Aus dem
Institut für Klinische Diabetologie
des Deutschen Diabetes-Zentrums
Leibniz-Zentrum für Diabetes-Forschung
an der Heinrich-Heine-Universität Düsseldorf
Direktor: Univ. Prof. Dr. Michael Roden

Lipid and energy metabolism of white adipose tissue in insulin-resistant humans

Dissertation

zur Erlangung des Grades eines Doktors der Medizin
der Medizinischen Fakultät der Heinrich-Heine-Universität Düsseldorf

vorgelegt von
Kálmán Benedikt Bódis
2021

Als Inauguraldissertation gedruckt mit Genehmigung der Medizinischen Fakultät der Heinrich-Heine-Universität

gez.:

Dekan: Univ. Prof. Dr. Nikolaj Klöcker

Erstgutachter: Univ. Prof. Dr. Michael Roden

Zweitgutachter: PD. Dr. med. Ralf Westenfeld

Drittgutachter: Prof. Dr. med. Robert Wagner

Widmung

Als Andenken an meinen Vater, für meine Mutter und Schwester.

Teile dieser Arbeit wurden veröffentlicht:

Bódis, K, Kahl S, Simon MC, Zhou Z, Sell H, Knebel B, Tura A, Strassburger K, Burkart V, Mussig K, Markgraf D, Al-Hasani H, Szendroedi J, and Roden M. Reduced expression of stearoyl-CoA desaturase-1, but not free fatty acid receptor 2 or 4 in subcutaneous adipose tissue of patients with newly diagnosed type 2 diabetes mellitus. *Nutrition & diabetes*. 2018;8(1):49.

Bódis, K, Jelenik T, Lundbom J, Markgraf DF, Strom A, Zaharia OP, Karusheva Y, Burkart V, Mussig K, Kupriyanova Y, Ouni M, Wolkersdorfer M, Hwang JH, Ziegler D, Schurmann A, Roden M, and Szendroedi J. Expansion and Impaired Mitochondrial Efficiency of Deep Subcutaneous Adipose Tissue in Recent-Onset Type 2 Diabetes. *The Journal of clinical endocrinology and metabolism.* 2020;105(4).

Zusammenfassung

Diabetes mellitus Typ 2 (T2D) ist durch eine zunehmende periphere Insulinresistenz und eine unzureichende kompensatorische Insulinsekretion charakterisiert. Eine gestörte Funktion des Fettgewebes scheint eine wichtige Rolle in der Entstehung der Insulinresistenz zu spielen. Sowohl die Zunahme der Fettmasse bei Adipositas als auch die Verringerung der Fettmasse bei Lipodystrophie können eine Insulinresistenz bewirken. Beiden Erkrankungen ist die Beeinträchtigung der Lipidspeicherung im Fettgewebe gemeinsam, die zu gesteigerten Lipolyseraten und damit zu erhöhter systemischer Lipidverfügbarkeit und Insulinresistenz in peripheren Geweben führen kann. Es gibt Hinweise aus klinischen Studien und präklinischen Modellen, dass die Rezeptoren für freie Fettsäuren (FFAR) 2 und 4 sowie die Stearoyl-Coenzym A-Desaturase-1 (SCD1), ein Schlüsselenzym der Synthese von Fettsäuren im Fettgewebe, an der Pathogenese von Insulinresistenz und gestörter Beta-Zell-Funktion (BCF) beteiligt sind. Ihre Relevanz für die Entstehung des T2D ist jedoch unklar. Während in der Skelettmuskulatur und der Leber die Störung der Mitochondrien-Funktion zur Insulinresistenz und Abnahme der BCF beiträgt, ist die Rolle des Energiestoffwechsels im weißen Fettgewebe für die Glukosehomöostase nicht geklärt.

Diese Arbeiten hatten zum Ziel, die Relevanz der FFAR2/4, der SCD1 und des Energiestoffwechsels im subkutanen Fettgewebe (SAT) für die Glukosehomöostase bei T2D zu untersuchen. Die Analysen wurden im Rahmen der Deutschen Diabetes-Studie (German Diabetes Study, GDS) durchgeführt, die Menschen mit kürzlich diagnostiziertem Diabetes prospektiv untersucht. Die erste Studie untersuchte die Rolle von FFAR2/4 und SCD1 im SAT für die Insulinsensitivität und BCF in Menschen mit neu diagnostiziertem T2D. Die zweite Studie untersuchte die Relevanz des Energiestoffwechsels in verschiedenen Schichten des SAT für die gewebespezifische Insulinresistenz bei neu diagnostizierten Patienten mit T2D.

Die erste Studie zeigte, dass Personen mit neu diagnostiziertem T2D im Vergleich zu glukosetoleranten Menschen (CON) eine geringere SCD1-, aber unveränderte FFAR2- oder 4-Expression im SAT aufweisen. Jedoch korrelierte nur die FFAR2-Expression negativ mit der BCF in CON und könnte daher durch eine verringerte Insulinsekretion einen negativen Einfluss auf die Glukosehomöostase haben. Die Ergebnisse deuten darauf hin, dass die SCD1-Expression an der frühen Entwicklung des T2D beteiligt sein könnte, aber die BCF nicht so effektiv wie FFAR2 beeinflussen kann. Die zweite Studie zeigte, dass das SAT bei T2D eine gestörte mitochondriale Effizienz, Störung der Kopplung der Atmungskette, Insulinresistenz und Vergrößerung seiner tiefen Schicht aufweist.

Die Ergebnisse dieser Studien deuten darauf hin, dass Störungen des Lipid- und Energiestoffwechsels im SAT von Patienten mit kürzlich diagnostiziertem T2D die systemische Insulinresistenz und die ektope Lipidspeicherung begünstigen. Diese Ergebnisse können sowohl für präventive als auch für therapeutische Strategien des T2D relevant sein und könnten als Grundlage für die Entwicklung neuartiger Insulin-sensibilisierender Medikamente dienen.

Summary

Type 2 diabetes (T2D) is characterized by increasing peripheral insulin resistance and insufficient compensatory insulin secretion. Impaired adipose tissue function is suggested to be involved in the development of T2D. Expansion of adipose tissue in obesity, but also decreased adipose tissue mass in lipodystrophy contribute to insulin resistance. Both conditions feature impaired lipid storage in adipose tissue, leading to increased systemic lipid availability, which can cause insulin resistance in peripheral tissues. Studies in animal models and humans suggest that free fatty acid receptors (FFAR) 2 and 4 as well as stearoyl-coenzyme A desaturase-1 (SCD1), a key enzyme in fatty acid metabolism, are relevant in the pathogenesis of insulin resistance and impaired beta-cell function (BCF), but their relevance for the development of T2D is less clear. Although in skeletal muscle and liver, abnormal mitochondrial function contributes to insulin resistance and impaired BCF, the role of energy metabolism of white adipose tissue for glucose homeostasis is not fully understood.

The aim of this thesis was to clarify the role of FFAR2/4, SCD1 and energy metabolism in adipose tissue for systemic glucose metabolism in T2D. Both enclosed studies were based on the observational German Diabetes Study (GDS) which prospectively includes patients with recently diagnosed diabetes. The first study elucidated the relevance of FFAR2/4 and SCD1 for insulin sensitivity and BCF in subcutaneous adipose tissue (SAT) of patients with recent-onset T2D. The second study examined the role of energy metabolism in distinct compartments of SAT for tissue-specific insulin resistance in patients with newly diagnosed T2D.

The first study showed that compared to glucose-tolerant humans (CON), patients with recent-onset T2D feature lower SCD1, but similar FFAR2 or 4 expression in SAT. Furthermore, FFAR2 expression correlated negatively with BCF in CON and may therefore negatively influence glucose homeostasis by decreasing insulin secretion. These findings imply that SCD1 expression could be important in early development of T2D, but is not as effective in modulating BCF as FFAR2. The second study showed that SAT of patients with T2D exhibit impaired mitochondrial coupling and efficiency, insulin resistance and increased expansion of its deep layer.

The findings of these studies imply that abnormalities of lipid and energy metabolism in SAT of patients with recent-onset T2D promote systemic insulin resistance and ectopic lipid deposition. These findings may be relevant for both preventive and therapeutic strategies of T2D and might help to identify possible targets for the development of novel insulin sensitizing drugs.

List of abbreviations

ACC	Acetyl-coenzyme A carboxylase
Adipo-IR	Adipose tissue insulin resistance index
ADP	Adenosine diphosphate
AdPLA2	Adipose-specific phospholipase A2
AK2	Adenylate kinase 2
AMPK	AMP-activated protein kinase
ANT2	Adenine nucleotide translocase 2
APP	Amyloid precursor protein
ATGL	Adipose triacylglycerol lipase
ATP	Adenosine triphosphate
ATP5J	ATP synthase-coupling factor 6
BCF	Beta-cell function
aP2	Adipocyte protein 2
BMI	Body Mass index
cAMP	Cyclic adenosine monophosphate
ChREBP	Carbohydrate-responsive element-binding protein
CoA	Coenzyme A
CON	Glucose-tolerant humans
CoVa	Cytochrome c oxidase polypeptide Va
COX6C	Cytochrome c oxidase subunit VIc
COX3	Cytochrome c oxidase subunit III
CPT1	Carnitine palmitoyltransferase 1
CYB5A	Cytochrome b5 type A
DAG	Diacylglycerols
DGAT	Diacylglycerol acyltransferase
DNL	De novo lipogenesis
DSAT	Deep subcutaneous adipose tissue
ELOVL6	Fatty acid elongase 6
EPIC	European Prospective Investigation into Cancer
ERR	Estrogen-related receptor
FA	Fatty acid
FACS1	Fatty acyl-CoA synthetase 1
FADH2	Hydroquinone form of flavin adenine dinucleotide
FAS	Fatty acid synthase
FATP1	Fatty acid transport protein 1
FFA	Free fatty acids
FFAR	Free fatty acid receptor
GDS	German Diabetes Study
GLUT4	Glucose transporter 4
GPAT	Glycerol-3-phosphate acyltransferase
GPR	G-protein coupled receptors
HbA_{1c}	Glycated hemoglobin A_{1c}
HCL	Hepatocellular lipids
HIF1α	Hypoxia-inducible factor 1α
HOMA	Homeostasis model assessment
HSL	Hormone-sensitive lipase
IGT	Impaired glucose tolerance
IL-1β	Interleukin-1β

IRS	Insulin receptor substrate
IR	Insulin receptor
KIHD	Kuopio Ischaemic Heart Disease
MAG	Monoacylglycerol
MARD	Mild age-related diabetes
MGAT	Monoacylglycerol acyltransferase
MGL	Monoacylglycerol lipase
MOD	Mild obesity-related diabetes
MRI	Magnetic resonance imaging
MRS	Magnetic resonance spectroscopy
mtDNA	Mitochondrial deoxyribonucleic acid
MTND1	Mitochondrial NADH dehydrogenase 1
mTORC1	Mammalian target of rapamycin
M-value	Whole-body insulin sensitivity
NAFLD	Non-alcoholic fatty liver disease
NGT	Normal glucose tolerance
NRF	Nuclear respiratory factor
n.a.	Not assessed
n.k.	Not known
n.r.	Not reported
OBE	Obese participants
OGTT	Oral glucose tolerance test
PGC1α	PPARγ coactivator 1-α
PI3K	Phosphatidylinositol 3-kinase
PKA	Protein kinase A
PKB, Akt	Protein kinase B
PLIN1	Perilipin 1
PPAR	Peroxisome proliferator-activated receptor
Rd	Rate of disappearance
ROS	Reactive oxygen species
SAID	Severe autoimmune diabetes
SAT	Subcutaneous adipose tissue
SCAP	SREBP cleavage-activating protein
SCD1	Stearoyl-coenzyme A desaturase-1
SEM	Standard error of the mean
SI	Insulin sensitivity index
SIDD	Severe insulin-deficient diabetes
SIRD	Severe insulin-resistant diabetes
SIRT2	Sirtuin 2
SREBP	Sterol regulatory element-binding protein
SSAT	Superficial subcutaneous adipose tissue
SSBP1	Single-stranded DNA-binding protein 1
TAG	Triacylglycerols
TFAM	Mitochondrial transcription factor A
TCA	Tricarboxylic acid
TFAM, TFB2M	Mitochondrial transcription factors A and B2
TNFα	Tumor-necrosis factor α
T1D	Type 1 diabetes
T2D	Type 2 diabetes
VAT	Visceral adipose tissue

Index of contents

1. Introduction

Increasing prevalence of obesity is a major global health problem in the industrialized world and its treatment and prevention represent one of the biggest challenges for the modern healthcare system. Overnutrition and reduced physical activity leading to excessive body fat accumulation results in imbalance of whole-body energy metabolism and glucose homeostasis (1, 2). In parallel with advancing obesity, the risk for cardiovascular diseases and type 2 diabetes mellitus (T2D) is increasing (3, 4). Nevertheless, humans with white adipose tissue deprivation or perturbation (e. g. lipodystrophy) are also characterized by insulin resistance (5). Although adipose tissue mass affects metabolic fluxes and participates in inter-organ crosstalk, the role of energy metabolism within white adipose tissue for insulin resistance is less clear.

1.1. Type 2 diabetes mellitus

1.1.1. Definition and description

Diabetes mellitus is defined by an increase in blood glucose levels (hyperglycemia) and can be subdivided in type 1 (T1D), T2D, gestational diabetes mellitus and other specific types of diabetes due to other causes, such as monogenic forms of diabetes (e. g. maturity-onset diabetes of the young and neonatal diabetes), diseases of the exocrine pancreas and chemical- or drug-induced diabetes (6).
According to the American Diabetes Association (6) and German Diabetes Society (Deutsche Diabetes Gesellschaft) (7) several diagnostic criteria define the diagnosis of diabetes and fulfilling one of the listed criteria below is sufficient for the diagnosis of diabetes:

(1) Hyperglycemia related symptoms (polydipsia, polyuria and unexplained weight loss) and plasma glucose ≥ 200 mg/dl (11.1 mmol/l)
(2) Fasting (defined by caloric restriction for at last 8 hours) plasma glucose ≥ 126 mg/dl (7.0 mmol/l)
(3) During an standardized 75-g oral glucose tolerance test (OGTT) 2-h plasma glucose ≥ 200 mg/dl (11.1 mmol/l) (6)
(4) glycated hemoglobin A_{1c} (HbA_{1c}) ≥ 6.5 % (General requirements for laboratory analysis of HbA_{1c}: Method certified by the National Glycohemoglobin Standardization Program and standardized by the assay according to the Diabetes Control and Complications Trial)

Of note, 'prediabetes' is defined by fasting plasma glucose levels between 100-125 mg/dl (5.6-6.9 mmol/l) and/or a HbA1c level between 5.7-6.4% and/or 2-h plasma glucose levels between 140-199

mg/dl (7.8-11.0 mmol/l) during an standardized 75-g OGTT (6, 7). T1D is characterized by autoimmune beta-cell destruction, which usually results in absolute insulin deficiency (6). T2D is characterized by insufficient insulin secretion from human beta-cells to compensate for peripheral insulin resistance (8). Usually the early period of T2D is characterized by insulin resistance and impaired first-phase insulin secretion and results in postprandial hyperglycemia. This is followed by abnormal second-phase beta-cell function (BCF). Together with insulin resistance this leads to persistent hyperglycemia in the fasting state. Gestational diabetes mellitus is characterized by diagnosis during the second or third trimester of pregnancy without overt diabetes prior to gestation (6).

Recent studies identified new subtypes (clusters) of diabetes mellitus, which are characterized by distinct clinical features and suggest that the traditional diabetes classification may call for revision (9, 10). Within this new classification patients can be classified in five clusters, of whom two feature mild - mainly obesity-related (MOD) and age-related (MARD) metabolic abnormalities, while the others consist of more severe alteration such as severe insulin-deficient diabetes (SIDD), a severe insulin-resistant diabetes (SIRD) or autoimmune diabetes (SAID). The SIRD cluster specifically associates with a higher prevalence of nonalcoholic fatty liver disease (NAFLD) and members of this cluster were more frequently carriers of the rs738409(G) variant in the patatin-like phospholipase domain–containing 3, which associates with increased risk and progression of NAFLD (11). Of note, NAFLD is characterized by excessive hepatocellular lipid (HCL) accumulation and is defined by >5% of lipid load in hepatocytes in liver biopsies (12) and as HCL >5.56% when measured by proton based magnetic resonance spectroscopy (MRS) (13). Furthermore, the SIRD cluster associates with surrogate markers of hepatic fibrosis (10) as well as with increased cardiovascular risk (14). The SIDD cluster features early decline of BCF and occurrence of diabetes-related complications and is associated with the need of early intensified treatment (9, 10). However, studies on the early BCF failure in these patients are lacking. Low prevalence of this cluster leads to underestimation of the risk for complications in this subtype. Detailed pathophysiological molecular mechanisms for the development and progression for these specific subtypes of patients and the role of adipose tissue mass and adipose tissue insulin resistance still remains to be investigated.

1.1.2. Epidemiology

According to the International Diabetes Federation in 2019 one in eleven adults (approximately 463 million adults) worldwide were estimated to have diabetes, thus almost 50 million more than in 2015 (15). More than 59 million in Europe and more than 9 million people in Germany are affected by the disease (15). If these trends continue, nearly 700 million humans worldwide will develop diabetes by the year 2045 (15). Due to the demographic of increasing aging in Germany, estimates for elderly humans are of particular interest. Indeed, one in five humans, aged >65 years, has diabetes (approximately 6 million elderly persons) (15). Behind China, USA and India, Germany ranks fourth

worldwide in this group of age (15). Thus, diabetes will continue to be one of the most important global health problems in the upcoming decades. Moreover, more than half of all patients with diabetes are currently undiagnosed (approximately 232 million) and the prevalence of undiagnosed diabetes could be comparable with the prevalence of diagnosed diabetes (15, 16). Most of these undiagnosed cases can be attributed to T2D, because symptoms during early manifestation are often not recognized as being related to this disease (17). Hence, patients with T2D are often only diagnosed when complications related to heart, kidneys, eyes, nerve system, specifically the diabetic foot syndrome with sensorimotor polyneuropathy or a combination of neural and vascular disorders occur (15, 18). Approximately 90% of all diabetes patients have T2D and 374 million humans more are estimated to have an increased risk of developing the disease (15). Especially early diagnosis and initiation of lifestyle modification as well as specific drug therapies are effective in preventing diabetes manifestation. Thus, the necessity to provide diabetes screening for patients with increased risk for diabetes is one of the biggest challenges for future healthcare systems. Along these lines, targeted strategies to manage hyperglycemia, dyslipidemia, hypertension and insulin resistance and diabetes-related complications are constantly updated to account for the changes in patients' phenotypes and clinical features (19). Despite optimized therapeutic options, cardiovascular mortality can only be moderately reduced - especially in young patients with T2D (20). In 2019, approximately 12% of global health care expenditure (760 billion dollar) was spent on diabetes and approximately 79% of adults with diabetes diagnosis currently live in countries with low and middle income (15). The enormous costs for care and treatment of these patients furthermore cause extreme financial burdens on present and even more on future society.

1.1.3. Risk factors

Multiple modifiable and non-modifiable factors increase the risk of T2D. The main non-modifiable risk factors are advanced age and specific genetic predisposition (21). Higher risk for T2D in elderly humans was explained by aging-related decrease of insulin sensitivity (22, 23). Mitochondrial oxidative capacity ex vivo (23) and mitochondrial activity in vivo (22) were impaired in elderly persons and partially accompanied by higher hepatic and intra-myocellular lipid contents (22) or increased whole-body adipose tissue mass (23). Despite age-related effects on mitochondrial deoxyribonucleic acid (mtDNA) abundance and nuclear transcription factor expressions and mitochondrial protein, endurance exercise can almost normalize age-related impairment in mitochondrial function (24). A positive family history for T2D was shown to be associated with increased risk for T2D (25) and 'prediabetes' (26). The risk for T2D onset was almost doubled in humans with a positive family history of diabetes, defined as diabetes in one or both natural parents (25). Ethnicity is associated with various genetic features, which determine the individual risk for T2D development. T2D prevalence was higher in South-Asian and African compared to European humans (27, 28) and higher in Hispanic than in non-Hispanic white persons (29). In addition, sex differences have been reported for diabetes prevalence. A previous study

showed that middle-aged European men are diagnosed with T2D at lower levels of body mass index (BMI) compared to women (30). Likewise, males have a higher risk for T2D (31, 32). In women, the polycystic ovary syndrome is associated with increased insulin resistance and impaired BCF ultimately increasing the risk for development of T2D (33). As another non-modifiable risk factor, gestational diabetes increases the risk for the mother and the unborn child to develop T2D (34). Furthermore, low birth weight of infants associated with increased risk for insulin resistance and T2D (35). Genome-wide association studies have increased the knowledge on genetic predisposition and helped to identify multiple genomic regions that associate with higher risk for T2D (36, 37). Although individual genetic variants only have a modest effect on the risk for T2D development, when combined into a polygenic score, they offer increasing accuracy to capture patterns of the disease predisposition. Polygenic scores may capture aspects of the etiological and clinical heterogeneity that contribute to variable clinical outcomes in patients with T2D and may help to predict individual disease progression, complication risk and response to pharmacological and behavioral interventions. A recent study found five novel clusters of genetic loci for T2D, three related to insulin resistance and two related to insulin deficiency (38). These genetic clusters seemed to overlap with clusters of clinical measures (9, 38). Specifically, one of the clusters encompassed a subgroup of insulin resistance-related variants mirroring lipodystrophy (38). This cluster was associated with increased fasting insulin levels and triacylglycerol (TAG) levels but decreased high-density lipoprotein cholesterol, adiponectin, and BMI (38). A study in Danish twins suggested that genetic predisposition is associated with the development of impaired glucose tolerance (39). Of note, both non-genetic and modifiable risk factors were shown to control if a genetic predisposition progresses to overt T2D (39). Furthermore, T2D is affected by both genetic and environmental determinants. Previous studies identified epigenetic factors in the pathogenesis of T2D, which link modifiable and non-modifiable at the gene regulatory level (40).

The most important modifiable risk factors for development of T2D are sedentary lifestyle and obesity (defined by a BMI $\geq$ 30 kg/m^2) (21). Especially because excess body weight, physical inactivity and inadequate nutrition are increasing in parallel with economic development and increasing urbanization, T2D is the most common type of diabetes. Apart from rare genetic predisposition, obesity results from energy surplus due to increased food intake and low physical activity. While the dietary intake of nutritional components with high glycemic index, saturated fatty acids (FA), red and processed meat increase the risk of T2D, a balanced diet with vegetables, fiber-rich foods and coffee were shown to decrease the risk of T2D (41-43). Cardiorespiratory fitness is reduced in patients with recent-onset T2D (44). Vice versa, higher physical activity was associated with reduced risk for T2D, cardiovascular disease and all-cause mortality (45). The main effect of increased physical activity on lowering T2D risk may be explained by improved skeletal muscle insulin action resulting in increased insulin sensitivity (46).

Obesity is characterized by increased whole-body fat mass and associates with both development of insulin resistance and impaired BCF (47). Nevertheless, the distribution of adipose tissue depots are

even more important than the total body fat mass for the risk of insulin resistance and T2D (48). Particularly, visceral adipose tissue (VAT), which includes mesenteric and omental adipose tissue, associates with increased risk for development of insulin resistance and T2D (49). In contrast to central fat accumulation, storage of fat in peripheral compartments (e. g. hips and thighs) was suggested to have rather protective effects on development of T2D (50, 51).

Lipid accumulation in non-adipose tissues and organs (i. e. in liver, skeletal muscle, heart) due to impaired lipid storage in adipose tissue cause insulin resistance [2]. The liver has a key role in interorgan metabolic crosstalk and integrative biology of insulin resistance and development of T2D (1, 52). The liver integrates peripheral signals from adipose tissue, gut, muscle and endothelial cells and affects systemic and hepatocyte-specific signaling pathways, cell growth and death (1, 52, 53). NAFLD promotes the development of hepatic insulin resistance and ultimately increases the risk for T2D and cardiovascular disease (54, 55). Vice versa, insulin resistance was suggested to be an underlying mechanism for development of NAFLD even in non-obese humans without diabetes (56, 57). In humans with recently diagnosed T2D, hepatic steatosis associated with cardiovascular autonomic neuropathy ultimately increasing the risk of mortality in patients with diabetes (58). Of note, insulin resistance was suggested to contribute to cardiac autonomic nervous system activity by early cardiovagal suppression rather than sympathetic predominance in both T1D and T2D (59). Ectopic lipid accumulation usually associates with both systemic and tissue-specific insulin sensitivity, but genetic susceptibility, family history of T2D, oxidative capacity and total fat mass influence this relationship (60, 61). Moreover, lipotoxic lipid species accumulation - such as ceramides and diacylglycerols (DAG) - inhibit insulin signaling in skeletal muscle (62, 63), liver (64) and the heart (65). Of note, differences in the subcellular distribution affect their inhibitory properties (66).

The incidence of T2D and its complications can be reduced via non-pharmacological and pharmacological interventions. Prognostic models and score-systems of all above mentioned risk factors to predict the individual risk for the development of T2D have been suggested (67-70). In addition to advances in genomics, other new prognostic factors from -omics technologies (e. g. metabolomics, lipidomics, proteomics, transcriptomics or epigenetic changes) have gained growing attention to identify novel associations between phenotypes, disease pathways and treatment response (71). Further advances in this area of research may have the potential to predict responses to preventive or therapeutic strategies. However, an optimized framework incorporating such –omics data into clinic records for evaluation of the validity, efficacy and cost-effectiveness of clinical testing as well as sufficient training and education of clinicians on how to use these data appropriately is required (72).

In addition, more research efforts are required to build the level of clinical evidence necessary for achieving consensus and developing guidelines (73) in the pursuit of precision medicine for making a comprehensive map of T2D-related risk factors. Precise diagnosis and correct classification of patients are essential for tailored treatment since metabolic characteristics differ between diabetes (sub)types (74). Precision medicine for T2D, therefore, will require even more accurate profiling of individuals

belonging to a given phenotype, by integrating genetic and -omics data and clinical monitoring. Of note, distinct metabolic phenotypes in patients with diabetes were recently found to have differential risk for diabetes complications at early stages of the disease (10). Targeted prevention and treatment for individualized precision medicine for diabetes and its comorbidities may characterize future therapy for diabetes (75). The prevalence of unhealthy lifestyle - accompanied by overweight, obesity and subsequently T2D as well as associated cardiovascular and neural co-morbidities - is increasing (76), but studies still failed to clarify whether distinct genetic and metabolic phenotypes can be attributed to a different pathogenesis.

1.2. Role of adipose tissue in type 2 diabetes

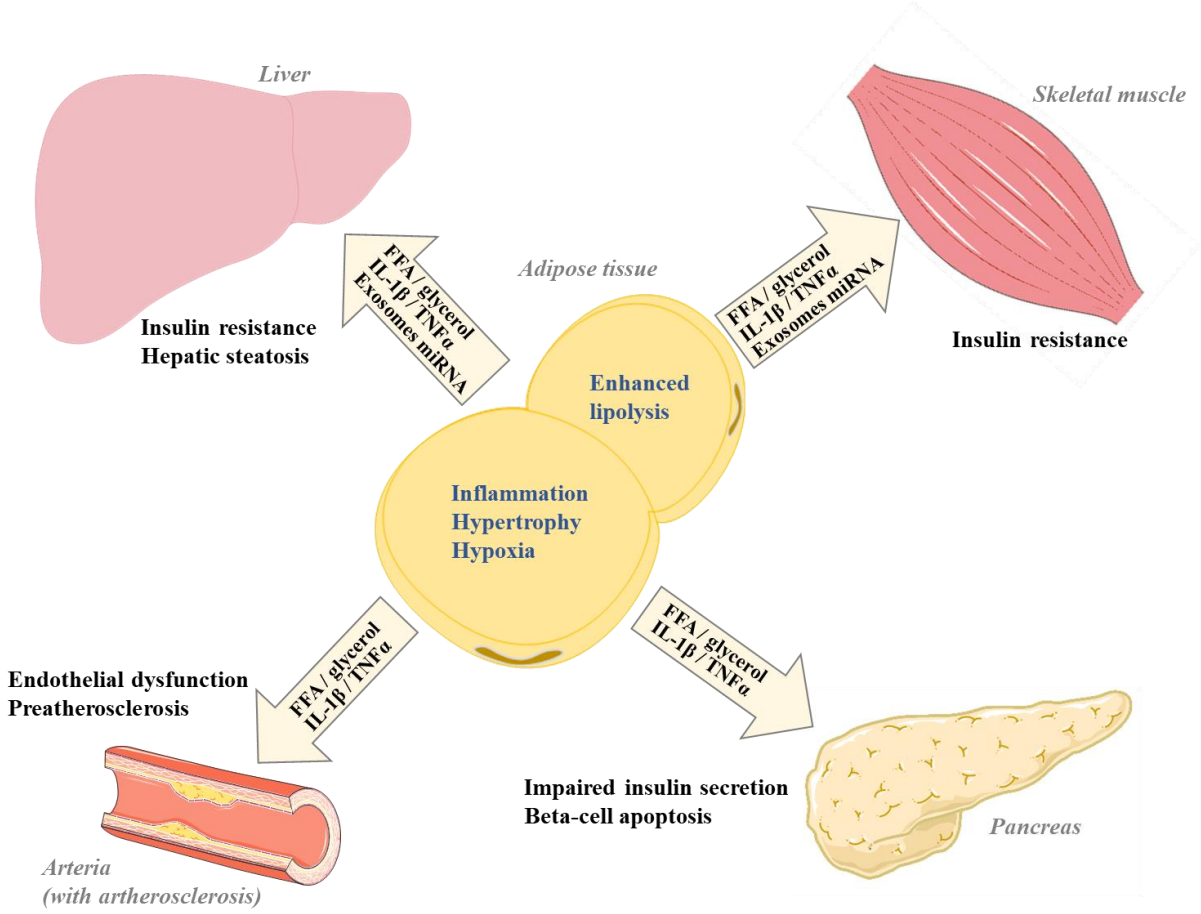
Already approximately 3000 years ago Hippocrates declared that a healthy diet can not only maintain good health but also prevent from sudden death, as observed more frequently in obese than in lean humans (77). The relevance of adipose tissue for whole-body energy metabolism was neglected for a long time and considered as a metabolically inert storage compartment for TAG. This notion changed at the latest in the late 1980s when a study showed that adipocytes are able to secrete proteins affecting systemic lipid and energy metabolism (78). The discovery of leptin, an adipokine that mainly regulates energy homeostasis by inhibiting hunger, almost one decade later ultimately recognized white adipose tissue as the biggest endocrine gland in humans (79, 80). Various adipokines are secreted directly from adipocytes or via extracellular vesicles, also known as exosomes, which include nucleic acids, lipids and proteins (81). Exosomes can regulate gene expression in distant tissues and modulate insulin sensitivity (82, 83). A recent study showed that exosome-specific proteins from adipocytes are mainly assigned to membrane-mediated processes and signaling pathways, thereby affecting interorgan crosstalk (84).

As an endocrine organ, the white adipose tissue has a central role by regulating systemic lipid and energy metabolism and controlling circulating levels of free fatty acids (FFA), glycerols and glucose (85). The main function of white adipose tissue is the storage of energy rich substrates in states of energy surplus. Vice versa, during scarcity of energy, adipose tissue supplies energy via TAG breakdown (86). During long periods of fasting, not glucose, but FFA from adipose tissue are the main energy source for whole-body energy metabolism. Impaired adipose tissue function is suggested to be involved in the development of T2D. Expansion of adipose tissue in obesity, caused by inadequate physical activity and chronic overnutrition (2, 87), but also decreased adipose tissue mass in lipodystrophy result in marked insulin resistance (5). Both conditions feature impaired lipid storage of neutral TAG in adipose tissue, leading to increased systemic lipid availability. This causes ectopic lipid deposition, perturbed insulin signaling (88, 89), oxidative stress (90, 91) and impaired mitochondrial function (92) leading to insulin resistance in distant tissues due to lipotoxic effects of specific lipid species (52) and thereby contribute to the onset of T2D (52, 93, 94). Vice versa, in a mouse model with enhanced hepatic lipogenesis, mice

only were protected from systemic insulin resistance when adipose tissue lipid storage capacity was preserved (95).

Expansion of adipose tissue in obesity not only associates with insulin resistance but also with progressive immune cell infiltration and low-grade inflammation in this tissue (2). Pro-inflammatory cytokines activate lipolysis (96) resulting in dyslipidemia (97). Increased lipolysis raises systemic availability of lipids, which cause insulin resistance in distant tissues (52), impaired BCF (98) and beta-cell apoptosis via lipotoxic effects of specific lipid species (99, 100). Furthermore, consistently high levels of circulating FFA that are also present in obesity resulted in impaired endothelial function in both lean and obese humans (101) and were suggested to promote atherosclerosis and cardiovascular disease (102) (Figure 1). Of note, approximately two-thirds of FFA used for TAG synthesis in the liver derive from adipose tissue (103). Thus, it is not surprising that the risk for diabetes and cardiovascular disease are increasing in parallel with progressive obesity (104). Previous studies showed that both an intravenous lipid infusion and even a single oral fat load induce insulin resistance (105, 106). Vice versa, pharmacological reduction of FFA levels improves insulin sensitivity in the skeletal muscle, pancreatic beta cells and the liver (107, 108).

Figure 1



The role of adipose tissue in development of type 2 diabetes. *Adipose tissue crosstalk model of lipotoxicity-induced type 2 diabetes. Adipocyte hypertrophy and hypoxia, as well as inflammatory pathways resulting in enhanced lipolysis and increased release of free fatty acids (FFA) and cytokines (interleukin-1β (IL-1β) and tumor-necrosis factor α (TNFα)) to distant tissues such as skeletal muscle, pancreatic beta cells and liver as well as to the vascular bed ultimately leading to peripheral insulin resistance, impaired insulin secretion from beta cells and increased risk for cardiovascular diseases. In obesity, exosomes secreted by adipose tissue macrophages transfer miRNAs to insulin target cells and cause insulin resistance in skeletal muscle and liver.*

Body weight loss and especially the reduction of whole-body fat mass leading to reversal of obesity both due to lifestyle intervention or bariatric surgery have been shown to have the strongest effects on glycemic control and even normalize blood glucose levels in patients with recently diagnosed T2D (109). The extent of body fat loss, duration of T2D and residual BCF were suggested to determine the response to remission strategies (110). While the beta cell recovery should be the major factor for restoration of normal glucose tolerance, impairment of adipose tissue function in recent-onset - yet reversible - T2D has gained growing interest.

White adipose tissue not only facilitates lipid storage by FA uptake or de novo lipogenesis (DNL), but also circulating FFA availability by lipolysis. FA can serve as substrates for energy metabolism via beta-oxidation in mitochondria. Mitochondria in turn generate energy, in the form of adenosine triphosphate (ATP) for a wide range of cellular processes, like cell growth, differentiation and signaling (111). As another kind of adipocytes, brown adipose tissue represents a quantitatively negligibly small amount in adult humans, but comprises more mitochondria than white adipose tissue. However, the rediscovery of brown adipocytes also in human adults has gained growing pharmacological attention for targeted induction of white adipose tissue, which may convert white into 'beige' adipocytes. These adipocytes feature similar characteristics as brown adipocytes and promote adaptive thermogenesis and energy expenditure and were suggested to be a potential target in anti-obesity treatment (112). Of note, although experimental induction of brown adipose tissue was associated with lower body weight and improved glycemic control (113), at present no evidence exist in humans for modification of brown adipose tissue mass or function under common physiological and pathophysiological conditions (114).
In summary, the development of tissue-specific insulin resistance in T2D could be explained by (i) generation and activation of intracellular lipotoxic lipid species (e. g. DAG, ceramides) (ii), by inflammatory processes (iii) by chronic energy overflow and (iv) impaired mitochondrial function (1).

1.2.1. Assessment of adipose tissue insulin resistance

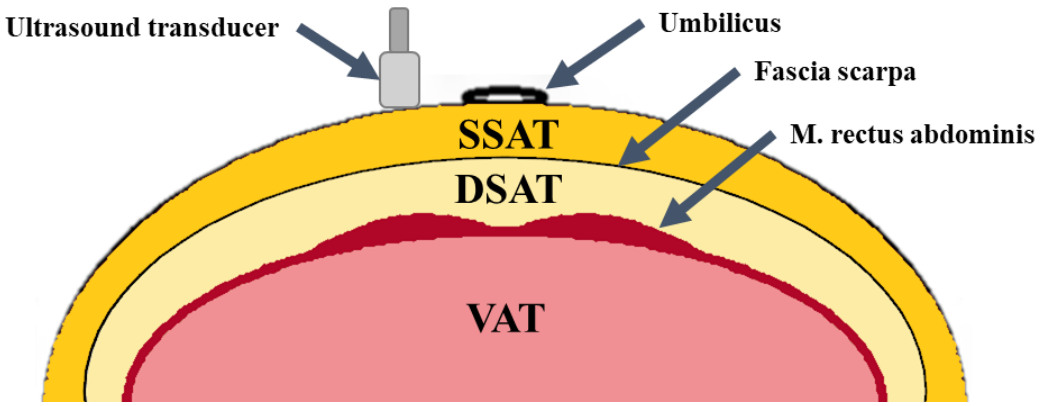
In vivo, the current gold-standard methods for quantification of insulin resistance in human adipose tissue are tracer-dilution techniques using stable isotope-labeled FA or glycerol tracers during an intravenous insulin infusion (115). Higher systemic FFA or glycerol appearance in plasma reflect deteriorated insulin-mediated suppression of lipolysis and thus reflects adipose tissue insulin resistance in vivo. Similarly, decreased ability of insulin to suppress plasma concentrations of endogenous (unlabeled) FFA during a multi-step hyperinsulinemic-euglycemic clamp test also characterize insulin resistance of adipose tissue. The multi-step euglycemic clamp technique additionally enables to measure hepatic and skeletal muscle insulin sensitivity and responsiveness. Combined with continuous infusion of a glucose tracer, such as deuterated glucose (D-[6,6-$^{2}H_{2}$]glucose), the clamp reveals skeletal muscle insulin sensitivity by assessment of insulin-stimulated rates of glucose disappearance and hepatic insulin sensitivity by measuring insulin-mediated suppression of rates of endogenous glucose production (116). Calculated from fasting plasma insulin and FFA levels, the adipose tissue insulin resistance index (Adipo-IR) represents a validated measure of adipose tissue insulin resistance (117). However, this surrogate parameter does not measure insulin action directly (118).
In vitro, studies using labeled glucose tracers, showed that lower glucose uptake after insulin stimulation represents insulin resistance in cultured adipocytes (119). Previous studies proposed that insulin resistance of adipose tissue could even develop prior to diabetes onset due to impaired intracellular insulin signaling (120) and insulin-stimulated glucose uptake into adipocytes (121). Additionally, the

membrane insulin receptor activation and the insulin receptor tyrosine kinase activity can be used as a measure of insulin sensitivity on receptor level (122, 123). Of note, most in vivo and in vitro studies did not completely assess the dose-response relationships for insulin action and functional studies in adipose tissue in human are still missing (118, 124).

1.2.2. Abdominal adipose tissue compartments

In humans, abdominal adipose tissue compartments are divided into VAT and subcutaneous adipose tissue (SAT), which is further separated by Scarpa's fascia into a deep (DSAT) and superficial (SSAT) layer (Figure 2). According to sex-dependent variations, VAT comprises 6-20%, SAT 80-90% of whole-body adipose tissue (125) and DSAT accounts for 66% of SAT in males and for 51% in females (126). Volume of VAT, but not of SAT, associates with whole-body and hepatic insulin resistance (127). Men have more VAT, but less SAT volume compared to females (128). The latter was suggested to have protective properties for glucose homeostasis (129). Comparing ethnical differences, White-Americans have higher VAT volume compared to Hispanics and African-Americans. In contrast, South-Asians have more VAT volume and an increased risk for T2D compared to Caucasians (130). Of note, independently of sex and ethnical differences, abdominal VAT increases during aging, which is accompanied by a higher risk for T2D in the elderly humans (49, 118).

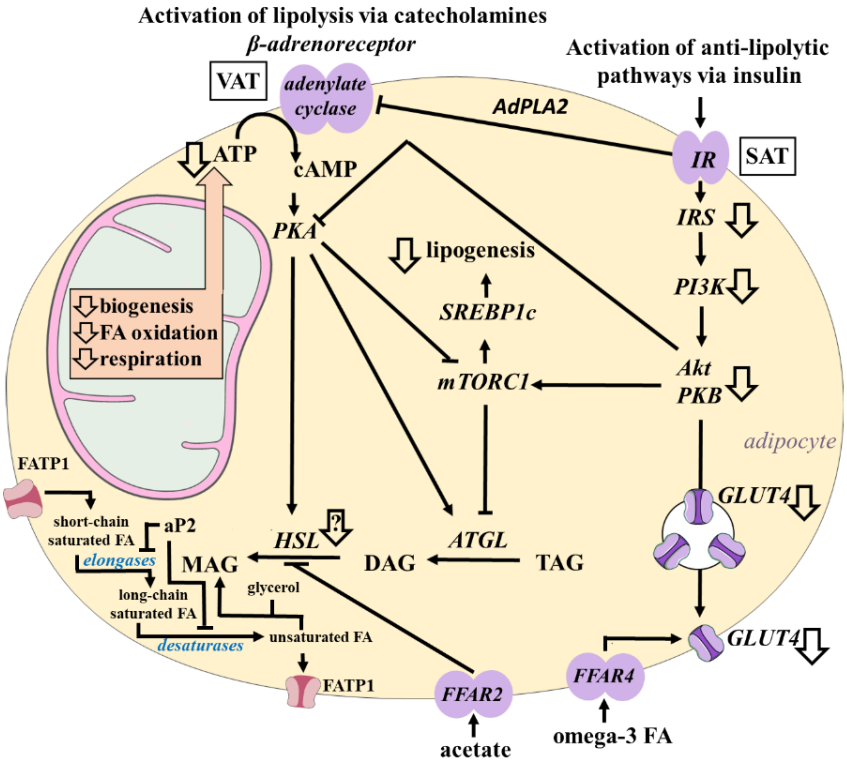
Figure 2



Scheme depicting adipose tissue layers of the abdominal wall. *Schematic figure presenting all compartments of the abdominal subcutaneous adipose tissue from the cutis to the visceral adipose tissue (VAT). The superficial (SSAT) and deep subcutaneous adipose tissue (DSAT) as well as Scarpa´s fascia (black line dividing SSAT and DSAT) between both adipose tissue layers. Figure modified from Bódis K and Roden M, Eur J Clin Invest, 2018 (118) and Bódis K et al., J Clin Endocrinol Metab, 2020.*

Previous studies provided evidence that compartments of human adipose tissue vary in their tissue-specific insulin sensitivity and lipolytic activity. While the lipolysis induced by catecholamines was higher, the antilipolytic effects of insulin were lower in VAT compared to SAT in insulin-resistant obese humans in vivo (49, 131) (Figure 3). Another study suggested that higher catecholamine-induced response in VAT increases portal plasma FFA transport to the liver, ultimately promoting hepatic and also systemic insulin resistance (132). Conversely, lower lipolysis and increased insulin sensitivity indicate an increased lipid storage capacity of SAT to store circulating FFA in TAG and thereby protect distant tissues from lipotoxicity (129). This is supported by findings that humans with decreased abdominal SAT, but higher VAT volume feature metabolic characteristics similar to patients with lipodystrophy with systemic insulin resistance and NAFLD (118, 133).

Figure 3



Lipolysis in adipose tissue of insulin-resistant obese persons. *Insulin-resistant obese human feature impaired antilipolytic and lipolytic pathways in both visceral and subcutaneous adipose tissue. Lipolysis is activated by catecholamine-induced activation of the β-adrenoreceptor signaling cascade resulting in production of cyclic adenosine monophosphate (cAMP) from adenosine triphosphate (ATP) by adenylate cyclase (134). Subsequent activation of protein kinase A (PKA) induces adipose triacylglycerol lipase (ATGL) and hormone-sensitive lipase (HSL) for triacylglycerol (TAG) breakdown into diacylglycerols (DAG) and monoacylglycerols (MAG) (135). Antilipolytic pathways are activated by insulin via various negative feedback signals resulting from insulin receptor (IR) activation. While IR activation of the signaling cascade via phosphorylation of the insulin receptor substrate (IRS) and downstream induction of phosphatidylinositol 3-kinase (PI3K) and protein kinase B (PKB, Akt) (136, 137) results in translocation of glucose transporter 4 (GLUT4) within the membrane of adipocytes, these pathways also lead to simultaneous activation of the mammalian target of rapamycin (mTORC1) (138, 139). While PKA inhibits mTORC1, the insulin-mediated activation of mTORC1 inhibits β-adrenoreceptor induced ATGL transcription and thereby reduces lipolysis (138). Of note, also lipogenic pathways are promoted by mTORC1 mediated induction of sterol regulatory element-binding protein (SREBP1c). Furthermore, lipolysis is also inhibited by the activation of the insulin signaling cascade via adipose-specific phospholipase A2 (AdPLA2) mediated inhibition of the adenylate cyclase (140, 141). Additionally, Akt induction reduces PKA activity via decreased cAMP levels due to phosphodiesterase induction (142). Stimulation of FFAR2 by acetate decreases the level of Ser563-phosphorylation of HSL, which results in suppression of lipolysis adipocytes. Stimulation of FFAR4 by omega-3 fatty acids (FA) increases GLUT4 translocation to the plasma membrane in adipocytes. FA binding protein adipocyte protein 2 (aP2) inhibits lipogenesis. The boxes beside adreno- and insulin receptors indicate the adipose tissue depot, where changes are most pronounced. Subcutaneous adipose tissue (SAT), visceral adipose tissue (VAT). The direction of changes in insulin-resistant humans is indicated by not filled arrows. Figure modified from Bódis K and Roden M, Eur J Clin Invest, 2018 (118).*

Previous studies also showed differences in metabolic activity between DSAT and SSAT (143, 144) (Figure 2). SSAT was suggested to have a similar protective function as the femoral fat depot (145), which was suggested to have a low rate of FA release and may play a major role in circulating FA uptake (146, 147). Increased SSAT volume correlated negatively with HbA1c and positively with high-density lipoprotein cholesterol in patients with T2D (148). In contrast, DSAT features similar inflammatory characteristics as VAT, including higher infiltration with macrophages than SSAT and only both DSAT and VAT volume was shown to associate with cardiovascular and liver diseases (126, 149). MRS techniques allow to quantify the degree of FA saturation non-invasively in adipose tissue and other human tissues (150, 151). Compared to SSAT, DSAT comprises more saturated FA (145), which were suggested to induce inflammation and insulin resistance (152-154). The unsaturation in DSAT revealed a negative association with intramyocellular lipid content, suggesting a possible cross-talk between both depots (155). A similar positive correlation was found between insulin resistance and DSAT volume as previously reported for VAT volume, but an association between insulin resistance and SSAT volume was lacking (144). However, unlike in VAT, an association of DSAT volume with hepatic and whole-body insulin resistance was found in males only (156). DSAT volume was even suggested to be a more precise predictor of fasting insulin concentrations than VAT volume (126). Higher lipolytic activity in DSAT than in SSAT was suggested to explain the correlation of DSAT and insulin resistance (157). Although no mechanistic studies analyzing insulin action between DSAT and SSAT were performed, increased glucose transporter 4 (GLUT4) protein expression suggest higher glucose uptake in SSAT (158). This is supported by previous studies showing that not only GLUT4 translocation, but also GLUT4 protein expression is inducible by insulin with consecutively increased glucose uptake in adipocytes (159, 160). Nevertheless, mechanistic studies assessing lipid metabolism in both depots to examine the role of FA turnover for development of T2D are missing. In summary, DSAT expansion may have unfavorable metabolic consequences, but its relation to tissue-specific insulin sensitivity has not been assessed yet. Furthermore, despite evidence for metabolic varieties between SSAT and DSAT, differences in mitochondrial function was not analyzed yet (118).

In summary, SAT features a lower lipolytic activity and higher insulin sensitivity than adipocytes from VAT compartments. Thus, SAT has a greater capacity to store FA in TAG and thereby protects distant insulin-sensitive tissues from lipotoxic effects. On the other hand, higher lipolytic activity of VAT contributes to the portal delivery of FA and glycerol for storage of TAG in the liver, ultimately resulting in hepatic and whole-body insulin resistance by perturbed insulin signaling. SSAT displays lower lipolysis and higher GLUT4 protein expression than adipocytes from DSAT. However, functional data analyzing lipid and glucose metabolism by tracer techniques in different tissues to explore the relevance for development of T2D and potential treatment targets in diabetes prevention strategies need to be performed in future studies.

1.2.3. Free fatty acid receptors in adipose tissue

Some lipids secreted from the adipose tissue - such as short and long chain FA - have insulin-sensitizing effects in peripheral tissues of humans (52). FFA serve as energy sources, but also induce receptor signaling and regulate whole-body energy homeostasis under various physiological conditions (161). In addition to FA binding proteins and peroxisome proliferator activated receptors (162), FA receptors regulate many physiological and pathophysiological processes (163). Studies in animal models and humans indicate that free fatty acid receptors (FFAR) - also named G-protein coupled receptors (GPR) - impair BCF and promote insulin resistance and T2D (164, 165). Until now, four FFARs - FFAR1, FFAR2, FFAR3 and FFAR4, also known as GPR40, GPR43, GPR41, GPR120 - have been identified having major roles in cellular processes, such as facilitating adipocyte differentiation, anti-inflammatory effects, insulin and incretin secretion and neuronal responses (163).

In adipose tissue, FFAR2 and FFAR4 function as receptors for acetate and long chain FA (166, 167), both inducing signaling pathways in the cell and thereby regulating systemic glucose homeostasis (168). In vitro, acetate signals via FFAR2 reduced the level of Ser563-phosphorylation of hormone-sensitive lipase (HSL), which resulted in suppression of lipolysis in cultured adipocytes (169) (Figure 3). Studies in rodents revealed that FFAR2-deficient mice were protected from high-fat diet induced obesity, adipose tissue inflammation and dyslipidemia and showed improved insulin sensitivity (170). Furthermore, during the insulin-resistant phase of pregnancy higher *FFAR2* gene expression was found in mouse islets (171, 172). Beta cell specific deletion of *FFAR2* in mice resulted in higher insulin secretion and improved glucose tolerance (165). A translational study provided evidence of *FFAR2* gene expression in islets of mice and also humans and indicated that FFAR2 mediates inhibition of insulin secretion by coupling to G_i-type G proteins (165).

In vitro, omega-3 FA-stimulation of FFAR4 resulted in increased GLUT4 translocation to the plasma membrane in cultured adipocytes (173) (Figure 3). While FFAR4 knock out attenuated omega-3 FA-related anti-apoptotic effects, FFAR4 activation protected human beta cells from palmitate-induced apoptosis (174). *FFAR4*-deficient mice on a high-fat diet developed more severe obesity, hepatic steatosis and insulin resistance than wild type mice (175, 176).

Studies in beta cells from mice and in vitro findings in humans suggest that FFAR2 and FFAR4 regulate glucose homeostasis and that these receptors may serve as a potential target for diabetes prevention strategies. Although results from previous studies suggested that FFAR2 and FFAR4 regulate glucose homeostasis in mice (165, 175, 176), the relevance of their expression in human SAT for glucose homeostasis was not elucidated. Of note, FFARs are expressed in various tissues, but they seemed to be particularly important in adipose tissue due to their important role in lipid metabolism.

1.3 Fatty acid turnover in adipose tissue

1.3.1. Lipolysis

Adipose tissue lipolysis is facilitated by enzymes catalyzing sequential breakdown of TAG. In each step of lipolysis, one FA chain is cleaved from the glycerol backbone. Initially, adipose triacylglycerol lipase (ATGL) catalyzes the conversion of TAG to DAG, HSL creates monoacylglycerols (MAG) and ultimately monoacylglycerol lipase (MGL) generates glycerol and FA. In postprandial state, insulin inhibits lipolysis and thereby reduces FFA and glycerol release from adipose tissue (Figure 3). On the other hand, low insulin concentrations during fasting, but also catecholamine-induced activation of the β-adrenoreceptor signaling promote breakdown of TAG. Both conditions result in increased glycerol and FA release from adipose tissue. Of note, catecholamine release is also stimulated by low blood glucose levels via induction of the sympathetic nervous system. On a cellular level, the induction of the β-adrenoreceptor signaling cascade results in activation of the adenylate cyclase. The latter catalyzes the conversion of ATP to cyclic adenosine monophosphate (cAMP), which in turn activates protein kinase A (PKA). This ultimately results in induction of the enzymes ATGL and HSL by PKA, which facilitates the breakdown of TAG in DAG (Figure 3) (118).

Obese humans feature low-grade inflammation and progressive immune cell infiltration in adipose tissue. Elevated FA further stimulate immune cells in adipose tissue to release pro-inflammatory cytokines, like interleukin-1β (IL-1β) and tumor-necrosis factor α (TNFα), both subsequently inducing adipocytes lipolysis (96). The increase of circulating lipids due to activation of lipolysis in adipose tissue cause insulin resistance in peripheral tissues, especially in the liver, skeletal muscle and heart (52). This is explained by FA-coenzyme A induced TAG synthesis, activation of inflammatory pathways and inhibition of insulin signaling (177). Even after surgically-induced weight loss, sustained elevation of adipose tissue lipolysis may be involved in the dynamic changes of muscle insulin sensitivity and epigenetic modifications of genes involved in muscle energy metabolism thereby affecting long-term glucose homeostasis (178). In parallel with the release of glycerol and FA, excessive lipolysis in adipose tissue also modifies cytokine secretion in insulin-resistant humans. These processes result in low-grade inflammation and ectopic lipid accumulation ultimately promoting hepatic and skeletal muscle insulin resistance (118).

While previous studies showed improved insulin sensitivity in HSL- and ATGL-deficient rodents with lower lipolysis (179, 180), evidence from studies in humans questioned the translational relevance of these animal models (181, 182). Humans carrying a mutation of the HSL gene showed dyslipidemia, hepatic steatosis, impaired glucose tolerance, decreased whole-body insulin sensitivity, partial lipodystrophy and higher T2D risk compared to healthy humans without the mutation (181). As a limitation of this study, changes in HSL expression will also appear in other than adipose tissue, which in turn makes it challenging to identify adipose tissue-specific roles in the observed alterations. Of note,

the latter study showed decreased basal and isoproterenol-stimulated lipolysis, but also lower inhibition of lipolysis by insulin in SAT. ATGL protein levels and expression of genes from DNL and TAG synthesis were lower in SAT. While the insulin receptor and insulin receptor substrate 1 protein expression were decreased, macrophage infiltration was increased in SAT. Ultimately, all persons homozygous for the HSL gene mutation featured T2D (118, 181).

A functional study analyzing the effect of an 8-week treatment with nicotinic acid to inhibit lipolysis in adipocytes revealed an upregulated expression of genes involved in lipogenesis in SAT of obese men (180). The results suggest that downregulation of adipocyte lipolysis reshapes energy fluxes into adipose tissue by DNL induction ultimately resulting in improved systemic insulin sensitivity (118).

A previous study displayed that hepatic glucose production is induced by lipolysis-dependent secretion of the FA binding protein adipocyte protein 2 (aP2) from adipocytes in lean mice and cultured hepatocytes (183). Furthermore, ATGL and HSL inhibition could prevent aP2 secretion from adipocytes (184). Thus, these findings could explain the relationship between augmented lipolysis from adipose tissue and systemic insulin resistance, particularly because the neutralization of secreted aP2 protected from diabetes in obese mice (118, 183).

1.3.2. Desaturases

FA from dietary intake partly reflect the FA profile in the circulation for several weeks and in adipose tissue from past months to years (185, 186). Nevertheless, the FA profile in humans also depends on the endogenous FA metabolism by DNL including the major enzymes from FA metabolism - elongases and desaturases (186) (Figure 3, 4 A). Elongases catalyze the subsequent extension of the acyl chain of FA. Desaturases are key enzymes removing two hydrogen atoms from the growing FA chain, thereby introducing a double-bond and converting saturated into unsaturated FA. To indicate at which position from the carboxyl end of a FA chain desaturases create the double bond, these enzymes are labeled with delta (Δ) and the targeted location in the FA chain. As an example, the Δ9 desaturase introduces a double bond between the ninth and tenth carbon atom from the carboxyl end of the FA. Humans have three desaturases. The Δ9 desaturase - also known as stearoyl-coenzyme A desaturase (SCD) - creates monounsaturated FA from short-chain FA, whereas the Δ5 desaturase and the Δ6 desaturase catalyze the synthesis of long-chain mono- and polyunsaturated FA (187).

A large prospective observational population-based study, the Kuopio Ischaemic Heart Disease (KIHD) Risk Factor Study showed that increased Δ5 desaturase activity in serum associates with a decreased T2D risk and that increased Δ6 desaturase activity correlates with an increased T2D risk among middle-aged and older Finish men (188). In contrast, another study showed lower risk for T2D was associated with increased Δ5 desaturase activity and the simultaneous improvement in insulin sensitivity was suggested to explain these findings (189). The European Prospective Investigation into Cancer (EPIC)

and Nutrition-Potsdam Study displayed that SCD1 activity - analyzed from product-to-precursor ratios - from the erythrocyte membrane was associated with increased T2D risk (190).

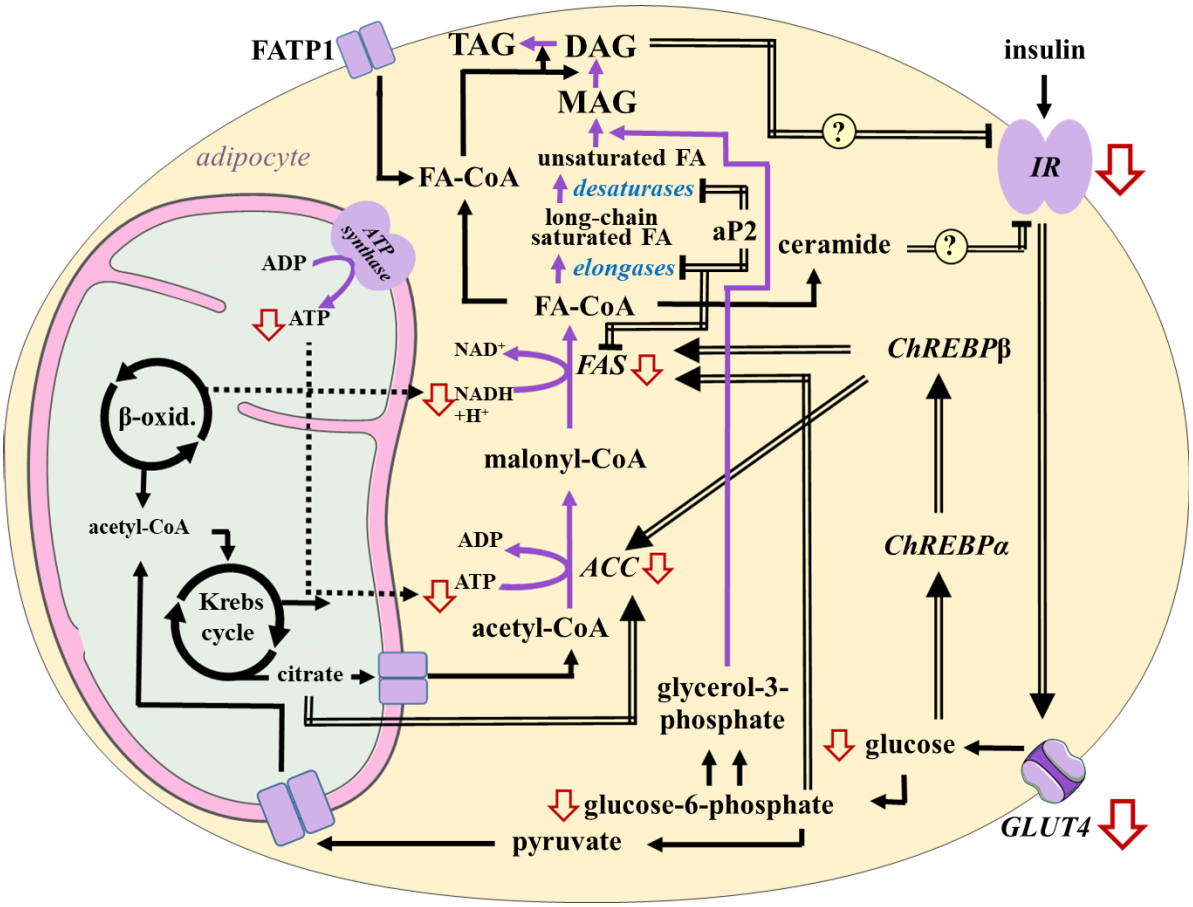
While humans feature two Δ9 desaturases (SCD1 and SCD5), four isoforms (SCD1-4) have been characterized in murine models (191-195). Nevertheless, SCD1 is the most expressed SCD isoform in adipose tissue, where it enables the conversion of lipotoxic lipids into protective species (saturated into monounsaturated FA). A previous study showed that circulating palmitoleate - an adipose tissue derived product of SCD1 - improved insulin signaling in both liver and skeletal muscle, increased BCF and improved whole-body glucose uptake in mice (196). In vitro, palmitoleate treated adipocytes showed reduced cytokine expression (196). SCD1 in adipose tissue induces the last step of DNL and enables FA esterification into TAG, which associate with improved systemic insulin sensitivity. Accordingly, thiazolidinedione treatment induced TAG production in cultured adipocytes (197) and promoted *SCD1* gene expression in SAT resulting in improved insulin sensitivity in patients with T2D (198). Although previous studies point to a prominent role of SCD1 in regulating glucose metabolism in mice (199), the relevance of its expression in human SAT for BCF and insulin sensitivity in patients with newly diagnosed T2D was not assessed.

1.3.3. Lipogenesis

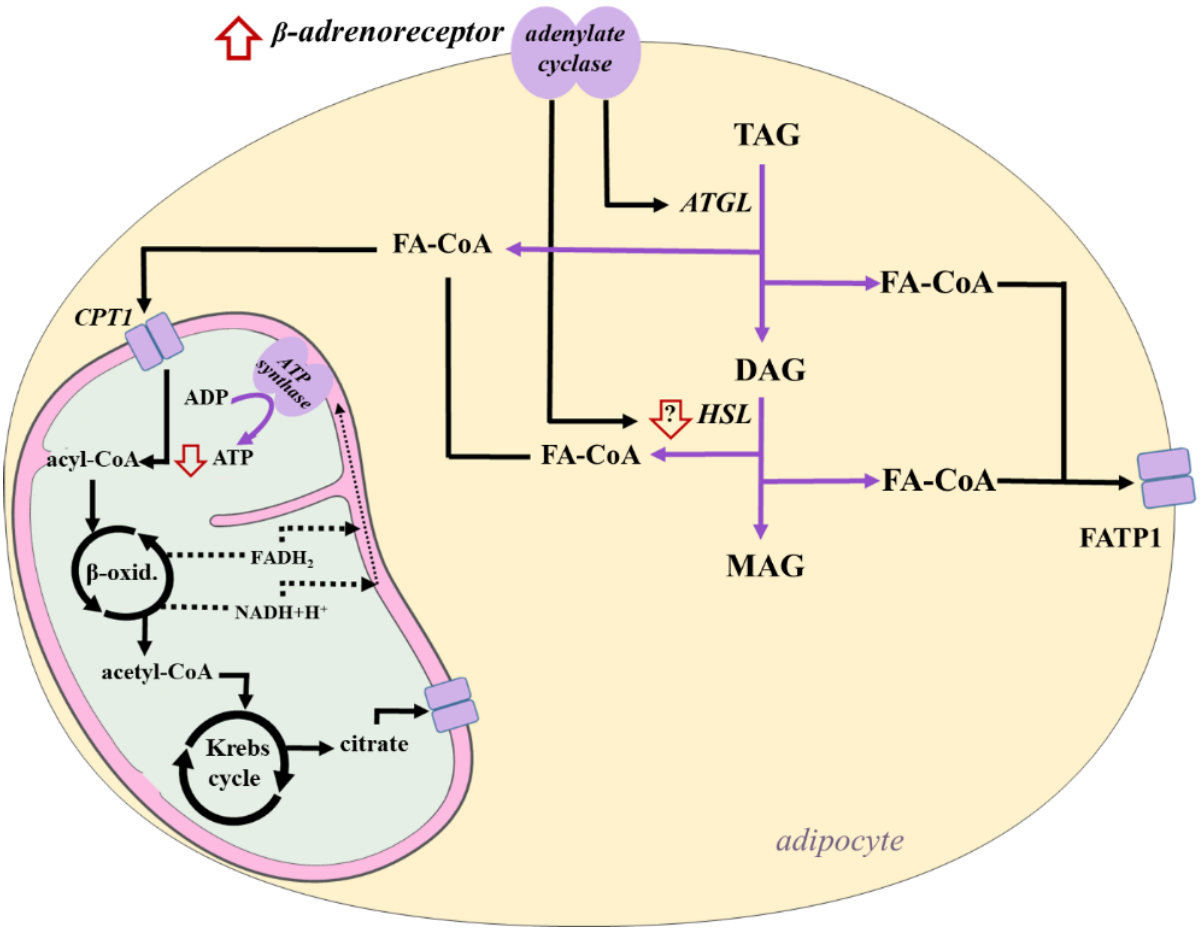
FFA in plasma and adipose tissue originate from dietary fat intake, but also from endogenous DNL. The rate limiting enzymes regulating FA synthesis are the acetyl-coenzyme A carboxylase (ACC) and fatty acid synthase (FAS) (Figure 4). Intake of carbohydrate rich food induces FAS activation in adipose tissue facilitating the conversion of glucose to FA (200). Of note, in the postprandial state, DNL in adipose tissue is quantitatively lower than in the liver. Nevertheless, when maximal glycogen storage capacity is reached, further glucose uptake in the liver results in activation of DNL and subsequent export of TAG for storage in adipose tissue of glucose-tolerant humans (CON) (201). The reciprocal interaction between both tissues is supported by an inverse association of DNL between adipose tissue and the liver (202). Additionally, when DNL in the liver is decreased, it is compensatory increased in adipose tissue, at least in a mouse model with hepatocyte-specific deficiency of an escort protein transporting sterol regulatory element-binding protein (SREBP), which is called SREBP cleavage-activating protein (SCAP) (118, 203).

Figure 4

A)


FATP1
TAG
DAG
MAG
insulin
adipocyte
FA-CoA
unsaturated FA
desaturases
long-chain saturated FA
aP2
elongases
ceramide
IR
ATP synthase
ADP
ATP
FA-CoA
NAD+
FAS
NADH +H+
ChREBPβ
β-oxid.
malonyl-CoA
acetyl-CoA
ADP
ATP
ACC
ChREBPα
Krebs cycle
citrate
acetyl-CoA
glycerol-3-phosphate
glucose
glucose-6-phosphate
pyruvate
GLUT4

B)



Insulin-resistant humans feature impaired lipid and energy metabolism in white adipocytes. *A) Normal mitochondrial function is required for lipogenesis: In states of adequate adenosine triphosphate (ATP) concentration, fatty acids (FA) can endogenously be produced de-novo from glucose via acetyl coenzyme A (CoA) by acetyl-coenzyme A carboxylase (ACC). Further upstream lipogenesis is preserved by the second key enzymes in lipogenic pathways, the fatty acid synthase (FAS) via malonyl-CoA in the presence of NADH+H$^+$. FA binding protein adipocyte protein 2 (aP2) inhibits lipogenesis. B) Lipolysis is induced by activation of the β-adrenoreceptor signaling cascade via catecholamines resulting in triacylglycerol (TAG) breakdown. This pathway provides energy rich substrates in forms of FA for beta (ß)-oxidation to produce energy sources in mitochondria. Adenosine diphosphate (ADP), adipose triacylglycerol lipase (ATGL), carbohydrate-responsive element-binding protein (ChREBP), carnitine palmitoyltransferase 1 (CPT1), diacylglycerols (DAG), hydroquinone form of flavin adenine dinucleotide (FADH2), fatty acid transport protein 1 (FATP1), glucose transporter 4 (GLUT4), hormone-sensitive lipase (HSL), insulin receptor (IR), monoacylglycerols (MAG). Dependency of respective pathway on presented substrate is indicated by dotted arrows. Metabolic pathways of biochemical substrates and products are shown by black arrows. Joined pathways of biochemical substrates resulting in the respective product are indicated by purple arrows. Regulatory functions are shown by double lined black arrows. The direction of change in the insulin-resistant state is indicated by not filled arrows in red. Figure modified from Bódis K and Roden M, Eur J Clin Invest, 2018 (118).*

In vitro, FAS expression and activity was induced by insulin via glucose 6-phosphate and carbohydrate responsive-element binding protein (ChREBP) in cultured human adipocytes (204-207). The transcription of ChREBPβ due to GLUT4-mediated augmentation of intracellular glucose levels and activation of the ChREBPα isoform resulted in higher adipose tissue DNL by activation of FAS (206, 207). ACC might be activated by its substrate citrate, independent of insulin, to induce DNL (208). On the other hand, low circulating insulin concentrations during fasting led to decreased adipose tissue glucose uptake and DNL (209). These findings suggest that insulin availability and efficient insulin signaling is not only crucial for glucose uptake into adipocytes, but also important for DNL. In agreement with previous results, insulin-resistant humans feature decreased lipogenic gene expression - indicated by lower FAS and ACC (202, 210, 211) (Table 1). In turn, this is accompanied by reduced insulin induced incorporation of carbons from labeled glucose in TAG in both SAT and VAT (182). Furthermore, mouse models support the concept that impaired DNL in adipocytes is involved in the pathogenesis of systemic insulin resistance (212). Decrease of aP2 led to higher adipose tissue DNL in a murine model and thereby induced insulin signaling in adipocytes and improved systemic insulin sensitivity (196) (Figure 3, 4 A). Palmitoleate - a product of DNL from adipocytes - might serve as a circulating lipokine suppressing hepatic steatosis and stimulating muscle insulin action (196). Apart from palmitoleate, other metabolites from DNL such as FA esters of hydroxy FA, also contributing to inter-organ crosstalk between adipose tissue and insulin-sensitive tissues regulating energy metabolism and insulin sensitivity were identified (118, 213).

In humans, systemic insulin sensitivity was associated with DNL in both VAT and SAT (182, 211, 214-216) (Table 1). Compared to non-obese humans, insulin-resistant obese humans featured higher fasting insulin and glucose levels as well as decreased ChREBPβ, FAS and GLUT4 expression in VAT (202). Vice versa, ChREBPβ and FAS mRNA levels were higher in the liver of insulin-resistant obese compared to non-obese controls (202). In contrast to several previous reports (182, 202, 210, 211, 214, 217), one study reported higher adipose tissue *FAS* gene expression in insulin-resistant than in insulin-sensitive humans (218) (Table 1). These contradictory results may be explained by differences in dietary intake (210) or adipose tissue mitochondrial function. Importantly, the relevance of the results of this study were weakened by further analyses in the study, which showed that differences in *FAS* gene expression between the groups were not supported by FAS protein expression (118, 218).

In summary, decreased lipogenesis and excessive lipolysis in adipose tissue promote FA and glycerol release and modify cytokine secretion in insulin-resistant persons. Ultimately these mediators cause insulin resistance in skeletal muscle and liver by ectopic lipid accumulation and low-grade inflammation.

Table 1

Reference	Cohort	Systemic insulin sensitivity	Insulin sensitivity of depot	Depot	Lipogenesis	Comment
Eissing, L., et al. Nat Commun, 2013 (202)	CON (n=19)			SAT & VAT		
	OBE (n=21)	↓ (HOMA) [vs CON]	↓ GLUT4 mRNA (only in VAT) [vs CON]		↓ ChREBP-β mRNA (only in VAT) [vs CON]	↑ ChREBP-β mRNA in liver [vs CON]
	OBE-T2D (n=21)	↓ (HOMA) [vs CON]	↓ GLUT4 mRNA & protein [vs CON] (protein only in VAT)		↓ FAS, GLUT4 mRNA [vs CON] ↓ FAS, GLUT4, ACC protein (all only in VAT) [vs CON]	↑ ChREBP-β, FAS, ELOVL6 mRNA in liver ↓ ChREBP-α in liver [vs CON]
Kursawe, R., et al. Diabetes, 2013 (182)	NGT (n=43)*			SAT		
	IGT (n=5) & T2D (n=5)**	↓ Liver *** ↓ muscle (Rd)	↔ (suppression of glycerol during lamp)		↓ insulin stimulated DNL ↓ ChREBP, SREBP1c, FAS, GLUT4 mRNA	↑ ChREBP, SREBP1c mRNA in liver
Ortega, F.J., et al., Obesity, 2010 (210)	lean CON (n=27)			VAT		↓ FAS, ACC mRNA in SAT [non-OBE vs OBE+OBE-T2D]
	overweight CON (n=24)	↔(HOMA) [vs lean CON]	n.a.		↓ FAS mRNA [vs lean CON]	
	OBE (n=49)	↓ (HOMA) [vs lean CON]	n.a.		↓ FAS, ACC mRNA [vs lean CON]	
	OBE-T2D (n=19)	↓ (HOMA) [vs lean CON]	n.a.		↓ FAS, ACC mRNA [vs lean CON]	
Mayas, M.D., et al., Nutr Metab, 2010 (214)	CON (n=n.k.)			VAT		
	high glycemia (n=n.k.)	↓ (HOMA)	n.a.		↓ FAS mRNA	FAS mRNA correlated negatively with insulin resistance
Kursawe, R., et al. Diabetes, 2010 (217)	↓VAT/[VAT+SAT] (n=20)			SAT		
	↑VAT/[VAT+SAT] (n=18)	↓ (M-value)	↑ insulin receptor gene expression		↓ ACC, FAS, PPARγ and SREBP1 mRNA	↑ liver fat content (MRI) Trend of p=0.08 for ↓ insulin stimulated DNL
Berndt, J., et al. Diabetologia 2007 (218)	NGT (n=129)			SAT & VAT		↑ FAS mRNA [VAT vs SAT]
	IGT & T2D (together n=67)	↓ (M-value)	n.a.		↑ FAS mRNA	
Ranganathan, G., et al. J Lipid Res, 2006 (211)	NGT (n=13)			SAT		
	IGT (n=37)	↓ (S_I) [vs NGT]	n.a.		↓ DGAT and FAS mRNA [vs NGT]	

In vivo and in vitro studies exploring the role of lipogenesis in human subcutaneous and visceral adipose tissue for insulin resistance. ** Persons with normal glucose tolerance (NGT) divided in humans with 2-h glucose levels <120 mg/dl (n=27) and between 120 and 140 mg/dl (n=16), ** humans with type 2 diabetes (T2D) and persons with impaired glucose tolerance (IGT) combined in one group. *** insulin sensitivity of the liver was assessed by suppression of hepatic glucose production during clamp. Acetyl-coenzyme A carboxylase (ACC), carbohydrate-responsive element-binding protein (ChREBP), de novo lipogenesis (DNL), diacylglycerol acyltransferase (DGAT), fatty acid elongase 6 (ELOVL6), fatty acid synthase (FAS), glucose transporter 4 (GLUT4), healthy controls (CON), homeostasis model assessment (HOMA), insulin sensitivity index (S_I), magnetic resonance imaging (MRI), not assessed (n.a.), not known (n.k.), obese participants (OBE), peroxisome proliferator-activated receptor (PPAR) γ, rate of disappearance (Rd), sterol regulatory element-binding protein 1 (SREBP1), subcutaneous adipose tissue (SAT), triacylglycerol (TAG), visceral adipose tissue (VAT), whole-body insulin sensitivity (M-value). Unless indicated all parameters were analyzed in fasted state. Table modified from Bódis K and Roden M, Eur J Clin Invest, 2018 (118).*

1.4. Mitochondrial function in adipose tissue

The integrated adaptation of whole-body substrate flux according to prevailing metabolic conditions in skeletal muscle, liver and white adipose tissue sustains physiologic levels in glucose homeostasis (219). The ability to switch from insulin stimulated lipid to carbohydrate oxidation defined as metabolic flexibility can be assessed by indirect calorimetry (220, 221). Reduced mitochondrial respiration in skeletal muscle, but also changes of mitochondrial function in adipose tissue was suggested to impair metabolic flexibility (222, 223). Insulin signaling and insulin-stimulated glucose uptake in adipocytes are dependent of mitochondrial efficiency (224, 225). Of note, mitochondrial efficiency can be induced by new compounds stabilizing PPARγ 1 coactivator α (PGC1α) in adipocytes in vitro (226) and by short-term dietary reduction of branched-chain amino acids in human white adipose tissue in vivo (227). Mitochondrial transcription factor A (TFAM) deficiency in white adipocytes of mice caused reduced expression and enzymatic activity of the electron transport chain proteins in complexes I, III and IV (228). This resulted in increased flux of lipid metabolites and lactate to the liver and skeletal muscle inducing ectopic lipid accumulation and tissue-specific insulin resistance (228). Insulin-resistant humans have been shown to feature lower expression of proteins involved in the regulation of mitochondrial function in abdominal SAT than insulin-sensitive persons (229). In conclusion, different features of impaired mitochondrial function in human white adipose tissue may alter metabolic adaptation, which contributes to insulin resistance and hepatic lipid accumulation and ultimately results in development of T2D (118). Although commonly used, the term ‘mitochondrial dysfunction’ or other simplifications and generalizations of ‘impaired mitochondrial function’ is misleading and in previous studies often not clearly defined or specified. Unless not defined or specified, these terms should be avoided as it implies only one unifying perturbation of mitochondria (230). Precise descriptions of different features of mitochondrial function such as respiration capacity for different substrates, ATP or ROS production, membrane potential, coupling and efficiency should rather be used to give more detailed information about the specific feature of mitochondrial malfunction (231). Furthermore, for even more detailed analyses additional features relevant for mitochondrial function such as tricarboxylic acid (TCA) cycle activity, mitochondrial content or density should be measured (232).
Only a few studies assessed mitochondrial function via direct measurements of respiration in SAT and these showed controversial results. While there is evidence for metabolic differences between both SAT compartments, the mitochondrial function has not been assessed in SSAT and DSAT yet.

1.4.1. Mitochondrial morphology and density

The human white adipocyte is spherical and consists of a single lipid droplet with a small surrounding cytosol containing quantitatively low amounts of mitochondria (233). The size of each adipocyte is variable and depends on the size of the lipid droplet storing TAG. White adipocytes have fewer

mitochondria than brown adipocytes and the amount of mitochondria is lower in large compared to small adipocytes (234). Adipocyte mitochondria are elongated, thin and have randomly oriented cristae (233). Mitochondrial density - assessed as the amount of mitochondria per milligram of tissue - is twofold higher in human VAT compared to SAT (235). Possible differences in mitochondrial density between DSAT and SSAT layers have not been elucidated yet.

Mitochondrial density is reduced in SAT of insulin-resistant humans with and without T2D (236, 237). Lower mitochondrial biogenesis was indicated by decreased gene expression of PGC1α in insulin-resistant persons with T2D (237). Previous studies showed that mitochondrial density in SAT associates with systemic insulin sensitivity (236, 238), but may be also affected by BMI and age (238). Of note, also a strong association between mitochondrial density and ex vivo lipogenesis in adipose tissue was reported previously (118, 238).

1.4.2. Mitochondrial lipid handling

During starvation, systemic energy homeostasis is maintained by a coordinated release of FA from adipose tissue serving as substrates for mitochondrial beta-oxidation to generate ATP (239). Sufficient ATP supply is essential for adipocytes insulin signaling (240) and thereby for induction of lipogenesis (241), but also for insulin stimulated inhibition of lipolysis (236).

The turnover of intracellular ATP to cAMP induces AMP-activated protein kinase (AMPK) and thereby activates ATP-producing pathways (242) like glucose uptake, glycolysis or beta-oxidation to maintain adequate energy levels in adipocytes (243). Vice versa, AMPK inhibition results in induction of ATP-dependent pathways like, glycogen, FA, protein or cholesterol synthesis (243). A previous study showed that AMPK is activated by inhibition of PKA via cAMP induction and followed by decreased HSL activity in adipocytes (244). In line with previous observations, lipolysis in adipocytes is decreased by AMPK induction by adrenoreceptor agonists (245). Furthermore, genetic deletion or inhibition of ATGL led to decreased lipolysis and was accompanied by reduced catecholamine-induced AMPK activation (118, 246).

These findings implicate a reciprocal AMPK activation, which is dependent on lipolytic activity and does not necessarily reduce lipolysis via downstream signaling from catecholamine-stimulated signaling. However, functional studies analyzing mechanisms of AMPK induction during chronic states of augmented lipolysis are still lacking. Thus, the interpretation of previous observations for systemic insulin resistance and possible implication for T2D development remain challenging (118).

1.4.3. Mitochondrial function in insulin resistance

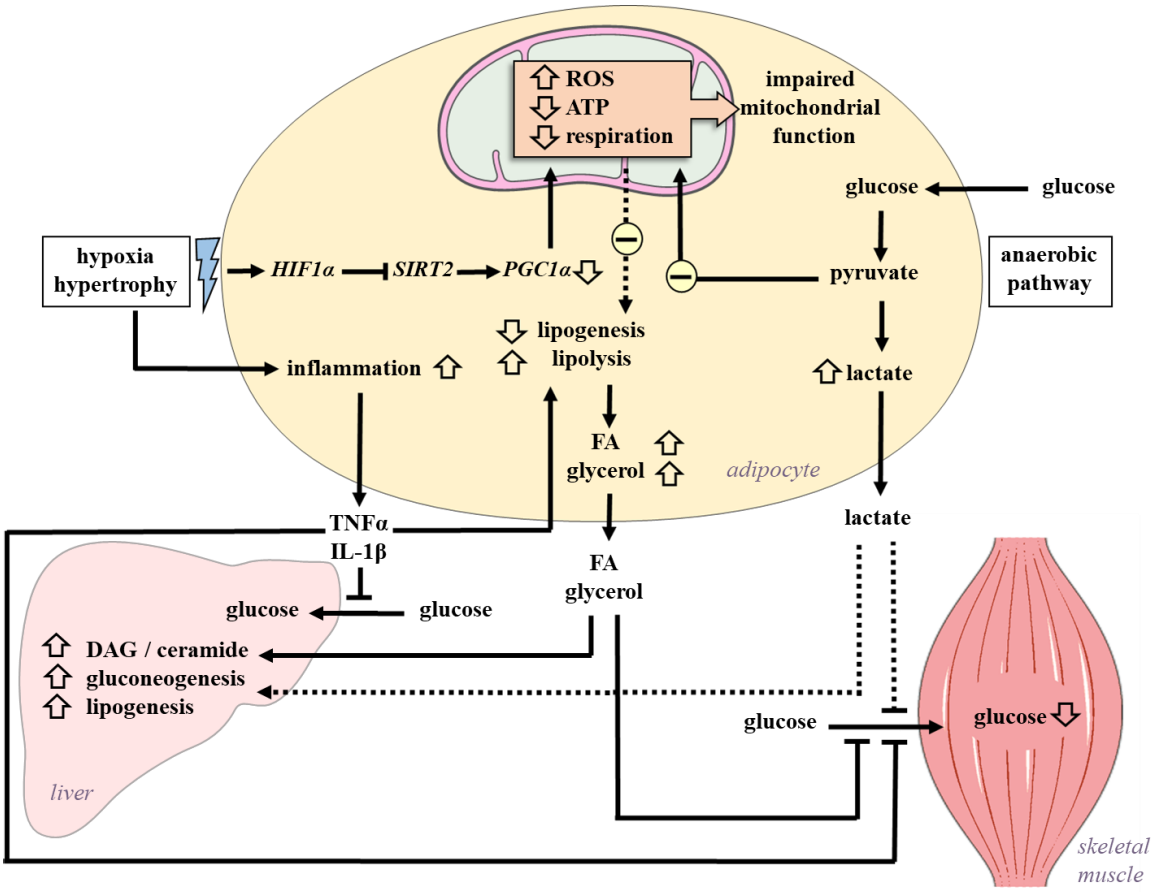
In humans, mitochondrial oxidative capacity, measured per milligram of tissue, was increased in VAT compared to SAT (235). Importantly, mitochondrial respiration was even decreased in VAT when

normalized for mitochondrial content, because mitochondrial density was higher in VAT than in SAT (235). Despite existing evidence for metabolic differences between DSAT and SSAT compartments, tissue segregated analyses for mitochondrial function and density have not been performed yet.

Compared to insulin-sensitive human, proteins regulating mitochondrial function are decreased in SAT of persons with insulin resistance (229). These observations were confirmed in mouse models, showing that systemic insulin resistance was induced by perturbation of the enzyme activity of electron transport chain proteins in adipose tissue (228). A wide range of metabolic pathways such as glucose uptake (225), insulin signaling (224), lipogenesis, lipolysis and secretion of adipokines (247) are dependent from sufficient energy sources. Thus, development of tissue-specific insulin resistance was suggested to result from perturbation of different features of mitochondrial function leading to lower production of energy sources for highly energy dependent pathways in adipocytes, ultimately resulting in systemic insulin resistance (118, 222).

In humans, systemic insulin resistance developed when augmented mitochondrial function in adipose tissue impaired the secretion of autocrine, paracrine and endocrine mediators regulating insulin sensitivity in distant tissues (248). Various impairments of mitochondrial function result in upregulated anaerobic energy metabolism in adipocytes, thereby compensating reduced pyruvate turnover in mitochondria. Both in vitro and in vivo studies revealed higher lactate production in hypertrophic adipocytes of insulin-resistant humans (249, 250). In agreement with the previous findings, insulin-resistant obese humans featured higher fasting plasma lactate levels, which positively correlated with whole-body insulin resistance (251). Another study showed that increased circulating lactate levels activate gluconeogenesis in the liver and perturb glucose uptake in muscle (118, 252) (Figure 5). Thus, decreased protein expression regulating mitochondrial function in adipose tissue of insulin-resistant humans may impair mitochondrial function and whole-body insulin sensitivity due to increased release of lactate and lipid metabolites.

Figure 5



Consequences of different features of impaired mitochondrial function in human white adipose tissue on systemic glucose homeostasis. *Different features of impaired mitochondrial function (e. g. increased production of reactive oxygen species (ROS), decreased mitochondrial respiration or production of energy sources) in human white adipose tissue alter the secretion of autocrine, paracrine and endocrine mediators affecting hepatic and skeletal muscle glucose uptake. In states of perturbation of various features of mitochondrial function anaerobic energy metabolism in white adipose tissue is compensatory upregulated to decreased pyruvate turnover in mitochondria. This leads to higher circulating fasting lactate levels, which might promote hepatic gluconeogenesis and decrease skeletal muscle insulin sensitivity. Lipolysis in white adipose tissue is enhanced by existing inflammatory processes in insulin-resistant obese humans also resulting in elevated cytokine release. Furthermore, inflammation and adipocyte hypertrophy can cause hypoxia in white adipose tissue resulting hypoxia-inducible factor 1 α (HIF1α) activation, followed by inhibition of sirtuin 2 (SIRT2) and subsequent diminished deacetylation of peroxisome proliferator-activated receptor-γ coactivator 1-α (PGC1α), which results in perturbation of various features of mitochondrial function. Different features of impaired mitochondrial function, increased lipolysis and decreased lipogenesis in white adipose tissue enhance the release of lipid metabolites (glycerol and fatty acids) and cytokines (TNFα, IL-1β), which both induce ectopic lipid accumulation and reduce hepatic and skeletal muscle glucose uptake. Fatty acids (FA), interleukin-1β (IL-1β) and tumor-necrosis factor α (TNFα). Suspected effects of presented substrates are shown by dotted arrows. The direction of change in insulin-resistant state is shown by not filled thick arrows. Figure modified from Bódis K and Roden M, Eur J Clin Invest, 2018 (118).*

In mouse models, tissue-specific inflammation and systemic insulin resistance is induced by perturbation of various features of mitochondrial function in adipose tissue. Increased adipocyte size and mass of white adipose tissue has been linked to insufficient vascularization, fibrosis, low-grade inflammation and hypoxia in adipose tissue (94). Hypoxia-inducible factor 1α (HIF1α), a protein of the inner mitochondrial membrane, was shown to be activated by hypoxia in VAT and results in inhibition of sirtuin 2 (SIRT2), subsequently leading to reduced deacetylation of PGC1α and finally decreased beta-oxidation (253). Compared to lean controls, insulin-resistant obese humans showed higher HIF1α and lower SIRT2 protein levels (253). Accordingly, insulin-resistant obese persons revealed lower expression of genes regulating mitochondrial biogenesis, respiratory chain, ATP synthase and FA oxidation in mitochondria of human adipose tissue (253) (Table 2, Figure 5). *HIF1α* deletion ameliorates obesity-induced adipocyte inflammation and insulin resistance in liver and muscle by facilitating lipid mobilization from the liver to adipose tissue (181). Adipocyte *HIF1α* deletion resulted in decreased hepatic inflammation suggesting another potential crosstalk between both tissue (181). This indicates that HIF1α activation might be an early process in adipocyte malfunction and a possible target for novel anti-obesity drugs or insulin sensitizers.

Increased mitochondrial oxidation by thiazolidinediones in adipose tissue of mice suggested that higher mitochondrial function in adipocytes induces whole-body insulin sensitization (254). While obese mice showed lower expression for genes coding for mitochondrial function, those genes were upregulated after treatment with rosiglitazone (254, 255). Lipid utilization in adipocytes of mice could be increased by higher mitochondrial mass and increased oxygen consumption, both resulting in whole-body insulin sensitization (255). Of note, thiazolidinediones improve whole-body insulin sensitivity by induction of genes regulating glycerol production, which improve TAG synthesis in adipocytes by initiation of FA esterification on the glycerol backbone (241, 254, 256). Nevertheless, a previous study showed that glycerol synthesis requires intact mitochondrial function (256). Thus, TAG production by glycerol synthesis in adipocytes may be disturbed by perturbation of various features of mitochondrial function.

In humans, only a few studies measured mitochondrial function directly by assessment of adipocyte respiration in SAT and these studies also reported controversial results. While one study reported decreased mitochondrial respiration in young obese and non-obese humans with T2D compared to lean healthy controls (257), another study displayed no differences between morbidly obese humans with and without T2D (258). However, conclusions from both studies for the relevance of mitochondrial function for the regulation of glucose homeostasis in T2D are limited because none of the studies provided data on insulin sensitivity.

Table 2

Reference	Cohort	Systemic insulin sensitivity	Insulin sensitivity of depot	Depot	Mitochondrial function	Comment
Xie, X., et al., Int J Obes, 2017 (236)	NGT (n=13)			SAT		
	IGT (n=7)	↓ (M-value)	↓ (FA suppression during clamp)		↓ mtDNA ↔ NADH cytochrome C reductase activity	
Xie, X., et al., Obesity, 2016 (229)	Insulin-sensitive (n=13)			SAT		
	Insulin-resistant (n=10)	↓ (M-value)	n.a.		↓ proteins of complexes I, III and IV ↓ ANT and other mitochondrial proteins	
Hansen M., et al., Obesity, 2015 (258)	OBE CON (n=27)			SAT		insulin sensitivity not reported, but gold-standard respirometry analysis for mitochondrial function in T2D
	OBE T2D (n=16)	n.r.	↓ (glycerol suppression during clamp)		↔ overall respiration [vs lean CON]	
Krishnan, J., et al., Genes Dev, 2012 (253)	lean CON (n=9)			VAT		HIF1α & SIRT2 protein expression inversely correlates ↑ adipocyte size [vs lean CON]
	OBE (n=9)	↓ (M-value)	n.a.		↓ SIRT2, CPT1, ↑ HIF1α protein ↓ FATP1, FACS1, Thiolase, AK2, CPT1 mRNA (FA oxidation) ↓ NRF1, ERR1β, TFAM, TFB2M, SSBP1 mRNA (mitochondrial biogenesis) ↓ ATP5J mRNA (ATP synthase) ↓ COX3, CYB5A, MTND1, COX6C, CoVa mRNA (mitochondrial respiratory chain)	
Chattopadhyay, M., et al., Metabolism, 2011 (257)	lean CON (n=10)			SAT		insulin sensitivity not assessed, but gold-standard respirometry analysis for mitochondrial function in T2D
	lean T2D (n=10)	n.a.	n.a.		↔ overall respiration [vs lean CON]	
	OBE CON (n=10)	n.a.	n.a.		↓ membrane potential, inorganic phosphate utilization, overall respiration [vs lean CON]	
	OBE T2D (n=10)	n.a.	n.a.		↓ membrane potential, inorganic phosphate utilization, overall respiration [vs lean CON] ↓ inorganic phosphate utilization, activities of several respiratory complexes [vs OBE CON]	

In vivo and in vitro studies exploring the role of mitochondrial function in human subcutaneous and visceral adipose tissue for insulin resistance. *Adenine nucleotide translocators (ANT), adenylate kinase 2 (AK2), adipose triacylglycerol lipase (ATGL), ATP synthase-coupling factor 6 (ATP5J), carnitine palmitoyltransferase 1 (CPT1), cytochrome b5 type A (CYB5A), cytochrome c oxidase polypeptide Va (CoVa), cytochrome c oxidase subunit VIc (COX6C), cytochrome c oxidase subunit III (COX3), estrogen-related receptor (ERR) 1β, fatty acid (FA), fatty acid transport protein 1 (FATP1), fatty acyl-CoA synthetase 1 (FACS1), healthy controls (CON), hypoxia-inducible factor (HIF) 1α, mitochondrial deoxyribonucleic acid (mtDNA), mitochondrial transcription factors A and B2 (TFAM and TFB2M respectively), mitochondrial NADH dehydrogenase 1 (MTND1), not assessed (n.a.), not reported [n.r.], obese participants (OBE), nuclear respiratory factor (NRF) 1/2, single-stranded DNA-binding protein 1 (SSBP1), sirtuin 2 (SIRT2), subcutaneous adipose tissue (SAT), humans with type 2 diabetes (T2D), visceral adipose tissue (VAT), whole-body insulin sensitivity (M-value). Unless indicated all parameters were analyzed in fasted state. Table modified from Bódis K and Roden M, Eur J Clin Invest, 2018 (118).*

2. Aims

The aim of this work was to elucidate the role of FFAR2/4, SCD1 and adipose tissue energy metabolism for whole-body glucose metabolism in T2D. Both enclosed studies were based on the multicenter prospective longitudinal GDS, which includes patients with T1D and T2D (< 1 year) and age-, BMI- and sex- matched CON. The GDS examines the natural course of the disease as well as comorbidities and complications by annual telephone interviews and 5-year follow-up visits. Metabolic features of whole-body and tissue-specific glucose and energy metabolism are measured by gold-standard methods. The GDS represents an ideal setting to characterize specific subphenotypes and to identify novel targets for the treatment of the disease. Furthermore, the GDS can clarify the link between clinical features and metabolic alterations by employing detailed metabolic phenotyping. The study is performed according to the Declaration of Helsinki, approved by the ethics board of the Heinrich Heine University of Düsseldorf in Germany (reference number 4508) and is registered at Clinicaltrials.gov (Identifier number: NCT01055093). More information on the study design and cohort description can be found elsewhere in detail (259). The findings of both studies will improve the understanding of potential pathophysiological regulators of whole-body insulin resistance and may serve as source of diagnostic and prognostic biomarkers for T2D. Thereby, these studies should provide new insights in the role of lipid and energy metabolism on glucose homeostasis in early-onset T2D and potential targets for preventive and therapeutic strategies.

2.1. Role of free fatty acid receptors 2/4 and stearoyl-CoA desaturase-1 in adipose tissue of humans with recent-onset type 2 diabetes

The first aim of the work was to assess the relevance of FFAR2/4 and SCD1 gene and protein expression in human SAT for insulin sensitivity and BCF in recently diagnosed humans with T2D. To this end, we performed mixed meal tests to assess insulin sensitivity as well as BCF and conducted biopsies of the abdominal SAT to measure gene and protein expression of SCD1 and FFAR2/4 in patients with T2D and sex-, age- and BMI-matched CON in a cross-sectional analysis of a subgroup of GDS participants. This study was performed at the Institute for Clinical Diabetology at the German Diabetes Center Düsseldorf.

2.2. Relevance of energy metabolism in adipose tissue of patients with newly diagnosed type 2 diabetes

The second aim of the work was to analyze mitochondrial respiration, coupling and efficiency in SSAT and DSAT separately and assess their relation to tissue-specific insulin sensitivity of the skeletal muscle, liver and adipose tissue as well as to hepatic steatosis and metabolic flexibility in patients with recently diagnosed T2D. To this end adipose tissue energy metabolism, liver fat content, metabolic flexibility and tissue-specific insulin sensitivity were measured in patients with T2D and sex-, age-, BMI-, total body fat mass- and whole SAT thickness-matched CON in a cross-sectional analysis of a subgroup of GDS participants. This study was also performed at the Institute for Clinical Diabetology at the German Diabetes Center Düsseldorf.

3. Publications

3.1. Reduced expression of stearoyl-CoA desaturase-1, but not free fatty acid receptor 2 or 4 in subcutaneous adipose tissue of patients with newly diagnosed type 2 diabetes mellitus

Bódis, K, Kahl S, Simon MC, Zhou Z, Sell H, Knebel B, Tura A, Strassburger K, Burkart V, Mussig K, Markgraf D, Al-Hasani H, Szendroedi J, and Roden M. Reduced expression of stearoyl-CoA desaturase-1, but not free fatty acid receptor 2 or 4 in subcutaneous adipose tissue of patients with newly diagnosed type 2 diabetes mellitus. *Nutrition & diabetes*. 2018;8(1):49.

Bódis et al. *Nutrition and Diabetes* (2018)8:49
DOI 10.1038/s41387-018-0054-9

Nutrition & Diabetes

ARTICLE Open Access

Reduced expression of stearoyl-CoA desaturase-1, but not free fatty acid receptor 2 or 4 in subcutaneous adipose tissue of patients with newly diagnosed type 2 diabetes mellitus

Kálmán Bódis[1,2], Sabine Kahl[1,2], Marie-Christine Simon[1,2], Zhou Zhou[2,4], Henrike Sell[1,2], Birgit Knebel[2,4], Andrea Tura[5], Klaus Strassburger[2,6], Volker Burkart[1,2], Karsten Müssig[1,2,3], Daniel Markgraf[1,2], Hadi Al-Hasani[2,4], Julia Szendroedi[1,2,3] and Michael Roden[1,2,3], for the GDS Study Group

Abstract

Background In subcutaneous adipose tissue (SAT), higher *stearoyl-CoA desaturase-1* (*SCD1*) expression has been related to improved insulin sensitivity in thiazolidinedione-treated type 2 diabetes mellitus patients. In animal models, deficiency of the *free fatty acid receptor* (*FFAR*) *2* associated with higher and *FFAR4*-deficiency with lower insulin sensitivity. We hypothesized that increased FFAR2 expression and reductions in FFAR4 and SCD1 expression in SAT of type 2 diabetes mellitus patients associate positively with insulin resistance and impaired beta cell function.

Methods Twenty-five type 2 diabetes mellitus patients and 25 glucose-tolerant humans (CON) matched for sex, age, and BMI underwent mixed-meal tests to assess insulin sensitivity (OGIS) and beta cell function ($\Delta AUC(C\text{-peptide})_{0-180\ min}/\Delta AUC(glucose)_{0-180\ min}$) in a cross-sectional study. Gene and protein expression of SCD1 and FFAR2/4 were quantified in SAT biopsies.

Results Insulin sensitivity was 14% and beta cell function 71% (both $p < 0.001$) lower in type 2 diabetes mellitus patients. In type 2 diabetes mellitus, *SCD1* mRNA was fivefold ($p < 0.001$) and protein expression twofold ($p < 0.01$) lower. While FFAR2/4 mRNA and protein expression did not differ between groups, FFAR2 protein levels correlated negatively with beta cell function only in CON ($r = -0.74$, $p < 0.01$). However, neither SCD1 nor FFAR2/4 protein expression correlated with insulin sensitivity in both groups.

Conclusions Type 2 diabetes patients have lower SCD1, which does not associate with insulin resistance. Only in non-diabetic humans, FFAR2 associated with impaired beta cell function.

Introduction

Type 2 diabetes is characterized by insufficient insulin secretion to compensate for peripheral insulin resistance[1]. Studies in animal models and humans implicate free fatty acid receptors (FFAR), also known as G-protein coupled

Correspondence: Michael Roden (michael.roden@ddz.uni-duesseldorf.de)
[1]Institute for Clinical Diabetology, German Diabetes Center, Leibniz Center for Diabetes Research at Heinrich Heine University, Düsseldorf, Germany
[2]German Center for Diabetes Research (DZD), München-Neuherberg, Germany
Full list of author information is available at the end of the article.
These authors contributed equally: Kálmán Bódis, Sabine Kahl, Marie-Christine Simon
Members of the GDS Study Group are listed below Acknowledgements.

receptors (GPR), as receptors for non-esterified fatty acids (NEFA) in the pathogenesis of beta cell dysfunction and progression to insulin resistance and type 2 diabetes mellitus[2,3].

FFAR2 and FFAR4 (also known as GPR43 and GPR120) serve as receptors for acetate and long-chain fatty acids (FA)[4,5], respectively, and are supposed to contribute to the regulation of glucose homeostasis through FA signaling pathways[6]. Recent studies provided evidence that *FFAR2*-deficient mice on high-fat diet are protected from the increase in body fat mass and dyslipidemia, accompanied by increased insulin sensitivity (IS)[7]. In addition, in mouse islets *FFAR2* gene expression was increased during the insulin-resistant phase of pregnancy[8,9]. One translational study further identified gene expression of *FFAR2* in mouse and human islets, and suggested FFAR2 to mediate inhibition of insulin secretion by coupling to G_i-type G proteins[3]. Beta cell-specific deletion of *FFAR2* in another mouse model led to increased insulin secretion and improved glucose tolerance[3]. These findings in beta cells from mouse models and human in vitro studies point to an involvement of FFAR2 in maintaining glucose homeostasis. Adipose tissue (AT) expansion in obesity associates with insulin resistance and progressive immune cell infiltration in AT[10]. Pro-inflammatory cytokines activate lipolysis[11] causing dyslipidemia[12], lipid-induced insulin resistance in peripheral tissues[13], and impairment of beta cell function[14]. In contrast, FFAR2 knock out mice were protected from high-fat diet-induced AT inflammation and obesity[7]. Thus, FFAR2 may serve as a potential target for diabetes prevention strategies via inhibition of lipid-induced insulin resistance.

A previous study showed that FFAR4 activation by omega-3 fatty acid protected human islets from palmitate-induced apoptosis, whereas FFAR4 knock out attenuated omega-3 fatty acid-related anti-apoptotic effects[15]. Compared to wild-type mice, high-fat fed *FFAR4*-deficient mice developed more severe obesity, liver fat accumulation, and insulin resistance[16,17]. However, these findings were accompanied by lower *stearoyl-CoA desaturase-1* (*SCD1*) gene expression in AT[18]. In murine models, four isoforms (SCD1, SCD2, SCD3, and SCD4) have been identified, whereas humans express only two Δ9 desaturases (SCD1 and SCD5). Our study focused on SCD1 as the most highly expressed SCD isoform in AT. SCD1 in AT facilitates the protective conversion of lipotoxic lipid species (saturated into monounsaturated FA). Circulating palmitoleate, an AT derived product of SCD1, increased insulin signaling in both skeletal muscle and the liver, increased insulin secretion from beta cells, and improved whole-body glucose uptake in mice[19]. Furthermore, palmitoleate treatment reduced cytokine expression in cultured adipocytes[19]. SCD1 in AT facilitates the last step of de novo lipogenesis and induces incorporation of FA into triglycerides (TG), both associating positively with whole-body insulin sensitivity. Accordingly, thiazolidinedione treatment promoted TG esterification in cultured adipocytes[20] and increased *SCD1* gene expression in subcutaneous adipose tissue (SAT) with subsequent improvement of IS in patients with type 2 diabetes mellitus, suggesting a potential role of SCD1 in AT on systemic glucose homeostasis[21].

Although FFAR2/4 and SCD1 seem to be involved in maintaining glucose homeostasis in mice[3,16,17,22], the relevance of their expression in human SAT for glucose homeostasis has not yet been elucidated. FFARs and SCD1 are expressed in various tissues, but might be especially important in AT due to its prominent role in lipid turnover. Here, we hypothesized that increased FFAR2 expression and reduced FFAR4 and SCD1 expression in SAT of patients with type 2 diabetes mellitus in the fasted state associate positively with insulin resistance and inversely with beta cell function. Furthermore, we hypothesized that increased FFAR2 and reduced FFAR4 expression in AT of type 2 diabetes patients associate with parameters of dyslipidemia. Finally, we hypothesized that higher SCD1 expression in AT of type 2 diabetes patients associates negatively with high-sensitivity C-reactive protein (hsCRP) in plasma. To this end, we analyzed FFAR2 as well as FFAR4 and SCD1 mRNA and protein expression in SAT of 25 metabolically well-characterized patients with newly diagnosed type 2 diabetes mellitus and 25 age-matched, sex-matched, and BMI-matched glucose-tolerant humans (CON).

Materials and methods

Study participants

The study population comprised 25 patients with recently diagnosed type 2 diabetes mellitus and 25 age-matched, sex-matched, and BMI-matched CON. All participants gave their written informed consent before inclusion into the study (ClinicalTrial.gov registration no: NCT01055093), which was performed according to the Declaration of Helsinki and approved by the ethics board of Heinrich Heine University, Düsseldorf, Germany. Participants were recruited via general practitioners, internet, or advertisements in newspapers. For three days prior to each visit, participants refrained from physical activity and alcohol ingestion and fasted for 10 h on the day before the metabolic studies. Exclusion criteria comprised medical history of acute or chronic diseases including cancer, insulin or thiazolidinedione treatment, medication affecting the immune system and/or a $HbA_{1c} > 9.0\%$ ($75\ mmol\ mol^{-1}$), diabetes other than type 2 diabetes mellitus. Patients with type 2 diabetes mellitus were treated with metformin only ($n = 15$), sulfonylurea only ($n = 2$), metformin and sulfonylurea ($n = 2$), glucagon-like peptide-1 receptor agonist and metformin ($n = 1$), or diet

only ($n = 5$). They withdrew their oral glucose-lowering medication for at least 3 days before all measurement to exclude acute effects on glucose metabolism[23]. All patients with type 2 diabetes mellitus also participated in the baseline cohort of the ongoing German Diabetes Study (GDS), a prospective observational study investigating the natural course of recently diagnosed diabetes and the development of diabetes-associated complications. The study design and cohort profile of the GDS are described in detail elsewhere[23]. Age-matched, sex-matched, and BMI-matched glucose-tolerant participants were recruited as control group (CON).

Mixed-meal test (MMT)

All participants underwent a standardized MMT to assess whole-body IS and beta cell function. For the MMT, each participant consumed 360 ml of Boost High Protein (Nestlé Nutrition, Vervey, Switzerland) containing 41 g of glucose, 9 g of fat, and 23 g protein before 10 am within 5 min followed by defined blood sampling for specific parameters described elsewhere[23,24]. Dynamic IS was assessed by the oral glucose insulin sensitivity index (OGIS), which allows calculating whole-body IS during both oral glucose tolerance test (OGTT) and MMT, provided that the dose of glucose administered during the test is taken into account[25,26]. Beta cell function was assessed from incremental AUC of plasma glucose, insulin, and C-peptide concentrations during MMT. The insulinogenic index from $\Delta AUC(C\text{-peptide})_{0-180\,min}/\Delta AUC(glucose)_{0-180\,min}$, and $\Delta AUC(insulin)_{0-180\,min}/\Delta AUC(glucose)_{0-180\,min}$ was used to describe insulin secretion in relationship to glucose as a measure of beta cell function[27].

Oral glucose tolerance test (OGTT)

All CON underwent a 75 g-OGTT (Accu-Chek Dextro O.G-T., Roche, Basel, Switzerland) after at least 10 h overnight fasting to assess glucose tolerance and exclude participants with (pre-) diabetes mellitus. Blood samples were taken at time points −5, 30, 60, 120, and 180 min and glucose tolerance was categorized according to internationally accepted criteria[28].

Laboratory analyses

Plasma glucose, HbA_{1c}, NEFA, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, TG and hsCRP, insulin, and C-peptide were measured as previously described[23]. Plasma glucagon was measured using radioimmunoassay (Millipore, St. Charles, MO, USA).

Adipose tissue analyses

A biopsy was obtained from abdominal SAT at the level of umbilicus by needle suction technique after administration of local anesthesia (5–10 ml of 1% lidocaine) under fasting conditions. Fat tissue specimen were immediately frozen in liquid nitrogen, and stored −80 °C until analysis. For analyses of mRNA expression, total RNA was isolated (miRNeasy Mini Kit, Qiagen, Hilden, Germany) including on-column DNase digestion. For gene expression analyses, RNA quantity and quality were determined by Nanodrop (Peqlab, Erlangen, Germany) and RNA 6000 Nano Kit (Agilent Technologies, Böblingen, Germany). The complementary DNA equivalent of 20 ng RNA was analyzed by RT-qPCR using predesigned assays with gene-specific hydrolysis probes (*FFAR2*: Hs00271142_s1; *FFAR4*: Hs00699184_m1; *SCD1*: Hs01682761_m1; *peptidylprolyl isomerase A (PPIA)*: Hs04194521_s1; Thermo Fisher Scientific, Darmstadt, Germany). Data were analyzed for relative expression differences using *PPIA* as reference gene with standard C_t method as previously described[29]. For protein expression analyses, reagents for SDS-PAGE were supplied by GE Healthcare (Freiburg, Germany). Complete protease inhibitor cocktail and PhosStop phosphatase inhibitor cocktail were provided by Roche (Mannheim, Germany). All other chemicals were of the highest analytic grade commercially available and purchased from Sigma-Aldrich. The following antibodies were used: anti-G-protein coupled receptor (GPR) 43 (FFAR2) (sc-293202) from Santa Cruz (Dallas, TX, USA), anti-GPR120 (FFAR4) (NBP1-00858) from Novus Biologicals (Abingdon, UK), anti-SCD1 (ab39969), and anti-actin (ab6276) from Abcam (Cambridge, UK). Horseradish peroxidase (HRP)-conjugated goat anti-rabbit and goat anti-mouse IgG antibodies were supplied by Promega (Mannheim, Germany). AT biopsies were lysed in a buffer containing 50 mmol l^{-1} HEPES, pH 7.4, 1% Triton X-100, complete protease inhibitor, and PhosStop phosphatase inhibitor cocktail. After incubation for 2 h at 4 °C, the suspension was centrifuged at 10,000×*g* for 15 min. Thereafter, 10 µg of the lysates were separated by SDS-PAGE using gradient horizontal gels and transferred to polyvinylidene fluoride filters in a semidry blotting apparatus. Filters were blocked with Tris-buffered saline containing 0.1% Tween and 5% nonfat dry milk and subsequently incubated overnight with a 1:1000 dilution (1:40,000 for anti-actin) of the appropriate antibodies. After washing, filters were incubated with secondary HRP-coupled antibody and processed for enhanced chemiluminescence detection using Immobilon HRP substrate (Millipore, Billerica, MA, USA). Signals were visualized and evaluated on a ChemiDoc workstation (Bio-Rad Laboratories, Munich, Germany).

Statistical analyses

Results are given as median [first and third quartiles] or mean ± SEM. Data were compared using Mann–Whitney

U test for unpaired samples to determine differences between groups. Relations between variables were investigated using Spearman rank correlation analyses. The total AUC for a specific variable was calculated as the integral of the time course of such variable during the test, while the incremental AUC (ΔAUC) was calculated by subtracting the basal area from the respective total AUC. All statistical tests were two-sided and a p-value ≤ 5% was accepted to indicate significant differences. All statistical analyses were performed using SPSS for Windows 23.0 (SPSS Inc., Chicago, IL, USA). All graphs were generated using GraphPad Prism, Version 7.01 (GraphPad Software, Inc., La Jolla, CA, USA).

Results

Participants' characteristics

CON and patients with type 2 diabetes mellitus had similar age, BMI, waist circumference, waist-to-hip ratio, and relative body fat mass (Table 1). Patients with type 2 diabetes mellitus had 34% higher fasting plasma glucose, 28% higher fasting insulin, and 29% higher fasting C-peptide. Additionally, serum hsCRP levels were 56% higher in patients with type 2 diabetes mellitus compared to CON. Furthermore, HDL cholesterol was 25% lower and systolic blood pressure 8% higher in patients with type 2 diabetes mellitus (Table 1). Of note, one female patient with type 2 diabetes was in the luteal phase. However, exclusion of this patient did not affect the results. All other females investigated were either postmenopausal or studied in the follicular phase (day 1–14) of their menstrual cycle. Furthermore, one woman in the follicular phase was controlled by an oral contraceptive.

Mixed-meal test (MMT)

In patients with type 2 diabetes mellitus, the glucose concentrations were higher at all time points during the MMT and $AUC(glucose)_{0-180\,min}$ was also 31% higher (Table 2, Supplementary Figure 1a). Insulin at 30 min and C-peptide at 90, 120, and 180 min were higher in patients with type 2 diabetes mellitus vs CON, while $AUC(insulin)_{0-180\,min}$ and $AUC(C\text{-}peptide)_{0-180\,min}$ did not differ between groups (Table 2, Supplementary Figure 1b, c). $AUC(NEFA)_{0-120\,min}$, $AUC(TG)_{0-180\,min}$, and $AUC(glucagon)_{0-180\,min}$ also did not differ between groups (data not shown). In patients with type 2 diabetes mellitus, OGIS was 14% lower and beta cell function assessed from $\Delta AUC(C\text{-}peptide)_{0-180\,min}/\Delta AUC(glucose)_{0-180\,min}$ as well as from $\Delta AUC(insulin)_{0-180\,min}/\Delta AUC(glucose)_{0-180\,min}$ was 71% and 78% lower than CON, respectively.

Gene and protein expression levels

FFAR2 mRNA and protein expression were similar in type 2 diabetes mellitus patients compared to CON (Fig. 1a, b, Supplementary Figure 2a). *FFAR4* mRNA expression did not differ, while FFAR4 protein expression tended to be lower in patients with type 2 diabetes mellitus (Fig. 1c, d, Supplementary Figure 2b). Patients with type 2 diabetes mellitus had fivefold lower mRNA expression and twofold lower protein expression of SCD1 compared to CON (Fig. 1e, f, Supplementary Figure 2c).

Correlation analyses

FFAR2 protein expression neither correlated with OGIS in type 2 diabetes mellitus patients nor in CON. Only in CON, protein expression levels of FFAR2 associated negatively with beta cell function, as assessed from $\Delta AUC(insulin)_{0-180\,min}/\Delta AUC(glucose)_{0-180\,min}$ ($r = -0.78$, $p < 0.01$) and $\Delta AUC(C\text{-}peptide)_{0-180\,min}/\Delta AUC(glucose)_{0-180\,min}$ (Fig. 2). Of note, fasted NEFA levels and $AUC(NEFA)_{0-120\,min}$ did not correlate with beta cell function. Only in patients with type 2 diabetes mellitus, protein levels of FFAR2 correlated positively with $AUC(TG)_{0-180\,min}$ ($r = 0.48$, $p < 0.05$) and negatively with HDL cholesterol ($r = -0.43$, $p < 0.05$). FFAR4 protein expression did not correlate with OGIS or beta cell function in both groups. Protein expression levels of FFAR4 associated negatively with fasting TG and $AUC(TG)_{0-180\,min}$ in patients with type 2 diabetes mellitus ($r = -0.62$ and $r = -0.59$, respectively, both $p < 0.01$), but not in CON ($r = 0.15$, $p = 0.48$ and $r = 0.25$, $p = 0.26$, respectively). FFAR2/4 did not associate with body weight, BMI, or relative body fat content in both groups.

SCD1 protein expression did not correlate with OGIS or beta cell function in both groups. However, SCD1 mRNA and protein levels associated positively with insulin sensitivity across the whole cohort ($r = 0.38$, $p < 0.01$ and $r = 0.31$, $p < 0.05$, respectively). SCD1 protein expression correlated positively with mRNA levels ($r = 0.60$, $p < 0.001$). In CON, protein levels of SCD1 associated negatively with fasting TG ($r = -0.57$, $p < 0.01$) and $AUC(TG)_{0-180\,min}$ ($r = -0.52$, $p < 0.05$). In patients with type 2 diabetes mellitus, these associations were absent (TG: $r = -0.17$, $p = 0.44$; $AUC(TG)_{0-180\,min}$: $r = 0.05$, $p = 0.85$). Only in patients with type 2 diabetes mellitus, SCD1 protein levels correlated negatively with hsCRP ($r = -0.45$, $p < 0.05$).

Discussion

This study found no differences in FFAR2/4 mRNA and protein expression between patients with type 2 diabetes mellitus vs CON of similar body weight. Our findings are in contrast to results in mice, which might be biased by differences in body weight explaining a large part of their phenotypes[30]. We found markedly lower gene and protein expression of SCD1 in subcutaneous AT of patients with type 2 diabetes mellitus when compared with sex-matched, age-matched, and BMI-matched glucose-tolerant

Table 1 Characteristics of study population

Variable	CON	T2D	*p*
Female/male [*n*]	6/19	6/19	
Age [years]	51 [42; 60]	49 [43; 58]	0.84
Weight [kg]	98 [91; 98]	99 [88; 112]	0.61
BMI [kg* m^{-2}]	33 [27; 41]	34 [27; 37]	0.81
HbA_{1c} [%]	5.3 [5.1; 5.5]	6.1 [5.5; 7.0]	**<0.001**
HbA_{1c} [mmol* mol^{-1}]	34 [32; 37]	43 [36; 52]	**<0.001**
Waist circumference [cm]	106 [99; 116]	107 [102; 118]	0.54
Hip circumference [cm]	111 [97; 127]	110 [102; 118]	0.97
WHR	0.98 [0.90; 1.04]	0.99 [0.93; 1.04]	0.54
Body fat [%]	34 [27; 42]	33 [28; 39]	0.87
Lean body weight [kg]	66 [57; 74]	65 [58; 72]	0.87
Systolic blood pressure [mmHg]	124 [114; 143]	144 [126; 159]	**<0.05**
Diastolic blood pressure [mmHg]	80 [69; 86]	80 [73; 94]	0.40
Fasting blood glucose [mmol* l^{-1}]	4.6 [4.4; 4.7]	6.7 [5.7; 8.0]	**<0.001**
Fasting insulin [μU* ml^{-1}]	11 [6; 17]	17 [11; 27]	**<0.05**
Fasting C-peptide [ng* ml^{-1}]	2.3 [1.5; 3.1]	3.4 [2.0; 4.7]	**<0.05**
Fasting glucagon [pg* ml^{-1}]	118 [98; 137]	110 [76; 138]	0.34
NEFA [μmol* l^{-1}]	603 [431; 655]	481 [333; 652]	0.21
TG [mg* dl^{-1}]	119 [82; 144]	117 [102; 252]	0.17
HDL cholesterol [mg* dl^{-1}]	53 [43; 66]	38 [33; 46]	**<0.001**
LDL cholesterol [mg* dl^{-1}]	135 [114; 156]	127 [111; 139]	0.21
hsCRP [mg* dl^{-1}]	0.13 [0.07; 0.33]	0.34 [0.14; 0.72]	**<0.05**
$OGIS_{0–180\ min}$ [ml* min^{-1}* m^{-2}]	362 [349; 385]	311 [293; 343]	**<0.001**

Data are median [first; third quartile], two-tailed Mann–Whitney *U* test ($n = 22–25$ for CON; $n = 16–25$ for type 2 diabetes patients). Significant differences between groups (p-value ≤ 5%) are indicated by bold values. Glucose-tolerant humans (CON), patients with type 2 diabetes mellitus (T2D), waist/hip ratio (WHR), diastolic and systolic blood pressure (BP), oral glucose insulin sensitivity index (OGIS). Triglycerides (TG), high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, non-esterified fatty acids (NEFA), and high-sensitivity C-reactive protein (hsCRP) were analyzed in fasted state

Table 2 Characteristics of beta cell function in study population

Variable	CON	T2D	*p*
AUC 0–180 min			
$AUC(glucose)_{0–180\ min}$ [mmol* l^{-1} min^{-1}]	842 [772; 901]	1 188 [1 080; 1 407]	**<0.001**
$AUC(insulin)_{0–180\ min}$ [nmol* l^{-1} min^{-1}]	48 [25; 68]	49 [31;65]	0.77
$AUC(C\text{-}peptide)_{0–180\ min}$ [nmol* l^{-1} min^{-1}]	350 [238; 405]	373 [290; 506]	0.17
Beta cell function			
$\Delta AUC(insulin)_{0–180\ min}/\Delta AUC(glucose)_{0–180\ min}$ [pmol* $mmol^{-1}$]	680 [408; 1367]	174 [92; 228]	**<0.001**
$\Delta AUC(C\text{-}peptide)_{0–180\ min}/\Delta AUC(glucose)_{0–180\ min}$ [nmol* $mmol^{-1}$]	46 [20; 78]	13 [8; 21]	**<0.001**

Data are median (first; third quartile), two-tailed Mann–Whitney *U* test ($n = 13–25$ for CON; $n = 18–25$ for patients with type 2 diabetes mellitus). Significant differences between groups (p-value ≤ 5%) are indicated by bold values. Glucose-tolerant humans (CON), patients with type 2 diabetes mellitus (T2D). Marker for beta cell function are $\Delta AUC(insulin)_{0–180\ min}/\Delta AUC(glucose)_{0–180\ min}$ in pmol* $mmol^{-1}$ and $\Delta AUC(C\text{-}peptide)_{0–180\ min}/\Delta AUC(glucose)_{0–180\ min}$ in nmol* $mmol^{-1}$

humans. Furthermore, FFAR2 protein expression correlated negatively with beta cell function in glucose-tolerant humans. However, this association was not found in patients with type 2 diabetes mellitus.

In mice, a recent study of whole-body or beta cell selective deletion of *FFAR2* also provided evidence that insulin secretion is increased, accompanied by improved glucose tolerance[3]. In the present study, FFAR2 expression did not differ between patients with type 2 diabetes mellitus and CON, while its protein levels negatively associated with beta cell function during MMT in CON. This points to a potential impact of the receptor on glucose homeostasis after food intake rather than in the fasted state. In addition, the association between *FFAR2*-deficiency and IS in mice[7] was not confirmed in humans with or without diabetes in the present study, indicating that reported findings in FFAR2-deficient mice cannot directly be translated to humans. Although previous studies indicated an assumed role in AT lipolysis[11] causing impaired beta cell function[14], we did not find a significant correlation of NEFA levels either during fasting or after the MMT with beta cell function. However, suppression of NEFA concentrations after the MMT might not be an optimal method to assess AT lipolysis, because of the exogenous oral intake of lipids and the individual variability of lipid absorption. Thus, the absence of a significant correlation between suppression of NEFA levels after the MMT and beta cell function does not exclude effects of insulin-mediated adipose tissue lipolysis.

In humans, a deleterious non-synonymous mutation (p. R270H) that inhibits *FFAR4* signaling was found to associate with increased risk of obesity in a European population[16]. Of note, development of severe obesity, liver fat accumulation, and insulin resistance in *FFAR4*-deficient mice under high-fat diet[16,17] were accompanied by lower *SCD1* gene expression[18]. We found no association between FFAR4 and body weight or differences in human FFAR4 expression between patients with type 2 diabetes mellitus and CON, but FFAR4 protein expression tended to be lower in type 2 diabetes mellitus. One possible explanation for the lack of differences between groups in FFAR4 expression might be that these receptors are dependent on acute increase in NEFAs triggering FFAR4 expression in AT. In the present study, fasted NEFA and TG levels did not differ between groups, mainly because our study only included patients with recently diagnosed and well controlled type 2 diabetes mellitus.

Interestingly, our study revealed markedly lower gene and protein expression of SCD1 in SAT of patients with type 2 diabetes mellitus. These findings are in line with the previously reported decrease in *SCD1* gene expression in FFAR4-deficient mice[18] and support a role of SCD1 (Δ9 desaturase) in glucose homeostasis. Desaturases are key enzymes in converting saturated to unsaturated FAs by

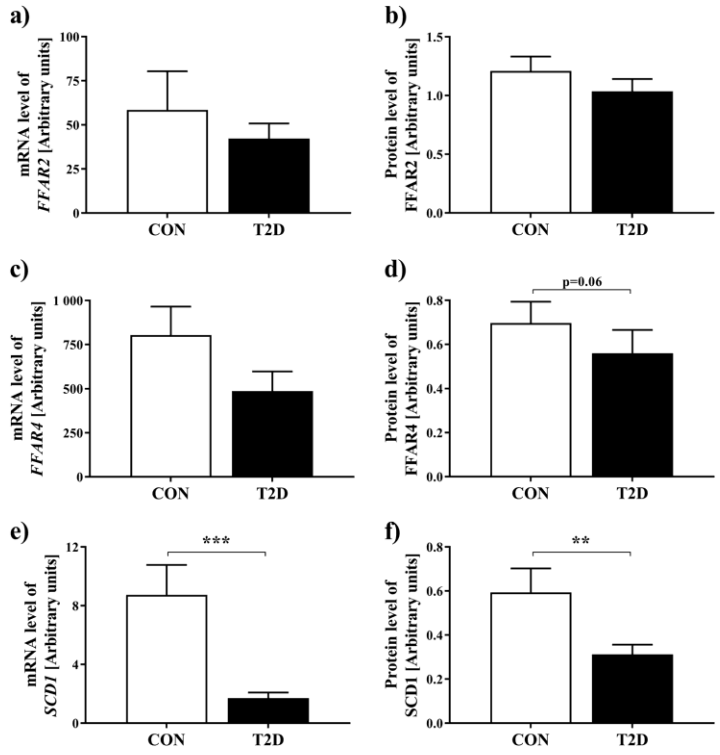


Fig. 1 mRNA and protein expression analyses for genes involved in fatty acid metabolism Data are mean ± SEM ($n = 23–25$ for CON; $n = 22–25$ for patients with type 2 diabetes mellitus), two-tailed Mann–Whitney *U* test. $^{**}p < 0.01$, $^{***}p < 0.001$. Data were analyzed for relative expression differences using *peptidylprolyl isomerase A (PPIA)* as reference gene with standard C_t method. Glucose-tolerant humans (CON), patients with type 2 diabetes mellitus (T2D). **a** mRNA expression levels of *free fatty acid receptor 2* (*FFAR2*), **b** protein expression levels of FFAR2, **c** mRNA expression levels of *free fatty acid receptor 4* (*FFAR4*), **d** protein expression levels of FFAR4, **e** mRNA expression levels of *stearoyl-CoA desaturase*-1 (*SCD1*), **f** protein expression levels of SCD1

introducing a double-bond in the growing FA chain. Of note, the large prospective population-based Kuopio Ischaemic Heart Disease (KIHD) Risk Factor Study indicated that higher serum Δ5 desaturase activity associate with a lower risk for type 2 diabetes mellitus and increased Δ6 desaturase activity with a higher risk among middle-aged and older Finish men[31]. A putative mechanism behind the negative association of higher Δ5 desaturase activity with lower risk of type 2 diabetes mellitus may be the simultaneous improvement in IS[32]. In the European Prospective Investigation into Cancer (EPIC) and Nutrition-Potsdam Study, SCD1 activity (assessed from product-to-precursor ratios) of erythrocyte membrane was positively associated with risk of type 2 diabetes mellitus[33]. In agreement with our findings and previously reported beneficial effects of SCD1 in type 2 diabetes mellitus[21,34], thiazolidinedione treatment led to increased *SCD1* expression in AT of patients with type 2 diabetes mellitus together with improved TG esterification and IS[20]. The mechanisms underlying insulin resistance are still not fully understood, but increased NEFA release from AT is generally recognized as an important factor for the development of insulin resistance[35,36]. Thus, dysfunctional AT as indicated by decreased SCD1 expression in type 2 diabetes mellitus compared to CON may contribute to the development of type 2 diabetes mellitus. Previously, we showed that an intravenous lipid infusion as well as a single oral fat load rich in long-chain polyunsaturated FAs can induce insulin resistance[29,37]. Especially, saturated FAs are thought to induce inflammation and insulin resistance[38–40]. Accordingly, *SCD1*-deficient mice exhibited increased inflammation[41]. In

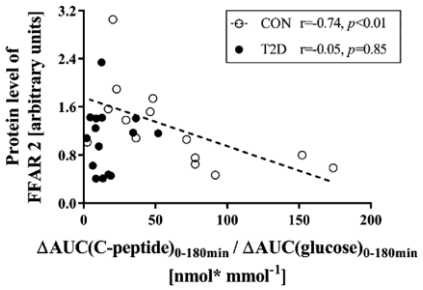


Fig. 2 Association of protein expression levels of FFAR2 with beta cell function *r*-Spearman's rank correlation coefficient. Glucose-tolerant humans (CON), patients with type 2 diabetes mellitus (T2D), free fatty acid receptor 2 (FFAR2), marker for beta cell function from $\Delta AUC(C\text{-peptide})_{0-180\ min}/\Delta AUC(glucose)_{0-180\ min}$ in nmol* $mmol^{-1}$

agreement with our results, AT-specific *SCD1* deletion in a mouse model induced glucose transporter 1 upregulation in AT, which was associated with increased tumor necrosis factor-alpha production[42]. The possible protective effect of SCD1 in CON is underlined by our observation that protein levels of SCD1 correlated negatively with hsCRP in patients with type 2 diabetes mellitus. Moreover, SCD1 protein levels in CON associated negatively with fasting TG and postprandial $AUC(TG)_{0-180\ min}$, respectively. The decrease in plasma TG levels with increasing SCD1 protein expression is in accordance with previous studies, where patients with type 2 diabetes mellitus exhibited enhanced TG esterification in SAT under thiazolidinedione treatment with subsequently increased *SCD1* gene expression[20].

The strength of our study lies in the deep phenotyping of patients with type 2 diabetes mellitus and well-matched glucose-tolerant controls. However, the conclusions from our study are limited by the small sample size and the lack of muscle samples. Due to the nature of a cross-sectional design, this study also does not allow to draw conclusions as to causality or temporal relationships.

In conclusion, patients with recent onset type 2 diabetes mellitus have lower SCD1, but not FFAR2 or 4 expression in SAT compared to CON. Our findings suggest that SCD1 expression may be important in early development of type 2 diabetes mellitus, but is not as effective in modulating beta cell function as FFAR2. Our study implies that FFAR2 could negatively influence glucose homeostasis by decreasing beta cell function in CON. Thus, both FFAR2 and SCD1 may be potential treatment targets in diabetes prevention strategies.

Data availability

To ensure data privacy of the study participants, the generated datasets of the currently still running GDS are not publicly available. Especially, since they are subject to national data protection laws and restrictions by the ethics committee. However, access to the data can be requested through an individual project agreement within the GDS.

Disclaimer

The funding sources had neither influence on design and conduct of this study, collection, analysis, and interpretation of the data; nor on the preparation, review, or approval of this article.

Acknowledgements
We would like to thank the staff of the Clinical Research Center, Institute for Clinical Diabetology at the German Diabetes Center, Düsseldorf, Germany for excellent help with the experiments. We thank the participants for their invaluable contributions to the study. Some of the data were presented as an abstract at the 52nd EASD Annual Meeting in 2016. This work was supported by the Ministry of Culture and Science of the State of North Rhine-Westphalia (MIWF NRW) and the German Federal Ministry of Health (BMG). This study was supported in part by a grant of the Federal Ministry for Research (BMBF) to the German Center for Diabetes Research (DZD e.V.).

Authors' contributions
M.R. designed the study. K.B. wrote the article and researched the data. S.K., M.S., and M.R. researched the data, contributed to the discussion, and reviewed and edited the article. D.M. performed laboratory analyses. J.S., K.S., V.B., K.M., Z.Z., B.K., H.S. and A.T. researched data, contributed to the discussion, and reviewed and edited the article. M.R. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. All authors gave final approval of this version to be published.

Members of GDS Group
A.E. Buyken, B. Belgardt, G. Geerling, H. Al-Hasani, C. Herder, A. Icks, J. Kotzka, O. Kuss, E. Lammert, D. Markgraf, K. Müssig, W. Rathmann, J. Szendroedi, D. Ziegler, and M. Roden (speaker).

Author details
[1]Institute for Clinical Diabetology, German Diabetes Center, Leibniz Center for Diabetes Research at Heinrich Heine University, Düsseldorf, Germany. [2]German Center for Diabetes Research (DZD), München-Neuherberg, Germany. [3]Division of Endocrinology and Diabetology, Medical Faculty, Heinrich Heine University, Düsseldorf, Germany. [4]Institute for Clinical Biochemistry and Pathobiochemistry, German Diabetes Center, Leibniz Center for Diabetes Research at the Heinrich Heine University, Düsseldorf, Germany. [5]Metabolic Unit, Institute of Biomedical Engineering, National Research Council, Padua, Italy. [6]Institute for Biometrics and Epidemiology, German Diabetes Center, Leibniz Center for Diabetes Research at Heinrich Heine University, Düsseldorf, Germany

Conflict of interest
The authors declare that they have no conflict of interest.

Supplementary Information accompanies this paper at (https://doi.org/10.1038/s41387-018-0054-9).

Received: 5 November 2017 Revised: 23 July 2018 Accepted: 3 August 2018
Published online: 07 September 2018

References

1. Prentki, M. & Nolan, C. J. Islet beta cell failure in type 2 diabetes. *J. Clin. Invest.* **116**, 1802–1812 (2006).
2. Miyamoto, J. et al. Nutritional signaling via free fatty acid receptors. *Int. J. Mol. Sci.* **17**, 450 (2016).
3. Tang, C. et al. Loss of FFA2 and FFA3 increases insulin secretion and improves glucose tolerance in type 2 diabetes. *Nat. Med.* **21**, 173–177 (2015).
4. Tazoe, H. et al. Roles of short-chain fatty acids receptors, GPR41 and GPR43 on colonic functions. *J. Physiol. Pharmacol.* **59**(Suppl 2), 251–262 (2008).
5. Hirasawa, A. et al. Free fatty acids regulate gut incretin glucagon-like peptide-1 secretion through GPR120. *Nat. Med.* **11**, 90–94 (2005).
6. Vangaveti, V., Shashidhar, V., Jarrod, G., Baune, B. T. & Kennedy, R. L. Free fatty acid receptors: emerging targets for treatment of diabetes and its complications. *Ther. Adv. Endocrinol. Metab.* **1**, 165–175 (2010).
7. Bjursell, M. et al. Improved glucose control and reduced body fat mass in free fatty acid receptor 2-deficient mice fed a high-fat diet. *Am. J. Physiol. Endocrinol. Metab.* **300**, E211–E220 (2011).
8. Layden, B. T. et al. Regulation of pancreatic islet gene expression in mouse islets by pregnancy. *J. Endocrinol.* **207**, 265–279 (2010).
9. Rieck, S. et al. The transcriptional response of the islet to pregnancy in mice. *Mol Endocrinol* **23**, 1702–1712 (2009).
10. Muoio, D. M. & Newgard, C. B. Obesity-related derangements in metabolic regulation. *Annu. Rev. Biochem.* **75**, 367–401 (2006).
11. Glass, C. K. & Olefsky, J. M. Inflammation and lipid signaling in the etiology of insulin resistance. *Cell Metab.* **15**, 635–645 (2012).
12. Tanaka, N. et al. Adipocyte-specific disruption of fat-specific protein 27 causes hepatosteatosis and insulin resistance in high-fat diet-fed mice. *J. Biol. Chem.* **290**, 3092–3105 (2015).
13. Gancheva, S., Jelenik, T., Alvarez-Hernandez, E. & Roden, M. Interorgan metabolic crosstalk in human insulin resistance. *Physiol. Rev.* **98**, 1371–1415 (2018).
14. Giacca, A., Xiao, C., Oprescu, A. I., Carpentier, A. C. & Lewis, G. F. Lipid-induced pancreatic beta-cell dysfunction: focus on in vivo studies. *Am. J. Physiol. Endocrinol. Metab.* **300**, E255–E262 (2011).
15. Taneera, J. et al. A systems genetics approach identifies genes and pathways for type 2 diabetes in human islets. *Cell Metab.* **16**, 122–134 (2012).
16. Ichimura, A. et al. Dysfunction of lipid sensor GPR120 leads to obesity in both mouse and human. *Nature* **483**, 350–354 (2012).
17. Waguri, T., Goda, T., Kasezawa, N. & Yamakawa-Kobayashi, K. The combined effects of genetic variations in the GPR120 gene and dietary fat intake on obesity risk. *Biomed. Res.* **34**, 69–74 (2013).
18. Ichimura, A., Hara, T. & Hirasawa, A. Regulation of energy homeostasis via GPR120. *Front. Endocrinol.* **5**, 111 (2014).
19. Cao, H. et al. Identification of a lipokine, a lipid hormone linking adipose tissue to systemic metabolism. *Cell* **134**, 933–944 (2008).
20. Tordjman, J. et al. Thiazolidinediones block fatty acid release by inducing glyceroneogenesis in fat cells. *J. Biol. Chem.* **278**, 18785–18790 (2003).
21. Riserus, U. et al. Rosiglitazone increases indexes of stearoyl-CoA desaturase activity in humans: link to insulin sensitization and the role of dominant-negative mutation in peroxisome proliferator-activated receptor-gamma. *Diabetes* **54**, 1379–1384 (2005).
22. Pinnamaneni, S. K., Southgate, R. J., Febbraio, M. A. & Watt, M. J. Stearoyl CoA desaturase 1 is elevated in obesity but protects against fatty acid-induced skeletal muscle insulin resistance in vitro. *Diabetologia* **49**, 3027–3037 (2006).
23. Szendroedi, J. et al. Cohort profile: the German Diabetes Study (GDS). *Cardiovasc. Diabetol.* **15**, 59 (2016).
24. Greenbaum, C. J. et al. Mixed-meal tolerance test versus glucagon stimulation test for the assessment of beta-cell function in therapeutic trials in type 1 diabetes. *Diabetes Care* **31**, 1966–1971 (2008).
25. Mari, A., Pacini, G., Murphy, E., Ludvik, B. & Nolan, J. J. A model-based method for assessing insulin sensitivity from the oral glucose tolerance test. *Diabetes Care* **24**, 539–548 (2001).
26. Pacini, G. & Mari, A. Methods for clinical assessment of insulin sensitivity and beta-cell function. *Best. Pract. Res. Clin. Endocrinol. Metab.* **17**, 305–322 (2003).
27. Alsalim, W. et al. Mixed meal ingestion diminishes glucose excursion in comparison with glucose ingestion via several adaptive mechanisms in people with and without type 2 diabetes. *Diabetes Obes. Metab.* **18**, 24–33 (2016).
28. American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care* **29**(Suppl 1), S43–S48 (2006).
29. Nowotny, B. et al. Mechanisms underlying the onset of oral lipid-induced skeletal muscle insulin resistance in humans. *Diabetes* **62**, 2240–2248 (2013).
30. Oh, D. Y. et al. GPR120 is an omega-3 fatty acid receptor mediating potent anti-inflammatory and insulin-sensitizing effects. *Cell* **142**, 687–698 (2010).
31. Yary, T. et al. Serum n-6 polyunsaturated fatty acids, Delta5- and Delta6-desaturase activities, and risk of incident type 2 diabetes in men: the Kuopio Ischaemic Heart Disease Risk Factor Study. *Am. J. Clin. Nutr.* **103**, 1337–1343 (2016).
32. Takkunen, M. J. et al. Longitudinal associations of serum fatty acid composition with type 2 diabetes risk and markers of insulin secretion and sensitivity in the Finnish Diabetes Prevention Study. *Eur. J. Nutr.* **55**, 967–979 (2016).
33. Jacobs, S. et al. Evaluation of various biomarkers as potential mediators of the association between Delta5 desaturase, Delta6 desaturase, and stearoyl-CoA desaturase activity and incident type 2 diabetes in the European Prospective Investigation into Cancer and Nutrition-Potsdam Study. *Am. J. Clin. Nutr.* **102**, 155–164 (2015).
34. Flowers, J. B. et al. Loss of stearoyl-CoA desaturase-1 improves insulin sensitivity in lean mice but worsens diabetes in leptin-deficient obese mice. *Diabetes* **56**, 1228–1239 (2007).
35. Krssak, M. et al. Intramyocellular lipid concentrations are correlated with insulin sensitivity in humans: a 1H NMR spectroscopy study. *Diabetologia* **42**, 113–116 (1999).
36. Petersen, K. F. & Shulman, G. I. Pathogenesis of skeletal muscle insulin resistance in type 2 diabetes mellitus. *Am. J. Cardiol.* **90**(5A), 11G–18GG (2002).
37. Roden, M. et al. Mechanism of free fatty acid-induced insulin resistance in humans. *J. Clin. Invest.* **97**, 2859–2865 (1996).
38. Kausch, C. et al. Skeletal muscle cells from insulin-resistant (non-diabetic) individuals are susceptible to insulin desensitization by palmitate. *Horm. Metab. Res.* **35**, 570–576 (2003).
39. Staiger, K. et al. Saturated, but not unsaturated, fatty acids induce apoptosis of human coronary artery endothelial cells via nuclear factor-kappaB activation. *Diabetes* **55**, 3121–3126 (2006).
40. Staiger, H. et al. Palmitate-induced interleukin-6 expression in human coronary artery endothelial cells. *Diabetes* **53**, 3209–3216 (2004).
41. MacDonald, M. L. et al. Despite antiatherogenic metabolic characteristics, SCD1-deficient mice have increased inflammation and atherosclerosis. *Arterioscler. Thromb. Vasc. Biol.* **29**, 341–347 (2009).
42. Hyun, C. K. et al. Adipose-specific deletion of stearoyl-CoA desaturase 1 up-regulates the glucose transporter GLUT1 in adipose tissue. *Biochem. Biophys. Res. Commun.* **399**, 480–486 (2010).

3.2. Expansion and impaired mitochondrial efficiency of deep subcutaneous adipose tissue in recent-onset type 2 diabetes

Bódis, K, Jelenik T, Lundbom J, Markgraf DF, Strom A, Zaharia OP, Karusheva Y, Burkart V, Mussig K, Kupriyanova Y, Ouni M, Wolkersdorfer M, Hwang JH, Ziegler D, Schurmann A, Roden M, and Szendroedi J. Expansion and Impaired Mitochondrial Efficiency of Deep Subcutaneous Adipose Tissue in Recent-Onset Type 2 Diabetes. *The Journal of clinical endocrinology and metabolism.* 2020;105(4).

Expansion and Impaired Mitochondrial Efficiency of Deep Subcutaneous Adipose Tissue in Recent-Onset Type 2 Diabetes

Kálmán Bódis,[1,2,3] Tomas Jelenik,[2,3] Jesper Lundbom,[2,3] Daniel F. Markgraf,[2,3] Alexander Strom,[2,3] Oana-Patricia Zaharia,[2,3] Yanislava Karusheva,[2,3] Volker Burkart,[2,3] Karsten Müssig,[1,2,3] Yuliya Kupriyanova,[2,3] Meriem Ouni,[3,4] Martin Wolkersdorfer,[5] Jong-Hee Hwang,[2,3] Dan Ziegler,[1,2,3] Annette Schürmann,[3,4] Michael Roden,[1,2,3] Julia Szendroedi[1,2,3]; for the GDS Study Group

[1]Division of Endocrinology and Diabetology, Medical Faculty, Heinrich Heine University, 40225 Düsseldorf, Germany; [2]Institute for Clinical Diabetology, German Diabetes Center, Leibniz Center for Diabetes Research at Heinrich Heine University, 40225 Düsseldorf, Germany; [3]German Center for Diabetes Research (DZD), 85764 München-Neuherberg, Germany; [4]Department of Experimental Diabetology, German Institute of Human Nutrition Potsdam-Rehbrücke, 14558 Nuthetal, Germany; and [5]Landesapotheke Salzburg, 5020 Salzburg, Austria

ORCiD number: 0000-0002-5185-8168 (J. Szendroedi).

Context/Objective: Impaired adipose tissue (AT) function might induce recent-onset type 2 diabetes (T2D). Understanding AT energy metabolism could yield novel targets for the treatment of T2D.

Design/Patients: Male patients with recently-diagnosed T2D and healthy male controls (CON) of similar abdominal subcutaneous AT (SAT)-thickness, fat mass, and age (n = 14 each), underwent hyperinsulinemic-euglycemic clamps with [6,6-$^2H_2$]glucose and indirect calorimetry. We assessed mitochondrial efficiency (coupling: state 3/4o; proton leak: state 4o/u) via high-resolution respirometry in superficial (SSAT) and deep (DSAT) SAT-biopsies, hepatocellular lipids (HCL) and fat mass by proton-magnetic-resonance-spectroscopy and -imaging.

Results: T2D patients (known diabetes duration: 2.5 [0.1; 5.0] years) had 43%, 44%, and 63% lower muscle insulin sensitivity (IS), metabolic flexibility ($P < 0.01$) and AT IS ($P < 0.05$), 73% and 31% higher HCL ($P < 0.05$), and DSAT-thickness ($P < 0.001$), but similar hepatic IS compared with CON. Mitochondrial efficiency was ~22% lower in SSAT and DSAT of T2D patients ($P < 0.001$) and ~8% lower in SSAT vs DSAT ($P < 0.05$). In both fat depots, mitochondrial coupling correlated positively with muscle IS and metabolic flexibility ($r \geq 0.40$; $P < 0.05$), proton leak correlated positively ($r \geq 0.51$; $P < 0.01$) and oxidative capacity negatively ($r \leq -0.47$; $P < 0.05$) with fasting free fatty acids (FFA). Metabolic flexibility correlated positively with SAT-oxidative capacity ($r \geq 0.48$; $P < 0.05$) and negatively with DSAT-thickness ($r = -0.48$; $P < 0.05$). DSAT-thickness correlated negatively with mitochondrial coupling in both depots ($r \leq -0.50$; $P < 0.01$) and

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in USA

Received 31 August 2019. Accepted 13 December 2019.
First Published Online 15 December 2019.
Corrected and Typeset 7 March 2020.

Abbreviations: ATP, adenosine triphosphate;AT, adipose tissue; Adipo IR, adipose tissue insulin resistance index; ANCOVA, analysis of covariance; BMI, body mass index; CON, controls (individuals with normal glucose tolerance); CSA, citrate synthase activity; DSAT, deep subcutaneous adipose tissue; EGP, endogenous glucose production; FFA, free fatty acids; GDS, German Diabetes Study; HbA_{1c}, glycated hemoglobin; HCL, hepatocellular lipid content; HDL, high-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein;MRI, magnetic resonance imaging, MRS, magnetic resonance spectroscopy; OGTT, oral glucose tolerance test; RQ, respiratory quotient; SAT, subcutaneous adipose tissue; SEM, standard error of the mean;SSAT, superficial subcutaneous adipose tissue; T2D, type 2 diabetes; TG; triglycerides; VAT, visceral adipose tissue; WSAT, whole subcutaneous adipose tissue.

muscle IS (r = –0.59; P < 0.01), positively with FFA during clamp (r = 0.63; P < 0.001) and HCL (r = 0.49; P < 0.01).

Conclusions: Impaired mitochondrial function, insulin resistance, and DSAT expansion are AT abnormalities in recent-onset T2D that might promote whole-body insulin resistance and increased substrate flux to the liver. **(*J Clin Endocrinol Metab* 105: 1–13, 2020)**

Key Words: Adipose tissue, humans, insulin resistance, metabolic flexibility, mitochondrial function, type 2 diabetes

Type 2 diabetes (T2D) is strongly related to weight gain and accumulation of excess fat within the liver. Abnormal adipose tissue (AT) function, which is induced by chronic overnutrition or is a result of lipodystrophy, promotes insulin resistance and ectopic lipid accumulation within skeletal muscle, liver, and heart (1). This likely contributes to the onset of T2D (2–4).

Weight loss and particularly the reduction of body fat content (as a result of lifestyle intervention or bariatric surgery), have been shown to achieve normalization of blood glucose levels in people with recently-diagnosed T2D (5). The major determinants of this response to the remission strategies are the extent of body fat loss, duration of T2D, and residual beta cell function (6). While the restoration of normal glucose tolerance has been allocated to beta cell recovery, the metabolic disturbance of AT in recent-onset, yet reversible, T2D has gained growing interest.

Glucose homeostasis is maintained by the integrated adaptation of substrate flux to prevailing metabolic conditions in skeletal muscle, white AT, and the liver (7). Metabolic flexibility refers to the ability of insulin-sensitive individuals to switch from lipid to carbohydrate oxidation in response to insulin (8, 9). Impairment of metabolic flexibility strongly relates to the reduction of mitochondrial plasticity in the skeletal muscle, but might also involve AT mitochondrial function (10, 11). Adipocyte insulin signaling and insulin-stimulated glucose uptake depend on mitochondrial efficiency (12, 13). Accordingly, the expression of proteins regulating mitochondrial function in abdominal subcutaneous AT (SAT) is lower in insulin-resistant compared with insulin-sensitive humans (14). In mice, impaired mitochondrial function in white AT directs the flux of lipid metabolites and lactate to the skeletal muscle and the liver, which results in ectopic lipid deposition and insulin resistance (15). Similarly, the discoordination of metabolic adaptation and energy metabolism in AT might also contribute to the onset of T2D and hepatic steatosis in humans who are at risk for impaired glucose homeostasis (16).

Human abdominal AT consists of SAT and visceral AT (VAT). In lean humans, SAT accounts for 80% to 90% of total body fat mass (17) and is divided by Scarpa's fascia into a superficial (SSAT) and a deep layer (DSAT). SSAT volume associates negatively with glycated hemoglobin (HbA_{1c}) in T2D (18), whereas DSAT volume-similar to VAT-relates to hepatic and whole-body insulin resistance (19, 20).

In general, DSAT expression profile was an intermediary to both SSAT and VAT, but reflected more of the VAT expression profile (21). Thus, the intracellular pool of glucose transporter type 4 proteins was lower in DSAT (21). Accordingly, lower protein expression of glucose transporter type 4 was found in DSAT as compared with SSAT, which likely reflects lower glucose uptake in DSAT (22). Moreover, DSAT showed a higher ratio of saturated than monounsaturated fatty acids (23). Thus, DSAT expansion could have adverse metabolic consequences, but its relation to tissue-specific insulin sensitivity has not been yet analyzed. Only a few studies have assessed mitochondrial function via direct measurements of respiration in SAT, yielding controversial results. While there is evidence for metabolic differences between both compartments, their mitochondrial function has not been assessed yet.

Our objective was to assess mitochondrial function in SSAT and DSAT separately and its relation to tissue-specific insulin sensitivity of the skeletal muscle, liver, and AT as well as steatosis and metabolic flexibility in recently-diagnosed T2D patients independently of total fat mass. We hypothesized that impaired mitochondrial function in DSAT associates with tissue-specific insulin resistance, impaired glucose homeostasis, lower metabolic flexibility, ectopic lipid deposition, and expansion of DSAT thickness.

Materials and Methods

Participants

From the German Diabetes Study (GDS), 14 male patients with T2D and 14 men with normal glucose tolerance (CON) were recruited. They were matched for sex, age, body mass

index (BMI), total body fat mass and whole SAT (WSAT) thickness. The GDS is a prospective observational study, which investigates the natural course of recently-diagnosed diabetes and the development of diabetes-associated complications compared with a cohort with normal glucose tolerance. The study design and cohort profile of GDS have been described before (24). All participants gave written informed consent before inclusion in the study (ClinicalTrial.gov registration no: NCT01055093), which was performed according to the Declaration of Helsinki and approved by the ethics board of Heinrich Heine University, Düsseldorf, Germany. In order to be eligible for the present study, the participants needed to have been diagnosed with T2D for a period of less than 7 years, to have a minimium BMI of 25 kg/m^2 and to be between the ages of 35 to 70 years. Specific exclusion criteria were the history of acute or chronic diseases including cancer, medication affecting the immune system, treatment with insulin or thiazolidinediones, and a HbA_{1c} level above 9.5% (80 mmol/mol) or a positive family history for diabetes in CON. The participating patients with T2D were prescribed with either lifestyle modification only (n = 2), metformin only (n = 9), sulfonylurea only (n = 1), metformin plus sulfonylurea (n = 1) or a dipeptidyl-peptidase-4-inhibitor plus metformin (n = 1). Those patients on oral glucose-lowering medication withdrew their treatment for at least 3 days before all measurements (24) and none of the recruited patients received thiazolidinediones. Therefore, a confounding effect of diabetes medication on mitochondrial function was unlikely (25). Three CON and 9 patients with T2D were treated with antihypertensive medication, 6 T2D patients received lipid-lowering medication and 3 T2D patients were treated with antiplatelet drugs.

Study protocol

For 3 days prior to each visit, participants refrained from physical activity and alcohol ingestion as well as adhered to a balanced isocaloric diet. For all visits, the participants arrived at the Clinical Research Center in the morning after 10 hours of overnight fasting. On the first day, each participant provided their medical history and underwent bioimpedance analysis and ultrasound imaging as well as physical examination and laboratory tests. On the second day, proton-based magnetic resonance spectroscopy (^{1}H MRS) and magnetic resonance imaging (MRI) measurements were followed by ultrasound imaging and SAT biopsies. On the third day, participants underwent a hyperinsulinemic-euglycemic clamp test with isotope dilution technique for an assessment of tissue-specific insulin sensitivity and with indirect calorimetry for an assessment of mitochondrial flexibility. All CON underwent a standardized 75-g oral glucose-tolerance test (OGTT) (Accu-Chek Dextro O.G-T., Roche, Basel, Switzerland) with blood sampling at time points of 5, 30, 60, and 120 minutes to assess glucose tolerance and to exclude persons with impaired glucose tolerance (24). Dysglycemia was categorized according to the current internationally accepted criteria (26).

Hyperinsulinemic-euglycemic clamp test

The clamp test was performed according to the Botnia protocol with an intravenous glucose-tolerance test and a subsequent clamp test (24). The latter was started with a priming dose of 10 mIU/(body weight [kg]*min) for 10 minutes, followed by a continuous infusion of short-acting human insulin (Insuman Rapid, Sanofi-Aventis, Frankfurt am Main, Germany) 1.5 mU/(body weight [kg]*min) for 3 hours to assess muscle and hepatic insulin sensitivity (24). Combined with continuous infusion of deuterated glucose (D-[6,6-$^2H_2$] glucose), the clamp yields insulin-stimulated rates of glucose disappearance as a measure of skeletal muscle insulin sensitivity (16). Hepatic insulin sensitivity was measured as difference between basal and insulin-suppressed endogenous glucose production (ΔEGP) using a continuous infusion of D-[6,6-$^2H_2$]glucose (24). Insulin sensitivity of AT was assessed by suppression of the plasma concentrations of endogenous free fatty acids (FFA) during the clamp test expressed as percent of FFA suppression from baseline and calculated as 1 – (average FFA during steady-state / baseline FFA) (27). Lower insulin-mediated suppression of lipolysis reflects insulin resistance of AT in vivo (16).

Whole-body substrate oxidation

The total energy expenditure is measured in vivo noninvasively via open circuit indirect calorimetry to assess glucose and lipid oxidation by oxygen uptake (VO_2) and the carbon dioxide output (VCO_2) under resting conditions in order to calculate the respiratory quotient (RQ). These methods have been described elsewhere in detail (24). To quantify metabolic flexibility of the substrate oxidation the measurement is repeated at the steady state period, generally during the last 30 minutes of the hyperinsulinemic-euglycemic clamp (28). The difference between basal and insulin-stimulated RQ is an estimate of metabolic flexibility.

Laboratory analyses

Plasma glucose, insulin, FFA, HbA_{1c}, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides (TG), and high-sensitivity C-reactive protein (hsCRP) were measured in the fasted state as described (24). The AT insulin resistance index (Adipo IR) was calculated as the product of the fasting plasma FFA concentration (mmol/L) and fasting plasma insulin concentration (pmol/L) and reflects AT insulin resistance (16, 29). Higher systemic FFA appearance during fasting reflects impaired insulin-mediated suppression of lipolysis and thereby determines insulin resistance of AT. Accordingly, the Adipo IR was also calculated as the product of mean plasma insulin and mean plasma FFA during the steady state of the clamp, which reflects AT insulin resistance in the postabsorptive state (16, 30).

^{1}H-based magnetic resonance

Localized proton spectra were obtained with a 3-T whole-body magnet (Philips X-series Achieva, Best, The Netherlands) from the liver. Hepatocellular lipid content (HCL) and whole-body fat mass were measured as described (24). In 19 participants, the thickness of SAT layers was measured by single-slice MRI acquired from the same defined paraumbilical position at the level of L4 to validate SSAT, DSAT, and WSAT thickness assessed from ultrasound images.

Ultrasound imaging of WSAT

The thickness of SSAT and DSAT was measured via 2-dimensional ultrasound imaging (Logiq S8 from GE

Healthcare, Munich, Germany) using a 12 MHz linear array probe on the first and second day of visit. Measurements were performed on both sides of the paraumbilical region 5 times repetitively to assess the thickness of WSAT layers as described elsewhere (31). The final SSAT and DSAT thickness was computed as the mean of the 5 measurements from both sides and both days. The thickness of WSAT layers was validated by single-slice MRI. In comparison between ultrasound imaging and MRI data, the thickness of both SAT layers measured by the 2 methods displayed a strong positive correlation (r = 0.98 for SSAT), (r = 0.99 for DSAT), and (r = 0.99 for WSAT) (all $P < 0.001$). Thus, this confirms the agreement between the 2 methods.

Adipose tissue biopsies

Ultrasound-guided biopsies from SSAT and DSAT were performed in the paraumbilical region above and beneath the fascia Scarpa under visual control at the level of rectus abdominis muscle by needle suction technique after administration of local anesthesia (5–10 mL of 1% lidocaine, B. Braun, Melsungen, Germany) (24). Video loops were recorded to document the correct location at each biopsy.

High-resolution respirometry

High-resolution respirometry allows direct assessment of tissue-specific mitochondrial function, including mitochondrial oxidative capacity and efficiency. Ex vivo analysis of mitochondrial oxidative capacity was performed in freshly digitonin-permeabilized DSAT and SSAT biopsy samples in a 2-chamber oxygraph (OROBOROS Instruments, Innsbruck, Austria) using a similar protocol as described before for skeletal muscle (32). Maximal fatty acid oxidative capacity (state 3) was measured in the presence of octanoyl-carnitine (50 µmol/L), ADP (1.0 mmol/L), glutamate (10.0 mmol/L) and succinate (10.0 mmol/L). Cytochrome C (10 µmol/L) was added to test the integrity of the outer mitochondrial membrane. Respiration due to proton leak and not coupled to adenosine triphosphate (ATP) synthesis (state 4o) was measured after addition of oligomycin. Finally, maximal uncoupled respiration capacity of the electron transport chain (state u) was assessed by incremental titration with carbonyl cyanide p-[trifluoromethoxyl]-phenyl-hydrozone (fccp) (0.1 mmol/L per step). Oxygen consumption was corrected for AT wet weight (2–4 mg) and given as oxygen flux expressed as pmol/mg/s. The respiratory control ratio and the leak control ratio as well as the markers of mitochondrial coupling and proton leak, were calculated as the ratios of state 3/state 4o and state 4o/state u respiration, respectively. A high respiratory control ratio and low leak control ratio indicate tight coupling and low proton leak of mitochondrial function, which characterize high mitochondrial efficiency. Mitochondrial content was estimated from citrate synthase activity (CSA) (33–35).

Statistical analyses

Results are given as median [first and third quartiles] or mean ± standard error of the mean (SEM). Variables were compared using Mann-Whitney U test and analysis of covariance (ANCOVA) model adjusted for BMI, age, body fat, and medication as indicated and presented for unpaired samples to determine differences between groups. Comparisons between abdominal SAT layers were performed using a paired Student t-test because these comparisons were made within the same individuum. Relationships between variables were investigated using partial Spearman rank correlation analyses adjusted for BMI, age, and body fat as indicated and presented. The standardized mean difference (Cohen's d) was used for power analyses, because preliminary estimates for mean and SEM of mitochondrial function in SAT were not available from the literature. Based on the 2-sample-2-sided t-test, the power calculation showed that a standardized mean difference of 1.25 (for a large effect size) can be detected in a sample size of n = 14 per group with a power of 90%. As our experiments showed later, the effect size was even larger. For example, we found a Cohen's d of 1.4 for the respiratory control ratio. All statistical tests were 2-sided and a P value less than 5% was accepted to indicate significant differences. All statistical analyses were performed using SPSS for Windows 23.0 (SPSS Inc., Chicago, IL). All graphs were generated using GraphPad Prism, Version 7.01 (GraphPad Software Inc., La Jolla, CA).

Table 1. Characteristics of recently-diagnosed type 2 diabetes patients and individuals with normal glucose tolerance

Variable	CON	T2D	*P*
Male / female, n	14 / 0	14 / 0	-
Diabetes duration, years	0	2.5 [0.1; 5.0]	-
Age, years	56 [46;60]	52 [46; 57]	0.847
BMI, kg/m^2	30 [29; 34]	32 [29; 34]	0.635
Fasting blood glucose, mmol/L	4.5 [4.3; 4.7]	7.1 [5.6; 9.6]	**<0.001**
HbA_{1c}, %	5.4 [5.1; 5.5]	7.0 [6.1; 7.8]	**<0.001**
HbA_{1c}, mmol/mol	(35 [33; 37])	(53 [43; 61])	**<0.001**
Triglycerides, mg/dL	81 [64; 120]	196 [135; 235]	**<0.001**
Total cholesterol, mg/dL	225 [181; 246]	207 [173; 232]	0.408
HDL cholesterol, mg/dL	54 [49; 77]	42 [35; 53]	**<0.01**
LDL cholesterol, mg/dL	148 [116; 173]	142 [107; 162]	0.475
hsCRP, mg/dL	0.13 [0.06; 0.21]	0.21 [0.10; 0.35]	0.394

Data are shown as median [first; third quartile], P values were computed via 2-tailed Mann-Whitney U test. HDL, LDL, and hsCRP were analyzed in fasted state. All variables were assessed in n = 14 T2D and n = 14 CON participants. Abbreviations: BMI, body mass index; CON, controls (individuals with normal glucose tolerance); HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; T2D, type 2 diabetes.

Results

Participant characteristics

Compared with CON, the T2D patients had 37% higher fasting glucose, 2.4-fold higher TG levels, 23% higher HbA_{1c}, and 22% lower HDL cholesterol levels; however, both groups were similar in age, BMI, hsCRP, and total and LDL cholesterol (Table 1).

Fat distribution

Both groups had similar waist circumference, total body fat mass and WSAT thickness (Table 2). Moreover, body fat mass, which was assessed by bioimpedance analysis, and the total volume of WSAT and VAT, which were measured by MRI, were comparable in both T2D and CON (Table 2). SSAT thickness was lower than DSAT thickness in both T2D ($P < 0.001$) and CON ($P < 0.05$). Despite similar total WSAT thickness (Table 2), T2D patients had lower SSAT/WSAT ratios and consequently higher DSAT/WSAT ratios than CON (Fig. 1A, 1B, 1C, 1D, and 1E). These differences were also present without normalizing for WSAT thickness (Table 2) and even after adjustments for participants' medications. Liver fat content was 73% higher in T2D patients compared with CON (Table 2). Of note, 8 T2D patients and 4 CON met the criteria for hepatic steatosis, defined as HCL > 5.6%.

Tissue-specific insulin sensitivity

Compared with CON, T2D patients had 43% lower muscle insulin sensitivity (Fig. 2A), but similar hepatic insulin sensitivity (Fig. 2B). As detected by clamp analysis, T2D patients had 37% higher FFA during hyperinsulinemia, reflecting AT insulin resistance (Table 3, Fig. 2C). Accordingly, Adipo IR during fasting and during clamp were respectively 29% and 37% higher in T2D compared with CON (Table 3, Fig. 2D). T2D patients showed 9% lower AT insulin sensitivity than CON, as assessed by suppression of FFA during the clamp compared with baseline (Table 3).

Whole-body substrate oxidation

Compared with CON, T2D patients had similar RQ under fasting conditions (0.79 [0.76; 0.84] vs 0.78 [0.76; 0.82]; $P = 0.55$). Nevertheless, T2D patients showed 5% lower insulin-stimulated RQ (0.88 [0.83; 0.94] vs 0.92 [0.91; 0.99]; $P < 0.05$) and 44% lower metabolic flexibility (Fig. 3).

Adipose tissue mitochondrial function

Both groups showed similar oxidative capacity in SSAT and DSAT, regardless of whether or not it was normalized for CSA as a marker of mitochondrial content (Fig. 4A). CSA was similar in both SSAT and DSAT in T2D patients and CON and comparable between T2D patients and CON in both layers (data not shown).

In T2D and CON, SSAT featured lower mitochondrial coupling than DSAT (Fig. 4B). Accordingly, proton leak was higher in SSAT compared with DSAT in CON, but not in T2D (Fig. 4C). Interestingly, mitochondrial coupling was lower and proton leak was higher in T2D compared with CON in both SAT depots (Fig. 4B and 4C). Thus, mitochondrial efficiency is lower in both SAT compartments in T2D and also slightly reduced in SSAT compared with DSAT. Of note, adjustment for the participants' medications did not change these results.

Correlation analyses

For all participants in this study, metabolic flexibility correlated positively with mitochondrial coupling

Table 2. Fat distribution in recently-diagnosed type 2 diabetes patients and individuals with normal glucose tolerance

Variable	CON	T2D	*P*
Waist circumference, cm	104 [97; 112]	107 [100; 113]	0.475
Body fat, %	27 [21; 37]	26 [23; 36]	0.504
Lean body weight, kg	68 [53; 80]	73 [59; 78]	0.812
SSAT thickness, cm	1.1 [0.9; 1.4]	0.8 [0.6; 1.0]	**<0.05**
DSAT thickness, cm	1.3 [1.2; 1.8]	1.9 [1.7; 2.5]	**<0.05**
WSAT thickness, cm	2.4 [2.1; 3.2]	2.7 [2.4; 3.5]	0.427
WSAT volume, $cm^{3§}$	25 569 [16 581; 30 777]	21 344 [16 804; 28 373]	0.586
VAT volume, $cm^{3§}$	4055 [2481; 4386]	5417 [4513; 5851]	**<0.05**
WSAT & VAT volume, $cm^{3§}$	54 769 [31 877; 65 621]	47 268 [37 845; 61 900]	0.894
HCL, % of water signal	2.5 [1.0; 8.1]	9.2 [5.2; 20.0]	**<0.05**

Data are shown as median [first; third quartile], *P* values were computed via 2-tailed Mann-Whitney U test. Body fat and lean body weight was assessed by bioimpedance analysis. The abdominal adipose tissue tickness was assessed by ultrasound and the adipose tissue volume by magnetic-resonance-imaging. Abbreviations: CON, controls (individuals with normal glucose tolerance); DSAT, deep subcutaneous adipose tissue; HCL, hepatocellular lipid content; SSAT, superficial subcutaneous adipose tissue; T2D, type 2 diabetes; VAT, visceral adipose tissue; WSAT, whole subcutaneous adipose tissue. §Two participants per group had no analyses of fat volume, because the measurements were not usable due to metallic implants. All other variables were assessed in n = 14 T2D and n = 14 CON participants.

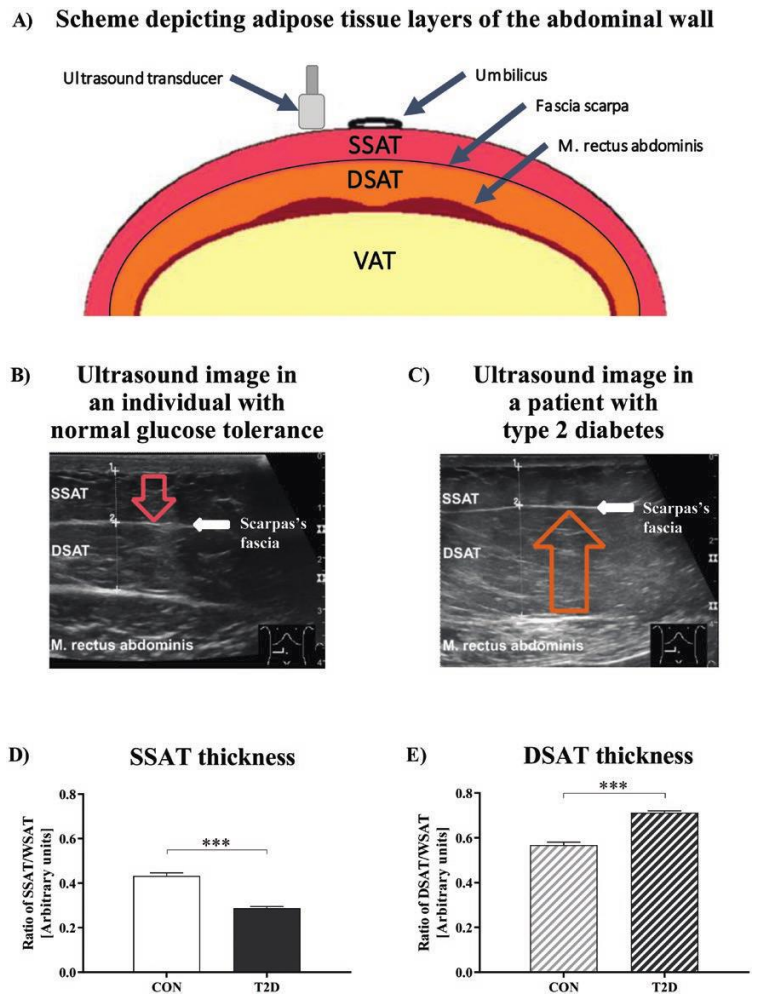


Figure 1. Subcutaneous adipose tissue layers of the abdominal wall. (A) Scheme depicting all adipose tissue layers of the abdominal wall from skin to the intestine: visceral adipose tissue (VAT), whole subcutaneous adipose tissue (WSAT) composed of superficial (SSAT) and deep subcutaneous layers (DSAT). **(B)** Ultrasound image at the level of musculus rectus abdominis showing SSAT, DSAT, and the Scarpa fascia (white line between SSAT and DSAT) dividing both adipose tissue depots in an individual with normal glucose tolerance (CON) and **(C)** a type 2 diabetes patient (T2D). The red arrow indicates the increase of SSAT thickness in CON and the orange arrow indicates the increase of DSAT thickness in T2D. **(D)** Ratio of SSAT/WSAT and **(E)** DSAT/WSAT thickness. Data are shown as mean ± SEM. ***$P < 0.001$, data were compared by 2-tailed Mann-Whitney U test. All variables were assessed in n = 14 T2D patients and n = 14 CON.

(Fig. 5A and 5B) and negatively with proton leak (Fig. 5C and 5D) in both SSAT and DSAT. Metabolic flexibility correlated positively with oxidative capacity in both SAT depots (SSAT: $r = 0.48$; $P < 0.05$; DSAT: $r = 0.52$; $P < 0.01$) and negatively with DSAT thickness ($r = –0.48$; $P < 0.05$). Mitochondrial coupling correlated positively with muscle insulin sensitivity in both SSAT and DSAT (Fig. 5E and 5F). Proton leak correlated positively (SSAT: $r = 0.51$ and DSAT: $r = 0.55$, both $P < 0.01$) and oxidative capacity negatively with fasting FFA (SSAT: $r = –0.47$; $P < 0.05$; DSAT: $r = –0.48$; $P < 0.01$). Mitochondrial coupling correlated negatively with FFA during clamp in SSAT (SSAT: $r = –0.49$; $P < 0.01$). In both depots, mitochondrial coupling correlated negatively (SSAT: $r = –0.72$; $P < 0.001$; DSAT: $r = –0.50$; $P < 0.01$) and proton leak positively with HbA_{1c} levels (SSAT: $r = 0.53$; $P < 0.01$; DSAT: $r = 0.44$; $P < 0.05$). Furthermore, mitochondrial coupling in both depots

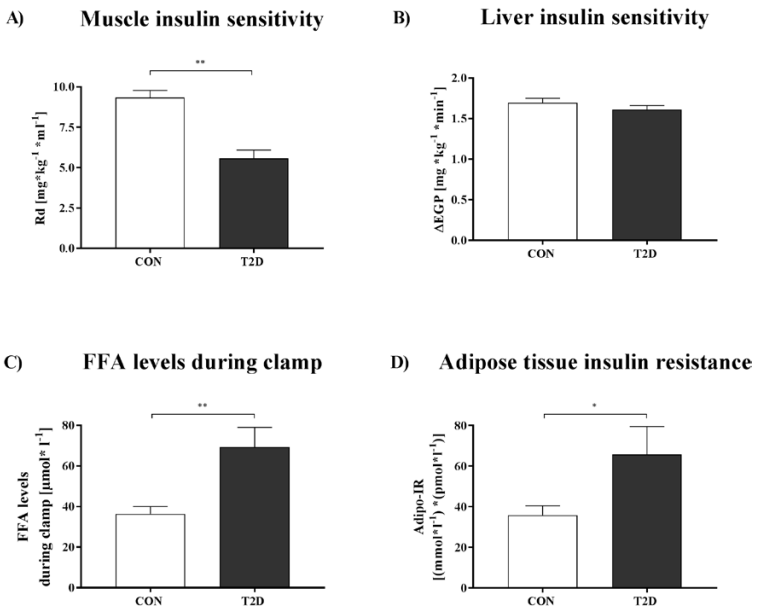


Figure 2. Muscle **(A)** and liver insulin sensitivity **(B)**, free fatty acid (FFA) levels during clamp **(C)** as a basis to assess adipose tissue insulin resistance **(D)**. Individuals with normal glucose tolerance (CON), rate of disappearance (Rd) for muscle insulin sensitivity, type 2 diabetes patient (T2D). The adipose tissue insulin resistance index (Adipo IR) was calculated as the product of the plasma FFA and insulin levels during the clamp and reflects adipose tissue insulin resistance. Hepatic insulin sensitivity was assessed by the difference between basal and insulin-suppressed endogenous glucose production (ΔEGP). Data are shown as mean ± SEM. *$P < 0.05$, **$P < 0.01$, data were compared by 2-tailed Mann-Whitney U test. All variables were assessed in n = 14 T2D patients and n = 14 CON.

Table 3. Adipose tissue insulin sensitivity in recently-diagnosed type 2 diabetes patients and individuals with normal glucose tolerance

Variable	CON	T2D	*P*
Fasting plasma insulin, pmol/L	50 [39; 87]	77 [61; 132]	0.122
Fasting plasma FFA, µmol/L	480 [376; 576]	497 [420; 710]	0.511
Clamp plasma insulin, pmol/L	972 [845; 1083]	788 [674; 1046]	0.198
Clamp plasma FFA, µmol/L§	34 [24; 46]	54 [43; 97]	**<0.01**
Indices of adipose tissue insulin resistance and sensitivity			
Adipo IR basal [(mmol/L) *(pmol/L)]	24 [16; 35]	34 [29; 53]	**<0.05**
Adipo IR clamp [(mmol/L) *(pmol/L)]	32 [22; 47]	51 [32; 82]	**<0.05**
FFA suppression, % from fasting§	93 [87; 94]	85 [81; 89]	**<0.05**

Data are shown as median [first; third quartile], *P* values were computed via 2-tailed Mann-Whitney U test and ANCOVA adjusted for age, BMI, and total body fat. The Adipo IR was calculated as the product of the fasting plasma FFA and insulin levels as well as the product of the plasma FFA and insulin levels during the clamp and reflects adipose tissue insulin resistance. Insulin sensitivity of adipose tissue was assessed by suppression of the plasma concentrations of FFA during the clamp expressed as percent of FFA suppression from baseline and calculated as 1 – (average FFA during steady-state / baseline FFA). All variables were assessed in n = 14 T2D and n = 14 CON participants. Abbreviations: Adipo IR, adipose tissue insulin resistance index; CON, controls (individuals with normal glucose tolerance); FFA, free fatty acids; T2D, type 2 diabetes. §Results are still significant after adjustment for age BMI and body fat.

correlated negatively (SSAT: $r = -0.39$; $P < 0.01$; DSAT: $r = -0.41$; $P < 0.01$) and proton leak in SSAT positively with TG levels (SSAT: $r = 0.38$; $P < 0.01$; DSAT: $r = 0.36$; $P = 0.06$). Neither mitochondrial coupling (SSAT: $r = -0.25$; $P = 0.39$; DSAT: $r = 0.12$; $P = 0.68$) nor proton leak (SSAT: $r = 0.37$; $P = 0.24$; DSAT: $r = 0.11$; $P = 0.71$) correlated with diabetes duration in both depots.

DSAT thickness correlated negatively with mitochondrial coupling in both depots (SSAT: $r = -0.58$ and DSAT: $r = -0.50$, both $P < 0.01$) and muscle insulin sensitivity ($r = -0.59$; $P < 0.01$). DSAT thickness had a positive correlation with FFA during clamp ($r = 0.63$; $P < 0.001$), TG levels ($r = 0.48$; $P < 0.01$), liver fat ($r = 0.49$; $P < 0.01$), and HbA_{1c} levels ($r = 0.70$; $P < 0.001$). DSAT thickness did not correlate with diabetes duration ($r = -0.11$;

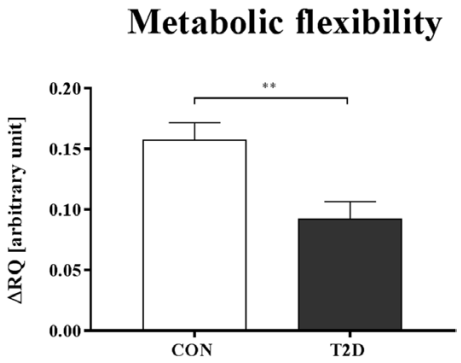


Figure 3. Metabolic flexibility Data are shown as mean ± SEM. ** $P < 0.01$, data are compared by 2-tailed Mann-Whitney U test. Difference between basal and insulin-stimulated respiratory quotient (ΔRQ) to assess metabolic flexibility, individuals with normal glucose tolerance (CON) and with type 2 diabetes (T2D). In 1 participant of the control group, metabolic flexibility was not assessable, because the insulin-stimulated measurement of respiratory quotient was not performed due to technical problems. Thus, variables were assessed in n = 14 T2D patients and n = 13 CON.

$P = 0.71$). Of note, these associations remained significant after adjusting for age, BMI, and body fat.

Discussion

Our study shows that recently-diagnosed T2D patients with muscle and AT insulin resistance and impaired metabolic flexibility have expanded DSAT and impaired mitochondrial efficiency similarly in both SAT layers. Independently of total fat mass, muscle insulin sensitivity and metabolic flexibility associates positively with AT mitochondrial efficiency and negatively with DSAT thickness. DSAT thickness was also related to impaired suppression of lipolysis and liver fat. Thus, impaired mitochondrial function in SAT, AT insulin resistance and expansion of DSAT are defects in recent-onset T2D, which might promote muscle insulin resistance and increased substrate flux to the liver.

Impaired lipid metabolism triggers a number of negative consequences in skeletal muscle, liver, and AT itself, such as sustained excessive FFA availability, impaired insulin signaling (36, 37), oxidative stress (38, 39), and impaired mitochondrial function (40). In dysfunctional AT, inefficient fatty acid oxidation promotes ectopic lipid deposition and lipotoxicity. Fasting FFA were not elevated in our T2D patients. One possible explanation for this result is that excessive FFA are stored ectopically in T2D, as reflected by elevated liver fat content preceding the development of insulin resistance in the liver. Our cohort of recently-diagnosed T2D

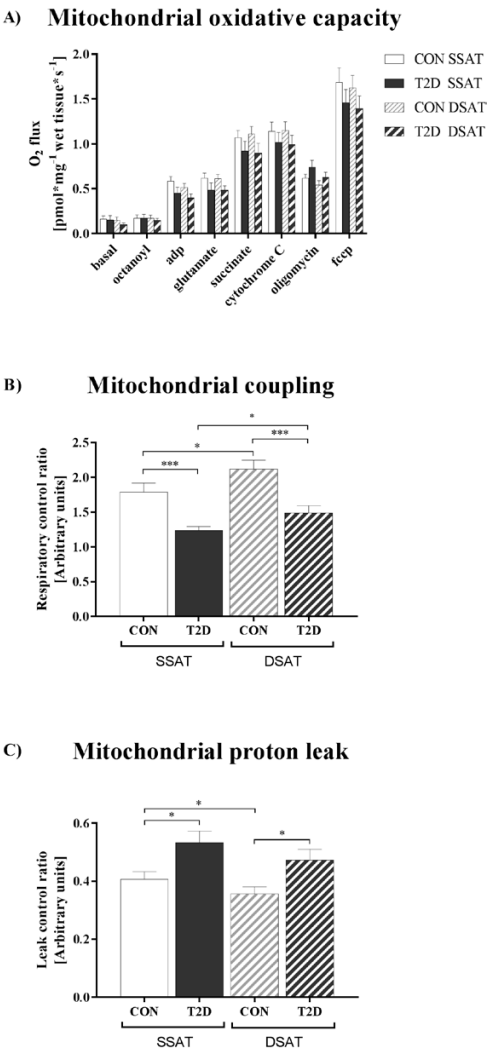


Figure 4. Mitochondrial oxidative capacity (A), coupling (B) and proton leak (C) in subcutaneous adipose tissue layers. Data are shown as mean ± SEM. * $P < 0.05$, *** $P < 0.001$, data were compared by ANCOVA adjusted for age, BMI, and total body fat and by paired Student *t*-test. All variables were assessed in n = 14 T2D patients and n = 14 CON. Abbreviations: CON, controls (individuals with normal glucose tolerance); DSAT, deep subcutaneous adipose tissue; fccp, carbonylcyanide-4-trifluoromethoxy phenylhydrazone; LCR, leak control ratio (LCR = state 4o/state u) to assess mitochondrial proton leak; RCR, respiratory control ratio (RCR = state 3/state 4o) to assess mitochondrial coupling; SSAT, superficial subcutaneous adipose tissue (SSAT); T2D, patients with type 2 diabetes.

patients showed increased TG levels and insulin resistance in AT as assessed by the Adipo IR during fasting and during the clamp as well as by suppression of FFA

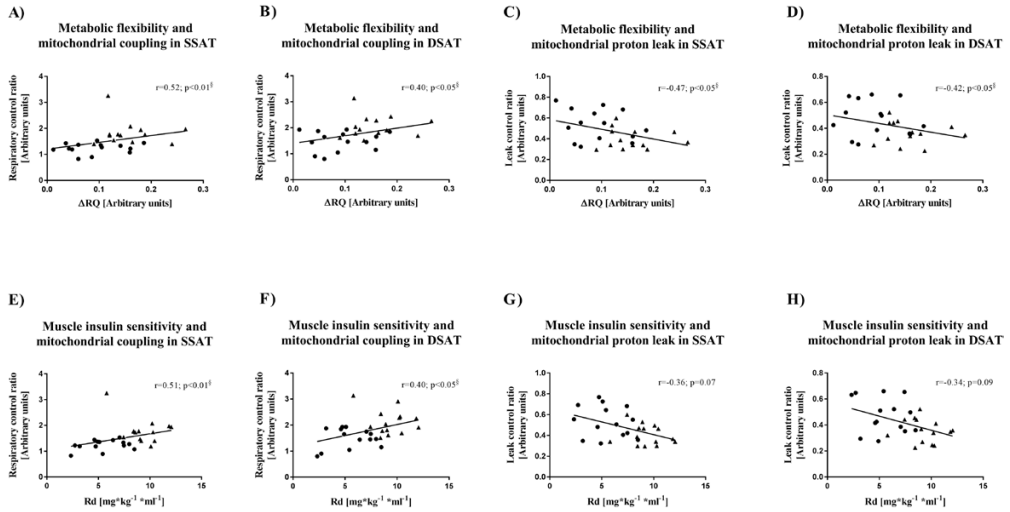


Figure 5. Association of metabolic flexibility and muscle insulin sensitivity with mitochondrial coupling and proton leak in SSAT/DSAT. Mitochondrial coupling from respiratory control ratio (RCR = state 3/state 4o respiration) and proton leak from leak control ratio (LCR = state 4o/state u) in superficial (SSAT) and deep subcutaneous adipose tissue (DSAT). Circles indicate type 2 diabetes patients (T2D) and triangles indicate individuals with normal glucose tolerance (CON). Of note, after bonferroni correction no correlation remaind significant. § indicates that the results are still significant after adjusting for age, BMI, and body fat still significant. In 1 CON participant, metabolic flexibility was not assessable, because the insulin-stimulated measurement of respiratory quotient was not performed due to technical problems. Thus, variables of metabolic flexibility were assessed in n = 14 T2D patients and n = 13 CON. All other variables were assessed in n = 14 T2D patients and n = 14 CON.

during hyperinsulinemia. These results are in agreement with accepted models linking dysfunctional and insulin-resistant AT to ectopic fat deposition in recent-onset T2D (41).

Although the human white adipocyte has a small cytosolic volume with few mitochondria compared with other tissues (ie, muscle, liver), abnormal mitochondrial function in white AT may contribute to insulin resistance in peripheral tissues (16,42,43). In adipocytes, efficient mitochondrial function that results in sufficient mitochondrial ATP production is essential for balanced storage of lipid metabolites, which is related to insulin sensitivity in peripheral tissues (16). In mouse-models, impaired mitochondrial function in AT leads to systemic insulin resistance, hypertension and cardiac dysfunction (15) and may be restored by new compounds that can induce proteins relevant for mitochondrial efficiency in AT (44). A previous study showed lower expression of proteins regulating mitochondrial function in abdominal SAT of insulin-resistant compared with insulin-sensitive humans without diabetes, but these differences were less pronounced, when both groups were matched for BMI and percent body fat (14). Although the authors of this study did not provide information on the total number of humans meeting the criteria for prediabetes, some of those insulin-resistant participants had prediabetes according to their HbA_{1c} and 2-hour plasma glucose levels after a 75-g OGTT (14). Another study showed that enzyme activities, reflecting complex I and complex III activities were similar between humans with normal and impaired glucose tolerance (45). In contrast, mitochondrial function assessed by high-resolution respirometry in SAT biopsies showed controversial results in previous studies. One study reported lower mitochondrial function in obese and nonobese young patients with T2D compared with lean healthy controls (46). Another study showed similar mitochondrial oxidative capacity and coupling in patients with and without T2D following bariatric surgery-induced weight loss (47). However, these studies did not report tissue-specific insulin sensitivity, ectopic lipid deposition or depot-specific analyses in SSAT and DSAT. Additionally, they did not match the participants for body fat mass. Thus, variances in the fat mass of the participants included in the 2 studies could explain these differences. While 1 study did not report fat mass of participants (46), another showed higher fat mass in obese patients with T2D than in obese humans without T2D (47). Furthermore, both studies did not report the diabetes duration, which is an important parameter, because longstanding diabetes is associated with impaired mitochondrial function. Additionally, only 1 study excluded individuals with normal glucose tolerance with positive family history of diabetes, although multiple

genes of oxidative metabolism are downregulated in healthy humans with a family history of diabetes (48). In our study, maximal oxidative capacity was not only similar in both SAT layers but also similar in patients with recently-diagnosed T2D compared with CON of similar fat mass.

The findings of a lower respiratory control ratio indicate that the electron transport system is not tightly coupled to ATP synthesis both in SSAT and DSAT in patients with T2D. Leak control ratio was higher in these patients, which suggests impaired mitochondrial integrity in both compartments. Of note, the respiratory control ratio and leak control ratio are independent of mitochondrial content and thereby allow for assessment of intrinsic mitochondrial efficiency in both SAT depots. Although mitochondrial function plays a prominent role in carbohydrate and lipid metabolism, depot-specific analyses of mitochondrial function have not been performed yet. Even though we found distinctly reduced mitochondrial efficiency in T2D compared with CON, we found only slightly lower mitochondrial efficiency in SSAT than in DSAT, which might not be of clinical significance. Previous studies indicated that SSAT and DSAT have different metabolic features, but the impairment of mitochondrial function observed in our study appeared to be equivalent in both SAT depots in T2D patients. Furthermore, mitochondrial function was also similar in both SAT depots in CON. During periods of prolonged fasting, fatty acids derived from adipose tissue serve as substrates for mitochondrial β-oxidation and ATP generation to maintain whole-body energy homeostasis (49). In turn, a sufficient amount of ATP is required for insulin signaling in adipocytes (50) and thereby crucial for insulin-stimulated antilipolytic effects (45). Development of whole-body insulin resistance was suggested to result from decreased ATP production in mitochondria for highly energy-dependent pathways in adipose tissue (10). However, segregated analyses of mitochondrial function in both SAT depots have not been performed so far. The present study observed similar mitochondrial impairments in both SAT depots in T2D and changes in both layers similarly associated with insulin resistance. Our finding that both SAT depots exhibited lower mitochondrial efficiency in T2D indicate a more general abnormality of AT energy metabolism in these patients. Furthermore, the similar negative association of mitochondrial coupling and similar positive association of proton leak in both SAT depots with HbA_{1c} may also suggest an overall contribution of mitochondrial efficiency to glucose homeostasis.

In our study, higher mitochondrial efficiency in both SAT depots related to improved metabolic flexibility. To maintain whole-body energy metabolism and homeostasis, mitochondrial function has to adapt to lipid supply, which is increased in obesity and insulin resistance (35). Similar to previous reports (8), our patients with T2D exhibit an impaired ability to switch from lipid to carbohydrate oxidation in response to insulin. Furthermore, previous data showed that ex vivo mitochondrial oxidative capacity in muscle predicts the degree of metabolic flexibility (8). Therefore, impaired mitochondrial function and plasticity in skeletal muscle might promote the lack of insulin-stimulated increase in substrate oxidation in insulin-resistant participants (8). Previously, we showed that metabolic flexibility is an independent determinant of whole-body insulin sensitivity, but is affected by AT dysfunction in recent-onset T2D patients of the GDS (11). In this cohort, metabolic flexibility associates positively with both mitochondrial oxidative capacity and efficiency in SSAT and DSAT. This indicates that not only muscle, but also SAT mitochondrial function predicts the degree of metabolic flexibility.

Despite comparable total body fat mass and abdominal SAT thickness, as assessed by ultrasound, patients with T2D feature lower SSAT and higher DSAT thickness than CON. Higher DSAT thickness in T2D patients supports findings from previous studies of a positive association between DSAT thickness and insulin resistance indices (31) and of a positive association between higher abdominal distribution of fat in DSAT and glycemic control in T2D (18, 23). Furthermore, a previous study showed that DSAT volume is associated with impaired fasting blood glucose levels in men (51). Therefore, the authors concluded that DSAT could be considered as a target for early intervention in these patients. A positive family history for T2D was shown to be associated with increased risk for T2D (52) and prediabetes (53). Interestingly, family history for T2D is not only associated with adipose tissue dysfunction, like adipocyte hypertrophy, inflammation, and fibrosis (54), but also with increased VAT and DSAT volume (55). In a tissue-specific analysis of insulin sensitivity, we found that DSAT thickness associates with circulating TG levels, muscle insulin resistance, and hepatic steatosis. Furthermore, DSAT thickness also associated negatively with metabolic flexibility and mitochondrial coupling in both SAT layers. This may indicate that higher DSAT content contributes to impaired AT energy metabolism and insulin resistance. Accordingly, the positive association of DSAT thickness with HbA_{1c} levels may indicate a contribution of this fat depot to systemic glucose homeostasis.

The present findings underline the importance of mitochondrial function in abdominal SAT for recent-onset T2D. The strengths of this study are the simultaneous

analyses using gold-standard methods for assessing mitochondrial function in AT and measurements of tissue-specific insulin sensitivity in well-phenotyped individuals with T2D and well-matched CON with similar fat mass. As a gold-standard method, high-resolution respirometry allows precise measurement of AT depot-specific mitochondrial function and efficiency. As a limititation of our study, the AT samples may contain mitochondria from cell types other than adipocytes, such as stem cells, vascular endothelial cells, and smooth muscle cells. Nevertheless, these contaminations are quantitatively minor and distributed equally in all samples. However, future studies should analyse isolated mitochondria from purified adipocyte preparations. Our findings support the concept of a relevant physiological role of energy metabolism in both SAT depots for whole-body glucose homeostasis. Despite suitable statistical adjustments and drug withdrawal before our measurements, glucose-lowering therapy as well as antihypertensive and lipid-lowering medication may indirectly modulate insulin secretion and sensitivity (56) and thereby may have affected our analyses. The range of diabetes duration and the small sample size of the study group can affect the results on mitochondrial efficiency and DSAT thickness. However, in this cohort, the duration of T2D neither associated with DSAT thickness nor with mitochondrial efficiency in both SAT depots. We therefore assume that the bias introduced by the reported duration of T2D is negligible in our cohort. Furthermore, we did not assess adipocyte lipolysis directly, but indirectly via insulin-mediated suppression of lipolysis during clamp. The sample size and the cross-sectional design of the study do not allow us to draw conclusions as to a causal relationship between the abnormal mitochondrial efficiency in both SAT depots and T2D. Furthermore, the current study cannot clarify if lower mitochondrial efficiency in both SAT depots in T2D is a cause or consequence of systemic lipotoxicity and abnormal glucose homeostasis in these patients. However, recently published data challenge the latter and that study's authors suggested obesity as the primary driver of impaired mitochondrial respiration in SAT, because mitochondrial respiration was not further deteriorated by worsening of the glycemic control (57). Moreover, mitochondrial efficiency was not associated with increased obesity (57), which corresponds to our results in humans matched for total body fat with and without diabetes.

In conclusion, mitochondrial efficiency in SAT is lower in T2D patients and associates with muscle insulin resistance and reduced metabolic flexibility independently of total fat mass. The results imply that (i) impaired mitochondrial function in SAT, (ii) AT insulin resistance, and (iii) expansion of DSAT are defects in newly-diagnosed T2D. These features might promote muscle insulin resistance and increased substrate flux to the liver. Impaired AT energy metabolism may be involved in the development of impaired glucose homeostasis and ectopic lipid deposition independently of total body fat mass.

Acknowledgments

The authors would like to thank all participants for their voluntary contribution as well as the staff from the German Diabetes Center, Petra Domass, Monika Schulte, Gunhild Heitkamp, Robert Wischeidt, Petra Heidkamp, Silke Tosenovian, Agnieszka Sutkowski, Viola Klein-Piklaps, Alessandra Bierwagen, Maik Rothe, Andrea Nagel, Nicole Achterath, Ilka Rokitta, Fariba Zivehe, Daniela Seeger, Christina Preuß and Marie-Ninon Krahnke-Schoelzel for their excellent help with the experiments.

Preliminary data of this study were presented as an abstract at the Annual Meetings of the American Diabetes Association and European Association for the Study in 2016, 2017, and 2018, respectively.

Financial Support: This work was supported by the Ministry of Culture and Science of the State of North Rhine-Westphalia (MKW NRW) and the German Federal Ministry of Health (BMG). The study was supported by grants of the German Research Foundation (DFG, SFB 1116), by a project grant from German Center for Diabetes Research (DZD e.V.), and the Federal Ministry of Education and Research (BMBF). The funding sources had no influence on design and conduct of this study, collection, analysis and interpretation of the data; or on the preparation, review, or approval of this article.

Clinical Trial Information: ClinicalTrial.gov registration no: NCT01055093.

Author Contributions: K.B., J.L., J.S., and M.R. designed the study. K.B. wrote the article and researched the data. J.L., T.J., D.M., Al.S., O.P.Z., Y.K., M.W., Yu.K., M.R., and J.S. researched the data, contributed to the discussion, and reviewed and edited the article. D.M. performed laboratory analyses. V.B., K.M., M.O., H.J.H., D.Z., and A.S. contributed to the discussion, and reviewed and edited the article. K.B. and J.S. are the guarantors of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. All authors gave final approval of this version to be published.

The GDS Group consists of A.E. Buyken, B. Belgardt, G. Geerling, H. Al-Hasani, C. Herder, J.H. Hwang, A. Icks, J. Kotzka, O. Kuss, E. Lammert, D. Markgraf, K. Müssig, W. Rathmann, J. Szendroedi, D. Ziegler and M. Roden (speaker).

Additional Information

Correspondence: Dr. Julia Szendroedi, PhD, Division of Endocrinology and Diabetology, Medical Faculty, Heinrich Heine University, 40225 Düsseldorf, Germany, c/o Auf'm Hennekamp 65, 40225 Düsseldorf, Germany. E-mail: julia.szendroedi@ddz.de.

Disclosure Summary: The authors have no potential conflicts of interest relevant to this article.

Data availability: To ensure data privacy of the study participants, the generated datasets of the ongoing GDS are not publicly available and are subject to national data protection laws and restrictions by the ethics committee. However, they can be requested through an individual project agreement within the GDS.

References

1. Petersen MC, Shulman GI. Mechanisms of insulin action and insulin resistance. *Physiol Rev.* 2018;**98**(4):2133–2223.
2. Ferrannini E, Gastaldelli A, Iozzo P. Pathophysiology of prediabetes. *Med Clin North Am.* 2011;**95**(2):327–39, vii.
3. Scherer PE. The many secret lives of adipocytes: implications for diabetes. *Diabetologia.* 2019;**62**(2):223–232.
4. Gancheva S, Jelenik T, Álvarez-Hernández E, Roden M. Interorgan metabolic crosstalk in human insulin resistance. *Physiol Rev.* 2018;**98**(3):1371–1415.
5. Ferrannini E, Mingrone G. Impact of different bariatric surgical procedures on insulin action and beta-cell function in type 2 diabetes. *Diabetes Care.* 2009;**32**(3):514–520.
6. Taylor R, Al-Mrabeh A, Sattar N. Understanding the mechanisms of reversal of type 2 diabetes. *Lancet Diabetes Endocrinol.* 2019;**7**(9):726–736.
7. Cusi K. Role of obesity and lipotoxicity in the development of nonalcoholic steatohepatitis: pathophysiology and clinical implications. *Gastroenterology.* 2012;**142**(4):711–725.e6.
8. Phielix E, Mensink M. Type 2 diabetes mellitus and skeletal muscle metabolic function. *Physiol Behav.* 2008;**94**(2):252–258.
9. Kelley DE. Skeletal muscle fat oxidation: timing and flexibility are everything. *J Clin Invest.* 2005;**115**(7):1699–1702.
10. Patti ME, Corvera S. The role of mitochondria in the pathogenesis of type 2 diabetes. *Endocr Rev.* 2010;**31**(3):364–395.
11. Apostolopoulou M, Strassburger K, Herder C, et al.; GDS group. Metabolic flexibility and oxidative capacity independently associate with insulin sensitivity in individuals with newly diagnosed type 2 diabetes. *Diabetologia.* 2016;**59**(10):2203–2207.
12. Shi X, Burkart A, Nicoloro SM, Czech MP, Straubhaar J, Corvera S. Paradoxical effect of mitochondrial respiratory chain impairment on insulin signaling and glucose transport in adipose cells. *J Biol Chem.* 2008;**283**(45):30658–30667.
13. Wang CH, Wang CC, Huang HC, Wei YH. Mitochondrial dysfunction leads to impairment of insulin sensitivity and adiponectin secretion in adipocytes. *Febs J.* 2013;**280**(4):1039–1050.
14. Xie X, Yi Z, Sinha S, et al. Proteomics analyses of subcutaneous adipocytes reveal novel abnormalities in human insulin resistance. *Obesity (Silver Spring).* 2016;**24**(7):1506–1514.
15. Vernochet C, Damilano F, Mourier A, et al. Adipose tissue mitochondrial dysfunction triggers a lipodystrophic syndrome with insulin resistance, hepatosteatosis, and cardiovascular complications. *Faseb J.* 2014;**28**(10):4408–4419.
16. Bódis K, Roden M. Energy metabolism of white adipose tissue and insulin resistance in humans. *Eur J Clin Invest.* 2018;**48**(11):e13017.
17. Karastergiou K, Smith SR, Greenberg AS, Fried SK. Sex differences in human adipose tissues - the biology of pear shape. *Biol Sex Differ.* 2012;**3**(1):13.
18. Golan R, Shelef I, Rudich A, et al. Abdominal superficial subcutaneous fat: a putative distinct protective fat subdepot in type 2 diabetes. *Diabetes Care.* 2012;**35**(3):640–647.
19. Miyazaki Y, Glass L, Triplitt C, Wajcberg E, Mandarino LJ, DeFronzo RA. Abdominal fat distribution and peripheral and hepatic insulin resistance in type 2 diabetes mellitus. *Am J Physiol Endocrinol Metab.* 2002;**283**(6):E1135–E1143.
20. Kelley DE, Thaete FL, Troost F, Huwe T, Goodpaster BH. Subdivisions of subcutaneous abdominal adipose tissue and insulin resistance. *Am J Physiol Endocrinol Metab.* 2000;**278**(5):E941–E948.
21. Walker GE, Verti B, Marzullo P, et al. Deep subcutaneous adipose tissue: a distinct abdominal adipose depot. *Obesity (Silver Spring).* 2007;**15**(8):1933–1943.
22. Walker GE, Marzullo P, Prodam F, Bona G, Di Blasio AM. Obesity modifies expression profiles of metabolic markers in superficial and deep subcutaneous abdominal adipose tissue depots. *Endocrine.* 2014;**46**(1):99–106.
23. Lundbom J, Hakkarainen A, Lundbom N, Taskinen MR. Deep subcutaneous adipose tissue is more saturated than superficial subcutaneous adipose tissue. *Int J Obes (Lond).* 2013;**37**(4):620–622.
24. Szendroedi J, Saxena A, Weber KS, et al.; GDS Group. Cohort profile: the German Diabetes Study (GDS). *Cardiovasc Diabetol.* 2016;**15**:59.
25. Bogacka I, Xie H, Bray GA, Smith SR. Pioglitazone induces mitochondrial biogenesis in human subcutaneous adipose tissue in vivo. *Diabetes.* 2005;**54**(5):1392–1399.
26. American Diabetes Association. 2. Classification and diagnosis of diabetes: standards of medical care in diabetes-2019. *Diabetes care.* 2019;**42**(Suppl 1):S13–S28.
27. Kelsey MM, Forster JE, Van Pelt RE, Reusch JE, Nadeau KJ. Adipose tissue insulin resistance in adolescents with and without type 2 diabetes. *Pediatr Obes.* 2014;**9**(5):373–380.
28. Storlien L, Oakes ND, Kelley DE. Metabolic flexibility. *Proc Nutr Soc.* 2004;**63**(2):363–368.
29. Søndergaard E, Espinosa De Ycaza AE, Morgan-Bathke M, Jensen MD. How to measure adipose tissue insulin sensitivity. *J Clin Endocrinol Metab.* 2017;**102**(4):1193–1199.
30. Borel AL, Boulet G, Nazare JA, et al. Improved plasma FFA/insulin homeostasis is independently associated with improved glucose tolerance after a 1-year lifestyle intervention in viscerally obese men. *Diabetes Care.* 2013;**36**(10):3254–3261.
31. Marinou K, Hodson L, Vasan SK, et al. Structural and functional properties of deep abdominal subcutaneous adipose tissue explain its association with insulin resistance and cardiovascular risk in men. *Diabetes Care.* 2014;**37**(3):821–829.
32. Phielix E, Jelenik T, Nowotny P, Szendroedi J, Roden M. Reduction of non-esterified fatty acids improves insulin sensitivity and lowers oxidative stress, but fails to restore oxidative capacity in type 2 diabetes: a randomised clinical trial. *Diabetologia.* 2014;**57**(3):572–581.
33. Mantena SK, Vaughn DP, Andringa KK, et al. High fat diet induces dysregulation of hepatic oxygen gradients and mitochondrial function in vivo. *Biochem J.* 2009;**417**(1):183–193.
34. Satapati S, Sunny NE, Kucejova B, et al. Elevated TCA cycle function in the pathology of diet-induced hepatic insulin resistance and fatty liver. *J Lipid Res.* 2012;**53**(6):1080–1092.
35. Koliaki C, Szendroedi J, Kaul K, et al. Adaptation of hepatic mitochondrial function in humans with non-alcoholic fatty liver is lost in steatohepatitis. *Cell Metab.* 2015;**21**(5):739–746.
36. Wang XL, Zhang L, Youker K, et al. Free fatty acids inhibit insulin signaling-stimulated endothelial nitric oxide synthase activation through upregulating PTEN or inhibiting Akt kinase. *Diabetes.* 2006;**55**(8):2301–2310.
37. Belfort R, Mandarino L, Kashyap S, et al. Dose-response effect of elevated plasma free fatty acid on insulin signaling. *Diabetes.* 2005;**54**(6):1640–1648.
38. Chinen I, Shimabukuro M, Yamakawa K, et al. Vascular lipotoxicity: endothelial dysfunction via fatty-acid-induced reactive oxygen species overproduction in obese Zucker diabetic fatty rats. *Endocrinology.* 2007;**148**(1):160–165.
39. Inoguchi T, Li P, Umeda F, et al. High glucose level and free fatty acid stimulate reactive oxygen species production through protein kinase C–dependent activation of NAD(P)H oxidase in cultured vascular cells. *Diabetes.* 2000;**49**(11):1939–1945.

40. DeFronzo RA. Insulin resistance, lipotoxicity, type 2 diabetes and atherosclerosis: the missing links. The Claude Bernard Lecture 2009. *Diabetologia*. 2010;**53**(7):1270–1287.
41. Cusi K. Role of insulin resistance and lipotoxicity in non-alcoholic steatohepatitis. *Clin Liver Dis*. 2009;**13**(4):545–563.
42. Szendroedi J, Phielix E, Roden M. The role of mitochondria in insulin resistance and type 2 diabetes mellitus. *Nat Rev Endocrinol*. 2011;**8**(2):92–103.
43. Koliaki C, Roden M. Alterations of mitochondrial function and insulin sensitivity in human obesity and diabetes mellitus. *Annu Rev Nutr*. 2016;**36**:337–367.
44. Pettersson-Klein AT, Izadi M, Ferreira DMS, et al. Small molecule PGC-1α1 protein stabilizers induce adipocyte Ucp1 expression and uncoupled mitochondrial respiration. *Mol Metab*. 2018;**9**:28–42.
45. Xie X, Sinha S, Yi Z, et al. Role of adipocyte mitochondria in inflammation, lipemia and insulin sensitivity in humans: effects of pioglitazone treatment. *Int J Obes. (2005)*. 2017.
46. Chattopadhyay M, Guhathakurta I, Behera P, et al. Mitochondrial bioenergetics is not impaired in nonobese subjects with type 2 diabetes mellitus. *Metabolism*. 2011;**60**(12):1702–1710.
47. Hansen M, Lund MT, Gregers E, et al. Adipose tissue mitochondrial respiration and lipolysis before and after a weight loss by diet and RYGB. *Obesity (Silver Spring)*. 2015;**23**(10):2022–2029.
48. Patti ME, Butte AJ, Crunkhorn S, et al. Coordinated reduction of genes of oxidative metabolism in humans with insulin resistance and diabetes: Potential role of PGC1 and NRF1. *Proc Natl Acad Sci U S A*. 2003;**100**(14):8466–8471.
49. Wolfe RR, Evans JE, Mullany CJ, Burke JF. Measurement of plasma free fatty acid turnover and oxidation using [1-13C]palmitic acid. *Biomed Mass Spectrom*. 1980;**7**(4):168–171.
50. Häring HU, Rinninger F, Kemmler W. Decreased insulin sensitivity due to a postreceptor defect as a consequence of ATP-deficiency in fat cells. *FEBS Lett*. 1981;**132**(2):235–238.
51. Kim SH, Chung JH, Song SW, Jung WS, Lee YA, Kim HN. Relationship between deep subcutaneous abdominal adipose tissue and metabolic syndrome: a case control study. *Diabetol Metab Syndr*. 2016;**8**:10.
52. Wilson PW, Meigs JB, Sullivan L, Fox CS, Nathan DM, D'Agostino RB Sr. Prediction of incident diabetes mellitus in middle-aged adults: the Framingham Offspring Study. *Arch Intern Med*. 2007;**167**(10):1068–1074.
53. Wagner R, Thorand B, Osterhoff MA, et al. Family history of diabetes is associated with higher risk for prediabetes: a multicentre analysis from the German Center for Diabetes Research. *Diabetologia*. 2013;**56**(10):2176–2180.
54. Henninger AM, Eliasson B, Jenndahl LE, Hammarstedt A. Adipocyte hypertrophy, inflammation and fibrosis characterize subcutaneous adipose tissue of healthy, non-obese subjects predisposed to type 2 diabetes. *Plos One*. 2014;**9**(8):e105262.
55. Skårn SN, Eggesbø HB, Flaa A, et al. Predictors of abdominal adipose tissue compartments: 18-year follow-up of young men with and without family history of diabetes. *Eur J Intern Med*. 2016;**29**:26–31.
56. Stumvoll M, Goldstein BJ, van Haeften TW. Type 2 diabetes: principles of pathogenesis and therapy. *Lancet*. 2005;**365**(9467):1333–1346.
57. Wessels B, Honecker J, Schöttl T, et al. Adipose mitochondrial respiratory capacity in obesity is impaired independently of glycemic status of tissue donors. *Obesity (Silver Spring)*. 2019;**27**(5):756–766.

4. Discussion

The clinical-experimental studies of this thesis shall contribute to the better understanding of the role of adipose tissue metabolism for glucose homeostasis in early T2D. The first study showed that, compared to CON, patients with recent-onset T2D feature similar FFAR2 and 4 expression in SAT, but lower SCD1. FFAR2 expression correlated negatively with BCF in CON and may therefore negatively influence glucose homeostasis by decreasing insulin secretion. The second study showed that SAT of patients with T2D exhibit impaired mitochondrial coupling and efficiency, insulin resistance and expansion of the deep layer of SAT. These abnormalities of adipose tissue in recent-onset T2D might promote systemic insulin resistance and increased substrate flux to the liver. Perturbed energy metabolism in SAT may contribute to the early development of impaired glucose homeostasis and ectopic lipid deposition. Thus, these studies support the contention that impaired function of adipose tissue may be a very early determinant of the development of T2D (1).
Of note, this thesis focused exclusively on the role of lipid and energy metabolism in white adipose tissue for glucose homeostasis in humans. Nevertheless, possible differences in energy metabolism in adipose tissue could concurrently also occur in brown adipose tissue. Effects of brown adipose tissue in humans have only been shown under specific experimental conditions of brown adipose tissue induction so far (113). Under normal physiological conditions, human white but not brown adipose tissue forms the main adipose tissue mass.

4.1. Lipid metabolism in subcutaneous adipose tissue and insulin resistance

The first study of this thesis examined the expression of FFAR2/4 and SCD in adipose tissue of patients with recently diagnosed T2D to clarify its role for insulin sensitivity and BCF. Patients with T2D have similar FFAR2/4, but lower SCD1 compared to CON. FFAR2 associated negatively with BCF in CON, but not in humans with T2D. This suggests that SCD1 may be important in development of early T2D and FFAR2 may negatively influence glucose homeostasis by decreasing BCF in CON.

4.1.1. Unchanged free fatty acid receptor 2 expression in adipose tissue of early onset type 2 diabetes, but association with impaired beta-cell function in healthy humans

This study did not find differences in gene and protein expression of FFAR2 in SAT of humans with recently diagnosed T2D compared to glucose tolerant humans of similar weight. In contrast, FFAR2 expression was increased in mouse models of diabetes and obesity, and reduced expression improved BCF and insulin sensitivity (165, 170). To exclude the effects of obesity in this study, patients with newly diagnosed T2D were compared to sex-, age- and BMI-matched glucose tolerant humans of similar

body weight and total body fat content. In glucose tolerant humans, protein expression of FFAR2 associated negatively with BCF during a mixed meal test, but this correlation was missing in patients with T2D. These results implicate a potential impact of FFAR2 on glucose homeostasis in postprandial rather than in the fasted state.

A previous study showed enhanced insulin secretion and improved glucose tolerance in mice with whole-body or beta cell selective deletion of *FFAR2* (165). While the findings of a negative association between *FFAR2* deletion and BCF in mice are in line with our data in humans without diabetes, correlations between *FFAR2*-deficiency and insulin sensitivity as observed in mice (170) were not confirmed in the first study. This indicates that previously reported associations between *FFAR2* and glucose homeostasis in mice cannot be directly translated into humans. Of note, high fiber intake protects against obesity and T2D via short-chain FA production from colonic bacterial fermentation (261, 262). Mice with adipocyte-specific overexpression of *FFAR2* on high fat diet developed lower body weight compared to wild-type mice (263).

Previous studies showed that enhanced adipose tissue lipolysis can cause decreased BCF (96, 98). However, our first study did neither show associations between fasted nor between postprandial FFA levels and BCF. Nevertheless, findings in our study during the mixed meal test do not exclude effects of insulin-mediated suppression of adipose tissue lipolysis. The mixed meal test comprises the intake of a liquid meal containing standardized quantities of glucose, protein and fat. Further methods for the determination of pancreatic BCF are the OGTT and the glucagon stimulation test. However, comparative studies have shown that the mixed meal test induces a stronger beta cell response and therefore is a more suitable method to assess human BCF (264). This can mainly be explained by increased beta cell sensitivity during the meal (265, 266), which contains proteins and FA besides glucose, both known to stimulate insulin secretion (267). Taken the dose of administered glucose into account, the mixed meal test allows to measure whole-body insulin sensitivity (268, 269) and the suppression of FFA. However, the FFA concentration as a surrogate parameter does not assess lipolysis directly and might thus not be the optimal method to measure the rate of adipose tissue lipolysis. Variability of individual gastrointestinal FA absorption might bias our results and therefore possible effects of insulin-mediated adipose tissue lipolysis cannot be excluded.

The first study implies that the FFAR2 expression could negatively modulate glucose homeostasis by decreasing insulin secretion from beta cells in humans without diabetes. This may point to a potential role of FFAR2 in the development of diabetes and could provide the basis for future research on new targets in diabetes prevention strategies.

4.1.2. Similar free fatty acid receptors 4 expression in adipose tissue of healthy humans and patients with newly diagnosed type 2 diabetes

Gene and protein expression of FFAR4 die not differ between humans with and without T2D of similar sex, age, BMI, body weight and total body fat content. Our findings in humans are in contrast to results from previous studies in mouse models of FFAR4 deficiency. These mice developed severe obesity, hepatic steatosis and insulin resistance (175, 176) under high-fat diet, which was accompanied by lower *SCD1* gene expression (270). This metabolic phenotype of *FFAR4*-deficient mice can also be found in human patients with T2D. Of note, Omega-3 FA treatment inhibited inflammation and improved whole-body insulin sensitivity in wild type mice, but had no effect in *FFAR4* knockout mice (173). A previous study in humans found that (175, 176) a deleterious non-synonymous mutation (p.R270H) inhibiting the signaling of *FFAR4* associates with higher risk of obesity in Europeans (175). In our study, FFAR4 mRNA or protein expression was not significantly different between humans with and without diabetes and FFAR4 expression did neither associate with body weight nor with insulin sensitivity or BCF. Of note, protein expression levels of FFAR4 tended to be lower in T2D.

The study included only patients with newly diagnosed T2D and these humans were characterized by well controlled glucose homeostasis. These patients featured similar FFA and TAG levels when compared to glucose tolerant humans. Since deleterious effects in mice with *FFAR4* deficiency were found during high-fat diet, one possible explanation for the lack of differences in FFAR4 expression might be explained by the lack of altered FFA levels in our patients. A possible dependency of the expression levels of these receptors on triggering mechanisms - such as enhanced levels of circulating FFA - may explain our results. We cannot exclude that further decrease of insulin sensitivity during the course of T2D, also leading to higher lipolysis in adipose tissue may change expression levels of FFAR4. However, the findings imply that at least in our cohort of patients with recently diagnosed T2D FFAR4 does not play a major role in diabetes progression.

4.1.3. Decreased stearoyl-CoA desaturase-1 expression in adipose tissue of humans with recent-onset type 2 diabetes

This study showed markedly lower SCD1 mRNA and protein expression in SAT of humans with recently diagnosed T2D compared to sex-, age- and BMI-matched glucose tolerant persons. These findings emphasize the relevance of SCD1 in early progression of T2D.

In vitro, SCD1 overexpression led to increased TAG storage and reduced palmitate-induced apoptosis as well as reduced ceramide and DAG synthesis, which was accompanied by improved insulin sensitivity (199, 271, 272). Furthermore, thiazolidinedione treatment led to increased SCD1 expression in adipose tissue, which was accompanied by improved TAG re-esterification in adipose tissue and insulin sensitivity of humans with T2D (197). In our study, protein levels of SCD1 in humans without

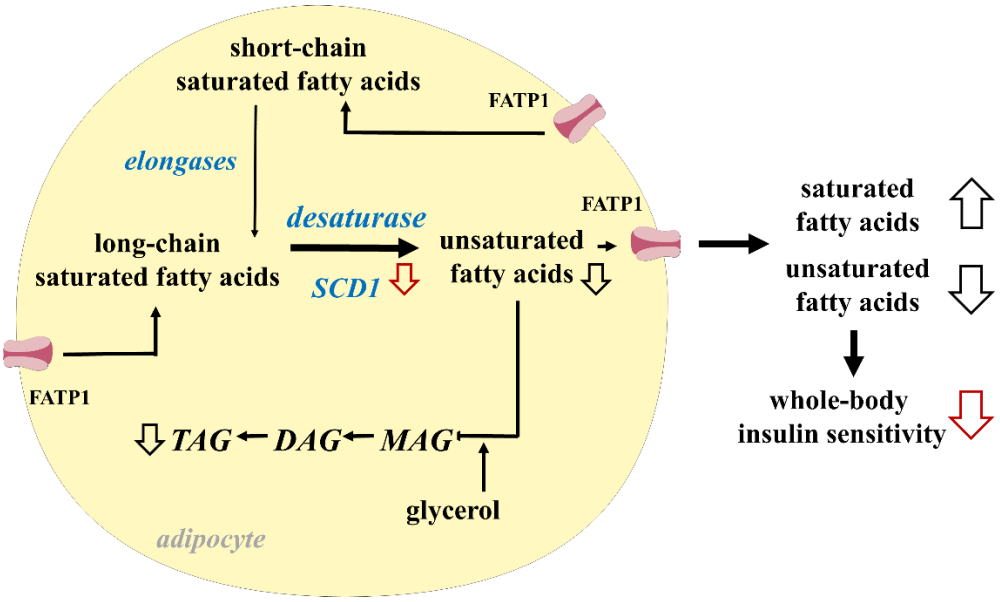
diabetes correlated negatively with pre- and postprandial TAG concentrations. Thus, the reduction in TAG levels in plasma and higher gene and protein expression of SCD1 are in agreement with previous studies, showing similar mechanisms under treatment with thiazolidinediones (197).

These findings are in line with results from a another study, showing reduced *SCD1* gene expression in mice with *FFAR4*-deficiency (270), thus supporting a relevance of SCD1 as a Δ9 desaturase in glucose homeostasis. As key enzymes, desaturases introduce double-bonds in the growing FA chain and thereby convert saturated into unsaturated FA. Although mechanisms underlying whole-body insulin resistance are still not fully understood, increased FA release from adipose tissue leading to increased lipid availability is generally recognized as a major factor in the development of T2D (273, 274). Although we cannot clarify if the observed differences in our study are a cause or a consequence of glucolipotoxicity, lower levels of SCD1 expression may reflect impaired adipose tissue function, which already exists in early T2D. Thus, the observed changes suggest that SCD1 contributes or is at least affected in the development of insulin resistance in early stages of the disease. Previous studies suggested that especially saturated FA induce inflammation and insulin resistance (152-154). Even a single oral fat load rich in saturated FA can simultaneously induce insulin resistance in skeletal muscle, liver and white adipose tissue and is accompanied by ~20% reduced glycogenolysis and ~70% increased gluconeogenesis in the liver of healthy humans (275). In adipocytes, hypoxia is induced by saturated FA-stimulated mitochondrial adenine nucleotide translocase 2 (ANT2), which triggers the transcription factor *HIF1α* and results in adipose tissue inflammation and perturbation (181). Deletion of adipocyte-specific ANT2 (also called Slc25a5) ameliorates obesity-induced hypoxia in adipocytes due to reduced demand of oxygen, even without changes of mitochondrial mass or number (276). This resulted in decreased adipose tissue inflammation as well as improved glucose tolerance and insulin resistance and underlines the relevance of saturated FA for the development of T2D (276). Lower stimulation of mitochondrial ANT2 via reduced saturated FA concentrations may be relevant for both preventive and therapeutic strategies of T2D and might be a possible treatment target for the development of novel anti-obesity drugs or insulin sensitizers (276).

Mice with *SCD1* deficiency - leading to decreased synthesis of unsaturated and ultimately higher availability of saturated FA - featured enhanced inflammation (277). The suggested protective effect of SCD1 in humans without diabetes is supported by our observation that SCD1 protein levels associated negatively with high sensitivity C reactive protein in patients with recent-onset T2D. Furthermore, a previous study in mice with an adipose tissue-specific *SCD1* deletion described the up-regulation of glucose transporter 1 expression in adipocytes, which was accompanied by enhanced TNFα production (278). Of note, after a single oral saturated fat load, healthy persons developed adipose tissue insulin resistance even without changes in circulating inflammatory markers (275). In primary human myotubes, overexpression of SCD1 even protected from inflammatory and endoplasmic reticulum stress response when exposed to palmitate (279). These previous findings in human muscle go along with our

results of a higher mRNA and protein expression of SCD1 in SAT in humans without T2D compared to patients with T2D.

Figure 6



Insulin-resistant humans with type 2 diabetes feature lower stearoyl-coenzyme A desaturase-1 in subcutaneous adipose tissue. *Diacylglycerol (DAG), fatty acid transport proteine 1 (FATP1), mono-acylglycerol (MAG), stearoyl-coenzyme A desaturase-1 (SCD1), triacylglycerol (TAG). Substrate flux and metabolic pathways of biochemical substrates and products are shown by black arrows. Direction of change in the insulin-resistant state suggested in previous studies is indicated by not filled arrows in black. The direction of change in the insulin-resistant state in this study is indicated by not filled arrows in red.*

In agreement with previously reported beneficial effects of SCD1 in T2D (198, 280), patients with recently diagnosed T2D in our study had 5-times lower mRNA expression of SCD1 and 2-times lower protein expression compared to glucose tolerant humans despite similar body weight and total fat mass. These results indicate that SCD1 might be of clinical relevance in diabetes prevention strategies.

4.2. Energy metabolism and morphology of subcutaneous adipose tissue in insulin resistance

The second study examined the role of energy metabolism in distinct compartments of SAT for tissue-specific insulin resistance in recent-onset T2D. This study shows that patients with newly diagnosed T2D with muscle and adipose tissue insulin resistance as well as altered metabolic flexibility feature both expanded DSAT and impaired mitochondrial efficiency similarly in both SAT layers. Furthermore, mitochondrial efficiency of adipose tissue relates to muscle insulin sensitivity and metabolic flexibility

and lower DSAT thickness independently of whole-body fat mass. Thus, impaired mitochondrial efficiency in SAT, adipocyte insulin resistance and expansion of DSAT might be defects of adipose tissue already in early T2D. These metabolic features of adipose tissue may promote increased substrate flux to the liver and skeletal muscle insulin resistance.

4.2.1. Increased deep subcutaneous adipose tissue thickness in recent-onset type 2 diabetes

This study showed increased DSAT in newly diagnosed humans with T2D with muscle and adipose tissue insulin resistance and impaired metabolic flexibility. DSAT thickness associated negatively with metabolic flexibility and positively with hepatic steatosis, muscle and adipose tissue insulin resistance. Thus, expansion of DSAT might characterize early T2D.

The relevance of mitochondria in white adipocytes has long been neglected, which might be explained by their lower occurrence compared to other tissues. Compared to the big lipid droplet, the relatively small cytosolic volume of the human white adipocyte contains less mitochondria than other tissues like skeletal muscle, liver or heart. Previous studies revealed positive correlations between DSAT thickness and insulin resistance indices (281) and between higher DSAT volume and glycemic control in T2D (148). Additionally, increased DSAT fat distribution associated with impaired fasting blood glucose levels in men (282). Thus, authors of the previous study suggested that DSAT may be a possible target for early intervention in these patients. In agreement to these results our study found higher DSAT and lower SSAT thickness in patients with recent-onset T2D compared to healthy humans despite similar whole-body fat mass and comparable abdominal whole SAT thickness, as assessed by ultrasound imaging.

A positive family history for T2D is associated with higher risk for prediabetes (26) and T2D (25). More detailed, a family history for T2D was associated with both adipocyte hypertrophy, inflammation and fibrosis (283) - and with expansion of VAT and DSAT volume (284). Our study showed that DSAT thickness relates to levels of circulating TAG, hepatic steatosis and skeletal muscle insulin resistance. Furthermore, both impaired metabolic flexibility and lower mitochondrial coupling in both SAT compartments associated with DSAT thickness. Additionally, we found an association of DSAT thickness and HbA_{1c} levels, which might point to a potential role of this fat depot for glycemic control. The findings imply that not mitochondrial content or adipocyte size, but higher distribution of fat in DSAT contributes to impaired energy metabolism and insulin resistance in both SAT depots and may play a major role in recent-onset diabetes.

4.2.2. Impaired mitochondrial efficiency in adipose tissue of early onset type 2 diabetes

This study showed increased leak control ratios in both SSAT and DSAT in patients with T2D, suggesting perturbed mitochondrial integrity in both depots. The lower respiratory control ratios in both

fat layers of humans with T2D indicate an attenuated coupling of the electron transport system to the synthesis of ATP. The leak control ratio and respiratory control ratio measure the intrinsic mitochondrial efficiency in both SAT compartments independent of the mitochondrial content. In contrast to the marked decrease of mitochondrial efficiency in patients with T2D compared to persons without T2D, this study found only slightly reduced mitochondrial efficiency in SSAT compared to DSAT, which may not be of clinical relevance. Although previous studies suggested different metabolic features of SSAT and DSAT, this study found similar perturbations of mitochondrial function in both SAT compartments in humans with T2D. In healthy humans, both SAT layers also featured similar mitochondrial function.

Besides the known role of mitochondria in adipogenesis, FA synthesis and esterification and lipolysis in adipose tissue (234, 285), different features of abnormal mitochondrial function in white adipocytes may contribute to insulin resistance in distant tissues (118, 286, 287). An efficient mitochondrial function for sufficient ATP production in adipocytes is vital for balanced storage of lipid metabolites, which can affect insulin sensitivity in peripheral tissues (118). Dysregulated mitochondrial function was reported previously in SAT of obese humans as assessed by lower activity of the respiratory chain complexes and decreased membrane potential (257). Additionally, protein levels of complexes III, IV and V of the respiratory chain were shown to be reduced in SAT of obese compared to lean co-twins (288). These results suggested that mitochondrial function in SAT is downregulated in acquired obesity. This study showed impaired mitochondrial efficiency in both SAT layers of patients with recently diagnosed T2D compared to body fat mass matched CON.

Mouse models revealed that impaired expressions and enzyme activities of the electron transport chain proteins, characterizing impaired mitochondrial function in adipose tissue results in hypertension, cardiovascular complications and also whole-body insulin resistance (228), but could be restored by new compounds inducing proteins which are relevant for adipocyte mitochondrial efficiency (226). A recent study showed that obesity-induced production of the amyloid precursor protein (APP) with subsequent enrichment in mitochondria impairs mitochondrial respiration and decreases ATP generation in adipocytes (289). Adipocyte-specific APP overexpression caused adipose tissue inflammation and adipocyte hypertrophy, loss of mitochondrial cristae structures and resulted in obesity, ectopic lipid accumulation and systemic insulin resistance (289). Knock-out of adipocyte APP improved mitochondrial respiration and protected from these obesity-associated metabolic perturbations (289). In line with our results from high-resolution respirometry analyses, insulin-resistant humans featured lower expression of proteins regulating mitochondrial function in SAT when compared to insulin-sensitive humans without diabetes (229). However, reported differences were less pronounced after matching for BMI and body fat mass. Of note, some of the included insulin-resistant humans met criteria for ‘prediabetes’ according to their 2-h plasma glucose concentration after an OGTT and HbA_{1c} (229), but the authors of this study did not provide information on the total number of persons with ‘prediabetes’. In summary, according to studies in mice (228), insulin-resistant humans and patients with T2D have

lower expression of genes regulating mitochondrial biogenesis, respiratory chain, ATP synthase and FA oxidation in mitochondria of their adipose tissue (229, 236).

In contrast to measurements of gene or protein expression levels as well as of enzyme activities, high-resolution respirometry analyses form this study give more detailed information about the physiological mitochondrial function by combined measurements of oxygen consumption and flux, mitochondrial membrane potential and ability to generate ATP (290). This method allows to follow the oxygen consumption over the time, which allows to monitor the respiration and assess the maximal respiratory capacity, the proton leak rate and coupling efficiency (291). Previous studies using this gold-standard method to measure mitochondrial function in SAT biopsies revealed controversial results in patients with insulin resistance or T2D. One study showed lower mitochondrial respiration in young obese and non-obese humans with T2D than in lean healthy persons (257). Another study reported comparable mitochondrial respiratory capacity and coupling in humans with and without T2D which were followed after bariatric surgery-induced weight loss (258). Both studies did not provide detailed information about the metabolic phenotype of the participants. In detail, these studies gave no information about tissue-specific insulin sensitivity of skeletal muscle, liver and adipose tissue.

Ectopic lipid deposition and analyses of mitochondrial function was not performed depot-specific in SSAT and DSAT. Thus, possible variances in mitochondrial function in both SAT compartments in the two studies could explain previous results. In contrast to this study, previous studies did not match the groups for total body fat mass and increased obesity - defined as increase in whole-body fat mass - associates with systemic insulin resistance (292) and decreased levels of proteins relevant for mitochondrial function (288). Thus, also differences in fat mass of included participants in previous studies could explain the variances. Whereas authors of one study did not report participants' body fat mass (257), the other revealed higher fat mass in obese humans with T2D than in obese persons without T2D (258). Although it is well known that longstanding diabetes associates with perturbation of various features of mitochondrial function, both previous studies did not adjust for diabetes duration. Healthy humans with a positive family history of diabetes feature downregulation of various genes from the oxidative metabolism (293). Nevertheless, only one study excluded healthy humans (with normal glucose tolerance) with a positive family history of diabetes from the study (257). In our study, maximal mitochondrial respiratory capacity was both comparable between humans without and with recently diagnosed T2D of similar fat mass and also similar between both SAT depots.

In times of prolonged fasting, whole-body energy homeostasis is sustained by FA release from adipose tissue serving as substrates for mitochondrial beta-oxidation and production of ATP (239). An adequate ATP generation is crucial for insulin signaling in adipocytes (240) and therefore also required for insulin stimulated suppression of lipolysis (236). In line with our results, previous studies suggested that development of whole-body insulin resistance results from perturbed mitochondrial ATP generation for energy dependent pathways in adipose tissue (222). Perturbation of various features of mitochondrial function and insulin resistance in adipose tissue was linked to ectopic lipid deposition in early T2D

(294). Of note, adipose tissue insulin resistance might start before whole-body insulin resistance (1, 121). Reduced fat storage of neutral TAG in adipose tissue due to both impaired FA metabolism and exhausted lipid storage capacity cause excessive secretion and circulation of lipotoxic metabolites ultimately promoting insulin resistance. In this study, fasting FFA were not elevated in humans with T2D. This may be explained by elevated ectopic lipid storage in these patients, as observed in the form of increased hepatic lipid storage. In agreement with previous results, newly diagnosed humans with T2D featured elevated TAG levels and adipose tissue insulin resistance as measured by both Adipo-IR during fasting and during hyperinsulinemia as well as by FFA suppression during the hyperinsulinemic-euglycemic clamp test.

Despite its central role in whole-body energy metabolism, only few studies analyzed mitochondrial function directly by respiration measurements in adipose tissue. Thus, it is not surprising that segregated assessment of mitochondrial function in both SAT layers was missing yet. Consistent with our findings of similar mitochondrial impairments in both SSAT and DSAT in T2D, changes in both depots similarly related to insulin resistance in this study. We conclude that similar reduction of mitochondrial efficiency in T2D in both SAT depots points to a more general impairment of adipose tissue energy metabolism in T2D. Additionally, the comparable positive association of proton leak and similar negative association of mitochondrial coupling in both adipose tissue compartments with glycemic control might also suggest an overall effect of mitochondrial efficiency on glucose homeostasis.

4.2.3. Association of mitochondrial function in adipose tissue and mitochondrial flexibility

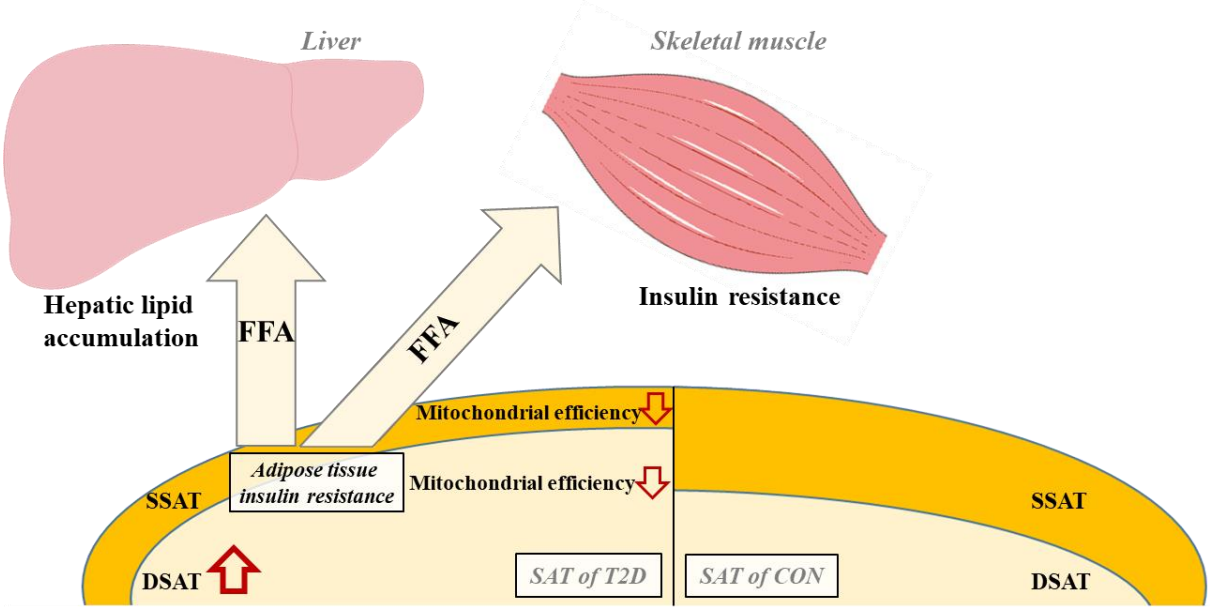
This study showed an augmented ability to switch from lipid to insulin stimulated carbohydrate oxidation in humans with T2D during the hyperinsulinemic-euglycemic clamp test. Furthermore, metabolic flexibility associates with mitochondrial efficiency and muscle insulin sensitivity independently of total fat mass.

Mitochondrial function can adapt to higher lipid availability in obesity and insulin resistance (295). An efficient mitochondrial flexibility is characterized by the ability of mitochondria to adapt to prevailing supply of various energy-rich substrates. This is vital to preserve systemic energy homeostasis and metabolism in health. A previous study reported that metabolic flexibility is predicted by ex vivo mitochondrial oxidative capacity in skeletal muscle (220). The authors therefore suggested that perturbed mitochondrial function and plasticity in skeletal muscle could explain reduced insulin-stimulated substrate oxidation in insulin-resistant humans (220). However, we found that increased mitochondrial efficiency similarly in both SSAT and DSAT associate with improved metabolic flexibility. Previously we reported that in newly diagnosed humans with T2D from the GDS, metabolic flexibility is an independent determinant of systemic insulin sensitivity as assessed by the hyperinsulinemic-euglycemic clamp test (223). This finding was still significant after adjusting for multiple possible metabolic confounders. However, the positive association was affected by markers of

impaired adipose tissue function (223) which indicated a role of adipose tissue for substrate dependent energy metabolism. The positive association of metabolic flexibility with both mitochondrial efficiency and maximal oxidative capacity in both SAT depots indicates that not only muscle, but also mitochondrial function in adipose tissue predicts the scope of metabolic flexibility.

In conclusion, the second study of this thesis revealed that humans with T2D feature reduced mitochondrial efficiency in SAT, which associates with muscle insulin resistance and reduced metabolic flexibility independently of total fat mass. We could show that impaired mitochondrial function in SAT and insulin resistance of adipose tissue are defects in early T2D. Both impairments of adipose tissue could promote elevated substrate fluxes to the liver and induce insulin resistance in skeletal muscle. Thus, impaired energy metabolism of adipose tissue might be involved in ectopic lipid deposition and in the development of impaired glucose homeostasis (Figure 7).

Figure 7



The role of subcutaneous adipose tissue in recent onset type 2 diabetes. *Compared to CON of similar fat mass, patients with recently diagnosed T2D show decreased mitochondrial efficiency in both deep (DSAT) and superficial subcutaneous adipose tissue (SSAT) as well as expansion of DSAT. Insulin resistance and impaired mitochondrial function in subcutaneous adipose tissue (SAT) may result in enhanced lipolysis and increased release of free fatty acids (FFA) to distant tissues such as skeletal muscle and liver leading to insulin resistance in the skeletal muscle and increased hepatic lipid accumulation.*

4.3. Strength and limitations

The first study underlines the role of both FFAR2/4 and SCD1 in abdominal SAT, while the second study emphasizes the relevance mitochondrial respiration, coupling and efficiency in distinct abdominal SAT depots for recent-onset T2D. The major strengths of this study are the simultaneous use of gold-standard methods to measure adipocytes mitochondrial function and tissue-specific insulin sensitivity in metabolically comprehensively phenotyped humans with and without T2D. To minimize possible confounding factors from previous studies, participants from both groups included in the second study were additionally matched for whole-body fat mass and whole SAT thickness. The use of high-resolution respirometry analyses - as state-of-the art method of ex vivo oxidative capacity - enabled precise measurement of adipose tissue depot-specific mitochondrial respiration, coupling and efficiency in the second study.

However, our studies have some limitations and reported findings must be interpreted carefully. Like other cross-sectional studies and despite suitable power calculations, the findings may not be generalizable as the sample size was limited and may not reflect results in other cohorts. The adipose tissue biopsy samples in the second study might contain mitochondria from non-adipose tissue cells like stem cells, smooth muscle cells and vascular endothelial cells. Even so, contaminations are quantitatively minor and should be distributed equally in all tissue samples. Nevertheless, future studies should use isolated mitochondria from purified adipocyte-preparations for their analyses. This study promotes the concept of a central physiological role of energy metabolism in both SAT compartments to maintain systemic energy and glucose homeostasis. Although both studies used appropriate statistical analyses and adjustments as well as drug withdrawal before the experiments, glucose-lowering therapy as well as lipid-lowering and antihypertensive medication might indirectly modify insulin secretion and sensitivity (296), which may have affected the measurements. The relatively small sample size and differences in diabetes duration in the second study could affect the findings. Of note, the duration of T2D in the cohort of the second study neither correlated with DSAT thickness nor with mitochondrial efficiency in both SSAT and DSAT. We therefore suppose that the bias from reported differences in the duration of T2D is negligible in this cohort. As another limitation, adipocytes lipolysis was not measured directly, but indirectly by insulin-stimulated suppression of FFA release from adipose tissue during hyperinsulinemia in the second study. Nevertheless, this method is the current gold-standard method to assess adipose tissue insulin sensitivity in vivo. Since mitochondrial respiratory capacity was not further aggravated by worsening of the glycemic control, a recent study proposed that obesity is the primary driver of perturbed mitochondrial function in SAT (297). However, in agreement with our results in total body fat matched humans with and without diabetes, mitochondrial efficiency did not associate with the degree of obesity in the previous study (297).

If decreased SCD1 expression and/or mitochondrial efficiency in abdominal SAT in T2D are a cause or consequence of abnormal glucose homeostasis and systemic lipotoxicity in these patients remains unclear. Due to the nature of a cross-sectional design and the lack of muscle samples both studies also do not allow to draw conclusions as to temporal relationships or causality between the findings and development of T2D.

5. Conclusions

The findings of the studies of this thesis revealed lower SCD1, but similar FFAR2 or 4 expression in SAT patients with recent-onset T2D when compared to CON. FFAR2 expression associated with perturbed BCF in CON and might therefore impair glucose homeostasis by decreasing insulin secretion. These findings point to a possible role of SCD1 expression in progression of early T2D, although SCD1 does not modulate BCF such effective as FFAR2. The second study revealed perturbed mitochondrial coupling and efficiency in SAT, insulin resistance and expansion of the deep layer of SAT in patients with T2D. These impairments of adipose tissue in early T2D could induce systemic insulin resistance and increased substrate flux to the liver. Thus, impaired energy metabolism in SAT may contribute to the early progression of perturbed glucose homeostasis and ectopic lipid deposition.

The findings of these studies imply that abnormalities of lipid and energy metabolism in SAT of patients with recent-onset T2D promote systemic insulin resistance and ectopic lipid deposition. Nevertheless, the necessity to define detailed pathophysiological molecular mechanisms for the development and progression for T2D forms the basis for future research.

6. Outlook

The concept that adipose tissue regulates metabolic flux and adaptation in the liver is supported by a large number of studies in humans and animal models. However, multiple mechanisms both on molecular and cellular levels, but also in humans must be proven. In humans, the initial events resulting in impaired adipose tissue lipid and energy metabolism are unclear. Growing availability of multi-omics approaches could improve the understanding of metabolic fluxes, ectopic lipid deposition and insulin sensitivity. The relevance for the initiation and reversal of insulin resistance in humans is still not fully understood. The understanding of pathophysiological regulators of lipid and energy metabolism in human adipose tissue regulating whole-body insulin sensitivity and lipid deposition could serve as a source for the identification of novel diagnostic and prognostic biomarkers for different T2D subtypes. The findings of these studies may be relevant for both preventive and therapeutic strategies of T2D and might help to identify possible targets for the development of novel insulin sensitizing drugs.

7. References

1. Roden M, and Shulman GI. The integrative biology of type 2 diabetes. *Nature.* 2019;576(7785):51-60.
2. Muoio DM, and Newgard CB. Obesity-related derangements in metabolic regulation. *Annual review of biochemistry.* 2006;75:367-401.
3. Wilson PW, D'Agostino RB, Sullivan L, Parise H, and Kannel WB. Overweight and obesity as determinants of cardiovascular risk: the Framingham experience. *Archives of internal medicine.* 2002;162(16):1867-72.
4. West KM, and Kalbfleisch JM. Influence of nutritional factors on prevalence of diabetes. *Diabetes.* 1971;20(2):99-108.
5. Grundy SM. Adipose tissue and metabolic syndrome: too much, too little or neither. *European journal of clinical investigation.* 2015;45(11):1209-17.
6. American Diabetes Association. 2. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes-2019. *Diabetes care.* 2019;42(Suppl 1):S13-S28.
7. Müller-Wieland PDmD, Nauck M, Petersmann A, Müller-Wieland D, Schleicher E, Müller UA, Landgraf R, Freckmann G and Heinemann L. Definition, Klassifikation und Diagnostik des Diabetes mellitus. Der Diabetologe. 2019;15(2):128-34.
8. Prentki M, and Nolan CJ. Islet beta cell failure in type 2 diabetes. *The Journal of clinical investigation.* 2006;116(7):1802-12.
9. Ahlqvist E, Storm P, Karajamaki A, Martinell M, Dorkhan M, Carlsson A, Vikman P, Prasad RB, Aly DM, Almgren P, Wessman Y, Shaat N, Spegel P, Mulder H, Lindholm E, Melander O, Hansson O, Malmqvist U, Lernmark A, Lahti K, Forsen T, Tuomi T, Rosengren AH, and Groop L. Novel subgroups of adult-onset diabetes and their association with outcomes: a data-driven cluster analysis of six variables. *The lancet Diabetes & endocrinology.* 2018;6(5):361-9.
10. Zaharia OP, Strassburger K, Strom A, Bonhof GJ, Karusheva Y, Antoniou S, Bódis K, Markgraf DF, Burkart V, Mussig K, Hwang JH, Asplund O, Groop L, Ahlqvist E, Seissler J, Nawroth P, Kopf S, Schmid SM, Stumvoll M, Pfeiffer AFH, Kabisch S, Tselmin S, Haring HU, Ziegler D, Kuss O, Szendroedi J, and Roden M. Risk of diabetes-associated diseases in subgroups of patients with recent-onset diabetes: a 5-year follow-up study. *The lancet Diabetes & endocrinology.* 2019;7(9):684-94.
11. Zaharia OP, Strassburger K, Knebel B, Kupriyanova Y, Karusheva Y, Wolkersdorfer M, Bódis K, Markgraf DF, Burkart V, Hwang J-H, Kotzka J, Al-Hasani H, Szendroedi J, and Roden M. Role of Patatin-Like Phospholipase Domain–Containing 3 Gene for Hepatic Lipid Content and Insulin Resistance in Diabetes. *Diabetes care.* 2020:dc200329.

12. Brunt EM, and Tiniakos DG. Histopathology of nonalcoholic fatty liver disease. *World journal of gastroenterology.* 2010;16(42):5286-96.
13. Szczepaniak LS, Nurenberg P, Leonard D, Browning JD, Reingold JS, Grundy S, Hobbs HH, and Dobbins RL. Magnetic resonance spectroscopy to measure hepatic triglyceride content: prevalence of hepatic steatosis in the general population. *American journal of physiology Endocrinology and metabolism.* 2005;288(2):E462-8.
14. Targher G, Lonardo A, and Byrne CD. Nonalcoholic fatty liver disease and chronic vascular complications of diabetes mellitus. *Nat Rev Endocrinol.* 2018;14(2):99-114.
15. Federation ID. IDF Diabetes Atlas, 9th edn. Brussels, Belgium: International Diabetes Federation. 2019.
16. Rathmann W, Haastert B, Icks A, Lowel H, Meisinger C, Holle R, and Giani G. High prevalence of undiagnosed diabetes mellitus in Southern Germany: target populations for efficient screening. The KORA survey 2000. *Diabetologia.* 2003;46(2):182-9.
17. Dall TM, Narayan KM, Gillespie KB, Gallo PD, Blanchard TD, Solcan M, O'Grady M, and Quick WW. Detecting type 2 diabetes and prediabetes among asymptomatic adults in the United States: modeling American Diabetes Association versus US Preventive Services Task Force diabetes screening guidelines. *Population health metrics.* 2014;12:12.
18. Bonhof GJ, Strom A, Puttgen S, Ringel B, Bruggemann J, Bódis K, Mussig K, Szendroedi J, Roden M, and Ziegler D. Patterns of cutaneous nerve fibre loss and regeneration in type 2 diabetes with painful and painless polyneuropathy. *Diabetologia.* 2017;60(12):2495-503.
19. Cosentino F, Grant PJ, Aboyans V, Bailey CJ, Ceriello A, Delgado V, Federici M, Filippatos G, Grobbee DE, Hansen TB, Huikuri HV, Johansson I, Jüni P, Lettino M, Marx N, Mellbin LG, Östgren CJ, Rocca B, Roffi M, Sattar N, Seferović PM, Sousa-Uva M, Valensi P, Wheeler DC, and Group ESD. 2019 ESC Guidelines on diabetes, pre-diabetes, and cardiovascular diseases developed in collaboration with the EASD: The Task Force for diabetes, pre-diabetes, and cardiovascular diseases of the European Society of Cardiology (ESC) and the European Association for the Study of Diabetes (EASD). *European heart journal.* 2019.
20. Giorgino F, Leonardini A, and Laviola L. Cardiovascular disease and glycemic control in type 2 diabetes: now that the dust is settling from large clinical trials. *Annals of the New York Academy of Sciences.* 2013;1281(1):36-50.
21. Paulweber B, Valensi P, Lindstrom J, Lalic NM, Greaves CJ, McKee M, Kissimova-Skarbek K, Liatis S, Cosson E, Szendroedi J, Sheppard KE, Charlesworth K, Felton AM, Hall M, Rissanen A, Tuomilehto J, Schwarz PE, Roden M, Paulweber M, Stadlmayr A, Kedenko L, Katsilambros N, Makrilakis K, Kamenov Z, Evans P, Gilis-Januszewska A, Lalic K, Jotic A, Djordevic P, Dimitrijevic-Sreckovic V, Huhmer U, Kulzer B, Puhl S, Lee-Barkey YH, AlKerwi A, Abraham C, Hardeman W, Acosta T, Adler M, AlKerwi A, Barengo N, Barengo

R, Boavida JM, Charlesworth K, Christov V, Claussen B, Cos X, Cosson E, Deceukelier S, Dimitrijevic-Sreckovic V, Djordjevic P, Evans P, Felton AM, Fischer M, Gabriel-Sanchez R, Gilis-Januszewska A, Goldfracht M, Gomez JL, Greaves CJ, Hall M, Handke U, Hauner H, Herbst J, Hermanns N, Herrebrugh L, Huber C, Huhmer U, Huttunen J, Jotic A, Kamenov Z, Karadeniz S, Katsilambros N, Khalangot M, Kissimova-Skarbek K, Kohler D, Kopp V, Kronsbein P, Kulzer B, Kyne-Grzebalski D, Lalic K, Lalic N, Landgraf R, Lee-Barkey YH, Liatis S, Lindstrom J, Makrilakis K, McIntosh C, McKee M, Mesquita AC, Misina D, Muylle F, Neumann A, Paiva AC, Pajunen P, Paulweber B, Peltonen M, Perrenoud L, Pfeiffer A, Polonen A, Puhl S, Raposo F, Reinehr T, Rissanen A, Robinson C, Roden M, Rothe U, Saaristo T, Scholl J, Schwarz PE, Sheppard KE, Spiers S, Stemper T, Stratmann B, Szendroedi J, Szybinski Z, Tankova T, Telle-Hjellset V, Terry G, Tolks D, Toti F, Tuomilehto J, Undeutsch A, Valadas C, Valensi P, Velickiene D, Vermunt P, Weiss R, Wens J, and Yilmaz T. A European evidence-based guideline for the prevention of type 2 diabetes. *Horm Metab Res.* 2010;42 Suppl 1:S3-36.

22. Petersen KF, Befroy D, Dufour S, Dziura J, Ariyan C, Rothman DL, DiPietro L, Cline GW, and Shulman GI. Mitochondrial dysfunction in the elderly: possible role in insulin resistance. *Science (New York, NY).* 2003;300(5622):1140-2.
23. Short KR, Bigelow ML, Kahl J, Singh R, Coenen-Schimke J, Raghavakaimal S, and Nair KS. Decline in skeletal muscle mitochondrial function with aging in humans. *Proceedings of the National Academy of Sciences of the United States of America.* 2005;102(15):5618-23.
24. Lanza IR, Short DK, Short KR, Raghavakaimal S, Basu R, Joyner MJ, McConnell JP, and Nair KS. Endurance exercise as a countermeasure for aging. *Diabetes.* 2008;57(11):2933-42.
25. Wilson PW, Meigs JB, Sullivan L, Fox CS, Nathan DM, and D'Agostino RB, Sr. Prediction of incident diabetes mellitus in middle-aged adults: the Framingham Offspring Study. *Archives of internal medicine.* 2007;167(10):1068-74.
26. Wagner R, Thorand B, Osterhoff MA, Muller G, Bohm A, Meisinger C, Kowall B, Rathmann W, Kronenberg F, Staiger H, Stefan N, Roden M, Schwarz PE, Pfeiffer AF, Haring HU, and Fritsche A. Family history of diabetes is associated with higher risk for prediabetes: a multicentre analysis from the German Center for Diabetes Research. *Diabetologia.* 2013;56(10):2176-80.
27. Snijder MB, Agyemang C, Peters RJ, Stronks K, Ujcic-Voortman JK, and van Valkengoed IG. Case Finding and Medical Treatment of Type 2 Diabetes among Different Ethnic Minority Groups: The HELIUS Study. *Journal of diabetes research.* 2017;2017:9896849.
28. Tillin T, Hughes AD, Godsland IF, Whincup P, Forouhi NG, Welsh P, Sattar N, McKeigue PM, and Chaturvedi N. Insulin Resistance and Truncal Obesity as Important Determinants of the Greater Incidence of Diabetes in Indian Asians and African Caribbeans Compared With Europeans. *The Southall And Brent REvisited (SABRE) cohort.* 2013;36(2):383-93.

29. Mayer-Davis EJ, Lawrence JM, Dabelea D, Divers J, Isom S, Dolan L, Imperatore G, Linder B, Marcovina S, Pettitt DJ, Pihoker C, Saydah S, and Wagenknecht L. Incidence Trends of Type 1 and Type 2 Diabetes among Youths, 2002-2012. *The New England journal of medicine.* 2017;376(15):1419-29.
30. Logue J, Walker JJ, Colhoun HM, Leese GP, Lindsay RS, McKnight JA, Morris AD, Pearson DW, Petrie JR, Philip S, Wild SH, Sattar N, and Scottish Diabetes Research Network Epidemiology G. Do men develop type 2 diabetes at lower body mass indices than women? *Diabetologia.* 2011;54(12):3003-6.
31. Lipscombe LL, and Hux JE. Trends in diabetes prevalence, incidence, and mortality in Ontario, Canada 1995-2005: a population-based study. *Lancet (London, England).* 2007;369(9563):750-6.
32. Choi YJ, Kim HC, Kim HM, Park SW, Kim J, and Kim DJ. Prevalence and management of diabetes in Korean adults: Korea National Health and Nutrition Examination Surveys 1998-2005. *Diabetes care.* 2009;32(11):2016-20.
33. Ehrmann DA, Barnes RB, Rosenfield RL, Cavaghan MK, and Imperial J. Prevalence of impaired glucose tolerance and diabetes in women with polycystic ovary syndrome. *Diabetes care.* 1999;22(1):141-6.
34. Gestational diabetes mellitus. *Diabetes care.* 2004;27 Suppl 1:S88-90.
35. Barker DJ. The developmental origins of chronic adult disease. *Acta paediatrica (Oslo, Norway : 1992) Supplement.* 2004;93(446):26-33.
36. Frayling TM. Genome-wide association studies provide new insights into type 2 diabetes aetiology. *Nature reviews Genetics.* 2007;8(9):657-62.
37. Morris AP, Voight BF, Teslovich TM, Ferreira T, Segrè AV, Steinthorsdottir V, Strawbridge RJ, Khan H, Grallert H, Mahajan A, Prokopenko I, Kang HM, Dina C, Esko T, Fraser RM, Kanoni S, Kumar A, Lagou V, Langenberg C, Luan Ja, Lindgren CM, Müller-Nurasyid M, Pechlivanis S, Rayner NW, Scott LJ, Wiltshire S, Yengo L, Kinnunen L, Rossin EJ, Raychaudhuri S, Johnson AD, Dimas AS, Loos RJF, Vedantam S, Chen H, Florez JC, Fox C, Liu C-T, Rybin D, Couper DJ, Kao WHL, Li M, Cornelis MC, Kraft P, Sun Q, van Dam RM, Stringham HM, Chines PS, Fischer K, Fontanillas P, Holmen OL, Hunt SE, Jackson AU, Kong A, Lawrence R, Meyer J, Perry JRB, Platou CGP, Potter S, Rehnberg E, Robertson N, Sivapalaratnam S, Stančáková A, Stirrups K, Thorleifsson G, Tikkanen E, Wood AR, Almgren P, Atalay M, Benediktsson R, Bonnycastle LL, Burtt N, Carey J, Charpentier G, Crenshaw AT, Doney ASF, Dorkhan M, Edkins S, Emilsson V, Eury E, Forsen T, Gertow K, Gigante B, Grant GB, Groves CJ, Guiducci C, Herder C, Hreidarsson AB, Hui J, James A, Jonsson A, Rathmann W, Klopp N, Kravic J, Krjutškov K, Langford C, Leander K, Lindholm E, Lobbens S, Männistö S, Mirza G, Mühleisen TW, Musk B, Parkin M, Rallidis L, Saramies J, Sennblad B, Shah S, Sigurðsson G, Silveira A, Steinbach G, Thorand B, Trakalo J, Veglia

F, Wennauer R, Winckler W, Zabaneh D, Campbell H, van Duijn C, Uitterlinden AG, Hofman A, Sijbrands E, Abecasis GR, Owen KR, Zeggini E, Trip MD, Forouhi NG, Syvänen A-C, Eriksson JG, Peltonen L, Nöthen MM, Balkau B, Palmer CNA, Lyssenko V, Tuomi T, Isomaa B, Hunter DJ, Qi L, Wellcome Trust Case Control C, Meta-Analyses of G, Insulin-related traits Consortium I, Genetic Investigation of ATC, Asian Genetic Epidemiology Network–Type 2 Diabetes C, South Asian Type 2 Diabetes C, Shuldiner AR, Roden M, Barroso I, Wilsgaard T, Beilby J, Hovingh K, Price JF, Wilson JF, Rauramaa R, Lakka TA, Lind L, Dedoussis G, Njølstad I, Pedersen NL, Khaw K-T, Wareham NJ, Keinanen-Kiukaanniemi SM, Saaristo TE, Korpi-Hyövälti E, Saltevo J, Laakso M, Kuusisto J, Metspalu A, Collins FS, Mohlke KL, Bergman RN, Tuomilehto J, Boehm BO, Gieger C, Hveem K, Cauchi S, Froguel P, Baldassarre D, Tremoli E, Humphries SE, Saleheen D, Danesh J, Ingelsson E, Ripatti S, Salomaa V, Erbel R, Jöckel K-H, Moebus S, Peters A, Illig T, de Faire U, Hamsten A, Morris AD, Donnelly PJ, Frayling TM, Hattersley AT, Boerwinkle E, Melander O, Kathiresan S, Nilsson PM, Deloukas P, Thorsteinsdottir U, Groop LC, Stefansson K, Hu F, Pankow JS, Dupuis J, Meigs JB, Altshuler D, Boehnke M, McCarthy MI, Replication DIG, and Meta-analysis C. Large-scale association analysis provides insights into the genetic architecture and pathophysiology of type 2 diabetes. *Nat Genet.* 2012;44(9):981-90.

38. Udler MS, Kim J, von Grotthuss M, Bonàs-Guarch S, Cole JB, Chiou J, Christopher DAoboM, the I, Boehnke M, Laakso M, Atzmon G, Glaser B, Mercader JM, Gaulton K, Flannick J, Getz G, and Florez JC. Type 2 diabetes genetic loci informed by multi-trait associations point to disease mechanisms and subtypes: A soft clustering analysis. *PLoS medicine.* 2018;15(9):e1002654-e.

39. Poulsen P, Kyvik KO, Vaag A, and Beck-Nielsen H. Heritability of type II (non-insulin-dependent) diabetes mellitus and abnormal glucose tolerance--a population-based twin study. *Diabetologia.* 1999;42(2):139-45.

40. Davegårdh C, García-Calzón S, Bacos K, and Ling C. DNA methylation in the pathogenesis of type 2 diabetes in humans. *Mol Metab.* 2018;14:12-25.

41. Ley SH, Hamdy O, Mohan V, and Hu FB. Prevention and Management of Type 2 Diabetes: Dietary Components and Nutritional Strategies. *Lancet (London, England).* 2014;383(9933):1999-2007.

42. Dyson PA, Kelly T, Deakin T, Duncan A, Frost G, Harrison Z, Khatri D, Kunka D, McArdle P, Mellor D, Oliver L, and Worth J. Diabetes UK evidence-based nutrition guidelines for the prevention and management of diabetes. *Diabetic medicine : a journal of the British Diabetic Association.* 2011;28(11):1282-8.

43. Barclay AW, Petocz P, McMillan-Price J, Flood VM, Prvan T, Mitchell P, and Brand-Miller JC. Glycemic index, glycemic load, and chronic disease risk--a meta-analysis of observational studies. *The American journal of clinical nutrition.* 2008;87(3):627-37.

44. Rohling M, Strom A, Bonhof G, Puttgen S, Bódis K, Mussig K, Szendrodi J, Markgraf D, Lehr S, Roden M, and Ziegler D. Differential Patterns of Impaired Cardiorespiratory Fitness and Cardiac Autonomic Dysfunction in Recently Diagnosed Type 1 and Type 2 Diabetes. *Diabetes care.* 2017;40(2):246-52.
45. Hamasaki H. Daily physical activity and type 2 diabetes: A review. *World J Diabetes.* 2016;7(12):243-51.
46. Lampman RM, and Schteingart DE. Effects of exercise training on glucose control, lipid metabolism, and insulin sensitivity in hypertriglyceridemia and non-insulin dependent diabetes mellitus. *Medicine and science in sports and exercise.* 1991;23(6):703-12.
47. Kahn SE, Hull RL, and Utzschneider KM. Mechanisms linking obesity to insulin resistance and type 2 diabetes. *Nature.* 2006;444(7121):840-6.
48. Kissebah AH, and Krakower GR. Regional adiposity and morbidity. *Physiological reviews.* 1994;74(4):761-811.
49. Wajchenberg BL. Subcutaneous and visceral adipose tissue: their relation to the metabolic syndrome. *Endocrine reviews.* 2000;21(6):697-738.
50. Snijder MB, Visser M, Dekker JM, Goodpaster BH, Harris TB, Kritchevsky SB, De Rekeneire N, Kanaya AM, Newman AB, Tylavsky FA, and Seidell JC. Low subcutaneous thigh fat is a risk factor for unfavourable glucose and lipid levels, independently of high abdominal fat. The Health ABC Study. *Diabetologia.* 2005;48(2):301-8.
51. Zhang X, Hu EA, Wu H, Malik V, and Sun Q. Associations of leg fat accumulation with adiposity-related biological factors and risk of metabolic syndrome. *Obesity (Silver Spring, Md).* 2013;21(4):824-30.
52. Gancheva S, Jelenik T, Alvarez-Hernandez E, and Roden M. Interorgan Metabolic Crosstalk in Human Insulin Resistance. *Physiological reviews.* 2018;98(3):1371-415.
53. Lorenz L, Axnick J, Buschmann T, Henning C, Urner S, Fang S, Nurmi H, Eichhorst N, Holtmeier R, Bódis K, Hwang JH, Mussig K, Eberhard D, Stypmann J, Kuss O, Roden M, Alitalo K, Haussinger D, and Lammert E. Mechanosensing by beta1 integrin induces angiocrine signals for liver growth and survival. *Nature.* 2018;562(7725):128-32.
54. Stefan N, Haring HU, and Cusi K. Non-alcoholic fatty liver disease: causes, diagnosis, cardiometabolic consequences, and treatment strategies. *The lancet Diabetes & endocrinology.* 2019;7(4):313-24.
55. Tilg H, Moschen AR, and Roden M. NAFLD and diabetes mellitus. *Nat Rev Gastroenterol Hepatol.* 2017;14(1):32-42.
56. Marchesini G, Brizi M, Morselli-Labate AM, Bianchi G, Bugianesi E, McCullough AJ, Forlani G, and Melchionda N. Association of nonalcoholic fatty liver disease with insulin resistance. *The American journal of medicine.* 1999;107(5):450-5.

57. Bugianesi E, Gastaldelli A, Vanni E, Gambino R, Cassader M, Baldi S, Ponti V, Pagano G, Ferrannini E, and Rizzetto M. Insulin resistance in non-diabetic patients with non-alcoholic fatty liver disease: sites and mechanisms. *Diabetologia.* 2005;48(4):634-42.
58. Ziegler D, Strom A, Kupriyanova Y, Bierwagen A, Bonhof GJ, Bódis K, Mussig K, Szendroedi J, Bobrov P, Markgraf DF, Hwang JH, and Roden M. Association of Lower Cardiovagal Tone and Baroreflex Sensitivity With Higher Liver Fat Content Early in Type 2 Diabetes. *The Journal of clinical endocrinology and metabolism.* 2018;103(3):1130-8.
59. Ziegler D, Strom A, Bonhof G, Puttgen S, Bódis K, Burkart V, Mussig K, Szendroedi J, Markgraf DF, and Roden M. Differential associations of lower cardiac vagal tone with insulin resistance and insulin secretion in recently diagnosed type 1 and type 2 diabetes. *Metabolism.* 2018;79:1-9.
60. Lettner A, and Roden M. Ectopic fat and insulin resistance. *Curr Diab Rep.* 2008;8(3):185-91.
61. Szendroedi J, and Roden M. Ectopic lipids and organ function. *Curr Opin Lipidol.* 2009;20(1):50-6.
62. Chavez JA, and Summers SA. A ceramide-centric view of insulin resistance. *Cell Metab.* 2012;15(5):585-94.
63. Szendroedi J, Yoshimura T, Phielix E, Koliaki C, Marcucci M, Zhang D, Jelenik T, Muller J, Herder C, Nowotny P, Shulman GI, and Roden M. Role of diacylglycerol activation of PKCtheta in lipid-induced muscle insulin resistance in humans. *Proceedings of the National Academy of Sciences of the United States of America.* 2014;111(26):9597-602.
64. Perry RJ, Samuel VT, Petersen KF, and Shulman GI. The role of hepatic lipids in hepatic insulin resistance and type 2 diabetes. *Nature.* 2014;510(7503):84-91.
65. Jelenik T, Flogel U, Alvarez-Hernandez E, Scheiber D, Zweck E, Ding Z, Rothe M, Mastrototaro L, Kohlhaas V, Kotzka J, Knebel B, Muller-Wieland D, Moellendorf S, Godecke A, Kelm M, Westenfeld R, Roden M, and Szendroedi J. Insulin Resistance and Vulnerability to Cardiac Ischemia. *Diabetes.* 2018;67(12):2695-702.
66. Preuss C, Jelenik T, Bódis K, Mussig K, Burkart V, Szendroedi J, Roden M, and Markgraf DF. A New Targeted Lipidomics Approach Reveals Lipid Droplets in Liver, Muscle and Heart as a Repository for Diacylglycerol and Ceramide Species in Non-Alcoholic Fatty Liver. *Cells.* 2019;8(3).
67. Abbasi A, Peelen LM, Corpeleijn E, van der Schouw YT, Stolk RP, Spijkerman AM, van der AD, Moons KG, Navis G, Bakker SJ, and Beulens JW. Prediction models for risk of developing type 2 diabetes: systematic literature search and independent external validation study. *BMJ (Clinical research ed).* 2012;345:e5900.
68. Noble D, Mathur R, Dent T, Meads C, and Greenhalgh T. Risk models and scores for type 2 diabetes: systematic review. *BMJ (Clinical research ed).* 2011;343:d7163.

69. Buijsse B, Simmons RK, Griffin SJ, and Schulze MB. Risk assessment tools for identifying individuals at risk of developing type 2 diabetes. *Epidemiologic reviews.* 2011;33:46-62.
70. Muhlenbruch K, Ludwig T, Jeppesen C, Joost HG, Rathmann W, Meisinger C, Peters A, Boeing H, Thorand B, and Schulze MB. Update of the German Diabetes Risk Score and external validation in the German MONICA/KORA study. *Diabetes research and clinical practice.* 2014;104(3):459-66.
71. Herder C, Kowall B, Tabak AG, and Rathmann W. The potential of novel biomarkers to improve risk prediction of type 2 diabetes. *Diabetologia.* 2014;57(1):16-29.
72. Floyd JS, and Psaty BM. The Application of Genomics in Diabetes: Barriers to Discovery and Implementation. *Diabetes Care.* 2016;39(11):1858-69.
73. Schully SD, Lam TK, Dotson WD, Chang CQ, Aronson N, Birkeland ML, Brewster SJ, Boccia S, Buchanan AH, Calonge N, Calzone K, Djulbegovic B, Goddard KA, Klein RD, Klein TE, Lau J, Long R, Lyman GH, Morgan RL, Palmer CG, Relling MV, Rubinstein WS, Swen JJ, Terry SF, Williams MS, and Khoury MJ. Evidence synthesis and guideline development in genomic medicine: current status and future prospects. *Genetics in medicine : official journal of the American College of Medical Genetics.* 2015;17(1):63-7.
74. Zaharia OP, Bobrov P, Strassburger K, Bódis K, Karusheva Y, Scholz M, Markgraf DF, Burkart V, Schloot NC, Mussig K, Szendroedi J, and Roden M. Metabolic Characteristics of Recently Diagnosed Adult-Onset Autoimmune Diabetes Mellitus. *The Journal of clinical endocrinology and metabolism.* 2018;103(2):429-37.
75. Bódis K, and Roden M. Diabetestherapie bei nichtalkoholischer Fettlebererkrankung. *Der Diabetologe.* 2016;12(7):486-93.
76. Ng M, Fleming T, Robinson M, Thomson B, Graetz N, Margono C, Mullany EC, Biryukov S, Abbafati C, Abera SF, Abraham JP, Abu-Rmeileh NM, Achoki T, AlBuhairan FS, Alemu ZA, Alfonso R, Ali MK, Ali R, Guzman NA, Ammar W, Anwari P, Banerjee A, Barquera S, Basu S, Bennett DA, Bhutta Z, Blore J, Cabral N, Nonato IC, Chang JC, Chowdhury R, Courville KJ, Criqui MH, Cundiff DK, Dabhadkar KC, Dandona L, Davis A, Dayama A, Dharmaratne SD, Ding EL, Durrani AM, Esteghamati A, Farzadfar F, Fay DF, Feigin VL, Flaxman A, Forouzanfar MH, Goto A, Green MA, Gupta R, Hafezi-Nejad N, Hankey GJ, Harewood HC, Havmoeller R, Hay S, Hernandez L, Husseini A, Idrisov BT, Ikeda N, Islami F, Jahangir E, Jassal SK, Jee SH, Jeffreys M, Jonas JB, Kabagambe EK, Khalifa SE, Kengne AP, Khader YS, Khang YH, Kim D, Kimokoti RW, Kinge JM, Kokubo Y, Kosen S, Kwan G, Lai T, Leinsalu M, Li Y, Liang X, Liu S, Logroscino G, Lotufo PA, Lu Y, Ma J, Mainoo NK, Mensah GA, Merriman TR, Mokdad AH, Moschandreas J, Naghavi M, Naheed A, Nand D, Narayan KM, Nelson EL, Neuhouser ML, Nisar MI, Ohkubo T, Oti SO, Pedroza A, Prabhakaran D, Roy N, Sampson U, Seo H, Sepanlou SG, Shibuya K, Shiri R, Shiue I, Singh GM, Singh JA, Skirbekk V, Stapelberg NJ, Sturua L, Sykes BL, Tobias M, Tran BX,

Trasande L, Toyoshima H, van de Vijver S, Vasankari TJ, Veerman JL, Velasquez-Melendez G, Vlassov VV, Vollset SE, Vos T, Wang C, Wang X, Weiderpass E, Werdecker A, Wright JL, Yang YC, Yatsuya H, Yoon J, Yoon SJ, Zhao Y, Zhou M, Zhu S, Lopez AD, Murray CJ, and Gakidou E. 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 (London, England).* 2014;384(9945):766-81.

77. Hippocrates. *The genuine works of Hippocrates.* Classics of Surgery Library; 1849.
78. Cook KS, Min HY, Johnson D, Chaplinsky RJ, Flier JS, Hunt CR, and Spiegelman BM. Adipsin: a circulating serine protease homolog secreted by adipose tissue and sciatic nerve. *Science (New York, NY).* 1987;237(4813):402-5.
79. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, and Friedman JM. Positional cloning of the mouse obese gene and its human homologue. *Nature.* 1994;372(6505):425-32.
80. Li MD. Leptin and beyond: an odyssey to the central control of body weight. *Yale J Biol Med.* 2011;84(1):1-7.
81. Tkach M, and Thery C. Communication by Extracellular Vesicles: Where We Are and Where We Need to Go. *Cell.* 2016;164(6):1226-32.
82. Thomou T, Mori MA, Dreyfuss JM, Konishi M, Sakaguchi M, Wolfrum C, Rao TN, Winnay JN, Garcia-Martin R, Grinspoon SK, Gorden P, and Kahn CR. Adipose-derived circulating miRNAs regulate gene expression in other tissues. *Nature.* 2017;542(7642):450-5.
83. Ying W, Riopel M, Bandyopadhyay G, Dong Y, Birmingham A, Seo JB, Ofrecio JM, Wollam J, Hernandez-Carretero A, Fu W, Li P, and Olefsky JM. Adipose Tissue Macrophage-Derived Exosomal miRNAs Can Modulate In Vivo and In Vitro Insulin Sensitivity. *Cell.* 2017;171(2):372-84.e12.
84. Hartwig S, De Filippo E, Goddeke S, Knebel B, Kotzka J, Al-Hasani H, Roden M, Lehr S, and Sell H. Exosomal proteins constitute an essential part of the human adipose tissue secretome. *Biochim Biophys Acta Proteins Proteom.* 2019;1867(12):140172.
85. Szendrodi J, and Roden M. [The adipose tissue as an endocrine organ]. *Acta Med Austriaca.* 2004;31(4):98-111.
86. Granneman JG, Li P, Zhu Z, and Lu Y. Metabolic and cellular plasticity in white adipose tissue I: effects of beta3-adrenergic receptor activation. *American journal of physiology Endocrinology and metabolism.* 2005;289(4):E608-16.
87. Anderwald C, and Roden M. Adipotoxicity and the insulin resistance syndrome. *Pediatr Endocrinol Rev.* 2004;1(3):310-9.
88. Wang XL, Zhang L, Youker K, Zhang MX, Wang J, LeMaire SA, Coselli JS, and Shen YH. Free fatty acids inhibit insulin signaling-stimulated endothelial nitric oxide synthase activation through upregulating PTEN or inhibiting Akt kinase. *Diabetes.* 2006;55(8):2301-10.

89. Belfort R, Mandarino L, Kashyap S, Wirfel K, Pratipanawatr T, Berria R, Defronzo RA, and Cusi K. Dose-response effect of elevated plasma free fatty acid on insulin signaling. *Diabetes.* 2005;54(6):1640-8.
90. Chinen I, Shimabukuro M, Yamakawa K, Higa N, Matsuzaki T, Noguchi K, Ueda S, Sakanashi M, and Takasu N. Vascular lipotoxicity: endothelial dysfunction via fatty-acid-induced reactive oxygen species overproduction in obese Zucker diabetic fatty rats. *Endocrinology.* 2007;148(1):160-5.
91. Inoguchi T, Li P, Umeda F, Yu HY, Kakimoto M, Imamura M, Aoki T, Etoh T, Hashimoto T, Naruse M, Sano H, Utsumi H, and Nawata H. High glucose level and free fatty acid stimulate reactive oxygen species production through protein kinase C--dependent activation of NAD(P)H oxidase in cultured vascular cells. *Diabetes.* 2000;49(11):1939-45.
92. DeFronzo RA. Insulin resistance, lipotoxicity, type 2 diabetes and atherosclerosis: the missing links. The Claude Bernard Lecture 2009. *Diabetologia.* 2010;53(7):1270-87.
93. Ferrannini E, Gastaldelli A, and Iozzo P. Pathophysiology of prediabetes. *The Medical clinics of North America.* 2011;95(2):327-39, vii-viii.
94. Scherer PE. The many secret lives of adipocytes: implications for diabetes. *Diabetologia.* 2019;62(2):223-32.
95. Jelenik T, Kaul K, Sequaris G, Flogel U, Phielix E, Kotzka J, Knebel B, Fahlbusch P, Horbelt T, Lehr S, Reinbeck AL, Muller-Wieland D, Esposito I, Shulman GI, Szendroedi J, and Roden M. Mechanisms of Insulin Resistance in Primary and Secondary Nonalcoholic Fatty Liver. *Diabetes.* 2017;66(8):2241-53.
96. Glass CK, and Olefsky JM. Inflammation and lipid signaling in the etiology of insulin resistance. *Cell metabolism.* 2012;15(5):635-45.
97. Tanaka N, Takahashi S, Matsubara T, Jiang C, Sakamoto W, Chanturiya T, Teng R, Gavrilova O, and Gonzalez FJ. Adipocyte-specific Disruption of Fat-specific Protein 27 Causes Hepatosteatosis and Insulin Resistance in High-fat Diet-fed Mice. *The Journal of Biological Chemistry.* 2015;290(5):3092-105.
98. Giacca A, Xiao C, Oprescu AI, Carpentier AC, and Lewis GF. Lipid-induced pancreatic beta-cell dysfunction: focus on in vivo studies. *American journal of physiology Endocrinology and metabolism.* 2011;300(2):E255-62.
99. Unger RH, and Zhou YT. Lipotoxicity of beta-cells in obesity and in other causes of fatty acid spillover. *Diabetes.* 2001;50 Suppl 1:S118-21.
100. Poitout V, and Robertson RP. Glucolipotoxicity: Fuel Excess and β-Cell Dysfunction. *Endocrine reviews.* 2008;29(3):351-66.
101. Kashyap SR, Belfort R, Cersosimo E, Lee S, and Cusi K. Chronic low-dose lipid infusion in healthy patients induces markers of endothelial activation independent of its metabolic effects. *J Cardiometab Syndr.* 2008;3(3):141-6.

102. Mathew M, Tay E, and Cusi K. Elevated plasma free fatty acids increase cardiovascular risk by inducing plasma biomarkers of endothelial activation, myeloperoxidase and PAI-1 in healthy subjects. *Cardiovascular Diabetology.* 2010;9(1):9.
103. Barrows BR, and Parks EJ. Contributions of Different Fatty Acid Sources to Very Low-Density Lipoprotein-Triacylglycerol in the Fasted and Fed States. *The Journal of Clinical Endocrinology & Metabolism.* 2006;91(4):1446-52.
104. Hu FB. Globalization of diabetes: the role of diet, lifestyle, and genes. *Diabetes care.* 2011;34(6):1249-57.
105. Roden M, Price TB, Perseghin G, Petersen KF, Rothman DL, Cline GW, and Shulman GI. Mechanism of free fatty acid-induced insulin resistance in humans. *The Journal of clinical investigation.* 1996;97(12):2859-65.
106. Nowotny B, Zahiragic L, Krog D, Nowotny PJ, Herder C, Carstensen M, Yoshimura T, Szendroedi J, Phielix E, Schadewaldt P, Schloot NC, Shulman GI, and Roden M. Mechanisms underlying the onset of oral lipid-induced skeletal muscle insulin resistance in humans. *Diabetes.* 2013;62(7):2240-8.
107. Santomauro AT, Boden G, Silva ME, Rocha DM, Santos RF, Ursich MJ, Strassmann PG, and Wajchenberg BL. Overnight lowering of free fatty acids with Acipimox improves insulin resistance and glucose tolerance in obese diabetic and nondiabetic subjects. *Diabetes.* 1999;48(9):1836-41.
108. Cusi K, Kashyap S, Gastaldelli A, Bajaj M, and Cersosimo E. Effects on insulin secretion and insulin action of a 48-h reduction of plasma free fatty acids with acipimox in nondiabetic subjects genetically predisposed to type 2 diabetes. *American journal of physiology Endocrinology and metabolism.* 2007;292(6):E1775-81.
109. Ferrannini E, and Mingrone G. Impact of different bariatric surgical procedures on insulin action and beta-cell function in type 2 diabetes. *Diabetes care.* 2009;32(3):514-20.
110. Taylor R, Al-Mrabeh A, and Sattar N. Understanding the mechanisms of reversal of type 2 diabetes. *The lancet Diabetes & endocrinology.* 2019.
111. Nicholls DG. Mitochondrial function and dysfunction in the cell: its relevance to aging and aging-related disease. *The international journal of biochemistry & cell biology.* 2002;34(11):1372-81.
112. Tam CS, Lecoultre V, and Ravussin E. Brown adipose tissue: mechanisms and potential therapeutic targets. *Circulation.* 2012;125(22):2782-91.
113. Peirce V, and Vidal-Puig A. Regulation of glucose homoeostasis by brown adipose tissue. *The lancet Diabetes & endocrinology.* 2013;1(4):353-60.
114. Marlatt KL, and Ravussin E. Brown Adipose Tissue: an Update on Recent Findings. *Curr Obes Rep.* 2017;6(4):389-96.

115. Klein S, and Wolfe RR. The use of isotopic tracers in studying lipid metabolism in human subjects. *Bailliere's clinical endocrinology and metabolism.* 1987;1(4):797-816.
116. Bischof MG, Bernroider E, Krssak M, Krebs M, Stingl H, Nowotny P, Yu C, Shulman GI, Waldhausl W, and Roden M. Hepatic glycogen metabolism in type 1 diabetes after long-term near normoglycemia. *Diabetes.* 2002;51(1):49-54.
117. Sondergaard E, Espinosa De Ycaza AE, Morgan-Bathke M, and Jensen MD. How to Measure Adipose Tissue Insulin Sensitivity. *The Journal of clinical endocrinology and metabolism.* 2017;102(4):1193-9.
118. Bódis K, and Roden M. Energy metabolism of white adipose tissue and insulin resistance in humans. *European journal of clinical investigation.* 2018;48(11):e13017.
119. Kashiwagi A, Verso MA, Andrews J, Vasquez B, Reaven G, and Foley JE. In vitro insulin resistance of human adipocytes isolated from subjects with noninsulin-dependent diabetes mellitus. *Journal of Clinical Investigation.* 1983;72(4):1246-54.
120. Carvalho E, Eliasson B, Wesslau C, and Smith U. Impaired phosphorylation and insulin-stimulated translocation to the plasma membrane of protein kinase B/Akt in adipocytes from Type II diabetic subjects. *Diabetologia.* 2000;43(9):1107-15.
121. Smith U. Impaired ('diabetic') insulin signaling and action occur in fat cells long before glucose intolerance--is insulin resistance initiated in the adipose tissue? *International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity.* 2002;26(7):897-904.
122. Freidenberg GR, Reichart D, Olefsky JM, and Henry RR. Reversibility of defective adipocyte insulin receptor kinase activity in non-insulin-dependent diabetes mellitus. Effect of weight loss. *The Journal of clinical investigation.* 1988;82(4):1398-406.
123. Kahn BB, and Flier JS. Obesity and insulin resistance. *The Journal of clinical investigation.* 2000;106(4):473-81.
124. Kahn CR. Insulin resistance, insulin insensitivity, and insulin unresponsiveness: a necessary distinction. *Metabolism.* 1978;27(12 Suppl 2):1893-902.
125. Karastergiou K, Smith SR, Greenberg AS, and Fried SK. Sex differences in human adipose tissues – the biology of pear shape. *Biology of Sex Differences.* 2012;3:13-.
126. Smith SR, Lovejoy JC, Greenway F, Ryan D, deJonge L, and de la Bretonne J. Contributions of total body fat, abdominal subcutaneous adipose tissue compartments, and visceral adipose tissue to the metabolic complications of obesity. *Metabolism: clinical and experimental.* 2001;50:425-35.
127. Item F, and Konrad D. Visceral fat and metabolic inflammation: the portal theory revisited. *Obesity reviews : an official journal of the International Association for the Study of Obesity.* 2012;13 Suppl 2:30-9.

128. Enzi G, Gasparo M, Biondetti PR, Fiore D, Semisa M, and Zurlo F. Subcutaneous and visceral fat distribution according to sex, age, and overweight, evaluated by computed tomography. *The American journal of clinical nutrition.* 1986;44(6):739-46.
129. Frayn KN. Adipose tissue as a buffer for daily lipid flux. *Diabetologia.* 2002;45(9):1201-10.
130. Gujral UP, Pradeepa R, Weber MB, Narayan KMV, and Mohan V. Type 2 diabetes in South Asians: similarities and differences with white Caucasian and other populations. *Annals of the New York Academy of Sciences.* 2013;1281(1):51-63.
131. Arner P, and Langin D. Lipolysis in lipid turnover, cancer cachexia, and obesity-induced insulin resistance. *Trends in endocrinology and metabolism: TEM.* 2014;25(5):255-62.
132. Bjorntorp P. "Portal" adipose tissue as a generator of risk factors for cardiovascular disease and diabetes. *Arteriosclerosis (Dallas, Tex).* 1990;10(4):493-6.
133. Taksali SE, Caprio S, Dziura J, Dufour S, Cali AM, Goodman TR, Papademetris X, Burgert TS, Pierpont BM, Savoye M, Shaw M, Seyal AA, and Weiss R. High visceral and low abdominal subcutaneous fat stores in the obese adolescent: a determinant of an adverse metabolic phenotype. *Diabetes.* 2008;57(2):367-71.
134. Fassina G, Dorigo P, and Gaion RM. Equilibrium between metabolic pathways producing energy: a key factor in regulating lipolysis. *Pharmacological Research Communications.* 1974;6(1):1-21.
135. Jaworski K, Sarkadi-Nagy E, Duncan RE, Ahmadian M, and Sul HS. Regulation of triglyceride metabolism. IV. Hormonal regulation of lipolysis in adipose tissue. *American journal of physiology Gastrointestinal and liver physiology.* 2007;293(1):G1-4.
136. Taniguchi CM, Emanuelli B, and Kahn CR. Critical nodes in signalling pathways: insights into insulin action. *Nature reviews Molecular cell biology.* 2006;7(2):85-96.
137. Cantley LC. The phosphoinositide 3-kinase pathway. *Science (New York, NY).* 2002;296(5573):1655-7.
138. Chakrabarti P, English T, Shi J, Smas CM, and Kandror KV. Mammalian target of rapamycin complex 1 suppresses lipolysis, stimulates lipogenesis, and promotes fat storage. *Diabetes.* 2010;59(4):775-81.
139. Czech MP, Tencerova M, Pedersen DJ, and Aouadi M. Insulin signalling mechanisms for triacylglycerol storage. *Diabetologia.* 2013;56(5):949-64.
140. Jaworski K, Ahmadian M, Duncan RE, Sarkadi-Nagy E, Varady KA, Hellerstein MK, Lee HY, Samuel VT, Shulman GI, Kim KH, de Val S, Kang C, and Sul HS. AdPLA ablation increases lipolysis and prevents obesity induced by high-fat feeding or leptin deficiency. *Nature medicine.* 2009;15(2):159-68.
141. Duncan RE, Sarkadi-Nagy E, Jaworski K, Ahmadian M, and Sul HS. Identification and functional characterization of adipose-specific phospholipase A2 (AdPLA). *The Journal of biological chemistry.* 2008;283(37):25428-36.

142. Kitamura T, Kitamura Y, Kuroda S, Hino Y, Ando M, Kotani K, Konishi H, Matsuzaki H, Kikkawa U, Ogawa W, and Kasuga M. Insulin-Induced Phosphorylation and Activation of Cyclic Nucleotide Phosphodiesterase 3B by the Serine-Threonine Kinase Akt. *Molecular and cellular biology.* 1999;19(9):6286-96.
143. Walker GE, Verti B, Marzullo P, Savia G, Mencarelli M, and Zurleni F. Deep subcutaneous adipose tissue: a distinct abdominal adipose depot. *Obesity.* 2007;15:1933-43.
144. Kelley DE, Thaete FL, Troost F, Huwe T, and Goodpaster BH. Subdivisions of subcutaneous abdominal adipose tissue and insulin resistance. *American journal of physiology Endocrinology and metabolism.* 2000;278(5):E941-8.
145. Lundbom J, Hakkarainen A, Lundbom N, and Taskinen MR. Deep subcutaneous adipose tissue is more saturated than superficial subcutaneous adipose tissue. *Int J Obes.* 2013;37(4):620-2.
146. Tan GD, Goossens GH, Humphreys SM, Vidal H, and Karpe F. Upper and lower body adipose tissue function: a direct comparison of fat mobilization in humans. *Obesity research.* 2004;12(1):114-8.
147. Yim J-E, Heshka S, Albu JB, Heymsfield S, and Gallagher D. Femoral-gluteal subcutaneous and intermuscular adipose tissues have independent and opposing relationships with CVD risk. *J Appl Physiol (1985).* 2008;104(3):700-7.
148. Golan R, Shelef I, Rudich A, Gepner Y, Shemesh E, Chassidim Y, Harman-Boehm I, Henkin Y, Schwarzfuchs D, Ben Avraham S, Witkow S, Liberty IF, Tangi-Rosental O, Sarusi B, Stampfer MJ, and Shai I. Abdominal superficial subcutaneous fat: a putative distinct protective fat subdepot in type 2 diabetes. *Diabetes care.* 2012;35(3):640-7.
149. Tordjman J, Divoux A, Prifti E, Poitou C, Pelloux V, Hugol D, Basdevant A, Bouillot JL, Chevallier JM, Bedossa P, Guerre-Millo M, and Clement K. Structural and inflammatory heterogeneity in subcutaneous adipose tissue: relation with liver histopathology in morbid obesity. *Journal of hepatology.* 2012;56(5):1152-8.
150. Lundbom J, Hakkarainen A, Fielding B, Soderlund S, Westerbacka J, Taskinen MR, and Lundbom N. Characterizing human adipose tissue lipids by long echo time 1H-MRS in vivo at 1.5 Tesla: validation by gas chromatography. *NMR Biomed.* 2010;23(5):466-72.
151. Lundbom J, Bierwagen A, Bódis K, Apostolopoulou M, Szendroedi J, Mussig K, Hwang JH, and Roden M. (1)H-MRS of femoral red and yellow bone marrow fat composition and water content in healthy young men and women at 3 T. *Magma.* 2019;32(5):591-7.
152. Kausch C, Staiger H, Staiger K, Krutzfeldt J, Matthaei S, Haring HU, and Stumvoll M. Skeletal muscle cells from insulin-resistant (non-diabetic) individuals are susceptible to insulin desensitization by palmitate. *Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme.* 2003;35(10):570-6.

153. Staiger K, Staiger H, Weigert C, Haas C, Haring HU, and Kellerer M. Saturated, but not unsaturated, fatty acids induce apoptosis of human coronary artery endothelial cells via nuclear factor-kappaB activation. *Diabetes.* 2006;55(11):3121-6.
154. Staiger H, Staiger K, Stefan N, Wahl HG, Machicao F, Kellerer M, and Haring HU. Palmitate-induced interleukin-6 expression in human coronary artery endothelial cells. *Diabetes.* 2004;53(12):3209-16.
155. Lundbom J, Bierwagen A, Bódis K, Szendrodi J, Kaprio J, Rissanen A, Lundbom N, Roden M, and Pietilainen KH. Deep subcutaneous adipose tissue lipid unsaturation associates with intramyocellular lipid content. *Metabolism.* 2016;65(9):1230-7.
156. Miyazaki Y, Glass L, Triplitt C, Wajcberg E, Mandarino LJ, and DeFronzo RA. Abdominal fat distribution and peripheral and hepatic insulin resistance in type 2 diabetes mellitus. *American journal of physiology Endocrinology and metabolism.* 2002;283(6):E1135-43.
157. Monzon JR, Basile R, Heneghan S, Udupi V, and Green A. Lipolysis in adipocytes isolated from deep and superficial subcutaneous adipose tissue. *Obesity research.* 2002;10(4):266-9.
158. Walker GE, Marzullo P, Prodam F, Bona G, and Di Blasio AM. Obesity modifies expression profiles of metabolic markers in superficial and deep subcutaneous abdominal adipose tissue depots. *Endocrine.* 2014;46(1):99-106.
159. Yu Z-W, Burén J, Enerbäck S, Nilsson E, Samuelsson L, and Eriksson JW. Insulin can enhance GLUT4 gene expression in 3T3-F442A cells and this effect is mimicked by vanadate but counteracted by cAMP and high glucose – potential implications for insulin resistance. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease.* 2001;1535(2):174-85.
160. Fasshauer M, Klein J, Ueki K, Kriauciunas KM, Benito M, White MF, and Kahn CR. Essential role of insulin receptor substrate-2 in insulin stimulation of Glut4 translocation and glucose uptake in brown adipocytes. *J Biol Chem.* 2000;275(33):25494-501.
161. Blad CC, Tang C, and Offermanns S. G protein-coupled receptors for energy metabolites as new therapeutic targets. *Nature reviews Drug discovery.* 2012;11(8):603-19.
162. Chawla A, Repa JJ, Evans RM, and Mangelsdorf DJ. Nuclear receptors and lipid physiology: opening the X-files. *Science (New York, NY).* 2001;294(5548):1866-70.
163. Kimura I, Ichimura A, Ohue-Kitano R, and Igarashi M. Free Fatty Acid Receptors in Health and Disease. *Physiological reviews.* 2020;100(1):171-210.
164. Miyamoto J, Hasegawa S, Kasubuchi M, Ichimura A, Nakajima A, and Kimura I. Nutritional Signaling via Free Fatty Acid Receptors. *International journal of molecular sciences.* 2016;17(4):450.
165. Tang C, Ahmed K, Gille A, Lu S, Grone HJ, Tunaru S, and Offermanns S. Loss of FFA2 and FFA3 increases insulin secretion and improves glucose tolerance in type 2 diabetes. *Nature medicine.* 2015;21(2):173-7.

166. Tazoe H, Otomo Y, Kaji I, Tanaka R, Karaki SI, and Kuwahara A. Roles of short-chain fatty acids receptors, GPR41 and GPR43 on colonic functions. *Journal of physiology and pharmacology : an official journal of the Polish Physiological Society.* 2008;59 Suppl 2:251-62.

167. Hirasawa A, Tsumaya K, Awaji T, Katsuma S, Adachi T, Yamada M, Sugimoto Y, Miyazaki S, and Tsujimoto G. Free fatty acids regulate gut incretin glucagon-like peptide-1 secretion through GPR120. *Nature medicine.* 2005;11(1):90-4.

168. Vangaveti V, Shashidhar V, Jarrod G, Baune BT, and Kennedy RL. Free fatty acid receptors: emerging targets for treatment of diabetes and its complications. *Therapeutic Advances in Endocrinology and Metabolism.* 2010;1(4):165-75.

169. Aberdein N, Schweizer M, and Ball D. Sodium acetate decreases phosphorylation of hormone sensitive lipase in isoproterenol-stimulated 3T3-L1 mature adipocytes. *Adipocyte.* 2014;3(2):121-5.

170. Bjursell M, Admyre T, Goransson M, Marley AE, Smith DM, Oscarsson J, and Bohlooly YM. Improved glucose control and reduced body fat mass in free fatty acid receptor 2-deficient mice fed a high-fat diet. *American journal of physiology Endocrinology and metabolism.* 2011;300(1):E211-20.

171. Layden BT, Durai V, Newman MV, Marinelarena AM, Ahn CW, Feng G, Lin S, Zhang X, Kaufman DB, Jafari N, Sorensen GL, and Lowe WL, Jr. Regulation of pancreatic islet gene expression in mouse islets by pregnancy. *The Journal of endocrinology.* 2010;207(3):265-79.

172. Rieck S, White P, Schug J, Fox AJ, Smirnova O, Gao N, Gupta RK, Wang ZV, Scherer PE, Keller MP, Attie AD, and Kaestner KH. The transcriptional response of the islet to pregnancy in mice. *Molecular endocrinology (Baltimore, Md).* 2009;23(10):1702-12.

173. Oh DY, Talukdar S, Bae EJ, Imamura T, Morinaga H, Fan W, Li P, Lu WJ, Watkins SM, and Olefsky JM. GPR120 is an omega-3 fatty acid receptor mediating potent anti-inflammatory and insulin-sensitizing effects. *Cell.* 2010;142(5):687-98.

174. Taneera J, Lang S, Sharma A, Fadista J, Zhou Y, Ahlqvist E, Jonsson A, Lyssenko V, Vikman P, Hansson O, Parikh H, Korsgren O, Soni A, Krus U, Zhang E, Jing XJ, Esguerra JL, Wollheim CB, Salehi A, Rosengren A, Renstrom E, and Groop L. A systems genetics approach identifies genes and pathways for type 2 diabetes in human islets. *Cell Metab.* 2012;16(1):122-34.

175. Ichimura A, Hirasawa A, Poulain-Godefroy O, Bonnefond A, Hara T, Yengo L, Kimura I, Leloire A, Liu N, Iida K, Choquet H, Besnard P, Lecoeur C, Vivequin S, Ayukawa K, Takeuchi M, Ozawa K, Tauber M, Maffeis C, Morandi A, Buzzetti R, Elliott P, Pouta A, Jarvelin MR, Korner A, Kiess W, Pigeyre M, Caiazzo R, Van Hul W, Van Gaal L, Horber F, Balkau B, Levy-Marchal C, Rouskas K, Kouvatsi A, Hebebrand J, Hinney A, Scherag A, Pattou F, Meyre D, Koshimizu TA, Wolowczuk I, Tsujimoto G, and Froguel P. Dysfunction

of lipid sensor GPR120 leads to obesity in both mouse and human. *Nature.* 2012;483(7389):350-4.

176. Waguri T, Goda T, Kasezawa N, and Yamakawa-Kobayashi K. The combined effects of genetic variations in the GPR120 gene and dietary fat intake on obesity risk. *Biomedical research (Tokyo, Japan).* 2013;34(2):69-74.

177. Roden M. Mechanisms of Disease: hepatic steatosis in type 2 diabetes--pathogenesis and clinical relevance. *Nat Clin Pract Endocrinol Metab.* 2006;2(6):335-48.

178. Gancheva S, Ouni M, Jelenik T, Koliaki C, Szendroedi J, Toledo FGS, Markgraf DF, Pesta DH, Mastrototaro L, De Filippo E, Herder C, Jahnert M, Weiss J, Strassburger K, Schlensak M, Schurmann A, and Roden M. Dynamic changes of muscle insulin sensitivity after metabolic surgery. *Nature communications.* 2019;10(1):4179.

179. Schoiswohl G, Stefanovic-Racic M, Menke MN, Wills RC, Surlow BA, Basantani MK, Sitnick MT, Cai L, Yazbeck CF, Stolz DB, Pulinilkunnil T, O'Doherty RM, and Kershaw EE. Impact of Reduced ATGL-Mediated Adipocyte Lipolysis on Obesity-Associated Insulin Resistance and Inflammation in Male Mice. *Endocrinology.* 2015;156(10):3610-24.

180. Girousse A, Tavernier G, Valle C, Moro C, Mejhert N, Dinel AL, Houssier M, Roussel B, Besse-Patin A, Combes M, Mir L, Monbrun L, Bezaire V, Prunet-Marcassus B, Waget A, Vila I, Caspar-Bauguil S, Louche K, Marques MA, Mairal A, Renoud ML, Galitzky J, Holm C, Mouisel E, Thalamas C, Viguerie N, Sulpice T, Burcelin R, Arner P, and Langin D. Partial inhibition of adipose tissue lipolysis improves glucose metabolism and insulin sensitivity without alteration of fat mass. *PLoS biology.* 2013;11(2):e1001485.

181. Albert JS, Yerges-Armstrong LM, Horenstein RB, Pollin TI, Sreenivasan UT, Chai S, Blaner WS, Snitker S, O'Connell JR, Gong D-W, Breyer RJ, Ryan AS, McLenithan JC, Shuldiner AR, Sztalryd C, and Damcott CM. Null Mutation in Hormone-Sensitive Lipase Gene and Risk of Type 2 Diabetes. *The New England journal of medicine.* 2014;370(24):2307-15.

182. Kursawe R, Caprio S, Giannini C, Narayan D, Lin A, D'Adamo E, Shaw M, Pierpont B, Cushman SW, and Shulman GI. Decreased transcription of ChREBP-alpha/beta isoforms in abdominal subcutaneous adipose tissue of obese adolescents with prediabetes or early type 2 diabetes: associations with insulin resistance and hyperglycemia. *Diabetes.* 2013;62(3):837-44.

183. Cao H, Sekiya M, Ertunc ME, Burak MF, Mayers JR, White A, Inouye K, Rickey LM, Ercal BC, Furuhashi M, Tuncman G, and Hotamisligil GS. Adipocyte lipid chaperone AP2 is a secreted adipokine regulating hepatic glucose production. *Cell Metab.* 2013;17(5):768-78.

184. Ertunc ME, Sikkeland J, Fenaroli F, Griffiths G, Daniels MP, Cao H, Saatcioglu F, and Hotamisligil GS. Secretion of fatty acid binding protein aP2 from adipocytes through a nonclassical pathway in response to adipocyte lipase activity. *Journal of lipid research.* 2015;56(2):423-34.

185. Hodson L, Skeaff CM, and Fielding BA. Fatty acid composition of adipose tissue and blood in humans and its use as a biomarker of dietary intake. *Progress in lipid research.* 2008;47(5):348-80.
186. Arab L. Biomarkers of fat and fatty acid intake. *The Journal of nutrition.* 2003;133 Suppl 3(3):925S-32S.
187. Nakamura MT, and Nara TY. Structure, function, and dietary regulation of delta6, delta5, and delta9 desaturases. *Annual review of nutrition.* 2004;24:345-76.
188. Yary T, Voutilainen S, Tuomainen TP, Ruusunen A, Nurmi T, and Virtanen JK. Serum n-6 polyunsaturated fatty acids, Delta5- and Delta6-desaturase activities, and risk of incident type 2 diabetes in men: the Kuopio Ischaemic Heart Disease Risk Factor Study. *The American journal of clinical nutrition.* 2016;103(5):1337-43.
189. Takkunen MJ, Schwab US, de Mello VD, Eriksson JG, Lindstrom J, Tuomilehto J, and Uusitupa MI. Longitudinal associations of serum fatty acid composition with type 2 diabetes risk and markers of insulin secretion and sensitivity in the Finnish Diabetes Prevention Study. *European journal of nutrition.* 2016;55(3):967-79.
190. Jacobs S, Schiller K, Jansen EH, Boeing H, Schulze MB, and Kroger J. Evaluation of various biomarkers as potential mediators of the association between Delta5 desaturase, Delta6 desaturase, and stearoyl-CoA desaturase activity and incident type 2 diabetes in the European Prospective Investigation into Cancer and Nutrition-Potsdam Study. *The American journal of clinical nutrition.* 2015;102(1):155-64.
191. Zheng Y, Prouty SM, Harmon A, Sundberg JP, Stenn KS, and Parimoo S. Scd3--a novel gene of the stearoyl-CoA desaturase family with restricted expression in skin. *Genomics.* 2001;71(2):182-91.
192. Tabor DE, Xia YR, Mehrabian M, Edwards PA, and Lusis AJ. A cluster of stearoyl CoA desaturase genes, Scd1 and Scd2, on mouse chromosome 19. *Mammalian genome : official journal of the International Mammalian Genome Society.* 1998;9(4):341-2.
193. Miyazaki M, Jacobson MJ, Man WC, Cohen P, Asilmaz E, Friedman JM, and Ntambi JM. Identification and characterization of murine SCD4, a novel heart-specific stearoyl-CoA desaturase isoform regulated by leptin and dietary factors. *J Biol Chem.* 2003;278(36):33904-11.
194. Wang J, Yu L, Schmidt RE, Su C, Huang X, Gould K, and Cao G. Characterization of HSCD5, a novel human stearoyl-CoA desaturase unique to primates. *Biochemical and biophysical research communications.* 2005;332(3):735-42.
195. Zhang L, Ge L, Parimoo S, Stenn K, and Prouty SM. Human stearoyl-CoA desaturase: alternative transcripts generated from a single gene by usage of tandem polyadenylation sites. *The Biochemical journal.* 1999;340 (Pt 1):255-64.

196. Cao H, Gerhold K, Mayers JR, Wiest MM, Watkins SM, and Hotamisligil GS. Identification of a lipokine, a lipid hormone linking adipose tissue to systemic metabolism. *Cell.* 2008;134(6):933-44.

197. Tordjman J, Chauvet G, Quette J, Beale EG, Forest C, and Antoine B. Thiazolidinediones block fatty acid release by inducing glyceroneogenesis in fat cells. *The Journal of biological chemistry.* 2003;278(21):18785-90.

198. Riserus U, Tan GD, Fielding BA, Neville MJ, Currie J, Savage DB, Chatterjee VK, Frayn KN, O'Rahilly S, and Karpe F. Rosiglitazone increases indexes of stearoyl-CoA desaturase activity in humans: link to insulin sensitization and the role of dominant-negative mutation in peroxisome proliferator-activated receptor-gamma. *Diabetes.* 2005;54(5):1379-84.

199. Pinnamaneni SK, Southgate RJ, Febbraio MA, and 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(12):3027-37.

200. Hillgartner FB, Salati LM, and Goodridge AG. Physiological and molecular mechanisms involved in nutritional regulation of fatty acid synthesis. *Physiological reviews.* 1995;75(1):47-76.

201. Postic C, and Girard J. Contribution of de novo fatty acid synthesis to hepatic steatosis and insulin resistance: lessons from genetically engineered mice. *The Journal of clinical investigation.* 2008;118(3):829-38.

202. Eissing L, Scherer T, Todter K, Knippschild U, Greve JW, Buurman WA, Pinnschmidt HO, Rensen SS, Wolf AM, Bartelt A, Heeren J, Buettner C, and Scheja L. De novo lipogenesis in human fat and liver is linked to ChREBP-beta and metabolic health. *Nature communications.* 2013;4:1528.

203. Kuriyama H, Liang G, Engelking LJ, Horton JD, Goldstein JL, and Brown MS. Compensatory increase in fatty acid synthesis in adipose tissue of mice with conditional deficiency of SCAP in liver. *Cell Metab.* 2005;1(1):41-51.

204. Claycombe KJ, Wang Y, Jones BH, Kim S, Wilkison WO, Zemel MB, Chun J, and Moustaid-Moussa N. Transcriptional regulation of the adipocyte fatty acid synthase gene by agouti: interaction with insulin. *Physiological genomics.* 2000;3(3):157-62.

205. Moustaid N, Jones BH, and Taylor JW. Insulin increases lipogenic enzyme activity in human adipocytes in primary culture. *The Journal of nutrition.* 1996;126(4):865-70.

206. Dentin R, Langin D, and Postic C. Hidden variant of ChREBP in fat links lipogenesis to insulin sensitivity. *Cell Metab.* 2012;15(6):795-7.

207. Herman MA, Peroni OD, Villoria J, Schon MR, Abumrad NA, Bluher M, Klein S, and Kahn BB. A novel ChREBP isoform in adipose tissue regulates systemic glucose metabolism. *Nature.* 2012;484(7394):333-8.

208. Locke GA, Cheng D, Witmer MR, Tamura JK, Haque T, Carney RF, Rendina AR, and Marcinkeviciene J. Differential activation of recombinant human acetyl-CoA carboxylases 1 and 2 by citrate. *Archives of biochemistry and biophysics.* 2008;475(1):72-9.
209. Kersten S. Mechanisms of nutritional and hormonal regulation of lipogenesis. *EMBO Reports.* 2001;2(4):282-6.
210. Ortega FJ, Mayas D, Moreno-Navarrete JM, Catalan V, Gomez-Ambrosi J, Esteve E, Rodriguez-Hermosa JI, Ruiz B, Ricart W, Peral B, Fruhbeck G, Tinahones FJ, and Fernandez-Real JM. The gene expression of the main lipogenic enzymes is downregulated in visceral adipose tissue of obese subjects. *Obesity (Silver Spring, Md).* 2010;18(1):13-20.
211. Ranganathan G, Unal R, Pokrovskaya I, Yao-Borengasser A, Phanavanh B, Lecka-Czernik B, Rasouli N, and Kern PA. The lipogenic enzymes DGAT1, FAS, and LPL in adipose tissue: effects of obesity, insulin resistance, and TZD treatment. *Journal of lipid research.* 2006;47(11):2444-50.
212. Solinas G, Borén J, and Dulloo AG. De novo lipogenesis in metabolic homeostasis: More friend than foe? *Mol Metab.* 2015;4(5):367-77.
213. Yilmaz M, Claiborn KC, and Hotamisligil GS. De Novo Lipogenesis Products and Endogenous Lipokines. *Diabetes.* 2016;65(7):1800-7.
214. Mayas MD, Ortega FJ, Macias-Gonzalez M, Bernal R, Gomez-Huelgas R, Fernandez-Real JM, and Tinahones FJ. Inverse relation between FASN expression in human adipose tissue and the insulin resistance level. *Nutrition & metabolism.* 2010;7:3.
215. Roberts R, Hodson L, Dennis AL, Neville MJ, Humphreys SM, Harnden KE, Micklem KJ, and Frayn KN. Markers of de novo lipogenesis in adipose tissue: associations with small adipocytes and insulin sensitivity in humans. *Diabetologia.* 2009;52(5):882-90.
216. Hoffstedt J, Forster D, and Lofgren P. Impaired subcutaneous adipocyte lipogenesis is associated with systemic insulin resistance and increased apolipoprotein B/AI ratio in men and women. *Journal of internal medicine.* 2007;262(1):131-9.
217. Kursawe R, Eszlinger M, Narayan D, Liu T, Bazuine M, Cali AM, D'Adamo E, Shaw M, Pierpont B, Shulman GI, Cushman SW, Sherman A, and Caprio S. Cellularity and adipogenic profile of the abdominal subcutaneous adipose tissue from obese adolescents: association with insulin resistance and hepatic steatosis. *Diabetes.* 2010;59(9):2288-96.
218. Berndt J, Kovacs P, Ruschke K, Kloting N, Fasshauer M, Schon MR, Korner A, Stumvoll M, and Bluher M. Fatty acid synthase gene expression in human adipose tissue: association with obesity and type 2 diabetes. *Diabetologia.* 2007;50(7):1472-80.
219. Cusi K. Role of obesity and lipotoxicity in the development of nonalcoholic steatohepatitis: pathophysiology and clinical implications. *Gastroenterology.* 2012;142(4):711-25 e6.
220. Phielix E, and Mensink M. Type 2 diabetes mellitus and skeletal muscle metabolic function. *Physiology & behavior.* 2008;94(2):252-8.

221. Kelley DE. Skeletal muscle fat oxidation: timing and flexibility are everything. *The Journal of clinical investigation.* 2005;115(7):1699-702.
222. Patti ME, and Corvera S. The role of mitochondria in the pathogenesis of type 2 diabetes. *Endocrine reviews.* 2010;31(3):364-95.
223. Apostolopoulou M, Strassburger K, Herder C, Knebel B, Kotzka J, Szendroedi J, Roden M, and group GDS. Metabolic flexibility and oxidative capacity independently associate with insulin sensitivity in individuals with newly diagnosed type 2 diabetes. *Diabetologia.* 2016;59(10):2203-7.
224. Shi X, Burkart A, Nicoloro SM, Czech MP, Straubhaar J, and Corvera S. Paradoxical effect of mitochondrial respiratory chain impairment on insulin signaling and glucose transport in adipose cells. *J Biol Chem.* 2008;283(45):30658-67.
225. Wang CH, Wang CC, Huang HC, and Wei YH. Mitochondrial dysfunction leads to impairment of insulin sensitivity and adiponectin secretion in adipocytes. *The FEBS journal.* 2013;280(4):1039-50.
226. Pettersson-Klein AT, Izadi M, Ferreira DMS, Cervenka I, Correia JC, Martinez-Redondo V, Southern M, Cameron M, Kamenecka T, Agudelo LZ, Porsmyr-Palmertz M, Martens U, Lundgren B, Otrocka M, Jenmalm-Jensen A, Griffin PR, and Ruas JL. Small molecule PGC-1α1 protein stabilizers induce adipocyte Ucp1 expression and uncoupled mitochondrial respiration. *Molecular metabolism.* 2018;9:28-42.
227. Karusheva Y, Koessler T, Strassburger K, Markgraf D, Mastrototaro L, Jelenik T, Simon MC, Pesta D, Zaharia OP, Bódis K, Barenz F, Schmoll D, Wolkersdorfer M, Tura A, Pacini G, Burkart V, Mussig K, Szendroedi J, and Roden M. Short-term dietary reduction of branched-chain amino acids reduces meal-induced insulin secretion and modifies microbiome composition in type 2 diabetes: a randomized controlled crossover trial. *The American journal of clinical nutrition.* 2019;110(5):1098-107.
228. Vernochet C, Damilano F, Mourier A, Bezy O, Mori MA, Smyth G, Rosenzweig A, Larsson NG, and Kahn CR. Adipose tissue mitochondrial dysfunction triggers a lipodystrophic syndrome with insulin resistance, hepatosteatosis, and cardiovascular complications. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology.* 2014;28(10):4408-19.
229. Xie X, Yi Z, Sinha S, Madan M, Bowen BP, Langlais P, Ma D, Mandarino L, and Meyer C. Proteomics analyses of subcutaneous adipocytes reveal novel abnormalities in human insulin resistance. *Obesity (Silver Spring, Md).* 2016;24(7):1506-14.
230. Koliaki C, and Roden M. Do mitochondria care about insulin resistance? *Mol Metab.* 2014;3(4):351-3.
231. Perry CGR, Kane DA, Lanza IR, and Neufer PD. Methods for Assessing Mitochondrial Function in Diabetes. *Diabetes.* 2013;62(4):1041-53.

232. Lanza IR, and Nair KS. Mitochondrial metabolic function assessed in vivo and in vitro. *Curr Opin Clin Nutr Metab Care.* 2010;13(5):511-7.
233. Cinti S. Transdifferentiation properties of adipocytes in the adipose organ. *American journal of physiology Endocrinology and metabolism.* 2009;297(5):E977-86.
234. De Pauw A, Tejerina S, Raes M, Keijer J, and Arnould T. Mitochondrial (dys)function in adipocyte (de)differentiation and systemic metabolic alterations. *Am J Pathol.* 2009;175(3):927-39.
235. Kraunsøe R, Boushel R, Hansen CN, Schjerling P, Qvortrup K, Støckel M, Mikines KJ, and Dela F. Mitochondrial respiration in subcutaneous and visceral adipose tissue from patients with morbid obesity. *The Journal of physiology.* 2010;588(Pt 12):2023-32.
236. Xie X, Sinha S, Yi Z, Langlais PR, Madan M, Bowen BP, Willis W, and Meyer C. Role of adipocyte mitochondria in inflammation, lipemia and insulin sensitivity in humans: effects of pioglitazone treatment. *International journal of obesity (2005).* 2017.
237. Bogacka I, Xie H, Bray GA, and Smith SR. Pioglitazone induces mitochondrial biogenesis in human subcutaneous adipose tissue in vivo. *Diabetes.* 2005;54(5):1392-9.
238. Kaaman M, Sparks LM, van Harmelen V, Smith SR, Sjolin E, Dahlman I, and Arner P. Strong association between mitochondrial DNA copy number and lipogenesis in human white adipose tissue. *Diabetologia.* 2007;50(12):2526-33.
239. Wolfe RR, Evans JE, Mullany CJ, and Burke JF. Measurement of plasma free fatty acid turnover and oxidation using [1-13C]palmitic acid. *Biomedical mass spectrometry.* 1980;7(4):168-71.
240. Haring HU, Rinninger F, and Kemmler W. Decreased insulin sensitivity due to a postreceptor defect as a consequence of ATP-deficiency in fat cells. *FEBS letters.* 1981;132(2):235-8.
241. Franckhauser S, Munoz S, Pujol A, Casellas A, Riu E, Otaegui P, Su B, and Bosch F. Increased fatty acid re-esterification by PEPCK overexpression in adipose tissue leads to obesity without insulin resistance. *Diabetes.* 2002;51(3):624-30.
242. Mihaylova MM, and Shaw RJ. The AMP-activated protein kinase (AMPK) signaling pathway coordinates cell growth, autophagy, & metabolism. *Nature cell biology.* 2011;13(9):1016-23.
243. Hardie DG. AMPK: a key regulator of energy balance in the single cell and the whole organism. *International journal of obesity (2005).* 2008;32 Suppl 4:S7-12.
244. Iwata T, Taniguchi H, Kuwajima M, Taniguchi T, Okuda Y, Sukeno A, Ishimoto K, Mizusawa N, and Yoshimoto K. The action of D-dopachrome tautomerase as an adipokine in adipocyte lipid metabolism. *PloS one.* 2012;7(3):e33402.
245. Yin W, Mu J, and Birnbaum MJ. Role of AMP-activated protein kinase in cyclic AMP-dependent lipolysis In 3T3-L1 adipocytes. *J Biol Chem.* 2003;278(44):43074-80.
246. MacPherson RE, Dragos SM, Ramos S, Sutton C, Frendo-Cumbo S, Castellani L, Watt MJ, Perry CG, Mutch DM, and Wright DC. Reduced ATGL-mediated lipolysis attenuates beta-

adrenergic-induced AMPK signaling, but not the induction of PKA-targeted genes, in adipocytes and adipose tissue. *American journal of physiology Cell physiology.* 2016;311(2):C269-76.

247. Koh EH, Park JY, Park HS, Jeon MJ, Ryu JW, Kim M, Kim SY, Kim MS, Kim SW, Park IS, Youn JH, and Lee KU. Essential role of mitochondrial function in adiponectin synthesis in adipocytes. *Diabetes.* 2007;56(12):2973-81.
248. Luo L, and Liu M. Adipose tissue in control of metabolism. *The Journal of endocrinology.* 2016;231(3):R77-r99.
249. Jansson PA, Smith U, and Lonnroth P. Evidence for lactate production by human adipose tissue in vivo. *Diabetologia.* 1990;33(4):253-6.
250. DiGirolamo M, Newby F, and Lovejoy J. Lactate production in adipose tissue: a regulated function with extra-adipose implications. *The FASEB Journal.* 1992;6(7):2405-12.
251. Lovejoy J, Newby F, Gebhart S, and DiGirolamo M. Insulin resistance in obesity is associated with elevated basal lactate levels and diminished lactate appearance following intravenous glucose and insulin. *Metabolism.* 1992;41(1):22-7.
252. Consoli A, Nurjhan N, Reilly J, Bier D, and Gerich J. Mechanism of increased gluconeogenesis in noninsulin-dependent diabetes mellitus. Role of alterations in systemic, hepatic, and muscle lactate and alanine metabolism. *The Journal of clinical investigation.* 1990;86(6):2038-45.
253. Krishnan J, Danzer C, Simka T, Ukropec J, Walter KM, Kumpf S, Mirtschink P, Ukropcova B, Gasperikova D, Pedrazzini T, and Krek W. Dietary obesity-associated Hif1alpha activation in adipocytes restricts fatty acid oxidation and energy expenditure via suppression of the Sirt2-NAD+ system. *Genes & development.* 2012;26(3):259-70.
254. Wilson-Fritch L, Nicoloro S, Chouinard M, Lazar MA, Chui PC, Leszyk J, Straubhaar J, Czech MP, and Corvera S. Mitochondrial remodeling in adipose tissue associated with obesity and treatment with rosiglitazone. *The Journal of clinical investigation.* 2004;114(9):1281-9.
255. Choo HJ, Kim JH, Kwon OB, Lee CS, Mun JY, Han SS, Yoon YS, Yoon G, Choi KM, and Ko YG. Mitochondria are impaired in the adipocytes of type 2 diabetic mice. *Diabetologia.* 2006;49(4):784-91.
256. Cadoudal T, Blouin JM, Collinet M, Fouque F, Tan GD, Loizon E, Beale EG, Frayn KN, Karpe F, Vidal H, Benelli C, and Forest C. Acute and selective regulation of glyceroneogenesis and cytosolic phosphoenolpyruvate carboxykinase in adipose tissue by thiazolidinediones in type 2 diabetes. *Diabetologia.* 2007;50(3):666-75.
257. Chattopadhyay M, GuhaThakurta I, Behera P, Ranjan KR, Khanna M, Mukhopadhyay S, and Chakrabarti S. Mitochondrial bioenergetics is not impaired in nonobese subjects with type 2 diabetes mellitus. *Metabolism - Clinical and Experimental.* 2011;60(12):1702-10.

258. Hansen M, Lund MT, Gregers E, Kraunsoe R, Van Hall G, Helge JW, and Dela F. Adipose tissue mitochondrial respiration and lipolysis before and after a weight loss by diet and RYGB. *Obesity (Silver Spring, Md).* 2015;23(10):2022-9.
259. Apostolopoulou M, Strassburger K, Herder C, Knebel B, Kotzka J, Szendroedi J, and Roden M. Metabolic flexibility and oxidative capacity independently associate with insulin sensitivity in individuals with newly diagnosed type 2 diabetes. *Diabetologia.* 2016;59(10):2203-7.
260. Yang H, Wu JW, Wang SP, Severi I, Sartini L, Frizzell N, Cinti S, Yang G, and Mitchell GA. Adipose-Specific Deficiency of Fumarate Hydratase in Mice Protects Against Obesity, Hepatic Steatosis, and Insulin Resistance. *Diabetes.* 2016;65(11):3396-409.
261. Flint HJ, Bayer EA, Rincon MT, Lamed R, and White BA. Polysaccharide utilization by gut bacteria: potential for new insights from genomic analysis. *Nature reviews Microbiology.* 2008;6(2):121-31.
262. Habib AM, Richards P, Rogers GJ, Reimann F, and Gribble FM. Co-localisation and secretion of glucagon-like peptide 1 and peptide YY from primary cultured human L cells. *Diabetologia.* 2013;56(6):1413-6.
263. Kimura I, Ozawa K, Inoue D, Imamura T, Kimura K, Maeda T, Terasawa K, Kashihara D, Hirano K, Tani T, Takahashi T, Miyauchi S, Shioi G, Inoue H, and Tsujimoto G. The gut microbiota suppresses insulin-mediated fat accumulation via the short-chain fatty acid receptor GPR43. *Nature communications.* 2013;4:1829.
264. Greenbaum CJ, Mandrup-Poulsen T, McGee PF, Battelino T, Haastert B, Ludvigsson J, Pozzilli P, Lachin JM, Kolb H, Type 1 Diabetes Trial Net Research G, and European CPTSG. Mixed-meal tolerance test versus glucagon stimulation test for the assessment of beta-cell function in therapeutic trials in type 1 diabetes. *Diabetes Care.* 2008;31(10):1966-71.
265. Rijkelijkhuizen JM, Girman CJ, Mari A, Alssema M, Rhodes T, Nijpels G, Kostense PJ, Stein PP, Eekhoff EM, Heine RJ, and Dekker JM. Classical and model-based estimates of beta-cell function during a mixed meal vs. an OGTT in a population-based cohort. *Diabetes research and clinical practice.* 2009;83(2):280-8.
266. Francque SM, van der Graaff D, and Kwanten WJ. Non-alcoholic fatty liver disease and cardiovascular risk: Pathophysiological mechanisms and implications. *Journal of hepatology.* 2016;65(2):425-43.
267. Guglielmi C, Del Toro R, Lauria A, Maurizi AR, Fallucca S, Cappelli A, Angeletti S, Lachin JM, and Pozzilli P. Effect of GLP-1 and GIP on C-peptide secretion after glucagon or mixed meal tests: Significance in assessing B-cell function in diabetes. *Diabetes/metabolism research and reviews.* 2017;33(6):10.1002/dmrr.2899.
268. Mari A, Pacini G, Murphy E, Ludvik B, and Nolan JJ. A model-based method for assessing insulin sensitivity from the oral glucose tolerance test. *Diabetes Care.* 2001;24(3):539-48.

269. Pacini G, and Mari A. Methods for clinical assessment of insulin sensitivity and beta-cell function. *Best practice & research Clinical endocrinology & metabolism.* 2003;17(3):305-22.
270. Ichimura A, Hara T, and Hirasawa A. Regulation of Energy Homeostasis via GPR120. *Frontiers in endocrinology.* 2014;5:111.
271. Listenberger LL, Han X, Lewis SE, Cases S, Farese RV, Jr., Ory DS, and Schaffer JE. Triglyceride accumulation protects against fatty acid-induced lipotoxicity. *Proceedings of the National Academy of Sciences of the United States of America.* 2003;100(6):3077-82.
272. Peter A, Weigert C, Staiger H, Rittig K, Cegan A, Lutz P, Machicao F, Haring HU, and Schleicher E. Induction of stearoyl-CoA desaturase protects human arterial endothelial cells against lipotoxicity. *American journal of physiology Endocrinology and metabolism.* 2008;295(2):E339-49.
273. Krssak M, Falk Petersen K, Dresner A, DiPietro L, Vogel SM, Rothman DL, Roden M, and Shulman GI. Intramyocellular lipid concentrations are correlated with insulin sensitivity in humans: a 1H NMR spectroscopy study. *Diabetologia.* 1999;42(1):113-6.
274. Petersen KF, and Shulman GI. Pathogenesis of skeletal muscle insulin resistance in type 2 diabetes mellitus. *The American journal of cardiology.* 2002;90(5A):11G-8G.
275. Hernandez EA, Kahl S, Seelig A, Begovatz P, Irmler M, Kupriyanova Y, Nowotny B, Nowotny P, Herder C, Barosa C, Carvalho F, Rozman J, Neschen S, Jones JG, Beckers J, de Angelis MH, and Roden M. Acute dietary fat intake initiates alterations in energy metabolism and insulin resistance. *The Journal of clinical investigation.* 2017;127(2):695-708.
276. Seo JB, Riopel M, Cabrales P, Huh JY, Bandyopadhyay GK, Andreyev AY, Murphy AN, Beeman SC, Smith GI, Klein S, Lee YS, and Olefsky JM. Knockdown of Ant2 Reduces Adipocyte Hypoxia And Improves Insulin Resistance in Obesity. *Nat Metab.* 2019;1(1):86-97.
277. MacDonald ML, van Eck M, Hildebrand RB, Wong BW, Bissada N, Ruddle P, Kontush A, Hussein H, Pouladi MA, Chapman MJ, Fievet C, van Berkel TJ, Staels B, McManus BM, and Hayden MR. Despite antiatherogenic metabolic characteristics, SCD1-deficient mice have increased inflammation and atherosclerosis. *Arteriosclerosis, thrombosis, and vascular biology.* 2009;29(3):341-7.
278. Hyun CK, Kim ED, Flowers MT, Liu X, Kim E, Strable M, and Ntambi JM. Adipose-specific deletion of stearoyl-CoA desaturase 1 up-regulates the glucose transporter GLUT1 in adipose tissue. *Biochemical and biophysical research communications.* 2010;399(4):480-6.
279. Peter A, Weigert C, Staiger H, Machicao F, Schick F, Machann J, Stefan N, Thamer C, Haring HU, and Schleicher E. Individual stearoyl-coa desaturase 1 expression modulates endoplasmic reticulum stress and inflammation in human myotubes and is associated with skeletal muscle lipid storage and insulin sensitivity in vivo. *Diabetes.* 2009;58(8):1757-65.

280. Flowers JB, Rabaglia ME, Schueler KL, Flowers MT, Lan H, Keller MP, Ntambi JM, and Attie AD. Loss of stearoyl-CoA desaturase-1 improves insulin sensitivity in lean mice but worsens diabetes in leptin-deficient obese mice. *Diabetes.* 2007;56(5):1228-39.
281. Marinou K, Hodson L, Vasan SK, Fielding BA, Banerjee R, Brismar K, Koutsilieris M, Clark A, Neville MJ, and Karpe F. Structural and functional properties of deep abdominal subcutaneous adipose tissue explain its association with insulin resistance and cardiovascular risk in men. *Diabetes care.* 2014;37(3):821-9.
282. Kim SH, Chung JH, Song SW, Jung WS, Lee YA, and Kim HN. Relationship between deep subcutaneous abdominal adipose tissue and metabolic syndrome: a case control study. *Diabetology & metabolic syndrome.* 2016;8:10.
283. Henninger AM, Eliasson B, Jenndahl LE, and Hammarstedt A. Adipocyte hypertrophy, inflammation and fibrosis characterize subcutaneous adipose tissue of healthy, non-obese subjects predisposed to type 2 diabetes. *PloS one.* 2014;9(8):e105262.
284. Skarn SN, Eggesbo HB, Flaa A, Kjeldsen SE, Rostrup M, Brunborg C, Reims HM, and Aksnes TA. Predictors of abdominal adipose tissue compartments: 18-year follow-up of young men with and without family history of diabetes. *European journal of internal medicine.* 2016;29:26-31.
285. Boudina S, and Graham TE. Mitochondrial function/dysfunction in white adipose tissue. *Exp Physiol.* 2014;99(9):1168-78.
286. Szendroedi J, Phielix E, and Roden M. The role of mitochondria in insulin resistance and type 2 diabetes mellitus. *Nat Rev Endocrinol.* 2011;8(2):92-103.
287. Koliaki C, and Roden M. Alterations of Mitochondrial Function and Insulin Sensitivity in Human Obesity and Diabetes Mellitus. *Annual review of nutrition.* 2016;36:337-67.
288. Heinonen S, Buzkova J, Muniandy M, Kaksonen R, Ollikainen M, Ismail K, Hakkarainen A, Lundbom J, Lundbom N, Vuolteenaho K, Moilanen E, Kaprio J, Rissanen A, Suomalainen A, and Pietilainen KH. Impaired Mitochondrial Biogenesis in Adipose Tissue in Acquired Obesity. *Diabetes.* 2015;64(9):3135-45.
289. An YA, Crewe C, Asterholm IW, Sun K, Chen S, Zhang F, Shao M, Funcke JB, Zhang Z, Straub L, Klein S, Kusminski CM, and Scherer PE. Dysregulation of Amyloid Precursor Protein Impairs Adipose Tissue Mitochondrial Function and Promotes Obesity. *Nat Metab.* 2019;1(12):1243-57.
290. Phielix E, and Roden M. Assessing multiple features of mitochondrial function. *Diabetes.* 2013;62(6):1826-8.
291. Brand MD, and Nicholls DG. Assessing mitochondrial dysfunction in cells. *The Biochemical journal.* 2011;435(2):297-312.
292. Patel P, and Abate N. Body fat distribution and insulin resistance. *Nutrients.* 2013;5(6):2019-27.

293. Patti ME, Butte AJ, Crunkhorn S, Cusi K, Berria R, Kashyap S, Miyazaki Y, Kohane I, Costello M, Saccone R, Landaker EJ, Goldfine AB, Mun E, DeFronzo R, Finlayson J, Kahn CR, and Mandarino LJ. Coordinated reduction of genes of oxidative metabolism in humans with insulin resistance and diabetes: Potential role of PGC1 and NRF1. *Proceedings of the National Academy of Sciences of the United States of America.* 2003;100(14):8466-71.

294. Cusi K. Role of insulin resistance and lipotoxicity in non-alcoholic steatohepatitis. *Clinics in liver disease.* 2009;13(4):545-63.

295. Koliaki C, Szendroedi J, Kaul K, Jelenik T, Nowotny P, Jankowiak F, Herder C, Carstensen M, Krausch M, Knoefel WT, Schlensak M, and Roden M. Adaptation of hepatic mitochondrial function in humans with non-alcoholic fatty liver is lost in steatohepatitis. *Cell Metab.* 2015;21(5):739-46.

296. Stumvoll M, Goldstein BJ, and van Haeften TW. Type 2 diabetes: principles of pathogenesis and therapy. *Lancet (London, England).* 2005;365(9467):1333-46.

297. Wessels B, Honecker J, Schottl T, Stecher L, Klingenspor M, Hauner H, and Skurk T. Adipose Mitochondrial Respiratory Capacity in Obesity is Impaired Independently of Glycemic Status of Tissue Donors. *Obesity (Silver Spring, Md).* 2019;27(5):756-66.

8. Acknowledgements

First and above all, I would like to thank my supervisor and mentor, Prof. Dr. Michael Roden, for giving me the opportunity to design the studies, to research the data and to write the manuscripts and thesis. I am extremely thankful to him for exceptional guidance, patience, enthusiasm, trusting me with scientific projects, giving me the opportunity to implement my ideas and for sharing your knowledge. This thesis would not have been possible without his continuous support, supervision, valuable input, encouragement and excellence.

My special gratitude also goes to PD Dr. Julia Szendrödi PhD, PD Dr. Volker Burkart and Prof. Dr. Karsten Müssig, for their continuous support, guidance, valuable input and great knowledge helping me to stay focused, structured and coordinated.

I would also like to express my deepest gratitude to Dr. Tomas Jelenik, Dr. Jesper Lundbom and Dr. Daniel Markgraf for their encouragement and great knowledge as well as the enthusiastic constructive discussions helping me to perform these studies.

I thank all group members and staff of the CRC for their help, team work, professionalism and for creating an excellent atmosphere for doing research. I would also like to acknowledge Dr. Klaus Strassburger and Dr. Pavel Bobrov for their help with statistical analyses. I also thank Prof. Dr. Annette Schürmann, Prof. Dr. Dan Ziegler, Prof. Dr. Hadi Al-Hasani, Dr. Alexander Strom, Dr. Meriem Ouni, Dr. Yuliya Kupriyanova, Dr. Alexandra Chadt, Dr. Henrike Sell, Dr. Birgit Knebel and Dr. Marie-Christine Simon for sharing their great knowledge and for enthusiastic and constructive discussions.

My special thanks go to my colleagues, Dr. Oana-Patricia Zaharia and Yanislava Karusheva, for supporting me in every way. I am thankful for all the beautiful memories we shared during our time working together.

Special thanks go to all volunteers for participating in the German Diabetes Study and for contributing to the progress in diabetes research. As the German Diabetes Study is a nation-wide mission, I would like to extend my gratitude to all centers across Germany involved in the conduct of this study and to the German Center for Diabetes Research (DZD) for their efforts.

Most importantly, I would like to thank my beloved family, Péter Jozsef and Edit Bódis as well as Katharina Emese Stommel and Susanne Julia Tolksdorf, who have been supporting me throughout writing this thesis and my life in general and for their unconditional love, inspiration, support and the continuously motivating words. This thesis would not have been possible without them.