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Low Physical Fitness and Low Thyroid Function as Cardiovascular and Metabolic Risk Factors in Diabetes Mellitus

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Zusammenfassung

Diabetes mellitus ist eine heterogene Erkrankung, bei der sich betroffene Personen hinsichtlich des Krankheitsverlaufs und des Risikos für die Entwicklung von Komplikationen unterscheiden. Kardiovaskuläre Erkrankungen und die metabolische Dysfunktion-assoziierte steatotische Lebererkrankung (MASLD) stellen dabei zwei der schwerwiegendsten Folgen des Diabetes mellitus dar. Eine Neueinteilung in Diabetes-Endotypen könnte daher zu präziseren Therapieansätzen und maßgeschneiderten Behandlungspläne führen.

Bereits bei gesunden Personen ist eine geringe körperliche Aktivität sowie mangelnde körperliche Fitness mit einem erhöhten kardiovaskulären Risiko assoziiert. Inwieweit sich die neuen Diabetes-Endotypen hinsichtlich ihrer körperlichen Aktivität und Fitness sowie ihres kardiovaskulären Risikos unterscheiden, ist jedoch fraglich. Auch unabhängig von der körperlichen Fitness, spielen reduzierte mitochondriale Funktion und dysfunktionales Fettgewebe eine entscheidende Rolle bei der Entwicklung und Progression von Diabetes und MASLD. Bislang ist die Bedeutung der oxidativen Kapazität des viszeralen (VAT) und subkutanen (SAT) Fettgewebes für die Entstehung und Progression von MASLD allerdings ungeklärt. Da Schilddrüsenhormone die mitochondriale Funktion regulieren, stellen auch Personen mit Hypothyreose eine Risikogruppe für Diabetes und MASLD dar. Der Zusammenhang zwischen geringerer Schilddrüsenfunktion und MASLD bei Personen mit Diabetes ist aber ebenso noch weitgehend unklar.

Die Ziele dieser Dissertation waren daher zu untersuchen, (i) ob sich die neuen Diabetes-Endotypen hinsichtlich ihrer körperlichen Fitness (bestimmt mittels Spiroergometrie) und des kardiovaskulären Risikos (bestimmt mittels etablierter Risiko-Scores) unterscheiden, (ii) ob sich die mitochondriale Atmung (bestimmt mittels hochauflösender Respirometrie) des VAT und des SAT von adipösen Menschen ohne und mit MASLD unterscheidet und (iii) ob ein erhöhtes Steatose-Risiko (bestimmt durch den Fatty Liver Index) bei Personen mit kürzlich diagnostizierten Diabetes mit der niedrig-normaler Schilddrüsenfunktion (bestimmt durch freies Thyroxin und Thyreotropin) assoziiert ist.

Die vorliegenden Untersuchungen zeigen, (i) dass Personen mit schwerem insulinresistenten Diabetes-Endotyp (SIRD) die geringste körperliche Fitness, aber das größte kardiovaskuläre Risiko haben, (ii) dass Personen mit Adipositas und progredienter MASLD eine verminderte oxidative Kapazität des VAT, nicht aber des SAT aufweisen und (iii) dass insbesondere bei Männern mit Typ 2 Diabetes eine niedrig-normale Schilddrüsenfunktion mit einem erhöhten Risiko für Leber-Steatose und verminderter Insulinsensitivität assoziiert ist.

Zusammenfassend, sollen diese Studien dazu beitragen, differenziertere Therapieansätze und Präventionsstrategien bei Diabetes mellitus und seinen Begleiterkrankungen zu entwickeln.

Summary

Diabetes mellitus is a heterogeneous disease and people with diabetes mellitus differ in terms of disease progression and the risk of complications. Cardiovascular diseases (CVD) and metabolic dysfunction-associated steatotic liver disease (MASLD) are two of the most severe complications of diabetes mellitus. A reclassification into diabetes endotypes could therefore lead to more precise therapy approaches and tailored treatment. Even in healthy individuals, low physical activity and physical fitness are associated with an increased CVD risk. However, it is unclear to what extent the new diabetes endotypes differ in terms of their physical activity, fitness, and CVD risk. Regardless of physical fitness, reduced mitochondrial function and adipose tissue dysfunction play a crucial role in the development and progression of diabetes and MASLD. The importance of the oxidative capacity of visceral (VAT) and subcutaneous (SAT) adipose tissue for the onset and progression of MASLD is still unclear. Since thyroid hormones regulate mitochondrial function, individuals with hypothyroidism are also at risk for diabetes and MASLD. However, the relationship between lower thyroid function and MASLD in people with diabetes is still largely unknown. The aims of this thesis were to assess (i) whether the new diabetes endotypes differ in terms of physical fitness (determined by spiroergometry) and CVD risk (determined by established risk scores), (ii) whether the mitochondrial respiration (determined by high-resolution respirometry) of VAT and SAT differs in obese individuals with and without MASLD, and (iii) whether an increased steatosis risk (determined by the fatty liver index) is associated with low-normal thyroid function (determined by free thyroxine and thyroid-stimulating hormone) in individuals with recent-onset diabetes. These studies show (i) that individuals with severe insulin-resistant diabetes endotype (SIRD) have the lowest physical fitness but the greatest cardiovascular risk, (ii) that individuals with obesity and progressive MASLD have reduced oxidative capacity of VAT but not SAT, and (iii) that low-normal thyroid function is associated with an increased risk of liver steatosis and reduced insulin sensitivity especially in men with type 2 diabetes.

Taken together, these studies are intended to contribute to the development of more differentiated therapy approaches and prevention strategies for diabetes mellitus and its comorbidities.

List of abbreviations

^{1}H-MRS - ^{1}H-Magnetic Resonance Spectroscopy

1-RM - One-Repetition Maximum

AMPK – 5´-AMP-Activated Protein Kinase

ASCVD - Atherosclerotic Cardiovascular Disease

ATP - Adenosine Triphosphate

BAT - Brown Adipose Tissue

BFRT- Blood Flow Restriction Training

BMI- Body Mass Index

CHD - Coronary Heart Disease

CRF - Cardiorespiratory Fitness

CS - Citrate Synthase

CVD - Cardiovascular Disease

DAG - Diacylglycerol

FA-CoA - Fatty Acyl-CoA.

FLI - Fatty Liver Index

FIB-4 - Fibrosis Index 4

FMD - Flow-Mediated Dilatation

FOXO1 - Forkhead box protein O1

FOXO3a - Forkhead box protein 3a

FRS - Framingham Risk Scores

fT3 - Free Triiodothyronine

fT4 - Free Thyroxine

FXR- Farnesoid X Receptor

GAD - Glutamic Acid Decarboxylase

GDS - German Diabetes Study

GLUT4 - Glucose Transporter 4

GSK3 - Glycogen Synthase Kinase 3

HbA1c - Glycated Hemoglobin

HCL - Hepatocellular Lipid Content

HDL- High-Density Lipoprotein

HK - Hexokinase

HOMA-B - Homeostasis Model Assessment of Beta-cell Function

HOMA-IR - Homeostasis Model Assessment of Insulin Resistance

IA-2 - Islet Antigen 2

IMCL - Intramyocellular Lipid Content

MARD - Mild Age-Related Diabetes

MASH - Metabolic Dysfunction-Associated Steatohepatitis

MASLD - Metabolic Dysfunction-Associated Steatotic Liver Disease

MHO - Metabolically Healthy Obesity

MR - Magnetic Resonance

MRI - Magnetic Resonance Imaging

MRI-PDFF - Magnetic Resonance Imaging estimated Proton Density Fat Fraction

MOD - Mild Obesity-Related Diabetes

MUO - Metabolically Unhealthy Obesity

NADH - Nicotinamide Adenine Dinucleotide

NAFLD - Nonalcoholic Fatty Liver Disease

NDUFB6 - NADH Dehydrogenase-1ß Subcomplex Subunit 6

NEFA - Non-Esterified Fatty Acids

NMD - Nitroglycerin-Mediated Dilatation

PKC - Protein Kinase C

PNPLA3 - Phospholipase Domain Containing 3

ROC - Receiver Operating Characteristic

ROS - Reactive Oxygen Species

SAID - Severe Autoimmune Diabetes

SAT- Subcutaneous Adipose Tissue

SCORE - European Society of Cardiology's Systematic Coronary Risk Evaluation

SIDD - Severe Insulin-Deficient Diabetes

SIRD - Severe Insulin-Resistant Diabetes

SNP - Single Nucleotide Polymorphism

T1DM - Type 1 Diabetes Mellitus

T2DM - Type 2 Diabetes Mellitus

TGR5 - Takeda G Protein-coupled Membrane Receptor 5

TM6SF2 - Transmembrane 6 Superfamily Member 2

TSH - Thyroid-Stimulating Hormone

VAT- Visceral Adipose Tissue

VLDL - Very-Low-Density Lipoprotein

VO_{2max} - Maximal Oxygen Consumption

VT1 - First Ventilator Threshold

WAT - White Adipose Tissue

WHO - World Health Organization

WHR - Waist-to-Hip Ratio

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1 Introduction

1.1 Diabetes Mellitus

The global prevalence of diabetes mellitus is steadily increasing. While it has nearly doubled between 1980 and 2014 (1), a recent estimation predicts a further increase of more than 50% between 2017 and 2045, leading to around 693 million people with diabetes mellitus (2). In 2019, diabetes mellitus likely contributed to 4.2 million deaths in adults between 20-79 years (3) and healthcare costs of about 760 billion US$ (4). The rising diabetes prevalence suggests that current therapeutic strategies are insufficient and points to the need for identification of more precise risk factors and/or biomarkers and for further elucidation of their role in the development of diabetes-related complications, in order to develop better targeted and cost-effective treatment strategies.

In general, diabetes mellitus is characterized by chronic hyperglycemia (5). The common diabetes classifications comprise four categories: type 1 diabetes mellitus (T1DM), type 2 diabetes mellitus (T2DM), other forms of diabetes due to specific causes and gestational diabetes (5). However, both T1DM and T2DM are heterogeneous diseases with variable clinical presentation, disease progression and diabetes-related complications. Therefore, recent studies proposed novel diabetes sub- or endotypes, which might facilitate a more precise diagnosis, prevention and tailored therapy (6).

1.1.1 Type 1 Diabetes

T1DM accounts for approximately 5–10% of all diabetes cases with a global prevalence of 5.9 per 10,000 people (7), the risk and rate of progression of T1DM being higher in Caucasian individuals compared with other ethnicities (8). Its pathogenesis is primarily based on insulin deficiency due to autoimmune destruction of the insulin-producing beta-cells, usually resulting in dependence on exogenous insulin (5; 7). In line, the presence of one or more islet cell-directed autoantibodies such as autoantibodies to glutamic acid decarboxylase (GADA), insulin, the tyrosine phosphatases islet antigen 2 (IA-2) and IA-2β, and zinc transporter, indicates autoimmune activity in T1DM. However, their absence does not necessarily exclude T1DM, as antibodies exhibit different time courses of appearance and disappearance (7; 9). T1DM typically occurs in young people (<35 years) with low body mass index

(BMI) (<25 kg/m^2), who often present symptoms like polyuria/polydipsia, diabetic ketoacidosis and weight loss (5). But T1DM can also manifest in adults, as the rate of beta-cell destruction is quite variable and the typical symptoms observed in children maybe missing in the presence of residual beta-cell function (5). In this context, slowly progressive autoimmune diabetes without requiring insulin therapy during the first 6–12 months after diagnosis onset in people aged ≥30 years, was formerly defined as latent autoimmune diabetes in adults (10), but it is doubtful whether this phenotype qualifies as independent subtype of T1DM (11; 12). Finally, people with T1DM are also prone to and can coexist with other autoimmune disorders such as autoimmune thyroid disease (M. Hashimoto), adrenal insufficiency (M. Addison), pernicious anemia and celiac disease being among others the most common (13).

1.1.2 Type 2 Diabetes

T2DM accounts for about 90% of all cases of diabetes mellitus, and its prevalence rose dramatically in countries of all income levels in the past three decades. As 462 million individuals were estimated to be affected by T2DM in 2017 (14), T2DM has become a major concern in healthcare worldwide (1; 5).

Among others, T2DM is linked to obesity, unhealthy lifestyle (i.e. hypercaloric nutrition and physical inactivity), socioeconomic and psychosocial conditions, smoking, sleep deprivation, environmental pollution, but also has a multigenic background (5; 15). T2DM is a heterogeneous disease defined by relative insulin deficiency by pancreatic islet beta-cells in the context of peripheral impaired insulin sensitivity, termed insulin resistance (5). There are several factors related to insulin resistance including genetic variations, lipotoxicity, abnormal mitochondrial function with oxidative stress, endoplasmic reticulum stress and low-grade inflammation (16). In most cases, insulin sensitivity declines years or decades before diabetes diagnosis, which is reflected by an impaired insulin-stimulated storage of ingested carbohydrate as skeletal muscle glycogen leading to a reduced non-oxidative glucose metabolism (17). During the early stages of disease, increased insulin secretion from pancreatic beta-cells compensated for the ambient insulin resistance resulting in hyperinsulinemia.

As positive energy balance can lead to insulin resistance, it is not surprising that most individuals with T2DM are overweight or obese (5; 15). Therefore, weight loss by intensive diet or bariatric surgery and increased physical activity are important treatment strategies to combat insulin resistance and T2DM (18).

Interestingly, despite the classification into T1DM and T2DM, both diseases exhibit a broad spectrum of insulin resistance and beta-cell dysfunction insulin secretion, indicating the presence of distinct endotypes of diabetes (6) and suggesting that advanced classification is highly needed **(Figure 1)**.

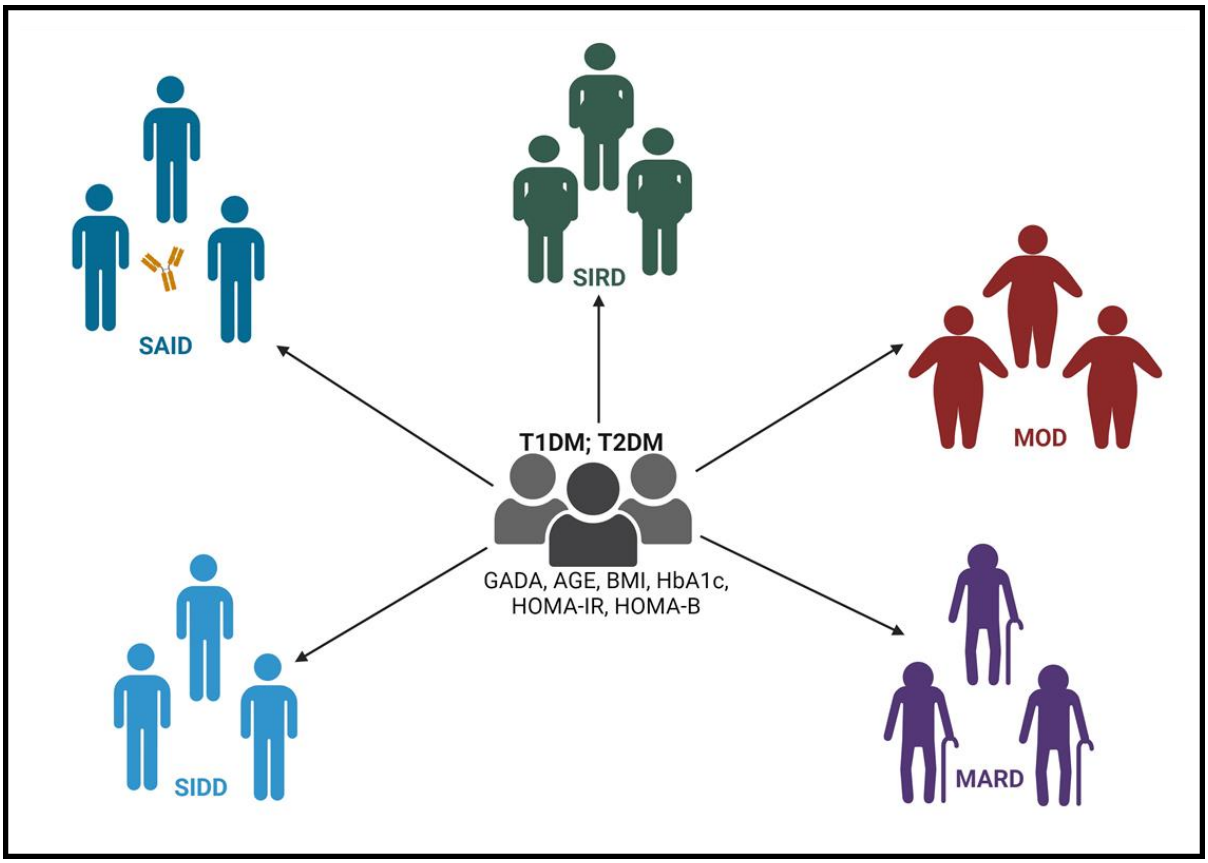

Figure 1. Heterogeneity of diabetes mellitus. Reclassification based on simple clinical parameters could help to determine an individual's absolute complication risk. Abbreviation: BMI, body mass index; GADA, autoantibodies to glutamic acid decarboxylase; HbA1c, glycated hemoglobin; HOMA-B, homeostasis model assessment of beta-cell function; HOMA-IR, homeostasis model assessment of insulin resistance, MARD, mild age-related diabetes; MOD, mild obesity-related diabetes; SAID, severe autoimmune diabetes; SIDD, severe insulin-deficient diabetes; SIRD, severe insulin-resistant diabetes; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus. Created with BioRender©.

1.1.3 Diabetes Endotypes

Recently, a Swedish cohort study challenged the existing paradigm of diabetes classification by reassessing adult-onset diabetes in five endotypes of disease that have different physiological profiles, rather than the traditional type 1 and 2 classification (19). This new classification should facilitate the development of stratified

prevention and treatment for individuals with diabetes, considering different risk profiles. This could further delay the onset of diabetes-related complications, reducing morbidity and mortality (6). The parameters used for the data-driven clustering approach based on autoimmunity, age at diagnosis, BMI, glycemic control and homeostasis model assessment of beta-cell function and insulin resistance (HOMA-B, -IR) (19; 20). Among others (6), this clustering approach has been validated by comprehensive phenotyping of 1105 humans with newly diagnosed diabetes within the German Diabetes Study (GDS) (20). Out of these five endotypes, one endotype (severe autoimmune diabetes (SAID)) overlaps with T1DM by the presence of GADA, early-onset disease, lower BMI, poor metabolic control and insulin deficiency, while the others (severe insulin-deficient diabetes (SIDD), severe insulin-resistant diabetes (SIRD), mild obesity-related diabetes (MOD), mild age-related diabetes (MARD) reflect the classical T2DM (19; 20). The SIDD endotype has the highest prevalence of retinopathy, distal sensorimotor polyneuropathy and cardiovascular autonomic neuropathy and shows similarities with individuals with SAID, but GADA are negative. Specially, the SIRD endotype, which includes people with the greatest degree of insulin resistance and high BMI, is associated with higher prevalence of metabolic dysfunction-associated steatotic liver disease (MASLD) (20) and diabetes-associated kidney disease (19), both of which are further related to increased risk of cardiovascular events (15; 21). Similar to SIRD, individuals with MOD are characterised by obesity but with a lower degree of insulin resistance and intermediate prevalence and risk of diabetes-related complications, while people with MARD are generally older than those other endotype and are at high risk for coronary events and stroke (6; 19; 20) **(Figure 2)**.

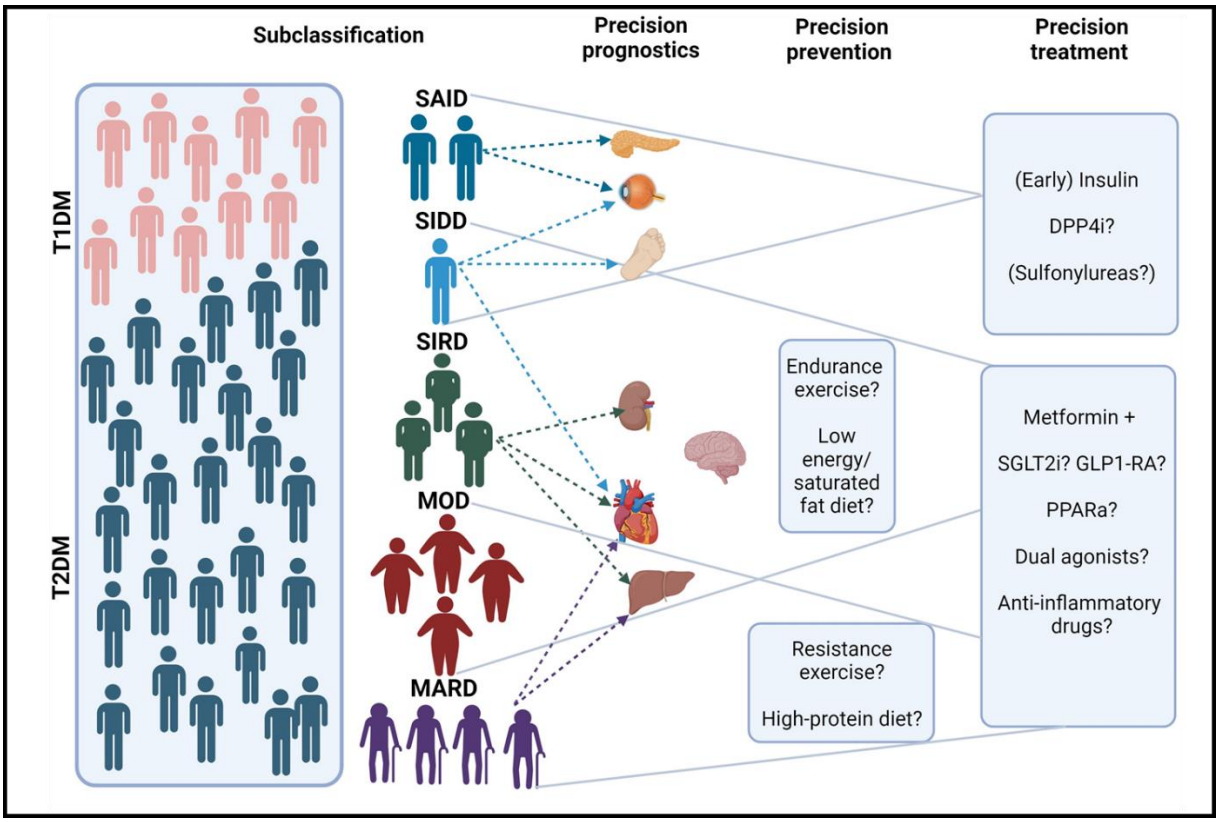

Figure 2. New subclassification of diabetes. Specific diabetes endotypes (clusters) exhibit distinct metabolic alterations and different risk patterns for the development of diabetes-related comorbidities and complications. The severe autoimmune diabetes (SAID) and severe insulin-deficient diabetes (SIDD) endotypes feature a high risk for retinopathy, while SIDD is also associated with distal sensorimotor polyneuropathy and cardiovascular autonomic neuropathy. Individuals with severe insulin-resistant diabetes (SIRD) are at high risk for metabolic dysfunction-associated steatotic liver disease and chronic kidney disease. People with mild obesity-related diabetes (MOD) show intermediate prevalence and risk of diabetes-related complications, while mild age-related diabetes (MARD is related to coronary events and stroke. This reclassification calls for precision prevention and treatment. Abbreviation: T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus. Adapted from Herder & Roden. Diabetologia. 2022 (6). Created with BioRender©.

Of note, adipose tissue dysfunction, insulin resistance and subsequent increases in glycemia, are important factors not only for the development of T2DM, but also for worsening cardiovascular risk (15). Furthermore, physical inactivity promotes insulin resistance and increases the risk of diabetes as well as cardiovascular diseases (22; 23). While distinct metabolic alterations and different risk patterns for the development of diabetes-related comorbidities and complications among the diabetes endotypes are already known, there is limited information about differences in physical activity

behavior, physical fitness, and cardiovascular risk in these endotypes.

1.2 Diabetes and Obesity

1.2.1 Definition of Obesity

Obesity is defined as a chronic disease characterized by excessive fat accumulation that poses a risk to health, resulting from a positive energy balance. The BMI - which is based on the ratio of weight in kilograms and the square of height in meters - is commonly used to determine the prevalence of overweight and obesity in adults (24). According to the definition of the World Health Organization (WHO), adults with a BMI ≥25 kg/m^2 are overweight, while a BMI ≥30 kg/m^2 indicates obesity (24). Although BMI is the most widely used measure to screen obesity, clinical limitations of BMI should be considered. Of note, BMI fails to distinguish between lean body mass and fat mass and more importantly, BMI is only modestly associated visceral adiposity (25; 26). Consequently, BMI overestimates obesity in people with high lean body mass due to increased muscle mass (e.g. athletes) and underestimates obesity in individual with high amounts of body fat but low lean body mass. Further, BMI differs across gender, ethnicities and age (25). Therefore, other measures like waist circumference and/or waist-to-hip ratio (WHR) have been suggested to identify people with obesity (27). Specifically, waist circumference might provide additional information on BMI, enhancing the prediction of morbidity and mortality (28).

1.2.2 Adipose Tissue Distribution

Adipose tissue is classically divided by morphology and function into white (WAT), brown (BAT), or brite/beige adipose tissue, whereby each fat depot has different biological function (29). In general, WAT is essential for the energy homeostasis, i.e. lipid storage and mobilization, while BAT is a thermogenic tissue responsible for heat production. In line, cold exposure, like submerged in ice water, has been shown to activate the BAT to generate heat, helping to maintain body temperature (30) . This process has gained interest in the context of metabolic health and weight regulation, as the activation of BAT can contribute to increased calorie expenditure (31). Histologically, BAT is characterized by adipocytes with multilocular lipid droplets and high mitochondrial density and is typically located in cervical, supraclavicular, axillary, paraspinal, mediastinal, and abdominal regions (32; 33). In addition, beige adipocytes

has been described as white adipocytes, which can be transformed to brown-like adipocytes (browning) under certain conditions like exercise or cold exposure. However, the origin of beige adipose tissue is still controversial as it is not observed in normal non-cooled humans (34).

Of note, almost 95% of the total adipose tissue is WAT, which can further be classified into subcutaneous (SAT) and visceral adipose tissue (VAT). As inferred from the name, SAT is located under the skin, mainly in abdominal and gluteofemoral depots, while VAT is located intra-abdominally (i.e. around internal organs) in omental and mesenteric depots (29; 35). Overall, SAT accounts for 80% to 90% of total body fat mass in lean, healthy individuals and represents a physiological buffer by storing excess energy, mainly in form triglycerides, during times of limited energy expenditure (33; 35). In context of caloric excess, adipose tissue expands either by increasing size of existing adipocytes (hypertrophy) or through the generation of new adipocytes due to the differentiation of resident precursor cells resulting in an increased number of adipocytes (hyperplasia) (36). However, the storage capacity of the SAT is limited and once exceeded, lipids start to accumulate within cells of non-adipose tissue (ectopic fat) such as the skeletal muscle, liver, pancreatic beta-cells, and visceral fat depots. (33). These conditions are termed as metabolically unhealthy obesity (MUO) as they promote systemic inflammation, insulin resistance and further increase the risk of T2DM (33). Although, the concept of MUO is still doubtful, the distribution of adipose tissue is a pivotal determinant of metabolic health associated with obesity than increased fat mass itself. There is, however, a paucity of data regarding differences between SAT and VAT in people with obesity. Interestingly, mitochondria respiration, which has an important role in regulating lipid homeostasis, seems to be higher in VAT compared to SAT in obese individuals (37). Conversely, SAT might exhibit greater activity in processes associated with vesicular transport and secretion, as well as increased lipid metabolism in obese individuals (38).

1.2.3 Obesity-Related Insulin Resistance

More than 80 % of individuals with obesity develop insulin resistance and have an increased risk of T2DM rises progressively with increasing BMI (39-41). This is even more alarming considering the frighteningly increasing rate of obesity worldwide caused by environmental factors (i.e. sedentary lifestyle, climate change, and inflation), aging and Western diet (24; 42).

There are numerous mechanisms by which the progression of obesity promotes systemic insulin resistance. In general, the reduced expandability of SAT and the consequent re-distribution of lipids to the VAT are associated with insulin resistance (43). In line, VAT adipocyte size seems to be positively correlated with insulin levels and the degree of insulin resistance (44).

First, hypertrophied adipocytes become dysfunctional and recruit monocytes - which then differentiate into macrophages - and various other immune cells. Moreover, M1-macrophages in dysfunctional adipocytes start to release cytokines, thereby promoting a pro-inflammatory state. Chronically, adipose dysfunction leads to imbalance of circulating inflammatory and anti-inflammatory adipokines, which is defined as low-grade inflammation. Second, dysfunctional adipose tissue is associated with elevated lipolysis, leading to increased flux of non-esterified fatty acids (NEFA) and glycerol to the liver, where both serve as substrates for triglyceride synthesis and contribute to hepatic triglyceride accumulation (hepatic steatosis). Of note, VAT can account up to 50% of the NEFA supplied to the liver (45) and therefore, dysfunction of the VAT has been suggested to be the major driver of MASLD (46). As mentioned earlier, mitochondria play a crucial role in fatty acid oxidation and energy production. Consequently, reduced hepatic mitochondrial function in the insulin-resistant state leads to an imbalance in lipid metabolism, which further promotes hepatic lipid accumulation (15). Of note, high amounts of ectopic lipids favour the production of toxic lipids such as ceramides and diacylglycerides (DAG) (42), which then interfere with the hepatocellular signaling resulting in increased risk of T2DM. Third, the increased delivery of NEFA and glycerol from dysfunctional adipose tissue to the liver stimulates hepatic gluconeogenesis (15; 47; 48). Fourth, increased transport of NEFA to skeletal muscle, together with decreased mitochondrial beta-oxidation, favours lipogenesis and elevation in intramyocellular lipid content (IMCL). Fifth, skeletal muscle insulin resistance leads to the diversion of ingested glucose from the skeletal muscle to the liver. Paired with compensatory hyperinsulinemia, this again favours hepatic *de novo* lipogenesis and triglyceride synthesis **(Figure 3)** (15; 49).

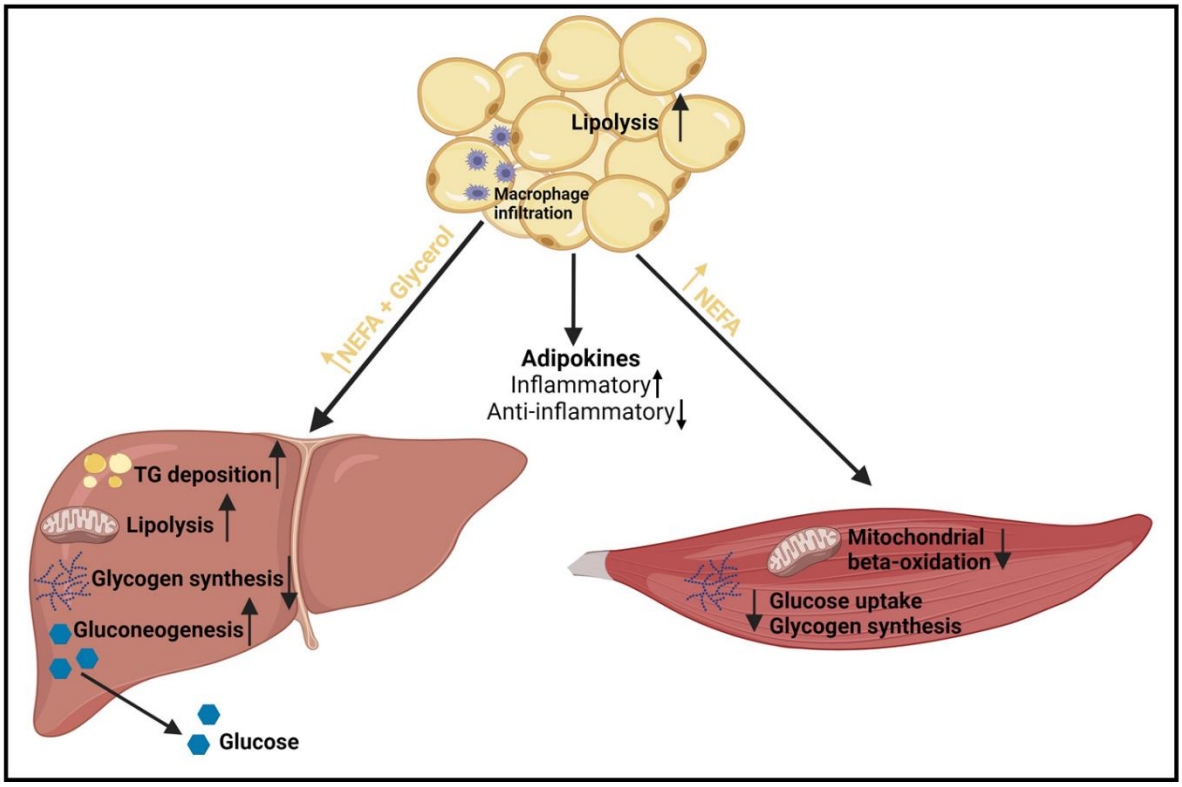

Figure 3. Development of insulin resistance during obesity. Elevated lipolysis in adipose tissue results in increased flux of non-esterified fatty acids (NEFA) and glycerol to the liver, contributing to triglyceride (TG) accumulation and increased lipolysis and gluconeogenesis. Further, lipid-induced hepatic insulin resistance results in lowering insulin-stimulated glycogen storage Simultaneously, NEFA are transported to skeletal muscle. Elevated skeletal muscle NEFA, combined with reduced mitochondrial beta-oxidation, promotes lipogenesis and increases intramyocellular lipid content. Ectopic fat-induced lipotoxic signaling reduces insulin-mediated glucose uptake and glycogen synthesis, causing skeletal muscle insulin resistance. Coupled with sedentary behaviour-associated reduction in non-insulin-stimulated glucose transport, postprandial plasma glucose levels increase. Chronically, adipose dysfunction leads to altered adipokine secretion and results in systemic low-grade inflammation. Created with BioRender©.

Finally, chronic excess availability of NEFA leads to decreased function of organelles like the endoplasmic reticulum, lysosomes and mitochondria and result in an excess release of reactive oxygen species (ROS) (50), pro-inflammatory cytokines and adipokines, thereby generating systemic low-grade inflammation. Whether reduced VAT mitochondrial function itself is also coupled to lower adipose tissue insulin sensitivity remains, unknown. Nevertheless, prolonged systemic inflammation impairs

insulin action and signaling, while enhanced ROS production induces lipid peroxidation in adipocytes, liver, and skeletal muscles (42).

Taken together, adipose tissue insulin resistance results in an increased lipolysis with elevated NEFA flux to the liver and skeletal muscle, which favours ectopic fat accumulation along with hepatic and myocellular insulin resistance.

While the described mechanisms of how obesity leads to the progression of T2DM are generally accepted, there is evidence suggesting that this hypothesis may not hold true for all individuals with T2DM. This is reflected by the variation among the diabetes endotypes, which encompass differences in HOMA scores, NEFA and triglyceride levels (19; 20).

1.2.4 Role of Physical Fitness

The terms "physical activity," "exercise," "physical fitness," and “cardiorespiratory fitness (CRF)” are frequently used synonymously, albeit they describe different concepts. **Table 1** briefly summarizes the precise definition of these terms.

Table 1. Definition of physical activity, exercise, physical fitness and cardiorespiratory fitness

Term	Definition
Physical Activity	Any voluntary bodily movement produced by skeletal muscles that requires energy expenditure
Exercise	Subcategory of physical activity that is planned, structured, repetitive, and improves or maintains one or more components of physical fitness
Physical Fitness	Set of abilities to perform aspects of sports, occupations and daily activities Health-related components of physical fitness are: - cardiorespiratory fitness - muscular endurance - muscular strength - body composition - flexibility
Cardiorespiratory Fitness	Capacity of the circulatory and respiratory systems to deliver oxygenated blood to skeletal muscles during exercise (VO_{2max}= maximal oxidative capacity)

Adapted from Caspersen et al. Public Health Rep. 1985 (51).

Habitual physical activity has not only been evaluated as treatment of overweight and obesity (52; 53), but also to increase glucose uptake and insulin sensitivity (54; 55). On the other hand, sedentary lifestyle is associated with higher risk of insulin resistance (22). Blair et al. underscored the importance of physical activity by indicating cardiovascular fitness as a better predictor for CVD and all-cause mortality than BMI (56). Therefore, the WHO (24) recommends that adults with chronic diseases to spend at least 150–300 minutes of moderate-intensity aerobic activity; or at least 75–150 minutes of vigorous aerobic activity; or an equivalent combination of moderate- and vigorous-intensity activity per week, for substantial health benefits (57). The adherence to these guidelines may result in a significant reduction in metabolic risk factors. Additionally, even a slight increase in physical activity, below the recommended 150 minutes of moderate-intensity aerobic activity, can be effective in reducing mortality risk in individuals with T2DM. The adherence to these guidelines may lead to a significant decrease in metabolic risk factors and even a minor increase in physical

activity below 150 minutes of moderate-intensity aerobic activity can also be effective in decreasing mortality risk in people with T2DM (58).

Physical activity has been prescribed as medicine for the treatment of 26 different diseases, including obesity and T2DM (59). However, several studies have shown that exercise alone may induce minimal or even no significant changes in body weight (60-62). These findings could be explained by behavioral changes, such as a reduction in non-exercise activity after exercise training due to negative energy balance (i.e., fatigue) and an increased energy intake after exercise to compensate for the exercise-induced increase in energy expenditure (i.e., increased hunger) (63). Alternatively, hedonic aspects of food, associated with the pleasure of eating and reward, may also contribute to these patterns (64).

Surprisingly, the effect of increased physical activity on total energy expenditure is not linear but seems to plateau at after a certain amount of exercise. Therefore, Ponzer et al. suggested a "constrained model" of energy expenditure during exercise. This model describes an increase in total energy expenditure with physical activity at low activity levels, but as the level of physical activity increases, the body appears to reach a point where it adapts to maintain its overall energy expenditure within a relatively consistent range, resulting in a plateau effect at higher activity levels (65).

In addition, activity-induced energy expenditure only account for 30-40% of the total energy expenditure in moderately active people (63). Although these data suggest that exercise alone is not sufficient to increase energy expenditure for compensating overnutrition, exercise should not be neglected for people with obesity, as it can be used as a treatment and/or prevention of obesity-related complications like MASLD and T2DM.

Moreover, exercise seems to induce a lager decrease in VAT compared to caloric restriction (66) and reduces ectopic fat in the liver (i.e, hepatocellular lipid content (HCL)) even without dietary modifications (67). Interestingly, even mild aerobic exercise training reduces HCL without changes in body weight (68). Furthermore, only exercise was found to decrease VAT and a reduction in waist circumference independent of changes in body weight (66; 69).

Acute exercise has been shown to increase skeletal muscle glucose uptake by up to 50-fold, achieved through tightly regulated processes involving glucose delivery, transport across the sarcolemma and intramyocellular glucose metabolism (70). First, exercise enhances blood flow in skeletal muscle, recruits capillaries to expand the

vessel surface area, which promotes an increased delivery of glucose and oxygen to working muscles. Second, the increased exercise-induced translocation of the glucose transporter 4 (GLUT4) to the plasma membrane enhances sarcolemma permeability to glucose, enabling greater glucose uptake. Three, this process is mediated by multiple intracellular signaling pathways, which become active due to an increased ATP turnover followed by contraction-induced mechanical stress. Four, once in the muscle, glucose becomes phosphorylated by hexokinase II to glucose-6-phosphate, which either enters glycolysis or is stored as glycogen **(Figure 4)** (70).

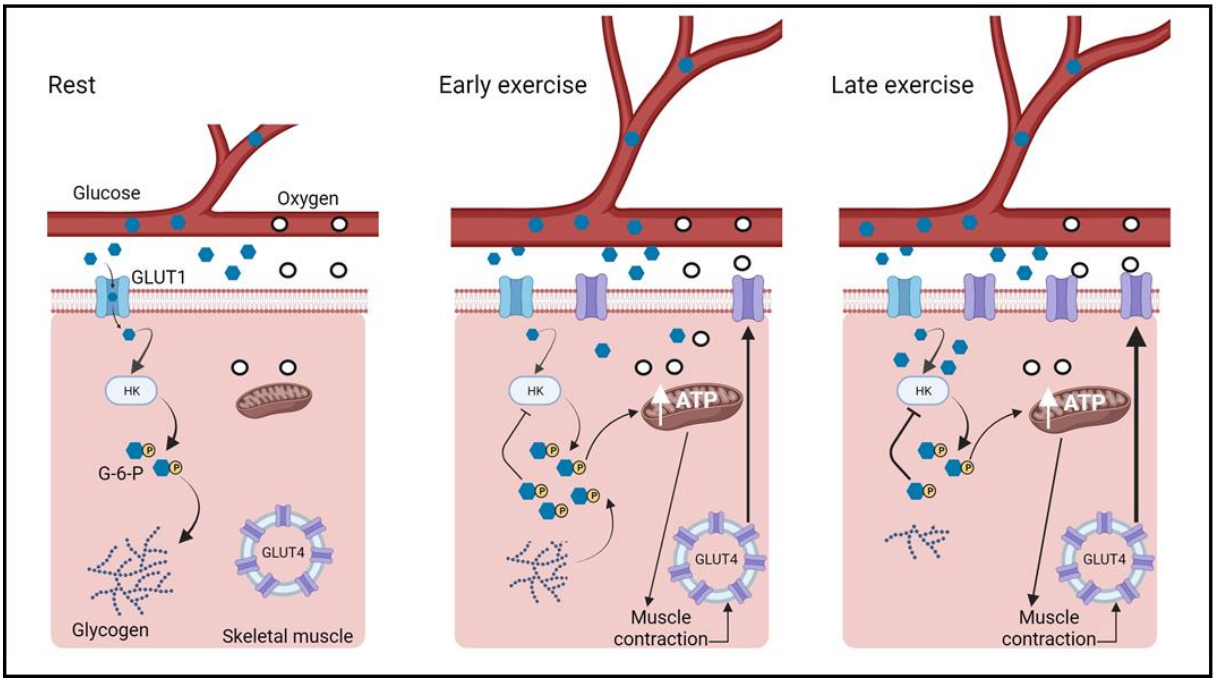

Figure 4. Exercise-induced insulin-independent glucose uptake. During rest, glucose demand is low, resulting in reduced glucose delivery due to decreased muscle fiber permeability. Once glucose that enters the muscle fiber, it is either stored as glycogen or used for ATP production through glycolysis and mitochondrial oxidative phosphorylation. At early stage of exercise, the stored glycogen is used for increased ATP production. This process leads to the accumulation of glucose 6-phosphate (G-6-P), which inhibits hexokinase (HK) II and reduces glucose phosphorylation. At later stage of exercise, glycogen is gradually depleted, and glucose supply shifts from glycogen to blood glucose uptake to meet ATP demands. This is induced by muscle contraction and increased ATP turnover. Adapted from Sylow et al. Nat Rev Endocrinol. 2017 (70). Created with BioRender©.

In addition to these insulin-independent processes, exercise also seems to upregulate insulin-stimulated glucose uptake as acute and chronic exercise sensitizes skeletal

muscle to insulin and, thereby, elevating insulin sensitivity in exercise-trained states (70).

Finally, exercise-induced improvements in insulin sensitivity protect skeletal muscle mass by suppressing muscle atrophy (71). This could be explained by the insulin-stimulated activation of AKT. AKT phosphorylates forkhead box protein O1 and O3a (FOXO1; FOXO3a), thereby excluding these proteins from the nucleus. As FOXO1 and FOXO3a are linked to increased autophagy and muscle atrophy by atrogin-1 expression, this mechanism prevents skeletal muscle mass. Further, activated AKT phosphorylates and activates mammalian target of rapamycin, leading to enhanced protein synthesis and cell growth in the skeletal muscle (72). This is particularly relevant for people with diabetes, as these individuals have an increased risk of sarcopenia and low skeletal muscle mass (73; 74). Of note, during weight loss, there is a natural tendency for a reduction in skeletal muscle mass, which can further elevate the risk of sarcopenia. Therefore, incorporating exercise, especially resistance exercise, into a weight loss regimen is essential not only for preserving lean muscle mass but also to contribute to the improvement of muscle strength (71; 75). Given that skeletal muscle is the primary organ for insulin-stimulated glucose uptake, the benefits of exercise on skeletal muscle mass become even more significant in the context of insulin resistance (17). In summary, exercise plays a multifaceted role in maintaining skeletal muscle mass, improving insulin sensitivity, and countering the adverse effects of weight loss on muscle health, making it a cornerstone in the management of conditions such as T2DM and obesity. However, individuals with T2DM have reduced exercise capacity compared to those without diabetes. This is linked to factors like insulin resistance, impaired muscle oxygen delivery, and inefficient energy substrate utilization. Besides, increased physical activity does not necessarily lead to a corresponding increase in physical fitness, possibly due to a resistance to exercise (76). Likewise, about 15-20% of individuals with T2DM failed to increase skeletal muscle mitochondrial density or improvements in glucose homeostasis and insulin sensitivity after supervised exercise interventions (77). Interestingly, genetic predisposition can also influence individual responses to exercise. In this context, a single nucleotide polymorphism in the nicotinamide adenine dinucleotide (NADH) dehydrogenase-1ß subcomplex subunit 6 (*NDUFB6*) gene, which encodes a mitochondrial complex I subunit, has been associated with ATP synthase flux in first-degree relatives of individuals with T2DM (78). The presence of the G/G single

nucleotide polymorphism (SNP) rs540467 has been linked to increased ATP synthase flux (78) and improved insulin sensitivity (79) through exercise, with those responding positively to exercise more frequently carrying the G allele of the *NDUFB6* rs540467 polymorphism. Finally, this polymorphism may also play a role in influencing the impact of physical activity in T2DM as the rs540467 SNP within the *NDUFB6* gene demonstrates an association with physical activity-induced alterations in insulin sensitivity, body composition and steatosis risk in people with T2DM (80). Notably, rather the type than the duration of exercise seems to be the determining factor to improve glycemic control in people with T2DM (76). Consequently, it is of interest to investigate the exercise respond in the novel diabetes endotypes. As SIRD features severe insulin-resistance, higher BMI and greatest risk of MASLD, regular physical activity might serve as specific tool for tailored prevention and treatment in this endotypes. Prior to my studies, it was unknown whether members of SIRD already show reduced levels of physical fitness and whether lower physical activity causes or least contributes to their metabolic phenotype.

1.3 Cardiovascular Risk in Diabetes

1.3.1 Cardiovascular Disease in Type 2 Diabetes

In 2017, more than 1 million global deaths were attributed to T2DM, thereby ranking T2DM as one of the main cause of global mortality (14). However, diabetes per se, but rather its related comorbidities are the reason for the observed excessive mortality rate associated with diabetes. Indeed, cardiovascular disease (CVD), which comprises a group of heart and blood vessels disorders including i.e. stroke, cerebrovascular disease, coronary artery disease and peripheral artery disease, remains the leading cause of death and disability among people with diabetes (81). Independent of other risk factors, diabetes confers an about doubled risk of cardiovascular outcomes and 32.2% of individuals with T2DM are affected by CVD globally (82; 83). These findings are supported by studies, which indicated chronic hyperglycemia as a contributor of increased cardiovascular risk by impairment of vascular function (84; 85). In line, rising fasting blood glucose levels increase the cardiovascular risk continuously (82). Beside hyperglycemia, other diabetes-related complications such as adipose tissue dysfunction and insulin resistance with subsequent dyslipidemia and hyperinsulinemia as well as hypertension and obesity are also worsen cardiovascular risk (15; 86; 87).

Additionally, endothelial dysfunction, characterized by reduced endothelial-dependent vasodilation due to decreased nitric oxide bioavailability (88), is another cardiovascular risk factor, which is associated with insulin resistance, dyslipidemia and T2DM (89) and further decreases during the early course of T2DM (90). Of note, reduced physical activity levels and decreased physical fitness further increase the risk of endothelial dysfunction in humans with recent-onset diabetes (91). However, it remains unknown if endothelial function is different in diabetes endotypes. Finally, unhealthy lifestyle such as smoking, alcohol intake, poor diet or low physical activity further increases cardiovascular risk in T2DM (92; 93). Therefore, lifestyle modifications and management like medical nutrition therapy, smoking cessation, and psychosocial care are recommended to people with T2DM (81). Moreover, regular physical activity reduces incidence of diabetes-related vascular complications (94) **(Figure 5)**.

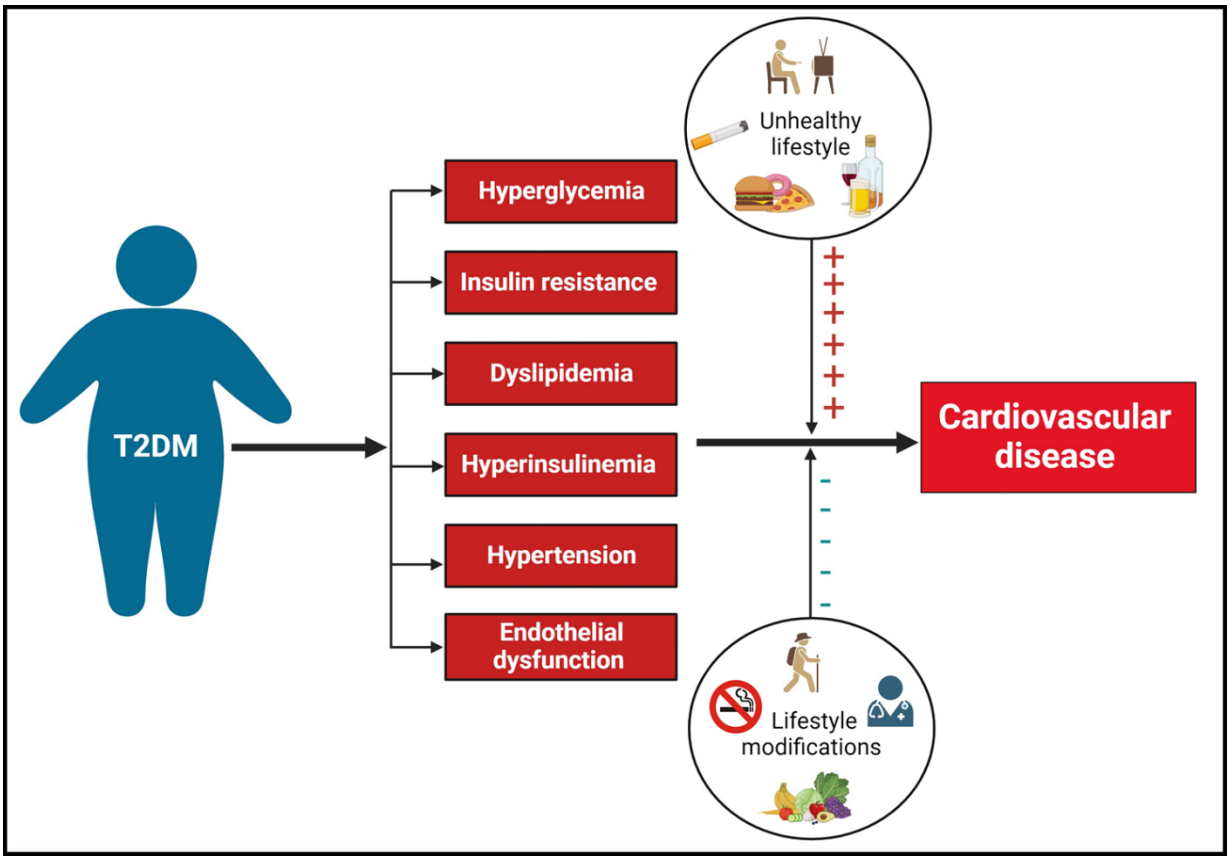

Figure 5. Cardiovascular Risk in Type 2 Diabetes. Diabetes-related complications like hyperglycemia, insulin resistance, dyslipidemia, hyperinsulinemia and hypertension increase the risk for cardiovascular diseases. This risk is worsen by an unhealthy lifestyle (i.e. smoking, alcohol intake, poor diet, and sedentary lifestyle), while lifestyle modifications (medical nutrition therapy, smoking cessation, psychosocial care and increased physical activity) reduce the risk for cardiovascular events in type 2 diabetes mellitus (T2DM). Created with BioRender©.

Taken together, CVD remains the leading cause of death and disability among people with diabetes (81). As such, it is of clinical relevance to assess differences in CVD risk in the diabetes endotypes to contribute to the road map of precision in medicine (95). Previous studies already reported different CVD risks between the endotypes, however those results are ambiguous. In Scandinavia cohorts, the SIRD and MARD endotype showed an at least nominally greater CVD risk than the other diabetes endotypes. Nevertheless, the differences did not remain statistically significant after adjustment for age and sex (19). In line, results from a Japanese cohort suggested the highest CVD risk for the SIRD endotype before, but not after adjustments for multiple confounders (96). Conversely, a high HbA1c and low BMI, which are typically found in the SIDD endotype, have been suggested as alternative explanation for the elevated risk of

major adverse cardiovascular events and death in the DEVOTE, LEADER and SUSTAIN-6 cohorts (97). Although the debate, whether poor glycemic control or severe insulin resistance represents the main risk factor for diabetes-related CVD, remains unsolved, the understanding of differences in cardiovascular risk in the novel diabetes endotypes could help to solve this issue.

1.3.2 Prediction of Cardiovascular Risk

Several different risk scores, based on multiple risk factors like age, sex, blood pressure, smoking, cholesterol, and diabetes status, have been developed to predict the individual cardiovascular risk (98-100). Although all prediction models are relevant for the prevention and management of CVD, each of these models have certain advantages and disadvantages and should be used with caution (101). The most commonly used risk models are the Framingham Risk Scores (FRS), European Society of Cardiology's Systematic Coronary Risk Evaluation (SCORE) and the American College of Cardiology/American Heart Association's Atherosclerotic Cardiovascular Disease (ASCVD) risk algorithms (99):

The FRS is one of the first predictive scores for coronary heart disease (CHD), which based on the Framingham Heart study. In 1998, Wilson et al. proposed a sex-specific prediction algorithm to estimate 10-year CHD risk (102), which has been validated by many studies in other populations (103-105). In 2008, D' Agostino et al. developed, based on a larger cohort of Framingham study, an extended sex-specific risk model to further estimate the 10-years risk of cardiovascular events like stroke, intermittent claudication and congestive heart failure (106).

The SCORE has been developed from 12 different cohort studies in several European countries and therefore benefits from a large study population and country-specific versions, which were expected to be more accurate. However, the SCORE includes only fatal cardiovascular outcomes and the total cardiovascular risk might therefore be underestimated (107; 108).

The ASCVD is a sex- and race-specific equations for African American and Caucasian men and women, which rather focus on risk of atherosclerotic cardiovascular disease rather on only hard CHD events (99).

1.4 Metabolic Dysfunction-Associated Steatotic Liver Disease

1.4.1 Definition and Epidemiology of Metabolic Dysfunction-Associated Steatotic Liver Disease

Recently, a multi-society Delphie consensus challenged the traditional nomenclature and diagnosis of nonalcoholic fatty liver disease (NAFLD) and suggested MASLD as a new nomenclature for fatty liver disease. In contrast to NAFLD, MASLD is diagnosed if at least one of five cardiometabolic risk factors is presented, which underline the strong connection between steatotic liver disease and metabolic risk factors and insulin resistance (109).

Of note, 60%-80% of people with T2DM and obesity concomitantly present with MASLD, which itself is considered as one of the leading causes of chronic liver disease with a current global prevalence of about 25% (15; 110-112). MASLD is diagnosed when HCL exceeds >5% by histology or >5.6% by magnetic resonance imaging (MRI) (113), without alcohol consumption being the primary cause (112). The spectrum of MASLD ranges from simple steatosis in the absence of inflammation to steatohepatitis, fibrosis, liver cirrhosis and hepatocellular carcinoma (114).

Several mechanisms have been discussed to promote hepatic lipid storage such as excessive substrate supply to the liver (i.e. NEFA, glucose); intrahepatic imbalance between lipogenesis and lipolysis; reduced export of lipids to peripheral tissue and/ or a combination of these mechanisms (115). Furthermore, aforementioned adipose tissue insulin resistance is linked to blunted insulin-mediated suppression of adipose tissue lipolysis, which results in an increased flux of NEFA and glycerol to the liver, where they serve as additional substrates for generation of lipid mediators and *de novo* lipogenesis, re-esterification or allosterically stimulate gluconeogenesis (116).

1.4.2 Diagnosis of Metabolic Dysfunction-Associated Steatotic Liver Disease

Histological evaluation of liver biopsies currently remains the gold standard method to diagnose MASLD, because it enables the identification of inflammation and classification of fibrosis stages (117). Nevertheless its invasiveness, side effects, costs, sampling and analytical variation limit the clinical practical use mainly to individuals at high risk of progressive MASLD. On the other hand, magnetic resonance (MR)-based methods such as magnetic resonance imaging estimated proton density fat fraction (MRI-PDFF) and ^{1}H-magnetic resonance spectroscopy (^{1}H-MRS) allow for accurate non-invasive quantification of even low amounts of liver fat concentrations at

or below the threshold for defining steatosis. However, these tools are not readily available in larger cohort studies. Consequently, ultrasonography, liver enzymes, or various indices are commonly used to diagnose MASLD in clinical studies (116).

Of note, serval large studies showed that ultrasonography-diagnosed MASLD or elevated liver enzymes predicted T2DM independent of age and obesity (118). Furthermore, MASLD indices have been established as surrogates for HCL after validation by MRI (119; 120). Among those non-invasive tests, the fatty liver index (FLI) is an established index with moderate sensitivity and specificity for diagnosing steatosis, which has been even recommended for clinical MASLD screening (119; 121; 122). The FLI has a sensitivity and specificity for diagnosing steatosis of 61% and 86%, respectively (122). Established cut-offs of the FLI further help to examine different degrees of steatosis risk. In detail, values <30 (low-steatosis category) rule out, while values ≥60 (high-risk steatosis category) rule in steatosis and values of 30-60 (intermediate-risk steatosis category) indicate the possible presence of steatosis (122). Moreover, the FLI not only correlates with insulin resistance (123) but also increases in severe insulin resistance (20). Recently, it has been shown that the FLI increased by 43% in T2DM and by 14% in T1DM after 5 years of disease onset which was confirmed by 2-fold rise in HCL (by ^{1}H-MRS) in people with T2DM (124).

One of the best evaluated non-invasive surrogate for assessing risk of advanced hepatic fibrosis is the fibrosis index 4 (FIB-4). The FIB-4 demonstrated a sensitivity of 65% and specificity of 97% for detecting advanced fibrosis. It also showed a 90% specificity with a sensitivity of 70% to exclude advanced fibrosis and has been validated in diverse populations. FIB-4 values <1.45 are considered to exclude, while values >3.25 are indicative of advanced fibrosis (125; 126).

1.4.3 Metabolic Dysfunction-Associated Steatotic Liver Disease Disease and Type 2 Diabetes

MASLD is associated with an approximate twofold greater risk of incident T2DM (127) and the risk of incident T2DM proceeds with the severity of MASLD (114). In this regard, lower HCL has been associated with a reduced risk of T2DM (128), while HCL accumulation associates with hepatic, adipose tissue and skeletal muscle insulin resistance (114). These findings indicate a close connection between insulin resistance and the progression of MASLD.

Although the molecular mechanisms describing how MASLD increases the risk of T2DM

are not completely understood, bile acid, lipid metabolites and gene variants have been discussed as potential risk factors of T2DM in MASLD.

DAG and ceramides are highly associated with hepatic insulin resistance and an increased risk of T2DM (47; 48). The most described mechanism by which DAG induces lipid-mediated hepatic insulin resistance is through the translocation of novel protein kinase C (PKC)ε. After its translocation to the plasma membrane, PKCε phosphorylates insulin receptor (INSR) Thr1160, followed by decreased distal INSR signaling. Ultimately, glycogen synthesis and gluconeogenesis suppression are reduced (129).

The mechanism of the ceramide-mediated hepatic insulin resistance is less explored, however, several hypotheses have been proposed. For example, ceramides have been shown to inhibit AKT though activation of PKCζ. AKT is a pivotal kinase within the insulin signaling pathway; which, after activation, promotes various cellular processes, including glucose uptake, glycogen synthesis as well as in the regulation of lipid metabolism, including the suppression of lipolysis. Therefore, ceramide-induced suppression of AKT results in reduced glycogen synthesis and increased HCL. However, these findings arise mainly from cell culture studies and are not thoroughly investigated (129) **(Figure 6)**.

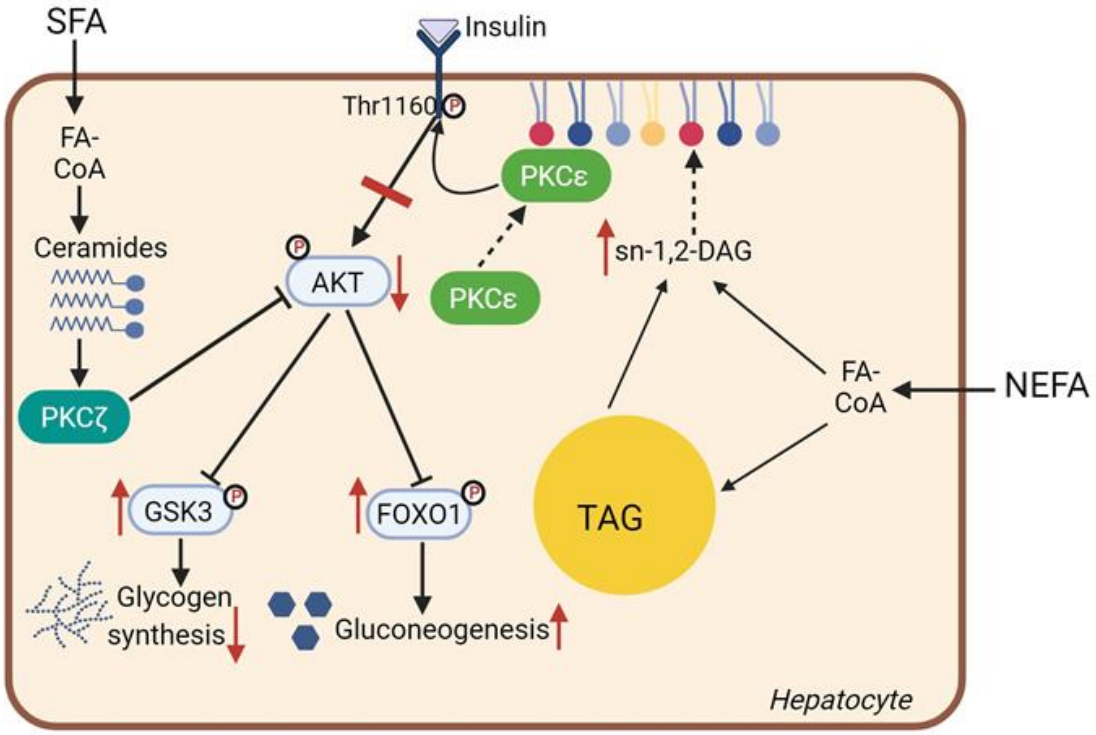

Figure 6. The DAG- and ceramide-induced hepatic insulin resistance. Increased flux of non-esterified fatty acids (NEFA) and saturated fatty acids (SFA) to the liver leads the accumulation of intrahepatic triglycerides (TAG), sn-1,2-diacylglycerol (DAG) and ceramides. After translocation to the plasma membrane, DAG activates protein kinase C (PKC)ε. Afterwards, the phosphorylation of insulin receptor (INSR) Thr1160 by PKCε, results in inhibition of INSR tyrosine kinase activity. This mechanism is expected to have an impact on all subsequent aspects of insulin signaling, including stimulation of glycogen synthesis and the transcriptional downregulation of genes associated with gluconeogenesis. Additionally, studies in cultured cells hypothesised that CER activates PKCζ, which results in the prevention of AKT from participating in insulin action. Dashed lines indicate translocation. Abbreviation: GSK3, Glycogen synthase kinase 3; FOXO1, Forkhead box protein O1, FA-CoA, fatty acyl-CoA. Created with BioRender©.

Also, ceramides can activate inflammatory pathways and induce oxidative stress within the liver, contributing to a pro-inflammatory environment that further exacerbates insulin resistance (15).

Furthermore, there is growing evidence that certain gene variants are related to hepatic lipid accumulation, leading to the assumption that MASLD could partly be inheritable (111; 130). In this context, patatin-like phospholipase domain containing 3 (*PNPLA3*), transmembrane 6 superfamily member 2 (*TM6SF2*), and glucokinase regulatory protein genes are three of the prevalent gene variants which promote the risk of MASLD by

altering lipid droplet formation, *de novo* lipogenesis or by reducing VLDL-cholesterol (131-134). It has been suggested recently, that the *TM6SF2*-polymorphism *rs58542926* has a protective effect for individuals with T2DM as this SNP dissociates hepatic lipid accumulation from whole-body insulin sensitivity in people with recent-onset diabetes (135). Interestingly, people with severe insulin resistance (SIRD endotype) exhibit the highest prevalence of MASLD and more frequently carry the rs738409(G) polymorphism of *PNPLA3.* Along these lines*,* G allele carriers demonstrate greater adipose tissue insulin resistance and excessive lipolysis, which may contribute to the elevated risk of MASLD in the SIRD endotype (111).

Finally, bile acids act as signaling molecules by interacting with the farnesoid X (FXR) receptor and Takeda G protein- coupled membrane receptor 5 (TGR5). Activation of hepatic FXR results in a reduced *de novo* lipogenesis with an additional decrease in gluconeogenesis and increase in glycogen synthesis (136), while activation with TGR5 increases the release of glucagon-like peptide 1 from L cells, which in turn, leads to insulin secretion by the pancreatic beta-cells (137). Therefore, bile acids might not only act as important mediators in the regulation of lipid but also glucose metabolism.

Taken together, MASLD, insulin resistance and obesity seem to be closely connected, but the underlying mechanisms of this connection remains incompletely understood.

1.5 Abnormal Thyroid Function

The close regulated relationship between the pituitary-derived thyroid-stimulating hormone (TSH) and the thyroid hormones thyroxine and triiodothyronine, renders TSH the more sensitive indicator of thyroid function. Because of the gradual alterations in thyroid function and its clinical presentation, overt hypothyroidism, defined by combined increased TSH with decreased free thyroxine (fT4), is distinguished from subclinical hypothyroidism, defined by moderately increased TSH along with euthyroid levels of free triiodothyronine (fT3) and fT4 (138) **(Table 2)**.

Table 2.Definition and diagnosis of primary hypothyroidism.

Primary hypothyroidism	
Definition	Abnormal function of the thyroid gland due to: - Autoimmune thyroiditis (M. Hashimoto) (positive anti-TPO or/and anti-TG auto-antibodies) - Other causes like thyroidectomy, thyreostatic drug treatment for hyperthyroidism, antidepressant treatment with lithium
Diagnosis	- Overt hypothyroidism: serum TSH >10 mIU/l, decreased serum fT3 and fT4 (prevalence: 0.2–2% in adults) - Subclinical hypothyroidism: serum TSH >4 mIU/l, serum fT3 and fT4 within the normal/euthyroid range (prevalence: 4–10% in adults)
Imaging	Thyroid ultrasound

Abbreviations: fT3, free triiodothyronine; fT4, free thyroxine; TG, thyroglobulin; TPO, thyroid peroxidase; TSH, thyroid stimulating hormone. Adapted from Hatziagelaki et al. Trends Endocrinol Metab. 2022 (138).

Interestingly, Chen et al. recently suggest to reevaluate the TSH reference range as they indicated low thyroid function as an independent risk factor for all-cause and cardiovascular mortality in euthyroid people with metabolic syndrome. Noteworthy, they further found mild subclinical (defined as TSH levels between 4.5- 10 mIU/L) as most detrimental impact on health (139).

Of note, thyroid hormones play a pivotal role in human growth, energy and lipid homeostasis. Consequently, reduced thyroid function may promote NEFA flux, accumulation of lipids in the liver, and an elevation in the levels of circulating pro-inflammatory adipokines (140; 141). This renders humans with hypothyroidism at greater risk of MASLD (126). In line, both hypothyroid and euthyroid individuals showed a positive correlation between TSH levels and MASLD, while higher fT4 levels may help to protect against MASLD (142). In addition, subclinical hypothyroidism and even low-normal thyroid function have been shown to predict steatohepatitis and advanced fibrosis (143). Hence, early detection of impaired thyroid function is of high clinical relevance, specifically for people at increased steatosis risk (144).

Interestingly, lower thyroid function is not only associated with steatosis but also correlates with adipose tissue and skeletal muscle insulin resistance, impaired insulin

secretion as well as reduced expression of glucose transporters (138; 145), which is present in T1DM and T2DM (146). In addition, the prevalence of hypothyroidism is higher in people with T1DM and T2DM in comparison to the general population (145). Notwithstanding, hypothyroidism has been shown to be associated with an increased risk of diabetes (147), while high and high-normal fT4 levels seems to decrease the risk of T2DM (148).

Finally, novel selective thyroid hormone receptor agonists such as Resmetirom have been identified as promising drugs to treat MASLD by demonstrating lipid-lowering and antisteatotic effects in a placebo-controlled phase 2 trial (149). These findings underscore the close relationship between thyroid function and MASLD. Although impaired thyroid function seems to be associated with MASLD and diabetes, little is known about the relationship between thyroid function and MASLD in people T1DM and T2DM.

1.6 Aims

The overall aims of the thesis were to assess to what extent alterations of energy metabolism affect the risk factor for the frequent diabetes-related complications, cardiovascular disease and MASLD in people with obesity and/or recent-onset diabetes. Thus, this thesis addressed three questions:

(i) Do the new diabetes endotypes exhibit differences in terms of physical fitness and CVD risk?

(ii) Is the mitochondrial respiration of VAT and SAT different in obese individuals with and without MASLD?

(iii) Is there an association between low-normal thyroid function and an increased risk of steatosis in individuals with recent-onset diabetes?

These studies are approved by the ethics boards of the Medical Faculty of Heinrich Heine University Düsseldorf (reference numbers: 4508 and 3516).

2 Publications

2.1 Physical fitness and cardiovascular risk factors in novel diabetes subgroups,

Saatmann, N, Zaharia, OP, Strassburger, K, Pesta, DH, Burkart, V, Szendroedi, J, Gerdes, N, Kelm, M, Roden, M., J Clin Endocrinol Metab., 107(4):1127-1139, (2022)

2.2 Mitochondrial respiration is decreased in visceral but not subcutaneous adipose tissue in obese individuals with fatty liver disease, Pafili, K, Kahl, S, Mastrototaro, L, Strassburger, K, Pesta, D, Herder, C, Pützer, J, Dewidar, B, Hendlinger, M, Granata, C, Saatmann, N, Yavas, A, Gancheva, S, Heilmann, G, Esposito, I, Schlensak, M, Roden, M., J Hepatol., 77(6):1504-1514, (2022)

2.3 Association of thyroid function with nonalcoholic fatty liver disease in recent-onset diabetes, Saatmann, N, Schön, M, Zaharia, OP, Huttasch, M, Strassburger, K, Trenkamp, S, Kupriyanova, Y, Schrauwen-Hinderling, V, Kahl, S, Burkart, V, Wagner, R, Roden, M, GDS Group., Liver Int., 44(1):27-38, (2024)

3 Discussion

3.1 Differences in Low Physical Fitness and Cardiovascular Risk Factor among Diabetes Endotypes

The first study observed that people with recent-onset diabetes show lower physical fitness compared to glucose-tolerant individuals and that physical fitness is lowest in humans with SIRD (150). These findings are partly surprisingly as physical activity from Baecke index is comparable among all diabetes endotypes. Despite those variations in physical fitness, endothelial function is similar across all diabetes endotypes, which is comparable even with glucose-tolerant individuals. Finally, this study extends the findings of recent analyses regarding diabetes endotypes by combining the assessment of physical fitness and CVD risk and found that the SAID endotype exhibits the lowest cardiovascular risk within the first year after diabetes diagnosis compared to the other diabetes endotypes (150).

Among other factors, VO_{2max} indicates mitochondrial function and density, as demonstrated by its positive relationship with skeletal muscle ATP production in humans (79). Of note, individuals with T2DM showed a reduced skeletal muscle mitochondrial function (i.e. reduced oxidative phosphorylation capacity and mitochondrial plasticity) (151). A reduction in mitochondrial plasticity to changes in insulinemia further results in an impaired metabolic flexibility, which is defined as the ability to switch from lipids (fasted state) to glucose oxidation (fed state) during insulin stimulation and is considered as independent determinants of insulin sensitivity in recent-onset T2DM (152). The shift from the oxidation of lipids to glucose is not only observed during the transition from the fasted to fed state but also represents a crucial physiological change during acute exercise. Limited data suggest that individuals with obesity and T2DM may also exhibit a reduced lipid oxidation and metabolic flexibility during moderate-intensity aerobic exercise (153). Therefore, it could be speculated that the dissociation of physical activity and physical fitness in the SIRD endotype results from a reduction in mitochondrial respiration and metabolic flexibility. However, it has been shown recently that metabolic inflexibility is not essential for the pathogenesis of skeletal muscle insulin resistance (154). Further research is needed to clarify these different findings.

Interestingly, the SIRD endotype not only displayed the lowest VO_{2max} but also had the lowest peak power at the first ventilator threshold (VT1) and peak power output (150), which underline the correlation between muscle strength and CRF (155). In

agreement, another study of the GDS cohort indicated a lower skeletal muscle mass in individuals with the SIRD endotype compared to individuals with SAID, SIDD, and MARD (156). In contrast, a recently published prospective cohort study in Japanese males and females with diabetes, found an increased risk of sarcopenia for individuals of the SAID and SIDD endotype compared to the other endotypes (96). Differences between these studies could be explained by variation in ethnicity and the methods employed in investigating skeletal muscle mass versus sarcopenia. The current study extends those findings by including a glucose-tolerant control group (150). In this context, muscle strength (reflected in peak power at VT1 and peak power output) was also lower in all diabetes endotypes compared to glucose-tolerant individuals. Taken together, these findings (96; 150; 156) underscore the association between a sarcopenic phenotype and diabetes mellitus (157), which might be driven by insulin resistance (156) or poor glycemic control (158). However, further studies are needed to explain this interaction.

To conclude, the current data suggest that low physical fitness is not simply resulting from aging, obesity, or physical inactivity but rather from presence of distinct abnormalities in mitochondrial function, metabolic flexibility and skeletal muscle mass. Of note, sarcopenia further shows a bidirectional relationship with CVD (159), which could partly be explained by the release of myokines, as myokines promote an anti-inflammatory milieu, resulting in a decreased CVD risk (160). Along this line, this study showed an increased CVD risk for people with diabetes compared to the glucose-tolerant group (150). However, evidence suggest that rather sarcopenic obesity than sarcopenia (based on muscle strength) alone is modestly associated with CVD risk (161). In line, individuals with a higher WHR and lower muscle strength (SIRD) had a higher CVD risk compared to individuals with lower WHR and higher muscle strength (SAID) (150). Nonetheless, studies in context of sarcopenia and/or sarcopenic obesity show heterogeneous results which might be explain by inconsistent definitions of sarcopenia (162).

Another link between reduced physical fitness and increased CVD risk could be related to adipose tissue distribution. As mentioned earlier, triglycerides are not only stored in adipose tissue but also as ectopically, e.g. in skeletal muscle (36). Strong evidence supports that physical fitness is an important modulator of the association between IMCL and insulin sensitivity in recent-onset T2DM. Moreover, there might be a tight relationship between IMCL and CVD risk in T2DM (163). The current results support

these associations by indicating the lowest CRF for the SIRD endotype, which renders these individuals at increased cardiometabolic risk and potential higher mortality. This interpretation was underlined by a strong association between HOMA-IR and FRS-CHD, FRS-CVD and ASCVD pointing to a higher risk of CVD/CHD in insulin-resistant individuals (150).

Moreover, CVD risk might be linked to age, sex and BMI as estimated differences between endotypes were no longer present after those adjustments (150). For example, FRS-CHD seemed to be higher in humans with SIRD than in the SAID, SIDD and MOD endotypes, but after adjustments for age, sex and BMI, the SIRD endotypes only differed to SAID. With regard to previous works, it could be speculated that BMI is the major driver for this correlation (164-167). Thus, obesity might be an important factor for cardiovascular health at least in the SIRD endotype.

Finally, the observed similarity in endothelial function among the diabetes endotypes (150) was particularly surprising for the MOD and SIRD endotypes as ectopic lipid deposition, dysregulated fatty acid metabolism and/or the release of inflammatory adipokines and hepatokines have been suggested to be related to endothelial dysfunction (15; 21; 168). However, previous studies already challenged the concept of a direct relationship between FMD and diabetes by reporting no association between FMD and diabetes (169) or only for overt T2DM, but not in those with impaired glucose metabolism (88). Nevertheless, poor glycemic control, insulin resistance and reduced physical fitness have been suggested as leading factors for the development of endothelial dysfunction (90). These findings render the SAID, SIDD, and SIRD endotypes at the highest risk of developing endothelial dysfunction at a later stage of disease progression.

Taken together, the new diabetes endotypes exhibit differences in cardiovascular risk right from the onset of the disease. Notably, the SAID subgroup demonstrates lower risk scores for CVD compared to other diabetes endotypes. Conversely, the SIRD subgroup exhibits the lowest levels of physical fitness, despite having similar levels of physical activity and endothelial function compared to the other endotypes.

3.2 Visceral Adipose Tissue Energy Metabolism in Individuals with Obesity and Metabolic Dysfunction-Associated Steatotic Liver Disease

The second study provides evidence that VAT dysfunction is associated with adipose tissue insulin resistance and MASLD. This conclusion is based on the reduced mitochondrial respiration in the VAT of obese people with MASLD compared to similarly obese individuals without liver steatosis, which is further associated with adipose tissue insulin resistance. Thus, this study reveals a crucial role of compartment-specific adipose tissue energy metabolism for MASLD progression and insulin resistance in people with obesity (170).

Dysregulation of adipose tissue mitochondrial function implicates an impaired lipid and glucose metabolism (171; 172). These data extended these findings in observing a compartment-specific mitochondrial function in VAT and SAT in humans with obesity and MASLD (170). In contrast to SAT, VAT mitochondrial respiration was downregulated in obese humans with hepatic steatosis and in those with MASH compared to individuals without MASLD (170), which support the tight relationship between decreased WAT oxidative phosphorylation capacity and increased NEFA flux to the liver (173).

Further adipose tissue insulin sensitivity has been shown to be positively associated with VAT mitochondrial respiration (170). Of note, fatty acid oxidation refers to the process of breaking down fatty acids into acetyl-CoA units and therefore, mitochondria function plays a crucial role in the regulating the storage and expenditure of lipids (172). However, abnormal fatty acid oxidation results in enhanced NEFA release into the portal vein at least in individuals with obesity (174) The increased flux of NEFA to the liver finally results in the described lipid-induced hepatic insulin resistance (15). Therefore, the association between adipose tissue insulin sensitivity and VAT mitochondrial respiration could be explained by the observed reduced fatty acid-linked (F-) NADH-linked (N-) and succinate-linked (S-) (FNS-pathway) maximal oxidative capacity ($[ETF]_P$) in VAT of obese people with MASLD compared to those without MASLD in this study (170).

In contrast to VAT, $[ETF]_P$ was similar in the SAT among all three groups in SAT. In line, recent studies showed an adequate SAT expandability in individuals with obesity and MASLD (175) and indicated that SAT inflammation and elevated NEFA release in relation to fat-free mass and plasma NEFA concentration were unrelated to insulin resistance (176).

Recently it has been shown that mitochondrial oxygen consumption was higher in VAT compared to SAT (38), while oxidative capacity was negatively associated with BMI in SAT but not in VAT in obese individuals (177). In line, the present study identified a higher mitochondrial respiration in VAT than in SAT in people with obesity, but without MASLD. Indeed, mitochondrial respiration was similar in both compartments in obese humans with MASLD. Further, oxidative capacity in VAT of people with MASLD was similar to that assessed in SAT of individuals without MASLD (170).

Taken together, these results support the hypothesis of an impaired insulin-mediated suppression of VAT lipolysis due to downregulated VAT mitochondrial respiration, specifically in the FNS-pathway, which results in an excess delivery of NEFA to the liver and MASLD progression in people with obesity (116). In regard to the fact, that there is currently no licensed drug therapy for MASH (178), these findings might be important to contribute to the development of new treatments to prevent the manifestation and progression of MASLD by indicating VAT mitochondrial function as a possible target. Still, the thyroid receptor beta agonist Resmetirom has been shown to be a promising candidate for treating MASH by targeting thyroid receptors in the liver without affecting thyroid function elsewhere. Interestingly, Resmetirom seems to improve lipid homeostasis by enhanced mitochondrial respiration (149), which further supports the close relationship between MASLD and mitochondrial function.

3.3 Low Thyroid Function and Metabolic Dysfunction-Associated Steatotic Liver Disease in Diabetes Mellitus

The third study indicates that low fT4 but not high TSH levels are associated with increased steatosis risk and reduced insulin sensitivity in T2DM. However, this relationship has not been found in T1DM or glucose-tolerant people. Interestingly, specifically men but not women with T2DM show a correlation between low-normal thyroid function and steatosis risk and insulin sensitivity. Further, the association between TSH and steatosis risk in male T2DM might be driven by body mass. Finally, the findings of the study indicate a sex-specific correlation between low-normal thyroid function and steatosis risk in individuals with T2DM. (179).

Of note, in both T2DM and MASLD gender differences has been shown before (180; 181). For example, T2DM and fasting hyperglycemia occur more frequently in men than in women, while slightly more women are diagnosed with impaired glucose tolerance. Moreover, men often develop T2DM at a younger age and lower BMI than

women. In contrast, risk factors such as excessive weight gain and hypertension are more frequently observed in women, particularly at a younger age, at the time of diabetes diagnosis (180). Yet, MASLD prevalence is higher in men than in women in the general population (181), which is in line with the present findings (179). In line, lower TSH or subclinical hypothyroidism was found to be independently associated with the presence of T2DM or increased risk of metabolic syndrome in glucose-tolerant men but not in women in previous published studies (182; 183). The current study further extends these findings by observing a relationship between serum triglyceride levels and insulin sensitivity with fT4 in males, whereas such a correlation was not observed in females with T2DM (179). In contrast to that, subclinical hypothyroidism was associated with several parameters of the metabolic syndrome only in Japanese women (184), which might be explained by different ethnicity.

Taken together, the detection of sex differences in the relationship between MASLD and lower thyroid function underscores the need to explore modifications in the hypothalamic–pituitary–thyroid axis and sex hormone regulation. In this regard, it might be interesting to investigate the treatment responses of novel liver-specific thyroid hormone receptor agonists like Resmetirom, which have shown to reduce HCL (185). A placebo-controlled phase 3 trial over 52 weeks demonstrated that Resmetriom does not observe treatment-emergent adverse events, however subanalyses in people with different glucometabolic control, grade of obesity or sex remain still open (186).

Of note, the association between TSH and steatosis risk was different between males and females before, but not after adjusting for BMI (179). This suggests that the sex-specific association between TSH and steatosis risk in T2DM is primarily driven by body mass. In line, the incremental rise in body weight and waist circumference have been demonstrated to correlate with increasing TSH levels, even within references ranges (187). Interestingly, previous research suggest leptin as a modulator of fine-tuning thyroid function in hepatic metabolism and obesity (188-190). As an endocrine organ, adipose tissue produces and secretes leptin, to regulate energy balance and body weight by regulating appetite and energy expenditure. Besides its role in appetite regulation, leptin also affects thyroid deiodinase activities and is involved in a feedback loop with the hypothalamic-pituitary-thyroid axis by regulating thyrotropin-releasing hormone gene expression, which further controls the release of TSH from the pituitary (188; 189). In line, it has been shown that serum TSH affects leptin secretion in individuals with obesity while it further correlates with body fat mass in case of thyroid

diseases (189; 190). Additionally, there are evidences that plasma leptin concentrations are increased in obese individuals with hypothyroidism and insulin resistance (190). In recent years, research has increasingly focused on the role of mitochondrial function in the development and progression of MASLD (191) and indicated abnormal mitochondrial energy production and lipid metabolism as a pivotal link between overall metabolic health, insulin resistance, and steatosis risk (173; 192). Therefore, reduced mitochondrial function might be an additional pathogenic mechanism responsible for the coexistence of insulin resistance, obesity, MASLD and abnormal thyroid function.

Finally, the present results suggest that hypothyroidism is associated with an increased risk of developing MASLD in T2DM, even when fT4 and TSH levels are within the reference range and highlight the relevance of the early detection of reduced thyroid function at the onset of T2DM to reduce the risk of hepatic steatosis. Furthermore, these findings initiate the discussion on the reconsideration of reference ranges, as both fT4 and TSH levels (179). In line, slight increases in serum TSH concentrations within the reference range are already associated with body weight in males and females (187). Strong evidences from the NHANES III study further support this concern by identifying low thyroid function as independent risk factor of MASLD even within the normal TSH range. Importantly, this study also found an association between low thyroid function and an increased risk of all-cause and cardiovascular mortality in the MASLD population. Despite a higher mortality risk for individuals with MASLD, subclinical hypothyroidism has been identified as a predictor of increased mortality risk also in the general population (139).

To conclude, even low-normal thyroid function increase mortality in individuals with MASLD. Therefore, there is an urgent need of thyroid function screening as early as possible in T2DM and individuals at higher risk for MASLD. Moreover, higher age further seems to be positively associated with subclinical hypothyroidism (184), which underscores the significance of screening in individuals of advanced age. However, these findings call for further validation in other cohorts and mechanistic clinical studies.

3.4 Prevention of Diabetes-Related Complications

It has been shown that regular physical activity and exercise training reduce the risk of T2DM (143), CVD (23; 193), and simultaneously increase mitochondrial content and

function (194), which further might reduce the risk of the development and progression of MASLD. As extensively discussed elsewhere, a combination of aerobic and resistance exercise is recommended for individuals with T2DM (195). Indeed, resistance training enhances the overall metabolic health of individuals with T2DM by enhancing skeletal muscle mitochondrial function and promoting increases in skeletal muscle mass, which may improve insulin sensitivity and glucose control (72). In line, it has been demonstrated recently, that resistance training is more effective for reducing HbA1c than aerobic training alone in normal-weight individuals with T2DM (196). However, we showed that people with T2DM, especially those with severe insulin resistance, featured a reduced physical fitness compared to glucose-tolerant humans (150). Consequently, traditional resistance training with loads of ~70 % of the one-repetition maximum (1-RM) may not be feasible for those individuals. Interestingly, resistance training with low loads (20–40 % 1-RM) but mild blood flow restriction training (BFRT), via inflation cuffs, has recently become a popular and effective alternative training method. Despite considerably less intensity, BFRT seems to be similarly effective as traditional resistance training regarding improvements in muscle strength and muscle mass in healthy individuals (197). However, it remains unknown if BFRT also improves glycemic control and insulin sensitivity in individuals with T2DM and if BFRT alone prevent sarcopenia in people with T2DM. Although the mechanisms are underinvestigated, there are serval proposed mechanisms by which of BFRT could also improve skeletal muscle metabolism in T2DM. In this regard, BFRT might increases glucose uptake by initiating the Ca^{2+}/ calmodulin-dependent protein kinase II-pathway followed by activation of 5´AMP-activated protein kinase (AMPK), which finally leads to the translocation of the GLUT4 to the plasma membrane. Additionally, an increasing ADP:ATP ratio results in the phosphorylation and activation of AMPK, a cellular energy sensor that detects low ATP levels. This is followed by the inhibition of acetyl-CoA carboxylase, which promotes mitochondrial fatty acid oxidation. Enhanced mitochondrial respiration further induces oxidation of IMCL and can reduce lipotoxic metabolites like DAG and ceramides thereby improving insulin sensitivity. Moreover, BFRT might activate specific signaling cascades, which imitate protein synthesis and mitochondrial biogenesis. Finally, the reduced oxygen tension can induce angiogenesis and support arteriogenesis, which is beneficial in cases of endothelial dysfunction and CVD (198). In line, exercise per se can reduce prevalent hypertension (199), has a protective effect against the development of atherogenic dyslipidemia

(200) and lowers the risk of stroke (201).

Taken together, exercise training, especially BFRT, seems to be a promising approach to prevent the onset and/ or the progression of T2DM and diabetes-related complications like CVD. Enhanced mitochondrial fatty acid oxidation might reduce ectopic lipid accumulation, while exercise-induced improvements in insulin sensitivity (54; 55) might further improves insulin-mediated restriction of adipose tissue lipolysis, which results in a decreased flux of NEFA and glycerol to the liver, thereby reducing the risk of MASLD.

On the other hand, precision medicine could help to delay the onset of diabetes and its complications. While the traditional classification of diabetes into type 1 and type 2 has proven effective in distinguishing precise pathophysiological mechanisms with therapeutic implications, it still inadequately accounts for the diverse clinical manifestations of diabetes. Precision diabetology represents a new approach to establish stratified prevention and treatment strategies tailored to specific subgroups with specific genetic, metabolic, or molecular, features (6). These methods comprise enhanced screening and monitoring schedules, personalized recommendations for lifestyle interventions, and the identification of new treatment targets. Furthermore, these algorithms enable the prediction and prevention of stratified complications associated with diabetes (95). While these results show promise in advancing the roadmap of precision medicine, further studies are necessary to test these classifications in more diverse ancestries and confirm their responsiveness to interventions.

In recent years, research has increasingly focused on the role of altered mitochondrial energy production as a central element in the development and progression of MASLD and T2DM (173; 191). Dysregulation of mitochondrial energy production and lipid metabolism in liver, skeletal muscle and adipose tissue appears to be a pivotal link between insulin resistance, and the development of MASLD (173) and highlights the importance of adequate mitochondrial respiration, coupling and efficiency in regulating energy and lipid metabolism (202; 203). Of note, HCL is negatively correlated with mitochondrial oxidative capacity in the skeletal muscle (204), which underscores the concept of an independent correlation between altered skeletal muscle and hepatic energy metabolism. In contrast to the skeletal muscle, hepatic mitochondrial respiration has been shown to adapt to rising lipid exposure in obese humans with and without MASLD (205). This phenomena has been described as “hepatic mitochondrial

flexibility". However, this adaptation is lost during the progression to MASH (205). On the other hand, mitochondrial respiration is reduced in adipose tissue in individuals with obesity compared to lean people (206), which likely involved in contributing to adipose tissue dysfunction (173). As mentioned earlier, compartment-specific alterations in mitochondria respiration have been observed in adipose tissue. In line, a recent study indicated smaller adipocyte cell sizes along with higher mitochondrial content and oxidative phosphorylation capacity in VAT compared to SAT in obese individuals. However, after normalization to mitochondrial content, the intrinsic respiration and uncoupling control ratio were lower in VAT than in SAT, despite higher substrate control ratios in VAT (37). Further, the results of the second study in this thesis suggested a decrease in mitochondrial respiration in the VAT of obese people with MASLD compared to similarly obese individuals without liver steatosis (170). In contrast to these findings, a recent study indicated that the reduction in adipose tissue mitochondrial respiration is independent of MAFLD status (206). Moreover, it could be proposed that the diminished hepatic mitochondrial respiration serves as an additional link between hypothyroidism, T2DM, and MASLD as all three conditions exhibit compromised lipid oxidation, ectopic lipid accumulation, and insulin resistance (179; 207). Thyroid hormones play a crucial role in regulating hepatic mitochondrial respiration by modulating mitochondrial gene expression. This includes key factors such as peroxisome proliferator-activated receptor gamma coactivator-1α, which contributes to mitochondrial biogenesis, and carnitine O-palmitoyltransferase 1, which represents the rate-limiting enzyme in beta-oxidation. Additionally, thyroid hormones are involved in regulating the mitochondrial turnover. (138). Finally, a recent cross-sectional clinical study in adult heart transplant recipients who undergo routine transcatheter ventricular endomyocardial biopsies showed that exposure to T2DM diminishes ventricular mitochondrial function in healthy human hearts. Interestingly, this effect occurs even in the absence of heart failure or coronary artery disease and emphasizing myocardial energy metabolism as a promising target for diabetes-related CVD (208).

Taken together, physical activity not only leads to weight loss but also promotes mitochondrial respiration and biogenesis (72). As alterations of energy metabolism affect diabetes-related complications like CVD and MASLD but are also linked to hypothyroidism, this is particularly important for individuals with T2DM. In the context of the roadmap for precision medicine, precise exercise training may contribute to

achieving these objectives.

4 Conclusion

In conclusion, physical fitness is reduced in people with recent-onset T2DM, which renders these individuals to a higher CVD risk. However, the novel diabetes endotypes feature differences in physical fitness and cardiovascular risk already shortly after disease onset. Despite comparable physicals activity levels and endothelial function, the SIRD endotype has the lowest physical fitness, while SAID have the lowest cardiovascular risk.

Moreover, mitochondrial respiration is reduced in VAT of obese individuals with MASLD compared to obese individuals without MASLD. Further, low mitochondrial respiration in VAT promotes whole-body adipose tissue insulin resistance. These results underline the need of an increased physical activity in obese individuals, especially in those with MASLD, as exercise not only promotes mitochondrial respiration but also enhance insulin sensitivity and reduces VAT content. Of note, alternative training methods are designed to facilitate individuals with overweight and low strength in initiating exercise. This is also important for humans with low-normal thyroid function and T2DM, as the relationship between lower thyroid function and steatosis risk in male T2DM, is not only mediated by insulin resistance but also by body mass. Further, the association between low thyroid function and liver steatosis seems to be sex-specific in humans with recent-onset diabetes **(Figure 7)**.

Taken together, precise exercise training may contribute to a decreased risk of CVD, not only by promoting weight loss and increased insulin sensitivity but also by enhancing whole-body energy metabolism. Since reduced mitochondrial function is also associated with MASLD and hypothyroidism, this is especially important for individuals with T2DM. Finally, these studies contribute to the development of more precise prevention and treatment for people with diabetes mellitus.

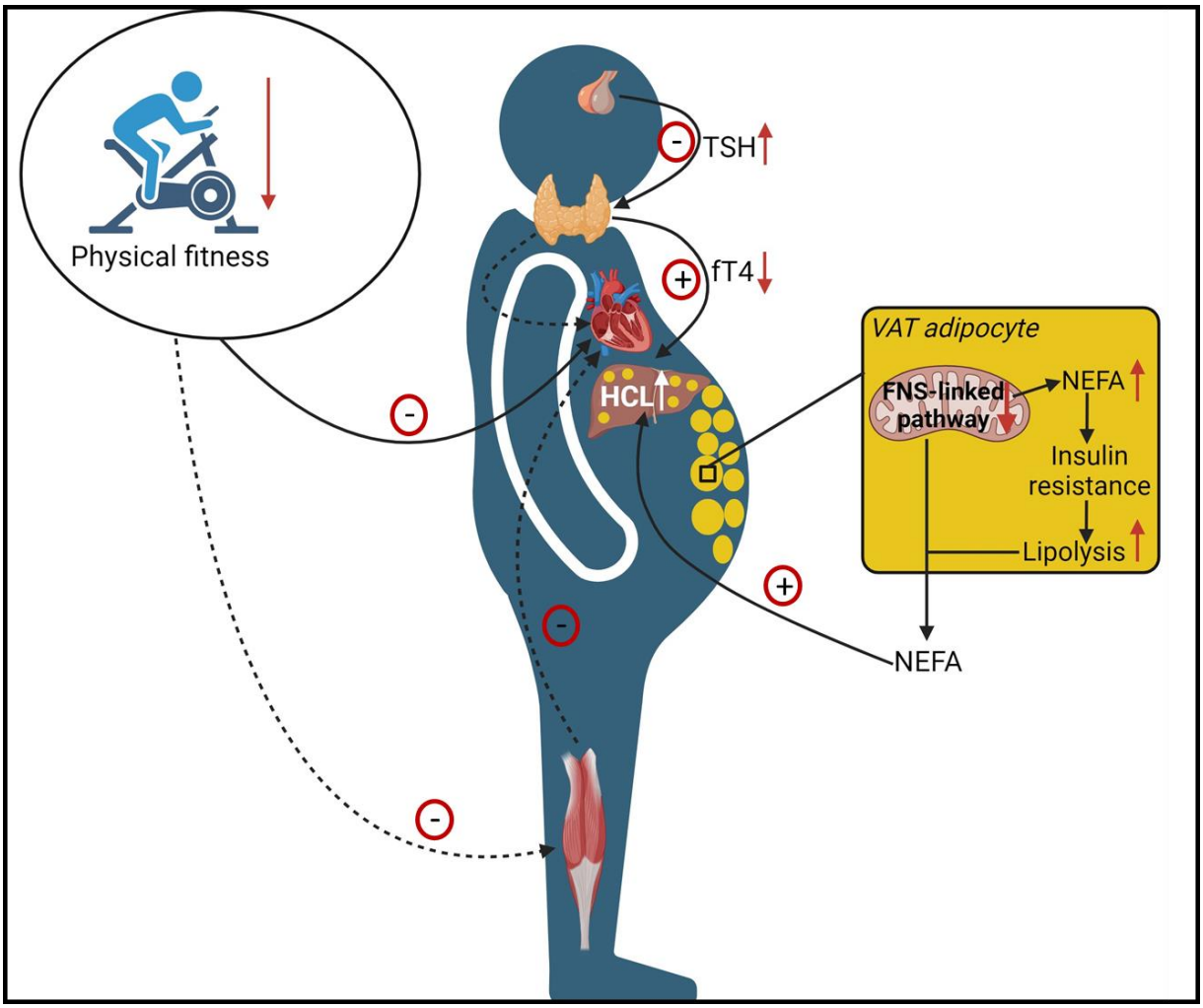

Figure 7. Diabetes-related complications. Physical fitness is reduced in people with insulin resistance, which increased the risk of cardiovascular diseases (CVD). It is tempting to speculate that individuals with severe insulin resistance further have low skeletal muscle mass, which even worsen CVD risk. Moreover, CVD risk might be increased in people with abnormal thyroid function, which is further associated with steatosis risk, especially in men with type 2 diabetes. Finally, reduced mitochondrial respiration in the visceral adipose tissue (VAT) promotes whole-body adipose tissue insulin resistance, which leads to an increased transport of non-esterified fatty acids (NEFA) to the liver resulting in hepatic steatosis. Abbreviation: FNS-pathway, fatty acid-linked (F-) NADH-linked (N-) and succinate-linked (S-) pathway. HCL, hepatocellular lipid content. Created with BioRender©.

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