 women) with metabolic syndrome or impaired glucose metabolism had a high-SFA mixed-meal test. These participants have been described elsewhere in more detail (18,25,26). From these participants, 10 obese insulin-resistant men also consumed two additional high-fat mixed meals, high in either monounsaturated fat (MUFA) or in PUFA (18). The local Medical Ethical Committee of Maastricht University Medical Center* approved the study protocols. All participants gave written informed consent before participation.

High-fat mixed-meal test

Participants were studied after an overnight fast and were asked to refrain from strenuous exercise and drinking alcohol 24 hours before the study day. In addition, they were asked to avoid food products naturally enriched with [^{13}C] for 7 days before the study day. Forearm muscle metabolism was calculated using arteriovenous concentration differences combined with measurements of forearm blood flow. Three catheters were inserted before the start of

the experiment. One catheter was placed retrogradely into a superficial dorsal hand vein, which was heated in a hot-box (60°C) to obtain an arterialized blood sample. In the same arm, another catheter was placed in an antecubital vein for the infusion of the [$^2\text{H}_2$]-palmitate tracer. A third catheter was placed retrogradely in a deep antecubital vein of the contralateral forearm to sample venous blood draining the forearm muscle. After taking an arterial and deep venous background sample at 90 minutes before meal ingestion (t -90), a continuous intravenous infusion of the stable isotope tracer [$^2\text{H}_2$]-palmitate (97% enrichment; Cambridge Isotope Laboratories) complexed to albumin was started ($0.035 \mu\text{mol} \cdot \text{kg body weight}^{-1} \cdot \text{min}^{-1}$). Baseline blood sampling was started after 1 hour of tracer infusion to allow isotopic equilibration to occur. Blood samples were taken simultaneously from the dorsal hand vein and the deep muscle vein at three time points during fasting: t -30, t -15, at meal ingestion (t 0). They were also taken at six time points postprandially (t 30, t 60, t 90, t 120, t 180, t 240) after consumption of a high-SFA mixed meal (t 0) containing 200 mg [$\text{U-}^{13}\text{C}$]-palmitate (98% enrichment; Cambridge Isotope Laboratories). The liquid meal test provided 2.6 MJ, consisting of 61 Energy % (E%) fat (35.5 E% SFA, 18.8 E% MUFA, and 1.7 E% PUFA), 33 E% carbohydrates, and 6.3 E% protein. Ten obese insulin-resistant participants also consumed in a randomized order a liquid high-fat mixed meal (2.6 MJ; 61 E% fat), which was high in MUFA (42.2 E%) or PUFA (34.8 E%) in a single-blinded randomized crossover design (18). Forearm blood flow was measured before each blood sampling and has been described previously (27).

Plasma ANGPTL4 concentration

ANGPTL4 was measured by ELISA, as described previously (12). Briefly, 96-well plates were coated with antihuman ANGPTL4 polyclonal goat IgG antibody (AF3485; R&D Systems) and incubated overnight at 4°C. Plates were washed extensively between each step. After blocking, 100 μL of 20-fold diluted human plasma was added, followed by 2-hour incubation at room temperature. A standard curve was prepared using recombinant ANGPTL4 (3485-AN; R&D Systems) at 0.3–2.1 ng/well. Next, 100 μL of diluted biotinylated antihuman ANGPTL4 polyclonal goat IgG antibody (BAF3485; R&D Systems) was added for 2 hours, followed by the addition of streptavidin-conjugated horseradish peroxidase for 20 minutes and tetramethyl benzidine substrate for 6 minutes. The reaction was stopped by the addition of 50 μL of 10% H_2SO_4 , and the absorbance was measured at 450 nm. Details on other biochemical analyses have previously been reported (25).

Skeletal muscle ANGPTL4 release and muscle lipid handling

Net fluxes of ANGPTL4 and metabolites (labeled and unlabeled) across the forearm were calculated by multiplying the arteriovenous concentration difference by forearm plasma flow. Plasma flow was measured using plethysmography and calculated by multiplying forearm blood flow with $(1 - \text{hematocrit} / 100)$. A positive flux indicates net uptake across forearm muscle, whereas a negative flux indicates net release. Labeled FFA and TAG concentrations were calculated as the product of the tracer-tracee ratio of $[^2\text{H}_2]$ - and $[\text{U-}^{13}\text{C}]$ -labeled palmitate and the concentration of palmitate in FFA and TAG, as reported before (25). *In vivo* LPL activity was based on net fluxes of $[^2\text{H}_2]$ - and $[\text{U-}^{13}\text{C}]$ -labeled palmitate. Postprandial areas under the curve (AUCs) of metabolites were calculated using the trapezium rule. Besides the total AUC (0–4 hours after meal ingestion), the “early” (0–2 hours) and “mid” (2–4 hours) AUCs were also calculated to obtain more detailed information on the time course of the postprandial responses. Likewise, ANGPTL4 changes from baseline (Δ) to the “total” (0–4 hours), “early” (0–2 hours), and “mid” (2–4 hours) postprandial phases were calculated.

Statistics

Correlations between clinical characteristics or postprandial responses and plasma ANGPTL4 concentrations were tested by Pearson’s correlation coefficients, partial regression analyses, and with multiple linear regression analyses. Repeated-measures ANOVA with time as within-subject and dietary fat quality as between-subject factors was performed to identify the effects of dietary fat quality on plasma ANGPTL4 concentrations. Student’s unpaired *t* test was used to examine whether ANGPTL4 was released from skeletal muscle into the circulation (ANGPTL4 flux *vs* “zero” flux). The data were analyzed using SPSS for Mac version 22.0 (SPSS Inc). Statistical significance was set at $p < 0.05$, and all data in this study are expressed as means \pm SEM.

Results

Determinants of fasting ANGPTL4 concentration

First, we investigated determinants of fasting plasma ANGPTL4 concentration in a cross-sectional study cohort of 150 participants (characteristics in Table 1). Participants had a wide range in body mass index (BMI; 20.8 – 49.3 kg/m²) and insulin resistance (homeostasis model of assessment for insulin resistance [HOMA-IR], 0.5–11.3). Plasma ANGPTL4 showed a

Table 1. Participants' characteristics

	Fasting	Meal test	Dietary fat quality
No. of participants	150	73	10
Male (n) / Female (n)	138 / 12	61 / 12	10 / 0
Age (y)	55.5 ± 0.8 (23 – 70)	58.6 ± 0.9 (36 – 70)	61.5 ± 1.6 (54 – 70)
BMI (kg/m ²)	30.8 ± 0.4 (20.8 – 49.8)	30.5 ± 0.4 (22.7 – 39.5)	33.8 ± 3.8 (28.6 – 38.6)
Waist-hip ratio	1.03 ± 0.01 (0.89 – 1.22)	1.02 ± 0.01 (0.89 – 1.17)	1.04 ± 0.01 (1.00 – 1.10)
Fasting plasma glucose (mmol/L)	5.7 ± 0.1 (4.4 – 8.4)	5.5 ± 0.1 (4.6 – 6.7)	5.6 ± 0.1 (4.8 – 5.6)
Fasting plasma insulin (mU/L)	14.9 ± 0.5 (2.1 – 38.0)	14.6 ± 0.7 (7.2 – 38.0)	13.7 ± 1.3 (7.8 – 24.1)
Fasting plasma FFA (μmol/L)	615 ± 16 (127 – 1265)	578 ± 17 (296 – 1002)	652 ± 47 (415 – 846)
Fasting plasma TAG (μmol/L)	1299 ± 53 (415 – 4571)	1276 ± 67 (415 – 3232)	1204 ± 154 (690 – 2481)
HOMA-IR	3.7 ± 0.2 (0.5 – 11.3)	3.6 ± 0.2 (1.7 – 11.3)	3.5 ± 0.4 (1.7 – 5.4)
Fasting plasma ANGPTL4 (ng/mL)	5.7 ± 0.2 (1.2 – 16.4)	5.2 ± 0.2 (2.4 – 11.1)	5.4 ± 0.6 (2.5 – 8.9)

Values are expressed as mean ± SEM (range) unless specified otherwise.

positive correlation with circulating FFA, fasting insulin, and markers of adiposity, including BMI and waist-hip ratio (Table 2). Plasma ANGPTL4 was inversely associated with age and fasting glucose. No significant relationship was observed between plasma ANGPTL4 and circulating TAG concentrations. A partial regression analysis demonstrated that these variables together accounted for 32.7% of the variance in fasting ANGPTL4 (adjusted R^2 , 0.327). These results might point toward higher ANGPTL4 concentrations when metabolic health is attenuated (Table 2).

Table 2. Pearson and partial regression coefficients between fasting plasma ANGPTL4, anthropometric and clinical characteristics

	<i>R</i>	<i>p</i> -value	Partial regression coefficients	<i>p</i> -value
Age (y)	-0.214	0.009	-0.200	0.016
BMI (kg/m ²)	0.420	< 0.001	0.137	0.103
Waist-hip ratio	0.292	< 0.001	0.167	0.045
Fasting plasma glucose (mmol/L)	-0.185	0.023	-0.318	< 0.001
Fasting plasma insulin (mU/L)	0.208	0.011	0.149	0.074
Fasting FFA (μmol/L)	0.355	< 0.001	0.334	< 0.001
Fasting TAG (μmol/L)	-0.041	0.617	-0.202	0.015
HOMA-IR	0.126	0.123		

High-fat meal intake alters postprandial plasma ANGPTL4

Next, we investigated the postprandial response of ANGPTL4 in 73 overweight or obese participants (characteristics can be found in Table 1), after consumption of a high-SFA meal. Arterialized ANGPTL4 concentrations significantly decreased during the postprandial phase (baseline, 5.2 ± 0.2 ng/mL, *vs* 4 hours after meal intake, 4.0 ± 0.2 ng/mL; ANOVA time $p < 0.001$) (Figure 1A). In the present study, fasting plasma FFAs, TAG, insulin, and glucose concentrations correlated with fasting plasma ANGPTL4. Therefore we investigated the relationship between the postprandial decline in ANGPTL4 concentrations and postprandial responses of these circulating factors after a high-SFA meal. Multiple regression analysis revealed that postprandial FFA concentration decline (iAUC_{0-4h}) was positively associated with the decline in ANGPTL4 (Δ_{0-4h}) after a high-SFA meal ($\beta = 0.002$; $p = 0.028$). This was independent from postprandial changes in plasma insulin, glucose, and TAG concentrations.

Plasma ANGPTL4 does not correlate with muscle LPL activity

Next, we examined whether fasting plasma ANGPTL4 concentrations or the postprandial (changes in) plasma ANGPTL4 concentrations after a high-SFA meal were associated with skeletal muscle LPL activity. Using a dual stable isotope methodology, we were able to determine forearm muscle TAG extraction from endogenous VLDL-TAG (TAG labeled with [²H₂]-palmitate) and from chylomicron TAG (TAG labeled with [U-¹³C]-palmitate), as previously reported (5,25). As expected, from the first fasting sample onward, the

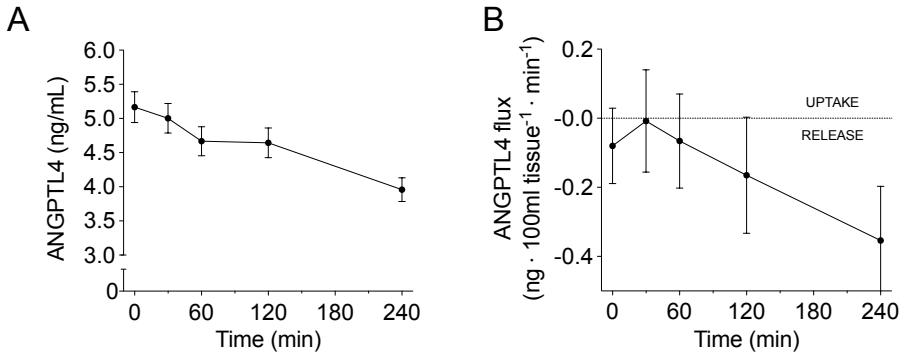


Figure 1. (A) Plasma ANGPTL4 concentrations (B) Net forearm muscle ANGPTL4 flux before and after a high-saturated fatty acid (SFA) meal. Values are mean \pm SEM; $n = 73$.

[$^2\text{H}_2$]-palmitate tracer could be measured in plasma TAG pool. This reflects the incorporation of the intravenously infused tracer into hepatically derived VLDL-TAG. The [$\text{U-}^{13}\text{C}$]-palmitate tracer (ingested together with the meal), representing exogenous chylomicron-TAG, appeared in the plasma TAG fraction 60 minutes after meal ingestion (Figure 2A). The net flux of [$\text{U-}^{13}\text{C}$]-palmitate in TAG, which reflects LPL-mediated hydrolysis of chylomicron-TAG, could be measured from 60 minutes onward until the end of the postprandial period (Figure 2B). This was greater than the net flux of [$^2\text{H}_2$]-palmitate in TAG, reflecting VLDL-TAG extraction. There was no relationship between fasting plasma ANGPTL4 and baseline VLDL-TAG flux ($r = -0.045$; $p = 0.717$) or the postprandial plasma ANGPTL4 concentrations and the SFA meal-induced VLDL-TAG flux ($\text{AUC}_{0-4\text{h}}$, $r = 0.117$; $p = 0.373$) and chylomicron-TAG flux ($\text{AUC}_{0-4\text{h}}$, $r = -0.186$; $p = 0.139$). No gender-related differences were observed in plasma ANGPTL4 concentrations, VLDL-TAG flux, and chylomicron-TAG flux or in the associations between plasma ANGPTL4 and skeletal muscle lipid handling. Moreover, when we divided the group into tertiles of [$^2\text{H}_2$]-palmitate and [$\text{U-}^{13}\text{C}$]-palmitate skeletal muscle TAG extraction, no differences were observed between postprandial plasma ANGPTL4 concentrations (ANOVA $p = 0.484$ and $p = 0.393$, respectively). These data suggest that plasma ANGPTL4 concentration does not play a major role in skeletal muscle fatty acid handling in humans.

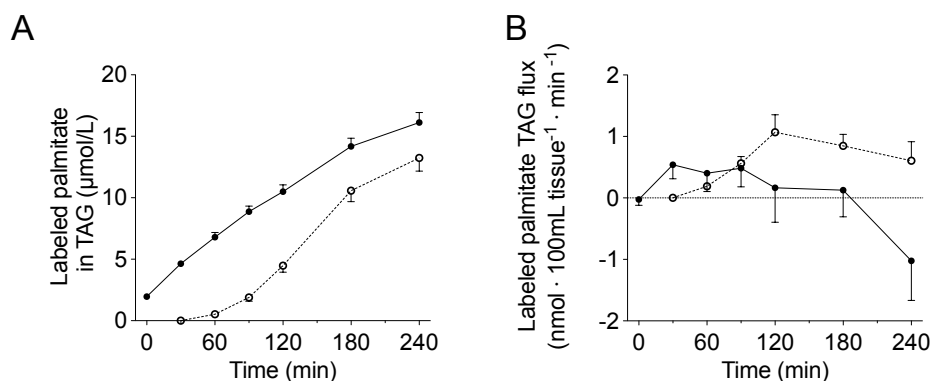


Figure 2. (A) [²H₂]- and [U-¹³C]-palmitate concentrations in the plasma TAG fraction (B) Net flux of [²H₂]- and [U-¹³C]-palmitate TAG across forearm muscle during fasting (*t* = 0) and after consumption of a high-SFA meal. Black symbols, [²H₂]-palmitate, *n* = 61; white symbols, dotted line [U-¹³C]-palmitate, *n* = 66. Values are mean ± SEM.

Dietary fat quality affects plasma ANGPTL4

Next, we investigated the effect of dietary fat quality on fasting and postprandial ANGPTL4 concentrations and examined postprandial fatty acid handling after meals varying in dietary fat quality (18). In 10 obese insulin-resistant participants (characteristics can be found in Table 1), plasma ANGPTL4 concentrations decreased after intake of the high-SFA meal (baseline, 5.6 ± 0.6 ng/mL; *vs* 4 hours after meal intake, 4.3 ± 0.4 ng/mL). This decrease was also evident after the high-MUFA meal (5.4 ± 0.8 *vs* 4.8 ± 0.6 ng/mL) but not after a high-PUFA meal in the mid postprandial phase (5.1 ± 0.7 *vs* 5.3 ± 0.6 ng/mL; Δ_{2-4h} *p* = 0.035) (Figure 3). Strikingly, the blunted decrease in plasma ANGPTL4 after the high-PUFA meal was accompanied by a lower TAG-derived fatty acid uptake (18).

ANGPTL4 is released from human skeletal muscle

Finally, it has been postulated that skeletal muscle may be a source of plasma ANGPTL4 in humans (21). In the present study, no significant release of ANGPTL4 from skeletal muscle after an overnight fast was found. Importantly, however, significant ANGPTL4 release from skeletal muscle was detectable after a high-SFA meal (AUC_{0-4h} ; -0.17 ± 0.09 ng · 100 mL tissue⁻¹ · min⁻¹ *vs* zero flux; *p* = 0.048) (Figure 1B), with the highest release observed in the mid postprandial phase (AUC_{2-4h} ; -0.26 ± 0.13 ng · 100 mL tissue⁻¹ · min⁻¹ *vs* zero flux; *p* = 0.045) (Figure 1B). No relationship was found between skeletal muscle ANGPTL4 release

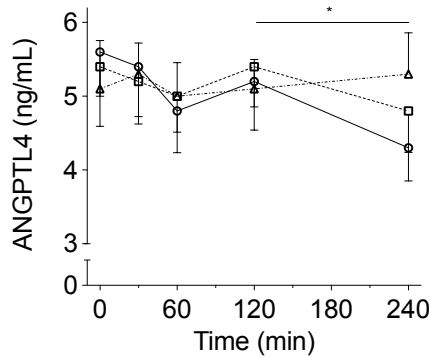


Figure 3. ANGPTL4 concentrations during fasting ($t = 0$) and after consumption of three high-fat mixed meals. Circles, SFA; squares, MUFA; triangles, PUFA. Values are mean \pm SEM, $n = 10$. *, Δ 2–4 h; $p < 0.05$.

and postprandial insulin sensitivity ($AUC_{\text{glucose}} \cdot AUC_{\text{insulin}} \cdot 10^{-6}$) ($p = 0.127$), BMI ($p = 0.568$), or waist-hip ratio ($p = 0.484$). Similarly, when we divided the group into tertiles of [$^2\text{H}_2$]-palmitate and [$\text{U-}^{13}\text{C}$]-palmitate TAG extraction, no differences were observed in skeletal muscle ANGPTL4 release (ANOVA $p = 0.944$ and $p = 0.895$, respectively).

Discussion

This study demonstrates that during fasting conditions and after intake of the high-SFA meal, plasma ANGPTL4 concentrations were not associated with *in vivo* skeletal muscle LPL activity. Moreover, under physiological postprandial conditions, we found that plasma ANGPTL4 was markedly decreased after a high-SFA and high-MUFA meal, but not after a high-PUFA meal. Strikingly, this was accompanied by a differential skeletal muscle fatty acid handling, with a reduced TAG extraction after the high-PUFA meal compared with the high-SFA meal, as reported previously (18). Finally, we demonstrate for the first time that ANGPTL4 was released from skeletal muscle into the circulation after high-SFA meal ingestion in humans.

Little is known about the determinants of plasma ANGPTL4. So far, a clear, positive relationship with plasma FFA has been shown (12,14,15,20). Here, we demonstrate in a group of participants with a broad range in BMI and insulin sensitivity that fasting plasma ANGPTL4 is positively related to waist-hip ratio and circulating FFA concentrations.

Furthermore, it is negatively related to circulating TAG and glucose concentrations. Overall, our data indicate that plasma ANGPTL4 is increased in participants with an attenuated metabolic health profile. Indeed, the progression of the metabolic syndrome toward type 2 diabetes mellitus has also been shown to increase plasma ANGPTL4 (28). Strikingly, after a high-SFA meal, plasma FFAs are the main determinants of circulating ANGPTL4 independently of insulin, glucose, and TAG, suggesting that FFAs are a key regulator of plasma ANGPTL4 in the postprandial state.

Our current understanding of the physiological role of ANGPTL4 indicates an important role in tissue-specific LPL inhibition (19). Nevertheless, we could not show a relationship between circulating ANGPTL4 and *in vivo* skeletal muscle LPL activity during fasting conditions and after a high-SFA meal. Importantly, these findings do not exclude the possibility that ANGPTL4 is a local regulator of LPL activity via autocrine and paracrine actions and that plasma ANGPTL4 does not reflect local effects in skeletal muscle on LPL activity. Indeed, tissue-specific LPL activity regulation by ANGPTL4 has been shown to take place in the subendothelial space rather than at the endothelium (7,29). In line with these findings, it was proposed that newly synthesized and locally produced ANGPTL4 might have more pronounced effects on LPL activity than circulating ANGPTL4 (30). Furthermore, data from a genetic study with carriers of a rare plasma TAG-lowering genetic variant of ANGPTL4, T266M, also suggest that ANGPTL4 at the cell surface rather than circulating ANGPTL4 might play a role in LPL inhibition (31). T266M carriers have significantly higher plasma ANGPTL4 concentrations compared with T266 carriers, and *in vitro* studies with cultured human cells have shown higher secretion of ANGPTL4 T266M compared with wild type. Nevertheless, this ANGPTL4 variant was not associated with plasma TAG (31). These data suggest that locally produced ANGPTL4 rather than plasma ANGPTL4 may mediate LPL inhibition after a high-SFA meal. Moreover, it is important to note that additional factors are involved in functional ANGPTL4 activity. For instance, it has been suggested that the locally produced glycosylphosphatidylinositol-anchored high-density lipoprotein-binding protein might compete with ANGPTL4 for LPL binding at either the interstitial space or capillary lumen (19). This indicates that there is a complex regulation of *in vivo* LPL activity with many factors involved. However, the exact inter-relationships remain unclear and need to be investigated in more detail in future research.

In addition to plasma FFA, dietary fat quality might be an important determinant of circulating ANGPTL4. Indeed, the present study found that a high-PUFA meal blunted the postprandial ANGPTL4 decline that was observed after a meal rich in SFAs or MUFAs.

This was accompanied by a reduced muscle TAG-derived fatty acid uptake (18). It is tempting to speculate from these data that ANGPTL4 may play a role in the reduced muscle LPL activity after a high-PUFA meal. For instance, it has been shown that incubation of murine C2C12 myocytes and human myotubes with long-chain fatty acids induced ANGPTL4 expression in a peroxisomal proliferator activator receptor- δ -dependent manner (14,16). Furthermore, infusion of lipid emulsions high in MUFA or PUFA under hyperinsulinemic conditions blunted the insulin-mediated ANGPTL4 decrease *in vivo* in humans (10). Our findings, together with earlier observations, indicate that meal fatty acid composition affects plasma ANGPTL4. However, it remains to be elucidated whether or not this finding is important in the previously reported differential effect of dietary fat quality on TAG-derived fatty acid uptake in skeletal muscle *in vivo*.

The contribution of different tissues to circulating ANGPTL4 concentration remains to be established. In the present study, we provide for the first time direct *in vivo* evidence that skeletal muscle contributes to plasma ANGPTL4 in humans. Furthermore, it has been shown that basal *in vitro* ANGPTL4 expression in human myotubes correlated positively with plasma ANGPTL4 of the donors (14). Moreover, increased skeletal muscle ANGPTL4 mRNA and protein expression and plasma ANGPTL4 have been shown after acute exercise in humans, indirectly suggesting the contribution of skeletal muscle to circulating ANGPTL4 (16,21). In our study, the relative muscle-derived contribution to circulating concentrations is difficult to estimate because information on ANGPTL4 half-life is lacking. The fact that plasma ANGPTL4 concentrations decline postprandially might indicate that skeletal muscle is not the major organ regulating postprandial plasma ANGPTL4 concentrations. In addition, we could not detect a significant release under fasting conditions, suggesting that other tissues such as adipose tissue, liver, and intestine contribute more to fasting plasma ANGPTL4 concentrations (16,19).

This study had some limitations. First, mainly overweight and obese people and/or subjects with impaired glucose metabolism were included. Therefore, we cannot exclude that the lack of association between *in vivo* LPL activity and plasma ANGPTL4 may have been due to the absence of a healthy, lean, normoglycemic group. Nevertheless, the participants covered a broad range in insulin sensitivity (HOMA-IR, 1.7 – 11.3) and adiposity (BMI, 22.7 – 39.5 kg/m²). Consequently, the present analyses do not support a major contribution of plasma ANGPTL4 concentration in the regulation of *in vivo* skeletal muscle LPL activity in humans. Secondly, we cannot exclude that plasma ANGPTL4 does not accurately reflect functional ANGPTL4. Moreover, we have measured full-length ANGPTL4 and the

C-terminal truncated fragment, but not the N-terminal truncated fragment of ANGPTL4 (20). However, both the full-length and the N-terminal fragment ANGPTL4 may influence plasma lipid concentration (32). Therefore, it remains to be established whether the results from the ANGPTL4 ELISA provide accurate reflection of functional ANGPTL4. Finally, in our forearm model, we cannot exclude the possibility that inter- and intramuscular fat contamination may have contributed to muscle-derived ANGPTL4 release in this study population (33) or the contribution of other cell types such as endothelial cells (34) and macrophages (28). However, previous data indicate that adipose tissue contribution is minor (27).

In conclusion, we provide *in vivo* evidence that ANGPTL4 is secreted from the human skeletal muscle after a high-SFA meal. Furthermore, our data indicate that plasma ANGPTL4 concentrations do not relate to *in vivo* muscle LPL activity after a high-SFA meal. However, we cannot exclude the possibility that ANGPTL4 could play a local role in skeletal muscle lipid metabolism in insulin resistant conditions after a high-SFA meal. Finally, we show a postprandial decline in plasma ANGPTL4 after a high-SFA and high-MUFA meal, but not after a high-PUFA meal, suggesting that dietary fat quality might regulate short-term muscle lipid handling via ANGPTL4. However, the mechanisms involved in the regulation of *in vivo* skeletal muscle LPL activity need to be investigated in more detail in future studies.

References

1. Stinkens R, Goossens GH, Jocken JWE, Blaak EE. Targeting fatty acid metabolism to improve glucose metabolism. *Obes Rev*. 2015;16:715–57.
2. Wang H, Eckel RH. Lipoprotein lipase: from gene to obesity. *Am J Physiol Endocrinol Metab*. 2009;297:E271–88.
3. Goldberg IJ, Merkel M. Lipoprotein lipase: physiology, biochemistry, and molecular biology. *Front Biosci*. 2001;6:D388–405.
4. Miles JM, Park YS, Walewicz D, Russell-Lopez C, Windsor S, Isley WL, et al. Systemic and forearm triglyceride metabolism: fate of lipoprotein lipase-generated glycerol and free fatty acids. *Diabetes*. 2004;53:521–7.
5. Bickerton AST, Roberts R, Fielding BA, Hodson L, Blaak EE, Wagenmakers AJM, et al. Preferential uptake of dietary Fatty acids in adipose tissue and muscle in the postprandial period. *Diabetes*. 2007;56:168–76.
6. Kersten S. Physiological regulation of lipoprotein lipase. *Biochim Biophys Acta*. 2014;1841:919–33.
7. Sukonina V, Lookene A, Olivecrona T, Olivecrona G. Angiopoietin-like protein 4 converts lipoprotein lipase to inactive monomers and modulates lipase activity in adipose tissue. *Proc Natl Acad Sci USA*. 2006;103:17450–5.
8. Yau M-H, Wang Y, Lam KSL, Zhang J, Wu D, Xu A. A highly conserved motif within the NH₂-terminal coiled-coil domain of angiopoietin-like protein 4 confers its inhibitory effects on lipoprotein lipase by disrupting the enzyme dimerization. *J Biol Chem*. 2009;284:11942–52.
9. Lichtenstein L, Mattijssen F, de Wit NJ, Georgiadi A, Hooiveld GJ, van der Meer R, et al. Angptl4 protects against severe proinflammatory effects of saturated fat by inhibiting fatty acid uptake into mesenteric lymph node macrophages. *Cell Metab*. 2010;12:580–92.
10. Brands M, Sauerwein HP, Ackermans MT, Kersten S, Serlie MJ. Omega-3 long-chain fatty acids strongly induce angiopoietin-like 4 in humans. *J Lipid Res*. 2013;54:615–21.
11. Ruge T, Sukonina V, Kroupa O, Makoveichuk E, Lundgren M, Svensson MK, et al. Effects of hyperinsulinemia on lipoprotein lipase, angiopoietin-like protein 4, and glycosylphosphatidylinositol-anchored high-density lipoprotein binding protein 1 in subjects with and without type 2 diabetes mellitus. *Metab Clin Exp*. 2012;61:652–60.
12. Kersten S, Lichtenstein L, Steenbergen E, Mudde K, Hendriks HFJ, Hesselink MK, et al. Caloric restriction and exercise increase plasma ANGPTL4 levels in humans via elevated free fatty acids. *Arterioscler Thromb Vasc Biol*. 2009;29:969–74.
13. van Raalte DH, Brands M, Serlie MJ, Mudde K, Stienstra R, Sauerwein HP, et al. Angiopoietin-like protein 4 is differentially regulated by glucocorticoids and insulin in vitro and in vivo in healthy humans. *Exp Clin Endocrinol Diabetes*. 2012;120:598–603.
14. Staiger H, Haas C, Machann J, Werner R, Weisser M, Schick F, et al. Muscle-derived angiopoietin-like protein 4 is induced by fatty acids via peroxisome proliferator-activated receptor (PPAR)-delta and is of metabolic relevance in humans. *Diabetes*. 2009;58:579–89.
15. Robciuc MR, Skrobuk P, Anisimov A, Olkkonen VM, Alitalo K, Eckel RH, et al. Angiopoietin-Like 4 Mediates PPAR Delta Effect on Lipoprotein Lipase-Dependent Fatty Acid Uptake but Not on Beta-Oxidation in Myotubes. Müller M, editor. *PLoS ONE*. 2012;7:e46212.

16. Norheim F, Hjorth M, Langleite TM, Lee S, Holen T, Bindesbøll C, et al. Regulation of angiopoietin-like protein 4 production during and after exercise. *Physiol Rep*. 2014;2.
17. González-Muniesa P, de Oliveira C, Pérez de Heredia F, Thompson MP, Trayhurn P. Fatty Acids and Hypoxia Stimulate the Expression and Secretion of the Adipokine ANGPTL4 (Angiopoietin-Like Protein 4/ Fasting-Induced Adipose Factor) by Human Adipocytes. *J Nutrigenet Nutrigenomics*. 2011;4:146–53.
18. Jans A, Konings E, Goossens GH, Bouwman FG, Moors CC, Boekschoten MV, et al. PUFAs acutely affect triacylglycerol-derived skeletal muscle fatty acid uptake and increase postprandial insulin sensitivity. *Am J Clin Nutr*. 2012;95:825–36.
19. Dijk W, Kersten S. Regulation of lipoprotein lipase by Angptl4. *Trends Endocrinol Metab*. 2014;25:146–55.
20. Jonker JT, Smit JW, Hammer S, Snel M, van der Meer RW, Lamb HJ, et al. Dietary modulation of plasma angiopoietin-like protein 4 concentrations in healthy volunteers and in patients with type 2 diabetes. *Am J Clin Nutr*. 2013;97:255–60.
21. Catoire M, Alex S, Paraskevopoulos N, Mattijssen F, Evers-van Gogh I, Schaart G, et al. Fatty acid-inducible ANGPTL4 governs lipid metabolic response to exercise. *Proc Natl Acad Sci USA*. 2014;111:E1043–52.
22. Köster A, Chao YB, Mosior M, Ford A, Gonzalez-DeWhitt PA, Hale JE, et al. Transgenic angiopoietin-like (angptl)4 overexpression and targeted disruption of angptl4 and angptl3: regulation of triglyceride metabolism. *Endocrinology*. 2005;146:4943–50.
23. Kersten S, Mandard S, Tan NS, Escher P, Metzger D, Chambon P, et al. Characterization of the fasting-induced adipose factor FIAF, a novel peroxisome proliferator-activated receptor target gene. *J Biol Chem*. 2000;275:28488–93.
24. Alex S, Lichtenstein L, Dijk W, Mensink RP, Tan NS, Kersten S. ANGPTL4 is produced by entero-endocrine cells in the human intestinal tract. *Histochem Cell Biol*. 2013;141:383–91.
25. van Hees AMJ, Jans A, Hul GB, Roche HM, Saris WHM, Blaak EE. Skeletal muscle fatty acid handling in insulin resistant men. *Obesity*. 2011;19:1350–9.
26. Moors CCM, van der Zijl NJ, Diamant M, Blaak EE, Goossens GH. Impaired insulin sensitivity is accompanied by disturbances in skeletal muscle fatty acid handling in subjects with impaired glucose metabolism. *Int J Obes (Lond)*. 2012;36:709–17.
27. Blaak EE, van Baak MA, Kemerink GJ. Beta-adrenergic stimulation of energy expenditure and forearm skeletal muscle metabolism in lean and obese men. 1994.
28. Tjeerdema N, Georgiadi A, Jonker JT, van Glabbeek M, Alizadeh Dehnavi R, Tamsma JT, et al. Inflammation increases plasma angiopoietin-like protein 4 in patients with the metabolic syndrome and type 2 diabetes. *BMJ Open Diabetes Res Care*. 2014;2:e000034–4.
29. Makoveichuk E, Vorrjö E, Olivecrona T, Olivecrona G. Inactivation of lipoprotein lipase in 3T3-L1 adipocytes by angiopoietin-like protein 4 requires that both proteins have reached the cell surface. *Biochim Biophys Acta*. 2013;441:941–6.
30. Nilsson SK, Anderson F, Ericsson M, Larsson M, Makoveichuk E, Lookene A, et al. Triacylglycerol-rich lipoproteins protect lipoprotein lipase from inactivation by ANGPTL3 and ANGPTL4. *Biochim Biophys Acta*. 2012;1821:1370–8.
31. Smart-Halajko MC, Robciuc MR, Cooper JA, Jauhainen M, Kumari M, Kivimäki M, et al. The relationship between plasma angiopoietin-like protein 4 levels, angiopoietin-like protein 4 genotype, and coronary heart disease risk. *Arterioscler Thromb Vasc Biol*. 2010;30:2277–82.

32. Yin W, Romeo S, Chang S, Grishin NV, Hobbs HH, Cohen JC. Genetic variation in ANGPTL4 provides insights into protein processing and function. *J Biol Chem*. 2009;284:13213–22.
33. Guo Z, Jensen MD. Arterio-venous balance studies of skeletal muscle fatty acid metabolism: what can we believe? *Am J Physiol Endocrinol Metab*. 2013;305:E925–30.
34. Zheng Z, Cheng C, Dong R, Li M, Xiong Z, Qian X, et al. Advanced glycation end products upregulate angiopoietin-like protein 4 expression by activating the renin-angiotensin system in endothelial cells. *Biomed Rep*. 2015;:1–5.

Chapter 4

Effect of diet-induced weight loss on Angiopoietin-like protein 4 and adipose tissue lipid metabolism in overweight and obese humans

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Submitted

Chapter 5

Plasma lipid profiling of tissue-specific insulin resistance in human obesity

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Chapter 6

Abdominal subcutaneous adipose tissue gene expression in relation to tissue-specific insulin resistance in human obesity

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In Preparation

This chapter is embargoed at request

Chapter 7

General Discussion

EMBARGOED



Addendum

Summary

Samenvatting

Valorization

Dankwoord

List of publications

About the author



Summary

Over the last decades, the prevalence of obesity has reached epidemic proportions and obesity and its metabolic consequences are major contributors to morbidity and mortality worldwide. Obesity is strongly linked to the development of insulin resistance, which in turn is a major risk factor in the development of cardiometabolic diseases. Insulin resistance can develop simultaneously in multiple organs and severity may vary between organs. Over the years, it has been convincingly shown that there is a complex interplay between metabolic insulin sensitive organs involved in lipid metabolism, such as adipose tissue, skeletal muscle and liver. In addition, impairments in lipid metabolism play an important role in the development and progression of insulin resistance. The present thesis therefore aimed to study multiple aspects of lipid metabolism in relation to whole-body and tissue-specific insulin resistance using a physiological approach.

In **chapter 2** we studied the contribution of endogenous and dietary fatty acid sources to skeletal muscle uptake and storage in overweight or obese individuals with a wide range of insulin resistance. To study the metabolic fate of dietary versus endogenous fatty acids, a dual stable isotope technique was applied. [U-¹³C]-palmitate was added to a high-saturated fatty acid mixed meal to label chylomicron-triacylglycerol (TAG) (i.e. exogenous or dietary TAG) in the circulation. Simultaneously, [²H₂]-palmitate was infused intravenously to label endogenous fatty acids. As [²H₂]-palmitate can be incorporated into newly synthesized TAG in the liver, [²H₂]-labelled TAG reflects very-low-density-lipoprotein (VLDL)-TAG (i.e. endogenous TAG) in the circulation. This stable isotope technique was combined with measurements of arterio-venous concentration differences across the forearm muscle to investigate skeletal muscle fatty acid handling and the specific contribution of the different fat sources to skeletal muscle fatty acid handling. We showed that insulin resistance is associated with increased postprandial VLDL-TAG extraction across the forearm muscle, despite a similar supply of TAG. In addition, distinct patterns of lipid composition were observed in the skeletal muscle lipid pools in insulin resistance, as reflected by an increased saturation of the intramyocellular non-esterified fatty acid pool. Ultimately, the elevated TAG extraction and an altered intramuscular lipid composition may lead to skeletal muscle insulin resistance, but exact underlying mechanisms remain to be defined.

In **chapter 3** and **4**, we investigated the role of plasma angiopoietin-like protein 4 (ANGPTL4) in skeletal muscle and in abdominal subcutaneous adipose tissue lipoprotein lipase (LPL) activity as reflected by *in vivo* TAG extraction across these tissues.

Chylomicron- and VLDL-TAG are hydrolyzed by LPL in the process of intravascular lipolysis and subsequently fatty acids are extracted by underlying tissues. Insulin is an important regulator of LPL activity, but a considerable part of the variation in LPL activity may also be explained by other factors. The LPL inhibitor ANGPTL4 is an interesting candidate and might be involved skeletal muscle and adipose tissue TAG extraction. In **chapter 3** and **4** we show that plasma ANGPTL4 concentrations were not associated with TAG extraction (e.g. a measure for *in vivo* LPL activity) across the forearm skeletal muscle and abdominal subcutaneous adipose tissue. Interestingly, the data we additionally present in **chapter 4**, indicated that plasma ANGPTL4 might play a role in adipose tissue intracellular lipolysis after weight loss and therefore possibly in lipid partitioning after weight loss. Finally, we provided for the first time *in vivo* evidence that ANGPTL4 is secreted from the human skeletal muscle and adipose tissue after a high-saturated fatty acid mixed meal (**chapter 3** and **4**).

In **chapter 5** and **6**, we acquired more information on distinct insulin resistant phenotypes, which is necessary to develop intervention strategies targeting different tissue-related or prediabetic phenotypes. Tissue-specific insulin resistance was estimated by using a 2 hours oral glucose tolerance test, with glucose and insulin measurements at five time-points. We included cross-sectional data from the Diet, Obesity and Genes (DiOGenes) project, which is a large, multicenter, randomized, controlled dietary intervention study and involved eight European countries. In **chapter 6**, we additionally included individuals from the Cohort on Diabetes and Atherosclerosis Maastricht (CODAM). Both cohorts comprised overweight and obese non-diabetic individuals who are at risk for developing cardiometabolic diseases.

In **chapter 5**, we investigated cross-sectional associations of tissue-specific insulin resistance with the plasma lipidome. 140 plasma lipids were quantified by liquid chromatography–mass spectrometry. We showed a positive association between muscle insulin sensitivity and plasma lysophosphatidylcholine levels in both sexes. Furthermore, we identified sex differences in the associations between hepatic insulin resistance and the plasma lipidome. Hepatic insulin resistance was higher in men compared to women. However, in women an increase of hepatic insulin resistance was associated with an increase in plasma TAG and diacylglycerol and a decrease in the relative abundance of odd-chain and very-long-chain TAG. In contrast, the degree of hepatic insulin resistance did not relate to alterations in the plasma lipidome in men. Combined with the observation that overweight/obese women had less hepatic insulin resistance and lower TAG levels than men, this would suggest that

the plasma lipidome may be more responsive to worsening of hepatic insulin resistance in women, or vice versa.

In **chapter 6** we further characterized tissue-specific insulin resistance phenotypes by studying the abdominal subcutaneous adipose tissue transcriptome by means of RNA sequencing. In individuals with primarily hepatic insulin resistance, extracellular matrix remodeling genes (e.g. collagens) were significantly upregulated, whilst in individuals with primarily muscle insulin resistance, genes related to inflammation (e.g. chemokines and complement activation) were significantly upregulated in abdominal subcutaneous adipose tissue. Subsequent analyses in the CODAM cohort showed that an increased systemic inflammatory profile comprised of eight plasma inflammatory markers may be specifically related to muscle insulin resistance. Based on these findings, we hypothesize that increased abdominal subcutaneous adipose tissue inflammatory gene expression in the muscle insulin resistant phenotype may translate into an increased systemic inflammatory profile, putatively linking subcutaneous adipose tissue inflammation to muscle insulin resistance.

In conclusion, the results from this thesis show distinct metabolic profiles in non-diabetic overweight and obese individuals in relation to (tissue-specific) insulin resistant phenotypes. We showed that insulin resistance is associated with increased postprandial VLDL-TAG extraction across the forearm muscle, despite a similar supply of TAG. As TAG extraction is mediated by LPL in skeletal muscle and adipose tissue, we showed that these results could not be explained by plasma levels of the LPL inhibitor ANGPTL4. In addition, a distinct lipid composition was observed in the skeletal muscle non-esterified fatty acid pool in insulin resistance, which were mainly related to an increased saturation of the intramyocellular non-esterified fatty acid pool. Finally, we showed distinct plasma lipidome and abdominal subcutaneous adipose tissue transcriptome profiles in overweight and obese individuals in relation to tissue-specific insulin resistance phenotypes. These unique metabolic profiles require further mechanistic exploration and may serve as starting point for developing intervention strategies targeting different tissue-related or prediabetic phenotypes.



Samenvatting

De prevalentie van obesitas heeft epidemische vormen aangenomen in de afgelopen decennia. Wereldwijd dragen obesitas en de metabole gevolgen ervan op grote schaal bij aan morbiditeit en mortaliteit. Obesitas gaat vaak gepaard met de ontwikkeling van insulineresistentie, wat op zijn beurt weer een grote risicofactor is voor de ontwikkeling van cardiometabole ziekten. Insulineresistentie kan zich in meerdere organen ontwikkelen en de ernst ervan kan variëren tussen verschillende organen. In de afgelopen jaren is het overtuigend bewezen dat, als het gaat om de vetstofwisseling, er een complexe samenwerking is tussen de verschillende metabole insulinegevoelige organen zoals vetweefsel, skeletspier en lever. Vetstofwisseling speelt dus een belangrijke rol in de ontwikkeling en het verloop van insulineresistentie. Het doel van het huidige proefschrift was daarom om meerdere aspecten van de vetstofwisseling in relatie tot insulineresistentie in het gehele lichaam of orgaanspecifiek te bestuderen. Daarbij werd gebruikt gemaakt van een fysiologische benadering.

In **hoofdstuk 2** onderzochten we *in vivo* de afzonderlijke bijdrage van vetten afkomstig van voeding (exogene vetten; chylomicronen) en vetten reeds aanwezig in het lichaam (endogene vetten; vrije vetzuren en vetten in de vorm van VLDL-deeltjes) aan de vetopname en opslag in de skeletspier bij personen met overgewicht of obesitas met een brede spreiding in insulineresistentie. Hiertoe gebruikten we twee verschillende stabiele isotopen van het vetzuur palmitaat, in combinatie met metingen van arterieel-veneuze concentratieverschillen over de onderarmspier. We voegden [U-¹³C]-palmitaat toe aan een vetrijke milkshake met veel verzadigde vetten om chylomicronen te kunnen traceren. Tegelijkertijd infuseerden we [²H₂]-palmitaat in de bloedbaan om vrije vetzuren en vetten in de vorm van VLDL-deeltjes te kunnen traceren. We vonden dat insulineresistentie was geassocieerd met een verhoogde extractie van VLDL-triacylglycerol (TAG) over de skeletspier bij insulineresistente personen, ondanks gelijke circulerende TAG-concentraties. De absolute hoeveelheid en de incorporatie van voedingsvetten in lipidenfracties van de spier (TAG, diacylglycerol, vrije vetzuren en fosfolipiden) was niet verschillend. Wel toonden we aan dat de vrije vetzuren fractie in de spier van insulineresistente personen een hoger gehalte aan verzadigde vetzuren bevatte. Deze data suggereren dat een verhoogde extractie van VLDL-TAG door de skeletspier na een maaltijd en een verhoogde verzadiging van de vrije vetzuren fractie in de spier geassocieerd zijn met insulineresistentie. Uiteindelijk kunnen deze verhoogde extractie van VLDL-TAG en veranderingen in samenstelling van lipidenfracties bijdragen aan de ontwikkeling van skeletspier insulineresistentie, maar de precieze onderliggende mechanismen moeten nog verder onderzocht worden.

In **hoofdstuk 3** en **4** onderzochten we de rol van het eiwit angiopoietin-like protein 4 (ANGPTL4) in lipoproteïne lipase (LPL) activiteit in de skeletspier en het abdominale onderhuidse vetweefsel. Vetten in de vorm van chylomicronen en VLDL-deeltjes worden gehydrolyseerd door het eiwit LPL in de intravasculaire ruimte en de vrijgekomen vetzuren kunnen worden geëxtraheerd door de onderliggende weefsels (TAG-extractie). Insuline is een belangrijke reguleerder van LPL activiteit, maar een deel van de variatie in TAG-extractie kan mogelijk ook verklaard worden door andere factoren. De LPL-remmer ANGPTL4 is daarom een interessante kandidaat en mogelijk betrokken bij de TAG-extractie in skeletspier en vetweefsel. In **hoofdstuk 3** en **4** laten we zien dat ANGPTL4-concentraties in het plasma niet gerelateerd zijn aan TAG-extractie over de skeletspier en het abdominale onderhuidse vetweefsel. Aanvullend laten onze data van **hoofdstuk 4** zien dat ANGPTL4 in het plasma mogelijk gerelateerd is aan intracellulaire lipolyse in het onderhuidse vetweefsel na gewichtsverlies. Eventueel kan dit erop wijzen dat ANGPTL4 een rol speelt in de verdeling van vetten over het lichaam na gewichtsverlies. Tot slot tonen we in **hoofdstuk 3** en **4** voor de eerste keer aan dat ANGPTL4 wordt uitgescheiden door de skeletspier en onderhuidse vetweefsel na een vette maaltijd met veel verzadigde vetten.

In **hoofdstuk 5** en **6** bestudeerden we verschillende weefselspecifieke insulineresistente fenotypes in meer detail. We bepaalden weefselspecifieke insulineresistentie met behulp van de orale glucosetolerantietest (OGTT), waarbij we gedurende twee uur vijf keer plasma glucose en insuline concentraties hebben gemeten. We maakten gebruik van cross-sectionele data van het 'Diet, Obesity and Genes' (DiOGenes) project. Dit is een groot, gerandomiseerd en gecontroleerd dieet interventie onderzoek, uitgevoerd in acht Europese landen. In **hoofdstuk 6** maakten we aanvullend ook gebruik van cross-sectionele data van het 'Cohort on Diabetes and Atherosclerosis Maastricht' (CODAM) project. Belangrijk om te vermelden is dat beide cohorten bestaan uit personen met overgewicht en obesitas zonder diabetes, maar een verhoogd risico hebben op het ontwikkelen van cardiometabole ziekten.

In **hoofdstuk 5** analyseerden we cross-sectionele associaties tussen weefselspecifieke insulineresistentie en het circulerende lipidenprofiel. 140 lipiden werden gekwantificeerd met behulp van vloeistofchromatografie met tandem massaspectrometrie (LC-MS). We toonden aan dat er een positieve relatie is tussen spier insulinegevoeligheid en lysophosphatidylcholine concentraties. Verder lieten we zien dat er geslachtsverschillen zijn in de associaties tussen lever insulineresistentie en het circulerende lipidenprofiel. Lever insulineresistentie was hoger in mannen dan in vrouwen. Maar we vonden bij vrouwen een relatie tussen een slechtere lever insulineresistentie en verhoogde concentraties van plasma TAG en diacylglycerol. Bovendien

hadden vrouwen ook een lagere dichtheid van oneven keten vetzuren en zeer lange keten vetzuren in TAG als ze een ongezondere lever insulineresistentie hadden. Daartegenover vonden we in mannen geen relatie tussen de mate van lever insulineresistentie en veranderingen in het lipidenprofiel. Als we deze waarnemingen combineren met de gegevens dat vrouwen met overgewicht of obesitas minder lever insulineresistentie hebben en lagere plasma TAG-concentraties, dan zou dit kunnen suggereren dat het vetprofiel in de circulatie bij vrouwen sneller reageert op een verslechtering van lever insulineresistentie, of vice versa.

In **hoofdstuk 6** hebben we het transcriptoom van het abdominale onderhuidse vetweefsel in weefsel specifieke insulineresistente fenotypes gekarakteriseerd. Dit hebben we gedaan met behulp van *RNA sequencing*. In personen met voornamelijk lever insulineresistentie waren extracellulaire matrix organisatie genen (bijvoorbeeld collagenen) significant opgereguleerd, terwijl genen die gerelateerd zijn aan ontstekingsfactoren waren opgereguleerd in personen met voornamelijk spier insulineresistentie, zoals chemokinen en complement factoren. Vervolganalyses in het CODAM-cohort lieten zien dat een verhoogd systemisch ontstekingsprofiel, bestaande uit acht plasma ontstekingsfactorenmarkers, mogelijk specifiek gerelateerd is aan spier insulineresistentie. Daarom hebben we de hypothese opgesteld dat een verhoogde genexpressie van ontstekingsfactoren in het abdominale onderhuidse vetweefsel kan leiden tot een verhoogd systemisch ontstekingsprofiel in het spier insulineresistentie fenotype. Dit kan eventueel de verbinding zijn tussen ontstekingen in het abdominale onderhuidse vetweefsel en spier insulineresistentie.

Concluderend kunnen we zeggen, op basis van de resultaten beschreven in dit proefschrift, dat er duidelijke onderscheidende metabole profielen zijn in (weefsel specifieke) insulineresistentie in personen met overgewicht en obesitas. We toonden aan dat insulineresistentie is geassocieerd met een verhoogde VLDL-TAG-extractie over de onderarmspier na een maaltijd, ondanks gelijke TAG-concentraties in de circulatie. Aangezien TAG-extractie door LPL wordt geregeld in de skeletspier en het vetweefsel, lieten we ook zien dat bovenstaande resultaten niet verklaard konden worden door plasmawaarden van de LPL-remmer ANGPTL4. Verder toonden we aan dat de vrije vetzuren fractie in de spier een hoger gehalte aan verzadigde vetzuren bevatte bij insulineresistente personen. Tot slot lieten we zien dat er een onderscheidend plasma lipidoom en abdominale onderhuidse vetweefsel transcriptoom zijn in relatie tot weefsel specifieke insulineresistentie in personen met overgewicht of obesitas. Deze unieke metabole profielen zullen nog verder onderzocht moeten worden, maar kunnen dienen als startpunt voor het ontwikkelen van nieuwe interventiestrategieën voor (weefsel specifieke) insulineresistentie of prediabetes.



Valorization

Knowledge valorization is described by the National Valorization Commission as “the process of creating value from knowledge, by making knowledge suitable and/or available for societal purposes, and suitable for translation into competitive products, services, processes, and new commercial activities”. In this addendum we describe how society may benefit from the work conducted in this thesis.

Our research focused on extending fundamental knowledge about the underlying multiple aspects of lipid metabolism in relation to whole-body and tissue-specific insulin resistance. The main outcomes of the present thesis can serve as starting point for further characterization of different tissue-specific insulin resistant or prediabetic phenotypes. Ultimately, this may provide directions for more targeted and personalized intervention strategies. The valorization potential of these studies will be described in terms of social and economic relevance of obesity research, relevance of measurements for targeted prevention and innovation and translation into practice.

Social and economic relevance of obesity research

The prevalence of obesity has risen to epidemic proportions over the last decades and affects people of all ages and socioeconomic groups (1). More than 39% of the world adult population was overweight (BMI > 25 kg/m²) and about 13% was obese (BMI > 30 kg/m²) in 2014 (2). In 2015, in the Netherlands, over 50% of the individuals over 20 years of age were overweight or obese (3).

The increasing prevalence of obesity is a major health concern. Obesity increases the risk for developing chronic diseases such as type 2 diabetes, cardiovascular diseases, mental disorders, several cancer types and reduces the quality of life. Overweight and obesity are the fifth leading risk for global deaths. 44% of the diabetes burden, 23% of the ischaemic heart disease burden and between 7 – 14% of certain cancers are attributable to overweight and obesity (4). In 2010, overweight and/or obesity was estimated to cause 3.4 million deaths worldwide (5). Additionally, obesity puts an enormous burden on patients, their families, and social health care systems. Therefore, it is important to explore underlying mechanisms that cause obesity and related chronic diseases.

Obesity is linked to the development of insulin resistance, which is a major contributor to hyperglycemia and hyperlipidemia. These are all risk factors towards the development of

type 2 diabetes and cardiovascular diseases. Strikingly, insulin resistance increases the risk for cardiovascular diseases even in the absence of hyperglycemia (6).

From 2011 – 2012, in the USA, the direct cardiovascular disease-related costs were estimated on 193.1 billion US dollars, which reflect e.g. hospital services, prescribed medication and home health care. In addition, indirect cardiovascular disease-related costs, such as loss of future productivity, were estimated to be about 123.5 billion US dollars (7). For diabetes, it has been estimated that the direct annual cost of diabetes to the world is more than 827 billion US dollar (8). Together, for cardiovascular disease and diabetes costs combined, this adds up to more than 1,143 billion US dollars in one year. In 2011, in the Netherlands, the total direct cardiovascular disease-related costs were 8.3 billion euro, which was 9.2% of the total Dutch healthcare costs (89.4 billion euro), while diabetes-related costs were estimated to be 1.7 billion euro (1.9%) (9). Although these numbers cannot only be subscribed to obesity-related cardiometabolic diseases, the expectation is that obesity and thus obesity-related morbidity will continue to increase in the coming years. Therefore, it will have major public health and socioeconomic consequences.

Relevance of measurements for targeted prevention

Recognition of symptoms at an early stage and early treatment of obesity and cardiometabolic diseases is important as it may prevent or delay the development of comorbidities. In addition, quality of life will be maintained or improved if weight and blood glucose concentrations can be well-controlled. However, obesity and cardiometabolic diseases are complex metabolic diseases whereby underlying mechanisms are yet not completely understood. Thus, understanding the etiology of obesity and insulin resistance is a key step in the prevention of cardiometabolic diseases. In this thesis, we focused on expanding fundamental knowledge about lipid metabolism in relation to early stages of insulin resistance. Although the findings may not lead to societal benefits at first glance, they generate improved insight into the pathophysiology of lipid metabolism in relation to whole-body and tissue-specific insulin resistance, which adds fuel for thought on future research initiatives.

The various measurements described in this thesis, e.g. plasma lipidomics and adipose tissue transcriptomics, may be future markers to detect the presence of insulin resistance at an early stage. One key finding in this thesis was that distinct plasma lipid profiles were associated with either muscle insulin sensitivity or hepatic insulin resistance in overweight and obese individuals. As an example: we showed that muscle insulin resistance was associated with lower plasma concentrations of the lipid lysophosphatidylcholine. Future clinical and

mechanistic studies will have to investigate whether monitoring lipid profiles as a risk marker will prove to be clinically useful in the treatment or prevention of cardiometabolic diseases.

Importantly, we also observed distinct plasma lipid profiles for men and women. Hepatic insulin resistance was higher in men compared to women. However, in women worse hepatic insulin resistance was associated with an increase in plasma TAG and diacylglycerol concentrations. This aspect of lipid metabolism in relation to sexes is currently very much underemphasized in research and underscores the importance for sex-specific research as this may improve targeted prevention for the individual sexes.

In addition to fasting lipid metabolism measurements, it is a given that the majority of the population in the Western world spends a significant part of the day in the postprandial state. Increasing evidence suggests that not only fasting lipid, lipoprotein and glucose concentrations, but also a disturbed postprandial lipid or glucose metabolism are important risk markers for cardiometabolic diseases. Postprandial measurements are therefore of clinical importance when cardiometabolic disease risk is studied. In chapter 2, we extended existing knowledge on postprandial lipid metabolism. We showed that insulin resistance is associated with increased postprandial very-low-density-lipoprotein (VLDL) triacylglycerol extraction across the forearm muscle, despite a similar supply of triacylglycerol. In addition, different lipid composition patterns were observed in the skeletal muscle lipid pools in insulin resistance, which were mainly related to an increased saturation of the intramyocellular non-esterified fatty acid pool. Ultimately, these events, e.g. elevated skeletal muscle VLDL-triacylglycerol extraction and changes in intramuscular lipid composition, may give rise to increased muscle storage of detrimental bio-active lipid intermediates. They may affect insulin sensitivity in a negative way and contribute to cardiometabolic complications.

Innovation and translation into practice

The results described in this thesis have and will become available to the scientific community via publication of scientific reports in international peer-reviewed journals in the field of obesity and diabetes. Additionally, results have been presented at (inter)national conferences to scientists as well as physicians, healthcare professionals and dieticians, working in the fields of obesity, diabetes and metabolism.

Changes in lifestyle are considered to play an important role in the etiology of obesity and cardiometabolic diseases. Manipulation of diet and physical activity are the first-choice treatment for these complications. Lifestyle interventions including combined diet and physical activity have been shown to be effective in the prevention of type 2 diabetes as they

reduce diabetes incidence by 47 - 57% over a 3 - 6 year timeframe (10). In addition to lifestyle interventions, pharmacological modulation may also prevent or delay the onset of cardiometabolic complications (11). Healthcare professionals (e.g. dietitians, physiotherapists and physicians) play an important role in stimulating a healthy lifestyle among individuals who are at increased risk of developing obesity and cardiometabolic diseases. Although the results from this thesis do not provide direct guidelines for healthcare professionals, they provide better insight in metabolic parameters that can be targeted through lifestyle and pharmacological interventions.

In chapter 5 and 6, we showed distinct metabolic profiles for muscle or liver insulin resistant conditions, based on the surrogate insulin sensitivity indexes MISI and HIRI. We estimated tissue-specific insulin resistance by using a 2 hours oral glucose tolerance test (OGTT), with glucose and insulin measurements at five time-points. Importantly, both indexes were developed and validated against the two-step gold standard hyperinsulinemic-euglycemic clamp in combination with a glucose tracer (12). In the future, these indexes may provide a relatively easy method to classify and identify tissue-specific insulin resistant subphenotypes as the OGTT is already widely used in the clinic to assess (gestational) diabetes.

In this thesis we revealed distinct metabolic profiles under different tissue-specific insulin resistant conditions, and the exact mechanisms behind this are possibly interesting for the food and pharmaceutical industry to guide development of more personalized targeted nutritional and lifestyle interventions. It should be noted that most associations presented here were based on cross-sectional data and therefore cannot distinguish cause and consequence. But the DiOGenes project is a valuable cohort study for follow-up measurements after diet-induced weight loss and weight regain. Therefore future follow-up of the data after the weight loss intervention may elucidate the temporality of the associations reported or elucidate potential interesting mechanisms. Still, more mechanistic studies are needed to find which pathway(s) need(s) to be targeted to maintain lipid homeostasis and insulin sensitivity.

Notably, key findings of this thesis, i.e. distinct metabolic profiles for muscle or liver insulin resistant conditions, have already served as starting point for developing personalized dietary intervention strategies. Recently, a new research project was started in collaboration with the public-private partnership Top Institute Food and Nutrition (TIFN) to investigate optimal diets to maintain postprandial blood glucose homeostasis in tissue-specific insulin resistant subphenotypes. The ambition of this private-public partnership is to provide the

knowledge base that is needed for more targeted nutritional interventions in the prevention of cardiometabolic diseases and high-impact innovations in food and nutrition. The close collaboration between industry and academia leads to demand-driven research with societal and industrial relevance.

References

1. Ng M, Fleming T, Robinson M, Thomson B, Graetz N, Margono C, et al. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980–2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet*. 2014;384:766–81.
2. World Health Organization. Fact sheet 311: obesity and overweight. <http://www.who.int/mediacentre/factsheets/fs311/en>. 2015
3. GGD'en G. CBS en RIVM. Gezondheidsmonitor GGD'en; 2016.
4. EASO, European Association for Study of Obesity. Obesity Facts & Figures. <https://easo.org/education-portal/obesity-facts-figures/>; Accessed 27 Feb 2018.
5. Lim SS, Vos T, Flaxman AD, Danaei G, Shibuya K, Adair-Rohani H, et al. A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet*. 2012;380:2224–60.
6. Bornfeldt KE, Tabas I. Insulin Resistance, Hyperglycemia, and Atherosclerosis. *Cell Metab*. 2011;14:575–85.
7. Writing Group Members, Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, et al. Heart Disease and Stroke Statistics–2016 Update: A Report From the American Heart Association. *Circulation*. 2016;133:e38–60.
8. World Health Organization. Global report on diabetes. 2016.
9. Rijksinstituut Volksgezondheid en Milieu (RIVM). Kosten van zorg voor diabetes mellitus. <https://www.volksgezondheidenzorg.info/onderwerp/diabetes-mellitus/kosten/kosten>; Accessed 19 Feb 2018.
10. Boer den AT, Herraets IJT, Stegen J, Roumen C, Corpeleijn E, Schaper NC, et al. Prevention of the metabolic syndrome in IGT subjects in a lifestyle intervention: results from the SLIM study. *Nutr Metab Cardiovasc Dis*. 2013;23:1147–53.
11. Pi-Sunyer X, Astrup A, Fujioka K, Greenway F, Halpern A, Krempf M, et al. A Randomized, Controlled Trial of 3.0 mg of Liraglutide in Weight Management. *N Engl J Med*. 2015;373:11–22.
12. Abdul-Ghani MA, Matsuda M, Balas B, DeFronzo RA. Muscle and liver insulin resistance indexes derived from the oral glucose tolerance test. *Diabetes Care*. 2007;30:89–94.



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Birgitta



List of publications

Published papers

- van der Kolk BW, Goossens GH, Jocken JW, Blaak EE. Altered skeletal muscle fatty acid handling is associated with the degree of insulin resistance in overweight and obese humans. *Diabetologia*. 2016;59:2686–96.
- van der Kolk BW, Goossens GH, Jocken JW, Kersten S, Blaak EE. Angiopoietin-Like Protein 4 and Postprandial Skeletal Muscle Lipid Metabolism in Overweight and Obese Prediabetics. *J Clin Endocrinol Metab*. 2016;101:2332–9.
- Vink RG, Roumans NJ, van der Kolk BW, Fazelzadeh P, Boekschoten MV, Mariman EC, et al. Adipose tissue meal-derived fatty acid uptake before and after diet-induced weight loss in adults with overweight and obesity. *Obesity*. 2017;25:1391–9.
- Stinkens R, van der Kolk BW, Jordan J, Jax T, Engeli S, Heise T, et al. The effects of angiotensin receptor neprilysin inhibition by sacubitril/valsartan on adipose tissue transcriptome and protein expression in obese hypertensive patients. *Sci Rep*. 2018;8:3933.

Submitted

- van der Kolk BW, Vogelzangs N, Jocken JW, Valsesia A, Hankemeier T, Astrup A, Saris WH, Arts IC, van Greevenbroek MM, Blaak EE and the DiOGenes consortium. Plasma lipid profiling of tissue-specific insulin resistance in human obesity.
- van der Kolk BW, Vink RG, Jocken JW, Roumans NJ, Goossens GH, Mariman EC, van Baak MA, Blaak EE. Effect of diet-induced weight loss on Angiopoietin-like protein 4 and adipose tissue lipid metabolism in overweight and obese humans.

In preparation

- van der Kolk BW^{*}, Kalafati M^{*}, Adriaens M, van Greevenbroek MM, Saris WH, Astrup A, Valsesia A, Langin D, van der Kallen CJ, Schalkwijk CG, Stehouwer CD, Goossens GH, Arts IC, Jocken JW, Evelo CT[§], Blaak EE[§] and the DiOGenes Consortium. Abdominal subcutaneous adipose tissue gene expression in relation to tissue-specific insulin resistance in human obesity.

^{*}, [§] Authors contributed equally to this work



About the author



Birgitta van der Kolk was born on 23 September 1987 in IJsselmuiden (the Netherlands) and was raised in Kamperveen. In 2005, she obtained her Gymnasium (VWO) diploma from Ichthus College in Kampen. In the same year, she started her studies (Medical) Biology at the Radboud University in Nijmegen. For her masters degree, she performed an internship at the department of Human Genetics, Radboud University Nijmegen, in which she studied the protein network in the Usher syndrome. Subsequently, she did an internship at the Division of Pathophysiology and Repair at Cardiff University in Cardiff (United Kingdom), in which she studied the role of the extracellular calcium-sensing receptor in vascular calcification. She graduated in 2012 with distinction *bene meritum*.

In November 2012, Birgitta started as a research assistant at the department of Human Biology, Maastricht University, under supervision of prof. dr. Ellen Blaak and dr. Gijs Goossens. This is where her love for human physiology grew. In September 2013, she started her PhD project at the department of Human Biology, Maastricht University, under supervision of prof. dr. Ellen Blaak, dr. Johan Jocken and dr. Marleen van Greevenbroek. During her graduation research, she investigated (tissue-specific) insulin resistance in human obesity with a special focus on lipid metabolism, as demonstrated in this thesis and publications in scientific journals. In addition, she presented her research findings at several national and international conferences, including the yearly European Congress on Obesity (ECO) meetings. In 2016, she was nominated for the Foppe ten Hoor Young Investigator award at the Dutch Nutritional Science Days (NSD). In 2017, she was awarded with the Publication Prize from the Netherlands Association for the Study of Obesity (NASO) for the best paper in the field of obesity.

Birgitta will continue her career as a postdoctoral research fellow at the Obesity Research Unit, University of Helsinki in Helsinki (Finland) under supervision of prof. dr. Kirsi Pietiläinen, MD. Here she will study twins who are discordant for obesity.

