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High-Intensity Interval Training in Diabetic Cardiomyopathy

Maxim Verboven

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DOCTORAL DISSERTATION

High-Intensity Interval Training in Diabetic Cardiomyopathy

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"Progress is made by trial and failure; the failures are generally hundred times more numerous than the successes; yet they are usually left unchronicled."

William Ramsay (1852 - 1916)

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ACE2	Angiotensin-converting enzyme 2
Ach	Acetylcholine
ADA	American Diabetes Federation
ADA	American Diabetes Association
AGE	Advanced glycated end-product
AMPK	Adenosine monophosphate-activated protein kinase
Ang II	Angiotensin II
AP	Arterial pressure
AP-1	Activating protein-1
AT1R	Angiotensin II receptor type 1
ATP	Adenosine triphosphate
AUC	Area under the curve
AWT	Anterior wall thicknesses
BMI	Body mass index
BRS	Baroreflex sensitivity
BW	Body weight
cGMP	Cyclic guanosine monophosphate
CAD	Coronary artery disease
CICR	Ca^{2+} - induced Ca^{2+} release
CO	Cardiac output
CRP	C-reactive protein
CTGF	Connective tissue growth factor
CVD	Cardiovascular diseases
DAB	3, 3-diaminobenzidine
DAG	Diacylglycerol
DCM	Diabetic cardiomyopathy
DIO	Diet-induced obesity
DM	Diabetes mellitus
Drp-1	Dynamin-related protein 1
eNOS	Endothelial nitric oxide synthase
ECL	Enhanced chemiluminescence

ECM	Extracellular matrix
EDP	Left ventricle end diastolic pressure
EDV	End-diastolic volume
EF	Ejection fraction
ER	Endoplasmic reticulum
ERK	Extracellular signal-regulated protein kinase
ESC	European Society of Cardiology
ESV	End-systolic volume
EXPERT	Exercise Prescription Everyday Practice and Rehabilitative Training
FAT	Fatty acid translocase
FATP	Fatty acid transport protein
FDA	Food and Drug Administration
FFA	Free fatty acid
FPG	Fasting plasma glucose
FS	Fractional shortening
GAD	Glutamic acid decarboxylase
GDM	Gestational diabetes mellitus
GLUT4	Glucose transporter 4
HbA_{1C}	Glycated haemoglobin
HBP	Hexosamine biosynthetic pathway
HDL	High-density lipoproteins
HF	Heart failure
HFD	High-fat diet
HFpEF	Heart failure with preserved ejection fraction
HFrEF	Heart failure with reduced ejection fraction
HIIT	High-intensity interval training
HIPA	High-intensity physical activity
HK	Hexokinase
HOMA	Homeostatic model assessment
HR	Heart rate
Hr_{max}	Maximal heart rate
HRV	Heart rate variability
HSP	Heat shock protein
iNOS	Inducible nitric oxide synthase

ICAM-1	Intercellular adhesion molecule 1
IFG	Impaired fasting glucose
IFN-γ	Interferon-gamma
IGT	Impaired glucose tolerance
IHF	Ischemic heart failure
IHR	Intrinsic heart rate
Iκβ	Inhibitor of κβ
IL-6	Interleukin-6
IRS-1	Insulin receptor substrate 1
IVRT	Isovolumic relaxation time
JNK	C-Jun N-terminal kinase
kcal	Kilocalories
L-ARG	L-arginine
LDL	Low-density lipoproteins
LIPA	Low-intensity physical activity
LOX	Lysyl oxidase
LV	Left ventricular
LVEDD	LV end-diastolic diameter
LVEDV	LV end-diastolic volume
LVESD	LV end-systolic diameter
LVESV	LV end-systolic volume
LVP	Left ventricle pressure
mTOR	Mammalian target of rapamycin
MAPK	Mitogen-activated protein kinase
MCP-1	Monocyte chemotactic protein-1
MDA	Malondialdehyde
MET	Metabolic equivalent
MIPA	Moderate-intensity physical activity
MIT	Moderate intensity training
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MR	Magnetic resonance
MRI	Magnetic resonance imaging
mtDNA	Mitochondrial DNA
MVC	Maximum voluntary contraction

NADPH	Nicotinamide adenine dinucleotide phosphate
NCX	Sodium-calcium exchanger
NEAT	Non-exercise activity thermogenesis
NEFA	Non-esterified fatty acids
NEPA	Non-exercise physical activities
NF-kB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NO	Nitric oxide
NOX	Nicotinamide adenine dinucleotide phosphate oxidase
O2·	Superoxide anion radical
O-GlcNAc	β-O-linkage of N-acetylglucosamine
OGTT	Oral glucose tolerance test
OLETF	Otsuka Long-Evans Tokushima Fatty
OXPHOS	Oxidative phosphorylation
PAI-1	Plasminogen activator inhibitor-1
PFK	Phosphofructokinase
PG	Plasma glucose
PGC-1a	Peroxisome proliferator-activated receptor gamma coactivator 1-alpha
PI3K	Phosphatidylinositol 3-kinase
PKC	Protein kinase C
PKG	Protein kinase G
PPAR-a	Peroxisome proliferator activated receptor-a
(P)RR	(Pro)renin receptor
PVDF	Polyvinylidene fluoride
PWT	Posterior wall thicknesses
RAAS	Renin-angiotensin II-aldosterone system
RAGE	Receptor for advanced glycated end-product
RCT	Randomised clinical trial
ROS	Reactive oxygen species
RWT	Relative wall thickness
RyR	Ryanodine receptor
S6K1	Ribosomal protein S6 kinase beta-1
SBP	Systolic blood pressure
SED	Sedentary
SEM	Standard error of the mean

SERCA	Sarco/endoplasmic reticulum Ca2+-ATPase
SIRT1	Sirtuin 1
SOD	Superoxide dismutase
SR	Sarcoplasmic reticulum
STZ	Streptozotocin
SV	Stroke volume
tPA	Tissue plasminogen activator
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus
TAG	Triacylglycerol
TAPSE	Tricuspid annular plane systolic excursion
TBS-T	Tris-buffered saline containing Tween-20
TGF-	Transforming growth factor-B
TLR	Toll-like receptor
TNF-a	Tumor necrosis factor alpha
TR	Twist rate
TTE	Transthoracic echocardiography
UCP	Uncoupling protein
UCP-GlcNAc	Uridine diphosphate N-acetylglucosamine
USA	United States of America
UTR	Untwist rate
vWF	von Willebrand factor
VCAM-1	Vascular cell adhesion molecule 1
VO_2max	Maximal oxygen consumption
WHO	World health organisation
ZnT8	Zinc transporter-8

1

Introduction

1 Introduction and aims

1.1 What is Diabetes Mellitus?

Diabetes mellitus (DM) is a chronic metabolic disease characterised by hyperglycemia, caused by insulin deficiency or insulin resistance (1). In insulin deficiency the pancreas is not able to produce a sufficient amount of insulin to regulate blood glucose, whereas insulin resistance is defined as the inability to effectively use the produced insulin (2). Currently, there are more than 420 million diabetic patients worldwide (3). As a chronic metabolic disease, DM can have severe complications affecting the kidneys, brain, eyes, blood vessels, nerves and the heart, resulting in premature death.

1.1.1 Diabetes classification and importance of insulin

Diabetes mellitus is defined as a group of metabolic disorders. The most accepted and conventional classification of diabetes is type 1 DM (T1DM), type 2 DM (T2DM) and gestational diabetes.

Type 1 diabetes, also known as insulin-dependent, juvenile or childhood-onset diabetes, is characterised by the inability of the pancreas to produce a sufficient amount of insulin (4). Generally, in T1DM, the pancreatic β-cells are destroyed by an immune-associated or direct immune-mediated response causing insulin defects (5). Therefore, a lifetime treatment with exogenous insulin is necessary for T1DM patients. While being able to be diagnosed with T1DM at any age, it is a common chronic disease of childhood (6). Mostly, diagnosis happens at the age of 5-7 or with the onset of puberty (7). Genetics play an essential role in the incidence and prevalence of diabetes, however the increase of T1DM in the last years can only be explained by lifestyle and environmental changes (8). In children, the development of two or more islet autoantibodies directed against insulin, glutamic acid decarboxylase (GAD), insulinoma-associated antigen 2 or zinc transporter-8 (ZnT8) shows an increased risk of developing diabetes, with 70% of children having these antibodies progressing into diabetic patients (9). Other factors that can trigger the development of T1DM are infections (congenital rubella, enteroviral infection), diet (early exposure to cereals, root vegetables and

eggs and cow's milk) and toxins (steroid treatment) affecting children in utero, perinatally or during early childhood (10).

Only 10 % of the diabetic population suffers from T1DM. The majority of diabetic patients, 87 – 91 %, are having **type 2 diabetes** (11). T2DM, or non-insulin-dependent or adult-onset diabetes, is caused by the ineffectiveness of the body to use insulin (4). Symptoms of T2DM are similar to those of T1DM. However they are often dormant or less profound, making it possible for the patient to remain undiagnosed for several years. T2DM is a complex, heterogeneous disease with social, behavioural and environmental risk factors combined with genetics and heredity (12, 13).

Gestational diabetes (GDM) is temporary diabetes diagnosed during pregnancy, usually developed during the 24th week, with increased blood glucose levels but below critical/diabetic values (14, 15). Women with GDM are more likely to develop T2DM in the years following pregnancy (16, 17) and are at increased risk during pregnancy and delivery, as is their offspring (16, 18, 19).

Insulin plays a crucial role in the pathophysiology of all types of diabetes. Insulin is a hormone secreted by the β-cells of the pancreatic islets of Langerhans, aiding to maintain blood glucose levels. After a meal, glucose levels will increase, resulting in a glucose-mediated insulin secretion out of the β-cells of the pancreas. This insulin secretion will cause the storage and metabolism of glucose via the insulin receptor in insulin-sensitive tissues. During fasting periods, insulin secretion is decreased, urging the body to go for other energy sources as lipids (adipose tissue) and amino acids (protein stores in muscle and other tissues). These will be used for oxidation and as precursors for gluconeogenesis (20, 21). The uptake of glucose into the cell is carried out by the glucose transporter 4 (GLUT4). When unstimulated, the GLUT4 receptor is situated intracellular. Upon stimulation by the insulin receptor, a rapid shift from the intracellular location towards the membrane is observed for GLUT4 (22). In diabetes, the physiological working of insulin is disturbed, as shown in figure 1. As already described above, in T1DM autoantibodies destroy the β-cells of the pancreas, resulting in insulin deficiency and hyperglycemia. In T2DM cells are unable to utilise the present insulin, resulting in insulin resistance and hyperglycemia.

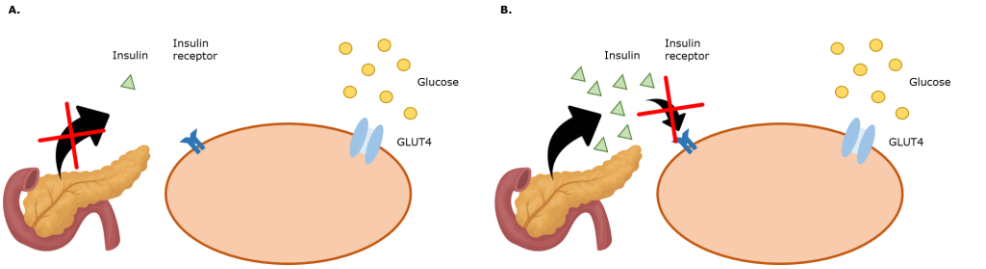

Figure 1: Insulin deficiency in diabetes mellitus A. Pathophysiology of insulin in Type 1 Diabetes Mellitus. B. Pathophysiology of Type 2 Diabetes Mellitus. GLUT 4: glucose transporter 4.

1.1.2 Prevalence and incidence of diabetes worldwide

Diabetes mellitus is a global pandemic of this time, with currently more than 422 million adult (> 18 years) diabetic patients (3). In the last decades, the number of diabetic patients has been risen due to multiple factors, such as population growth, increased life span and a higher diabetes prevalence at each age. Since 1980, the diabetic population has grown worldwide by a factor 4 (108 million in 1980; 422 million in 2014), showing the urge for a cure (4). A major concern is an increase in diabetic patients in low- and middle-income countries (23). Once considered as a disease from "Western countries", approximately 50 % of these patients are now located in the world health organisation (WHO) South-East Asia and Western Pacific Regions, with global spreading (4). The lowest numbers of diabetic patients are currently found in South and Central America and Africa, however it is estimated that in 2040 those will increase by 65 % in South and Central America and even double in sub-Saharan Africa (24). It should be taken into account that there are often inter- and intracountry differences, especially in Africa. There, 65 % of diabetic patients remain undetected, resulting in lower numbers as expected (25). Ethnicity is a significant risk factor in the development of T2DM. Chances of developing diabetes are significantly higher if one is a member of an ethnic minority group, compared with non-Hispanic whites (26). Immigration to developed countries contributes to an increased risk of T2DM (27). Minorities display a worsening in diabetic outcome with higher mortality rates, poor self-management and a higher burden of disease (27-29).

As stated before, diabetes was known as a "Western" disease. However, a remarkable increase in prevalence in developing countries is currently observed.

These countries experience rapid economic growth, conversing from low- to high-income economies rapidly, causing shifts in lifestyle habits and dietary structure (27, 30). Overnutrition is promoted by these changes, resulting in excess of energy. Animal fat and energy-dense foods are increasingly consumed, together with a decrease in fibres. Globalisation and global trade liberalisation led to increased accessibility of sugar and oils and made products more affordable (31). Fast food is on the rise, tilting the energy balance even further (30). Moreover, industrialisation causes an increasingly sedentary lifestyle, with more mechanisation and driving, replacing physical activity in daily life (32). This accumulating evidence emphasises the fact that diabetes is not only a "Western disease" anymore but a real global pandemic.

1.1.3 Prediabetes and diagnosis of diabetes

Between a healthy person and a diabetic patient, there is a diagnostic zone between 5.5 mmol/L (normal) and 7.0 mmol/L (diabetic) fasting plasma glucose, called prediabetes. Persons with prediabetes have an increase in their postprandial plasma glucose (PG) above the normal range (7.8 mmol/L) but below the borders of clinical diabetes (11.1 mmol/L) (33). Prediabetes includes patients with impaired fasting glucose (IFG) defined as a fasting plasma glucose (FPG) between 6.1 – 6.9 mmol/L. Patients with impaired glucose tolerance (IGT), defined as a postload plasma glucose of 7.8 – 11.0 mmol/L based on a 2h oral glucose tolerance test (OGTT) are also considered prediabetic. At last, patients suffering from both IFG and IGT are prediabetic (33). All these patients – prediabetic, IFG, IGT – have an increased risk to develop T2DM (34, 35). There is not only the increased risk for T2DM development, but prediabetes is also often associated with other comorbidities such as cardiovascular disease, microvascular diseases, blood pressure abnormalities, metabolic syndrome and fatty liver (33). In the United States, 1 out of 3 adults is prediabetic according to the International Diabetes Federation, while only 7% of them are aware they have the condition (24). These numbers go even up to 50 % in persons over 65 years of age (36). The annualised incidence rate of diabetes is on average approximately 10 % according to different studies in prediabetic patients (37, 38). Prediabetic patients that have both IGT and IFG are at even higher risk of developing diabetes (34). Eventually, up to 70% of prediabetic patients will develop diabetes, according to

the American Diabetes Federation (ADA) (35). However, prediabetic patients can reduce their risk in developing diabetes by interventions in lifestyle and with drug-based therapies (39-44).

Strict criteria are used to diagnose a person having diabetes. In table 1, the current WHO diagnostic criteria for diabetes are displayed, together with prediabetes criteria (45).

Table 1: Diagnostic criteria for both T2DM and prediabetic states.

	Fasting plasma glucose	***2h - Plasma glucose***	***HbA_{1C}***
T2DM	≥ 7.0 mmol/l (126 mg/dl)	≥ 11.1 mmol/l (200 mg/dl)	≥ 6.5 %
IGT	< 5.5 mmol/l (100 mg/dl)	7.8 – 11.1 mmol/l (140 – 200 mg/dl)	5.7 – 6.5 %
IFG	5.5 – 6.9 mmol/l (100 – 125 mg/dl)	< 7.8 mmol/l (140 mg/dl)	5.7 – 6.5 %
Normal	< 5.5 mmol/l (100 mg/dl)	< 7.8 mmol/l (140 mg/dl)	< 5.7 %

HbA_{1C}, glycated haemoglobin

As no specific biological marker for the diagnosis of diabetes is available, plasma glucose remains the primary diagnostic criteria. Glycated haemoglobin (HbA_{1C}) can be used as a diagnostic marker since 2009 for the diagnosis of T2DM and prediabetes (46). HbA_{1C} is a stable parameter as it reflects the average plasma glucose over 2-3 months. Furthermore, the advantage of HbA_{1C} is that a single measurement can be taken any time a day without any special preparation such as fasting (47). Other measurements that increase the predicted probability of T2DM are lipid metabolites (triglycerides, cholesterol, and lipoproteins), small-molecule intermediates (2-hydroxybutyrate), and creatinine. These, combined with information about lifestyle factors such as smoking, an unhealthy diet and no physical activity, increase the probability to develop T2DM (48). Clinical trials to discover new biomarkers to check the susceptibility of T2DM in the general population are currently being performed, however specificity for T2DM is not yet

established. On the other hand, the detection of these markers occurs rather late in the development of T2DM, when patients are already showing metabolic disturbances (49, 50).

1.1.4 Lifestyle factors in diabetes

Genetics, heredity and family history are risk factors in the development of T2DM. However, not all risk factors are independent of the lifestyle of the patient. The main risk factors for T2DM are thought to be an unhealthy (or Western) diet combined with physical inactivity (51). Both factors are contributing to the global obesity pandemic, closely associated with the rise of T2DM prevalence (52). Body mass index (BMI), often used as a predictor for T2DM, is not a proper tool to estimate the risk of developing T2DM, while an increased visceral fat deposition correlates better with T2DM (53-55). Food consumption and choice of diet are important in the association with the incidence of T2DM. In general, plant-based food has a positive effect on health and prevention of diabetes, while meats are associated with an increased risk at T2DM (56). Furthermore, high-density energy foods, non-fermented dairy products, refined grains and sugar-sweetened beverages all promote higher chances of obesity and T2DM (57-67). Low-fibre diet and food with a high glycemic index are positively correlated with T2DM development (68). Fatty acids play a role in insulin resistance and diabetic risk, as well as total and saturated fat intake (69, 70). Over the last few decades, the intake of soft drinks, sweetened carbonated beverages and fruit drinks with added sugar are dramatically increased, especially in youth (71, 72). Currently, various artificially sweetened beverages and fruit juices are available on the market as alternatives for these sugar-sweetened beverages. However, their association with T2DM has not yet been established. Recently, a systematic review by Imamura et al. showed that persons consuming artificially sweetened beverages are tended to be overweight or obese or hypertensive (73-77). Either an intake of one sugar-sweetened or artificially sweetened beverage per day already increase the risk of T2DM incidence, however data of artificially sweetened beverages could be biased as stated in this review (78). Furthermore, other lifestyle factors contributing to an increased risk of developing T2DM are a sedentary lifestyle and physical inactivity. Physical activity can decrease T2DM risk by approximately 30%, independent of the type of leisure time (79). Insulin

sensitivity and glycemic control have been proven to be improved after exercise (80-82). Independent of physical activity, an association is reported between obesity, diabetes, and sedentary time, with increased risk for diabetes incidence with prolonged sedentary time (83-87). Smoking is another independent risk factor for T2DM, but the mechanism is complex and not fully known yet (88-90). All these lifestyle factors increase diabetes incidence but are preventable by adaptations in personal habits. Preventing and treating diabetes should be a priority, as it is one of the major pandemics worldwide and healthcare expenditures for diabetic patients are assumed to be on average two-fold higher than non-diabetic persons. In 2015, more than 550 billion € was spent on healthcare due to diabetes (24).

1.2 Diabetic cardiomyopathy

Diabetic cardiomyopathy (DCM) is defined as diabetes-associated structural and functional changes in the myocardium, not directly attributable to other confounding factors such as coronary artery disease or hypertension, as first described by Rubler et al (91, 92). DCM is a major, chronic complication of T2DM whose prevalence is still increasing rapidly and being called a 'global emergency' (93). In diabetes, progressive structural and functional remodelling leads to gradual cardiac function impairment and ultimately compromises life expectancy. DCM is characterised by concentric hypertrophy, extracellular fibrosis, alterations at the cardiomyocyte level and changes in metabolism, leading to impaired cardiac output (94). Despite a lot of progress in understanding DCM aetiology during the last few decades, molecular mechanisms remain incompletely understood (95).

1.2.1 Myocardial substrate utilisation and cardiac lipotoxicity

In a healthy situation, the heart relies on the oxidation of substrates as free fatty acids (FFAs), glucose, lactate, ketone bodies and some amino acids to produce adenosine triphosphate (ATP). The choice of the used substrate depends mainly on availability, oxygen concentration and workload of the heart. Usually, >90 % of ATP is produced in the mitochondria, 70% using FFAs as oxidation substrate and approximately 30% glucose oxidation (96). These FFAs enter the cell via transporters, such as FA translocase/CD36 (FAT/CD36) and FA transport proteins (FATP1 and 6) (97). In the diabetic heart, despite the presence of hyperglycemia,

FFA oxidation remains the primary source of energy associated with reduced glucose oxidation (98-100). Due to failure of proper handling of insulin, it causes an abundance in circulating FFAs, which will in its turn lead to activation of the peroxisome proliferator activated receptor-α (PPARα). This latter is responsible for increased FA uptake, triacylglycerol (TAG) accumulation and reduced use of glucose (101). In addition, FFA delivery to the cardiomyocyte is increased, thereby increasing FA β-oxidation. Glucose uptake in the cardiomyocyte is mainly regulated by GLUT4, which will translocate from the intracellular compartment to the plasma membrane. However, insulin signal transduction is impaired, resulting in a decreased GLUT4 translocation and therefore less glucose uptake (102). The overutilization of fatty acids as energy substrate will result in reduced cardiac efficiency. Moreover, an altered cardiac metabolism is associated with the development of ventricular dysfunction in both animals and humans (98, 100, 102-104). Diacylglycerol (DAG) levels are elevated in cardiomyocytes of diabetic patients resulting in altered glucose metabolism through activation of protein kinase C (PKC) isoforms. This will affect insulin signalling and nitric oxide (NO) production (105, 106). Acyl-CoA dehydrogenases are enzymes helping in FFA oxidation. As cardiomyocytes are not specialised in storing lipids, an excess of acyl-CoA is stored as triacylglycerol, resulting in toxic accumulation of intermediates as diacylglycerol and ceramides, compromising cell viability and ATP production via stress kinases as PKC (107, 108).

1.2.2 Altered insulin signalling

Insulin resistance is one of the hallmarks of T2DM and DCM. Insulin signalling is mediated mainly through two different pathways. First, insulin binds to its receptor (insulin receptor substrate 1 (IRS-1)), resulting in activation of the phosphatidylinositol 3-kinase (PI3K) – protein kinase B (known as Akt) to cause metabolic responses. Eventually, activation of the Akt-pathway will lead to increased glucose uptake in the cardiomyocyte by GLUT4 translocation to the plasma membrane (109, 110). Alternatively, insulin can also use the mitogen-activated protein kinase (MAPK) pathway (110), resulting in myocardial hypertrophy by cellular growth and remodelling, increased collagen deposition and cell death of myocardial and endothelial cells (111). When insulin resistance occurs, a shift will take place towards the second MAPK-pathway, eliciting growth

factor-like responses. The primary controlling unit of this process is the docking protein IRS-1. Controlling the IRS-1 function occurs by phosphorylation by different types of kinases such as PKC, mammalian target of rapamycin (mTOR) and C-Jun N-terminal kinase (JNK) (112-114). In overnutrition or peripheral insulin resistance, an accumulation of FFAs and triglycerides occurs in the cardiomyocyte, resulting in increased levels of lipid molecules as DAG, FA and ceramides that affects the cell and contribute to even worse insulin resistance by activation of kinases that phosphorylase IRS-1. Phosphorylation of the serine kinase on IRS-1 leads to an impaired PI3K/Akt pathway stimulation, resulting in decreased glucose uptake causing diminished Ca^{2+} ATPase activity and make Ca^{2+} move back into the sarcoplasmic reticulum, eventually increasing intracellular Ca^{2+} (109, 115). Oxidative stress, caused by persistent hyperglycemia, triggers redox-sensitive kinases that on their turn increases phosphorylation of IRS-1. Hyperglycemia-induced oxidation activates PKC and upregulates angiotensin II (Ang II) signalling, and an abundance of glucose will active mTOR/ribosomal protein S6 kinase beta-1 (S6K1), resulting in further insulin resistance (112, 116-119). Obesity, closely associated with diabetes and a significant risk factor, and dysregulated adipocyte function, together with macrophage activation causes cytokine secretion (tumor necrosis factor (TNF)-α, interleukin 6 (IL-6)) and release of adipokines (resistin) while decreasing adiponectin. Resistin has a positive correlation with obesity and insulin resistance and plays a role in inducing insulin resistance (120). TNF-α and IL-6 activate MAPK, PKC and mTOR/S6K1 resulting in insulin resistance (113, 121-125). Altered insulin signalling also affects insulin-stimulated coronary endothelial NO synthase activity and NO production, resulting in an even higher increase in intracellular Ca^{2+} through the cyclic guanosine monophosphate/ protein kinase G (cGMP/PKG) pathway (109). Another factor contributing to insulin resistance is the activation of the renin-angiotensin II-aldosterone (RAAS) system. Ang II and aldosterone can activate membrane-bound nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX) enzyme complex. NOX emerged to be a major source of reactive oxygen species (ROS). Indeed, production of ROS will also result in the activation of redox-sensitive kinases as described previously, causing phosphorylation of IRS-1 leading to insulin resistance (126-128).

1.2.3 Hyperglycemia

Long-lasting states of hyperglycemia in diabetes can induce an increase in glycated proteins causing structural and physiological alterations in the cardiovascular system. These advanced glycated end-products (AGEs) are products derived from non-enzymatic glycosylation of lipids, lipoproteins and amino acids. AGEs can be found in cigarettes and in a Western diet, both risk factors for the development of T2DM (129, 130). AGEs can be obtained both exo- as endogenously, appear in plasma and accumulate in tissue such as cardiac tissue. Elevated levels of AGEs are associated with diabetes and can lead to increased connective tissue crosslinking, fibrosis, cardiac stiffness and impaired diastolic relaxation (109). AGEs contribute to the development of cardiovascular dysfunction by two mechanisms. They can bind to their cell surface-named receptor for AGE (RAGE) or by crosslinking tissue proteins (131, 132). The crosslinking of myocardial or vascular collagen by AGEs alters the properties of the tissue and diminishes vascular elasticity, cardiac flexibility and promotes stiffness, both on vascular and myocardial level, which can ultimately lead to diastolic dysfunction (133-135). In diabetes and diabetic cardiomyopathy AGEs will also affect the ryanodine receptor (RyR) leading to impaired cardiac contractility (136, 137). Moreover, AGEs can alter Ca^{2+} homeostasis by cross-links in the RyR domains and the sarco/endoplasmic reticulum Ca^{2+} - ATPase (SERCA) pump (136-138). AGEs play a role in heart failure not only by crosslinking but also by activation of RAGE inducing cardiac dysfunction in T2DM as showed by Nielsen et al (139). Other studies confirm the role of RAGE in heart failure (140, 141) and its negative effect on Ca^{2+} homeostasis (142). AGE-RAGE interaction is able to inhibit NO activity and can reduce endothelial NO synthase, which in turn causes a decrease in NO levels, all leading to increased cardiac stiffness (143). Additionally, AGE-RAGE can activate NADPH oxidase resulting in the production of ROS and increased oxidative stress, known to be deleterious for cardiac function (129).

Glycated low-density lipoproteins (LDL) are known to activate production of plasminogen activator inhibitor-1 (PAI-1) and reduce tissue plasminogen activator (tPA) in human vascular endothelial cells (144). PAI-1 is a serine-protease inhibitor that promotes fibrosis and thrombosis in cardiovascular and renal systems (145). In tissue, PAI-1 causes increased accumulation of the extracellular matrix, cardiac fibrosis and glomerulosclerosis (146-149). Patients with T2DM

have significantly higher levels of PAI-1 compared with controls, showing the link between PAI-1 and T2DM (150). Glycated high-density lipoproteins (HDL) affect the ability of monocyte adhesion in aortic endothelial cells (151). The study of Iribarren et al. showed that an increase of 1% in blood glycated haemoglobin (HbA_{1C}) results in an increased hospitalisation rate or heart failure-related death of 8% (152, 153). These data support the finding that hyperglycemia contributes to the development of DCM. Both hexokinase (HK) and phosphofructokinase (PFK) play a role in glycolysis as controllers, and their activity influences glycemic control (154-158). Both enzymes are inhibited in cardiac muscle of diabetic patients (156). Hyperglycemia in the heart can result in chronic activation of the hexosamine biosynthetic pathway (HBP) and an increase in oxidative stress (159). This increased oxidative stress is the result of NADPH produced by the pentose phosphate pathway due to hyperglycemia, leading to oxidative stress as described above (160, 161). On the other hand, HBP chronically activated by hyperglycemia is associated with insulin resistance (162, 163). Moreover, HBP will form a high-energy glycoside precursor (UDP-GlcNAc; Uridine diphosphate N-acetylglucosamine) for post-translational modification of proteins, resulting in β-O-linkage of N-acetylglucosamine (O-GlcNAc), which plays a role in altering expression, function and translation of proteins, and this O-GlcNAcylation protein is elevated in several diabetes models (164-171). Hyperglycemia and the resulting elevated O-GlcNAcylation attenuates the function of mitochondrial electron transport complexes I, III and IV in neonatal rat cardiac myocytes (172). Secondarily, O-GlcNAc plays a role in calcium handling and thus cardiac contractile function. O-GlcNAcylation may modify the relation between phospholamban and SERCA2a, as in diabetes O-GlcNAcylation at Ser16 on phospholamban is increased (173). The result is reduced phosphorylation of phospholamban with decreased association with SERCA2a and thus ultimately diminished cellular and cardiac pump function (164).

1.2.4 Inflammation and diabetic cardiomyopathy

Overweight and obesity are major contributors in the development of T2DM and DCM (174). Obesity is characterized by chronic inflammation in the visceral adipose tissue and is in many cases the result of an unhealthy diet. This visceral adipose tissue acts as an endocrine organ that secretes adipokines and produces

pro-inflammatory cytokines (e.g. IL-1β, TNF-α, IL-18) causing systemic inflammation (175). During this process, hypertrophied adipocytes secrete monocyte chemotactic protein-1 (MCP-1) which recruits macrophages. These macrophages infiltrate and expand, producing inflammatory cytokines including IL-6 and TNF-α. Furthermore, these cytokines enter the systemic circulation promoting the formation of C-reactive protein (CRP). Together, these molecules cause peripheral cellular dysfunction (i.e. endoplasmic reticulum and mitochondria) and interfere with insulin signalling (174). This is accompanied by lower adiponectin levels and higher levels of hs-CRP and FFAs, thus worsening insulin resistance. At cardiac level, arterial intima changes arise. Inflammatory molecules (i.e. CRP) activate monocytes which lead to the generation of ROS and oxidation of LDL-cholesterol. LDL-cholesterol and ROS are taken up by macrophages and transformed into lipid-loaden foam cells. In addition, injury and fibroblast migration further contribute to plaque formation (176). As a consequence, visceral adipose tissue distribution induces systemic inflammation in DCM.

The inflammatory process in cardiomyocytes generally occurs as an early response to myocardial injury. T2DM is characterised by insulin resistance, resulting in hyperglycemia (177). High glucose levels and dyslipidemia in diabetes directly increase the activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κβ), cytokines (TNF-α), chemokines (MCP-1), interleukins (IL-1β, IL-6) and adhesion molecules (ICAM-1, intercellular adhesion molecule 1; VCAM-1, vascular cell adhesion molecule 1) secretion in cardiac cells and migration of leukocytes into the myocardium (106). The secretion of cytokines and pro-fibrotic factors increases ROS production while leucocyte infiltration plays a role in the perpetuation of the inflammatory process.

Hyperglycemia, FFA and elevations in ROS activate NF-κβ, a transcription factor that regulates DNA transcription and the production of pro-inflammatory molecules (e.g. TNF-α, IL-6, IL-1β) in DCM (178, 179). NF-κβ is present in the cytoplasm as an inactive heterodimer bound to inhibitor of Kβ (IKβ), which is an inhibitor protein subunit of NF-κβ. Phosphorylation of IKβ relieves the inhibition of NF-κβ. NF-κβ can translocate to the nucleus where it binds to specific promotor sequences on its target genes to start the transcription machinery and thus the

production of pro-inflammatory cytokines. The produced cytokines contribute to a rapid degradation of IKβα, i.e. an IKβ subunit, which further enhances NF-κβ activation (180). NF-κβ activation and ROS overproduction cause activation of cardiac Toll-like receptors (TLRs) and the inflammasome complex (NLRP3) in myocardial cells which are key inducers for cardiac inflammation and play a central role in the pathogenesis of DCM (106, 181). TLRs are membrane-anchored proteins which work as pattern recognition receptors in the innate immune system and are present on several cell types including macrophages, T cells, B cells and cardiomyocytes. Evidence showed that TLRs elicit inflammatory pathways culminating in ROS-induced activation of NF-κβ and activating protein-1 (AP-1) (181). In cardiomyocytes of DCM patients, TLR2 and TLR4 are strongly upregulated and are upstream inducers of several pro-inflammatory molecules (TNF-α, ICAM-1, MCP-1, interleukins, chemokines, heat shock proteins (HSPs), interferon-γ (IFNγ) and inducible nitric oxide synthase (iNOS)) (181, 182). An inflammasome is a group of multimeric protein complexes responsible for the activation of inflammatory responses. NLRP3 inflammasome is the best characterized complex and can be found in macrophages, cardiomyocytes and cardiac fibroblasts (181). It is activated by hyperglycemia, hyperlipidemia and ROS. NLRP3 increases the number of proinflammatory cytokines (IL-1β, IFNγ, IL-18), promotes insulin resistance and takes part in apoptosis and macrophage transmigration. Therefore, TLRs and NLRP3 inflammasome both participate in DCM-associated inflammation of the heart (181).

Hyperglycemia, hyperlipidemia and oxidative stress also contribute to the formation of AGEs within cardiomyocytes. Increased AGE formation triggers the activation of NF-κβ (183). AGEs bind to their RAGE receptors and induce a signalling cascade via MAPKs and PI-3K to activate NF-κβ which in turn induces the production of inflammatory molecules (TNF-α, IL-6 and VCAM-1) and fibrosis. This contributes to the reduced cardiac contractility which is characteristic in DCM. In addition, RAGE itself is regulated in a positive feedback loop by NF- κβ (183). Once NF- κβ is activated, it drives growth factor expression of connective tissue growth factor (CTGF) and transforming growth factor-β (TGF-β). This in turn contributes to the production of extracellular matrix (ECM) proteins (i.e. collagen, fibronectin, proteoglycans) in the heart causing left ventricle remodelling, fibrosis and stiffness which impairs cardiac relaxation (179).

Besides NF-κβ, RAAS plays a role in the inflammatory process (184). Hyperglycemia in the diabetic heart increases tissue angiotensin II via p38-MAPK/ PPAR pathways which stimulate inflammation, endothelial damage, oxidative stress, vascular remodelling, vasoconstriction and myocardial hypertrophy (180, 185), all characteristics of DCM.

1.2.5 Calcium handling

Calcium and calcium handling are essential for excitation-contraction coupling of the cardiomyocytes. In physiological conditions, depolarization of the cardiomyocytes causes the opening of the voltage-dependent L-type Ca^{2+} channels resulting in a Ca^{2+} influx. Subsequently, Ca^{2+} - induced Ca^{2+} release mechanism (CICR) leads to more Ca^{2+} release from the sarcoplasmic reticulum (SR) by activation of the RyR. The increased intracellular Ca^{2+} concentration will ultimately activate the contractile machinery. During diastole, intracellular Ca^{2+} decreases due to the sodium-calcium exchanger (NCX) and sarcolemmal Ca^{2+}-ATPase (SERCA2a) resulting in cardiomyocyte relaxation (186-189). However, in diabetes, Ca-homeostasis is altered. This is attributed to the combination of the decreased sensitivity of contractile proteins to Ca^{2+} (190, 191) and the alterations in SERCA2a and NCX function or expression (190, 191). Impaired Ca^{2+} reuptake will lead to prolonged action potential duration and thus a slower diastolic relaxation (109). Defects in the SERCA2a will result in different alterations such as altered SR Ca^{2+} load, prolongation of the intracellular Ca^{2+} decay, accumulation of both free radicals and long-chain acyl carnitines, cardiac myosin heavy chain alterations, phosphorylation of troponin I, and changes in regulatory and contractile proteins (187, 192, 193). Accumulation of those can increase oxidative stress, leading in turn to alterations in SERCA2a and NCX (190). Altered calcium handling, together with oxidative stress and endoplasmic reticulum (ER) stress, can promote the cardiomyocytes to induce cell death via necrosis, apoptosis and autophagy (109).

1.2.6 Mitochondrial dysfunction

Several studies suggest that mitochondrial dysfunction, together with increased oxidative stress, is an important factor in DCM (188, 194-196). Injured mitochondria are considered the primary source of intracellular ROS in the diabetic

heart (197-200). Autophagy, the process in which long-living and injured organelles and proteins are degraded and recycled, is diminished in animal models of T2DM, further suggesting its contribution in the development of diabetic cardiomyopathy (201-204). Obesity and overfeeding can activate the mTOR-pathway, resulting in phosphorylation of the serine-threonine-protein kinase ULK1 and thus inhibiting the activation (205). Phosphorylation of ULK1 alters the relation between ULK1 and adenosine monophosphate-activated protein kinase (AMPK), critical for its function in autophagy and mitochondrial homeostasis (205-208). AMPK is also involved in GLUT4 translocation, GLUT4-independent glucose uptake and glycogen synthase phosphorylation (209-212). Sirtuin 1 (SIRT1) is one of the regulators of AMPK and PPAR-γ coactivator-1α (PGC-1α) and is involved in inflammatory processes and metabolic homeostasis (213). AMPK can improve mitochondrial biogenesis via activation of the peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) pathway (106). PGC-1α controls multiple cellular processes, such as mitochondrial biogenesis and respiration, gluconeogenesis and glucose transport, fatty acid oxidation, glycogenolysis, peroxisomal remodelling, muscle fibre-type switching and oxidative phosphorylation (214). In advanced stages of DCM, PGC-1α and AMPK signalling pathways involved in β-oxidation are impaired, again progressing mitochondrial dysfunction (106, 109). Hyperlipidemia, as previously described, can lead to apoptosis of the cardiomyocyte due to ROS production and ER stress (215). Nutrient overflow, as seen in diabetes, causes electron transfer to oxygen without ATP production, increasing ROS, leading to oxidative stress and damage within the mitochondria (216). Furthermore, mitochondria are associated with the ER and its release of Ca^{2+} via inositol triphosphate. Calcium handling is also affected by mitochondria, with an excess of Ca^{2+} uptake resulting in Ca^{2+} overload and mitochondrial permeability transition pore opening (217).

1.2.7 Oxidative stress in the diabetic heart

Oxidative stress and ROS are increased in T2DM and play a significant role in diabetic cardiomyopathy. Hyperglycemia leads to elevations in ROS synthesis in the diabetic heart (198). Furthermore, there are multiple sources for ROS in the diabetic heart, as the AGE-RAGE interaction, activation of PKC, mitochondrial electron transport chain leakage, activity of NADPH oxidase, lipoxygenase (LOX)

enzymes and uncoupling of NO synthase (218). ROS, together with PKC activation cause upregulation of NF-κβ, increasing iNOS expression and eventually resulting in elevated NO production (219-221). When NO reacts with superoxide anion radical ($O_2\cdot$), generation of peroxynitrite can occur which in turn can form 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and more ROS generation, cytochrome c leakage and apoptosis (220). Oxidative stress, ROS generation and mitochondrial dysfunction are associated with each other, with cardiac generated ROS causing further damage to mitochondria and altered mitochondrial DNA (222), with the latter leading to damaged synthesis of mitochondrial proteins resulting in turn in more ROS (220). Another pathway implicated in oxidative stress is with the redox-sensitive kinases extracellular signal-regulated protein kinase (ERK)1, ERK2 and JNK. Phosphorylation of these kinases is suggested to be involved in downstream signalling in the events of oxidative stress. ERK1/2 phosphorylation is showed to be increased in left ventricle hypertrophy, with increased phosphorylation correlating with more severe left ventricle hypertrophy (223). The activation of NF-κβ is involved in many pro-fibrotic, pro-inflammatory and pro-hypertrophic genes. As already discussed above, inflammation plays a crucial role in diabetic cardiomyopathy and is a possible mediator of ROS-induced fibrosis and hypertrophy (224, 225). Studies of Nakamura et al. and Suzuki et al. showed that treatment with anti-oxidants abolished partially the harmful effects of oxidative stress, supporting the hypothesis that at least oxidative stress is partially responsible for fibrosis and hypertrophy (219, 226).

1.2.8 The renin-angiotensin-aldosterone system

RAAS plays a vital role in the development of DCM, as it contributes to atherosclerosis, cardiomyocyte loss and myocardial fibrosis (227-231). In early phases of diabetes, plasma renin activity is increased, together with the mean arterial pressure and renal vascular resistance emphasizing the involvement of RAAS upregulation in diabetes (232). Hyperglycemia can promote the production of Ang II in cardiomyocytes, cardiac fibroblast and endothelial cells, both in animal and human diabetic hearts (233-236). An increase in Ang II makes cardiomyocytes more susceptible for apoptosis as seen by Fiordaliso et al (237). Hyperglycemia can stimulate the RAAS by increased intracellular Ang II, which could cause oxidative stress and apoptosis, together with an elevated RAAS

expression (238). Secondly, increased glucose concentrations can promote tissue response to Ang II and the other way around. In vascular smooth muscle cells, Ang II can activate glucose-induced transcription factors, while the contractile aortic response to Ang II can be influenced by hyperglycemia (239, 240). Hyperglycemia is also able to promote aldosterone secretion by elevated Ang II levels. Aldosterone, like Ang II, can induce oxidative stress, fibrosis and apoptosis suggesting a role for aldosterone in diabetic cardiomyopathy (241). AGEs also promote the AngII/ Angiotensin II receptor type 1 (AT1R) pathway by upregulation of AT1R expression (242-245). By promoting Ang II expression and decrease in Ang 1 – 7, an imbalance occurs in RAAS induced by downregulation of angiotensin-converting enzyme 2 (ACE2), as seen in diabetes (246). Recently, the increased pro-renin levels in diabetic patients have been unravelled as an exciting target. High levels of pro-renin are associated with an increased risk of microvascular complications in diabetes. Pro-renin can bind to the (pro)renin receptor ((P)RR), generating Ang I and stimulating its intracellular signalling. A study of Connely et al. showed that in diabetic cardiomyopathy, a 3-fold increase in (P)RR expression is observed (247).

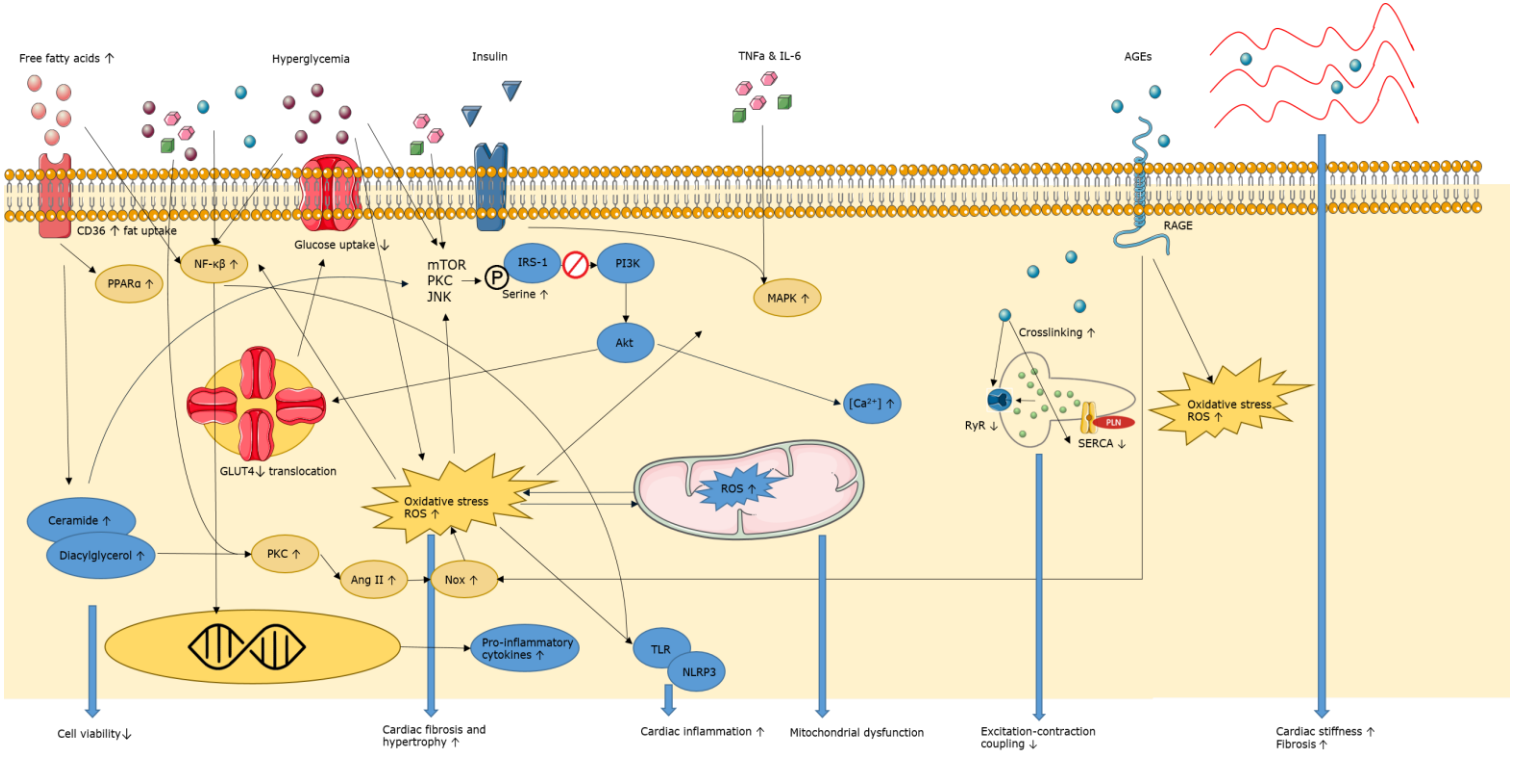

Figure 2: Overview of mechanisms in diabetic cardiomyopathy. As shown in the figure, DCM pathophysiology is complex as many factors interact with each other. PPARα, peroxisome proliferator activated receptor alpha; CD36, cluster of differentiation 36; NF-κβ, nuclear factor kappa-light-chain-enhancer of activated B cells; GLUT4, glucose transporter 4; PKC, protein kinase C; Ang II, Angiotensin II; mTOR, mammalian target of rapamycin; JNK, C-Jun N-terminal kinase; Nox, nicotinamide adenine dinucleotide phosphate oxidase; IRS-1, insulin receptor substrate 1; PI3K, phosphatidylinositol 3-kinase; TLR, Toll-like receptor; MAPK, mitogen-activated protein kinase; ROS, reactive oxygen species; RyR, ryanodine receptor; SERCA, Sarco/endoplasmic reticulum Ca2+-ATPase; PLN, phospholamban; AGE, advanced glycated end-product; RAGE, receptor for advanced glycated end-product

1.3 Exercise as a therapy for diabetic patients

Exercise is an effective, low-cost and safe strategy for the prevention and treatment of cardiovascular diseases (CVD), with the scientific evidence of the beneficial effects being indisputable (248). The risks of exercise therapy in most adults are by far outweighed by the benefits of exercise (249-251). Therapy in T2DM patients is designed to maintain optimal blood glucose, lipid and blood pressure levels and prevent diabetic complications (252). Exercise affects T2DM and obesity, diseases associated with diet and a sedentary lifestyle, by improving both cardiovascular health and metabolic control (253). While obesity and hypertension frequently occur in T2DM patients and are well-known risk factors, studies have shown that cardiorespiratory fitness is a more powerful predictor of CVD (253). Prevention of CVD in T2DM related to exercise is thought to be caused via improved vascular function and hereby related tissue perfusion (254). The beneficial effects of moderate endurance intensity training (MIT) in normal as well as in pathological situations are often described (255). Currently, adding physical exercise therapy as a prescribed medication is reviewed by the Food and Drug Administration (FDA) in the United States of America (USA). However, to be approved as prescriptive medicine, some factors have to be revised (efficacy, efficiency, dosing, and safety).

Most common types of exercise are aerobic exercise training, anaerobic exercise training and resistance training (256). In general, the effect of exercise in diabetes has been tested using aerobic exercise (257). In aerobic training, large muscle groups are involved doing dynamic activities resulting in an increased heart rate and energy expenditure, for example running, walking or cycling. Current American Diabetes Association (ADA) guidelines state that individual sessions of aerobic activities should be performed at least 30 minutes per day for at least three days per week, with no more than two consecutive days without exercise (258). Aerobic exercise will improve the cardiovascular system and skeletal muscles, increase endurance performances and patients' quality of life (256, 259). Furthermore, aerobic exercise improves HbA_{1C} levels in diabetic patients and reduces fasting plasma glucose, insulin resistance, fasting insulin levels and systolic blood pressure in T2DM patients (258, 260-266). Anaerobic exercise training on the other hand is exercise performed at very high-intensity levels using

the glycolytic and phosphagens pathways with glycogen and phosphocreatine stores as energy sources (256).

In the last 20 years, resistance training is gaining more interest as a therapy and training option in T2DM patients. In resistance training, a person moves using free weights, weight machines, elastic resistance bands or performs body weight exercises. Improvements are observed in diabetic patients in strength, bone mineral density, blood pressure, lipid profiles, muscle mass, insulin sensitivity, HbA_{1C}, glucose responses and cardiovascular health (258, 267-269). These results indicate the effectiveness of resistance training, and their potential to be included in the diabetes care plan. It is suggested that through resistance training, increased skeletal muscle mass improves glycemic control by enhanced muscle glycogen storage (269, 270). Therefore, guidelines were updated to include resistance training for treatment and prevention of T2DM (271). A combination of both aerobic and resistance training could be the preferable exercise program in T2DM for controlling glucose and lipid levels. Many studies investigated the effects of combination programs, showing the additive effect of combination training in T2DM. Indeed, insulin sensitivity, HbA_{1C}, fasting glucose levels, postprandial glucose levels and fasting insulin levels were all improved after combination training (272-275).

1.4 Exercise in cardiovascular diseases

Historically, heart failure patients were advised to avoid physical activity in order to prevent exercise-induced events and hemodynamic overload of the affected ventricle (276, 277). However, in 1988, Sullivan et al. showed that heart failure (HF) patients experience beneficial effects on exercise tolerance by exercise training (278). These findings were the cornerstone of research on exercise training and heart failure. It is shown that exercise training in HF patients protects against cardiac mortality, with even small exercise being beneficial for survival rates after cardiac injury (279). Nowadays, exercise is added as a therapy in HF patients, stated by the European Society of Cardiology (ESC) HF guidelines that 'patients with HF are recommended to perform properly designed exercise training' (280, 281). However, only 10% of HF patients receive a proper cardiac rehabilitation program after hospitalisation for HF, despite their proven advantages (282). The effects of exercise on disease are modulated by multiple

organ systems such as the heart, skeletal muscle, vascular functions, respiratory functions and neurohormonal systems (278, 283-286). Importantly, a distinction is being made in the HF spectrum between HF with preserved ejection fraction (HFpEF) and HF with reduced ejection fraction (HFrEF) (287-289). In DCM, diastolic dysfunction with normal ejection fraction (EF) is present at the early stage of the disease (11). This early onset of DCM often progresses to HF with normal ejection fraction and eventually to systolic dysfunction with reduced ejection fraction (290). The outcome of clinical comorbidities between HFpEF and HFrEF also differs, such as with diabetes mellitus. DM leads to worse clinical outcomes in HFpEF than in HFrEF according to a study of MacDonald et al (291). Several studies imply that HFpEF and HFrEF are different phenotypes of DCM, with different outcomes in DM (292, 293). The mechanisms in both phenotypes are variable, with hyperglycaemia, lipotoxicity and insulin resistance being important in HFpEF phenotype. In the HFrEF phenotypes however autoimmunity is relevant. In both phenotypes AGE deposition and microvascular rarefaction are playing a role (292, 293). Thus, DM therapy should be evaluated specifically in HFpEF and HFrEF patient populations. In patients with preserved EF, both cardiorespiratory fitness and quality of life are improved after exercise training (294). Furthermore, in a study by Edelmann et al., exercise training improved diastolic function in HFpEF patients (295). A meta-analysis by Pearson et al. confirmed these data, observing a significant post-training effect on diastolic function. However, the subject remains controversial as other meta-analysis cannot confirm these findings (294, 296). Furthermore, in HF patients with preserved EF, exercise did not alter systolic function (296). In HFrEF, exercise has been shown to improve maximal cardiac output, reverse left ventricular remodelling, improve EF and decrease left ventricle end-diastolic diameter (285, 286, 297). Moreover, resting blood pressure is reduced, with improved endothelial function and afterload reduction and direct effects are visible at myocardial hypertrophy and fibrosis (285, 298-301). In HFpEF rats, an improvement in endothelial dysfunction was observed after exercise intervention, as both endothelial nitric oxide synthase (eNOS) expression and endothelial-dependent vasodilatation were recovered after exercise (302). However, endothelial function was not altered in a study of Kitzman et al (303). Inflammation and inflammatory cytokines as IL-6, IL-11β and TNF-α are described to be upregulated in HF patients (304-311). In patients

with reduced EF, exercise training was able to exert a positive effect on cytokine levels (312-316). In HFpEF, exercise training was reported to increase ghrelin, but no effect was observed in other inflammatory parameters in a study of Trippel et al (317). In short, exercise effects on inflammation in diabetes remain controversial (318-331).

Altogether, data combining exercise and cardiac function further suggest the possibilities of exercise as an adjuvant therapy in T2DM patients and DCM, but require further research in specific populations, such as diabetes and HFpEF.

1.5 Exercise modalities

Nowadays, different exercise modalities are available. However, to ensure the best treatment for each patient, the optimal training modality/intensity/duration/frequency has to be selected. Aerobic exercise modalities can vary from activities requiring minimal skill and physical fitness (e.g. walking) to activities requiring both skill and physical fitness levels to perform the preferred exercise (e.g. swimming). At the basic level, frequency, duration and intensity can be defined as the exercise dose. Frequency and duration are parameters reflecting the total amount of time invested in the exercise program. Duration is the amount of time spent in a single exercise session, mostly minutes or hours. Frequency of exercise reflects the amount of exercise over a period of time (per week/month). Lastly, the exercise intensity is a variable parameter. The intensity of exercise can be quantified using metabolic equivalents (METs) or using kilocalories (kcal) burned per unit of time. However, using absolute values, no distinction can be made in variability of fitness across individuals. Alternatively, one could examine relative exercise intensity determined by maximal physiologic parameters, such as heart rate and oxygen consumption. To use exercise prescription in clinical practice, relative exercise intensity measurements are preferable, as they are adapted to the individual capacities of the patient (332). The applied exercise intensity can vary from very light exercise training to maximal or sub-maximal intensities (251).

At one end of the exercise intensity continuum, **sedentary activity** is located. Activities comprising sedentary behaviour refer to those equal to an energy expenditure of maximum 1.5 METs, such as riding a car or sitting down (333,

334). Sedentary activities are often replacing **non-exercise physical activities (NEPAs)**, activities performed at low intensity not perceived as exercise (334). High amounts of sedentary time are increasing the risk of developing metabolic diseases, such as T2DM (83). Moreover, the risk of developing cardiovascular disease and all-cause mortality is associated with a sedentary lifestyle (335, 336). Watching TV is a common cause of long sedentary time with non-contractive or inactive muscles. Prolonged sitting time during life appears to be associated with diabetes development, independent of BMI (83, 337, 338). The ADA encourages diabetic patients to reduce their sedentary time and to limit their sitting time to no more than 90 minutes (339). Replacing sedentary time with a large amount of non-exercise physical activity can exert beneficial effects at least similar to exercise. A study of Duvivier et al. reported that an increased NEPA-sedentary time ratio was more effective than exercise in reducing non-HDL cholesterol, postprandial insulin and plasma triglycerides (340). Furthermore, non-exercise activity thermogenesis (NEAT) scores – the energy expenditure of all physical activities other than volitional sporting-like exercise – were negatively correlated with insulin levels, waist circumference, both systolic and diastolic blood pressures, while a positive correlation was shown with HDL-C (341). In general, when an exercise program is prescribed, NEPA does not change during exercise training. However, exercise session duration can influence NEPA negatively increasing sedentary time, implicating that shorter exercise sessions could have less influence on physical activity outside of the exercise than when longer exercise bouts are implemented (342). Reductions in NEPA are often associated with less than expected weight loss during an exercise program, however these changes can occur in some but not all individuals following an exercise program (342, 343). These data suggest that when prescribing exercise, effects on NEPA time should be taken into account as in some patients, but not all, a reduction could be observed (342, 344, 345).

Low-intensity physical activity (LIPA) can be described as exercise performed until 3.0 METs, or at maximal heart rate (HR_{max}) of 63% (249). This exercise intensity is a major determinant of the total daily energy expenditure, and is inversely associated with the sedentary time as described above (346). Therefore, the recommendation to increase LIPA to counteract on sedentary behaviour and its deleterious effects on health outcomes should be supported (346, 347).

Moreover, according to the study of Balducci et al., low levels of LIPA are negatively associated with age, duration of diabetes, HbA_{1C}, FPG, BMI, fat-mass, inflammatory parameters and waist circumference (346). Even limited changes in LIPA already may provide beneficial effects on metabolic and cardiovascular risk factors in T2DM patients (346, 348-351). In a study performed by Aune et al., both walking and low-intensity activity was associated with a reduced relative risk of T2DM (79, 352-361). Furthermore, LIPA is capable of improving the metabolic and cardiovascular profile in T2DM patients (362).

Most commonly, **moderate-intensity physical activity (MIPA)** or MIT is used as prescribed exercise training. MIPA is performed at 55 – 70 % of HR_{max} or between 3 to 6 METs (333). According to the American Diabetes Association, adults with diabetes should perform at least 150 min of moderate- to vigorous-intensity activity weekly, on at least 3 or more days, with no more than two consecutive sedentary days (363). Endurance or cardiorespiratory exercise training has been extensively investigated in T2DM, showing the beneficial effects of this type of exercise in the patient population. MIPA has been associated with reduced risks of diabetic microvascular complications and all-cause mortality in the diabetic patient population (364, 365). Moreover, a meta-analysis performed by Boulé et al. demonstrated improvements in both cardiorespiratory fitness and HbA_{1C} after 20 weeks of moderate intensity exercise (261). Furthermore, a recent meta-analysis performed by De Nardi et al. showed that MIPA improved maximal oxygen consumption (VO_2max), fasting glucose, HbA_{1C}, homeostatic model assessment (HOMA) values, blood pressures, lipid profile (total cholesterol, HDL, LDL, triglycerides), BMI, waist-to-hip ratio and waist circumference in diabetic and prediabetic patients (366).

Furthermore, higher than moderate intensities are used nowadays, such as vigorous, near maximal, maximal and even supramaximal intensities. These intensities start at 6 METs, or 70 % of HR_{max}, and end at 'all-out' efforts.

Currently, **high-intensity interval training** or **HIIT** is gaining more and more interest. HIIT consists of short bouts of relatively high-intensity exercise alternated with recovery periods of low-intensity exercise or rest (367). High-intensity physical activity (HIPA) is generally performed at near maximal levels, above 90% of HR_{max}, or requires ≥9 METs (333). This kind of training can be

defined as anaerobic exercise, in which an intense physical activity is performed for a very short period of time, fuelled by the energy sources of the contracting muscles independently of inhaled oxygen (249). The cells therefore use the glycolysis and fermentation to provide themselves with the necessary ATP.

In the last decades, numerous reports indicate that HIIT could be more effective than MIT in patients with cardiometabolic diseases to improve their aerobic fitness (368-372). Moreover, bouts of higher intensity exercise induce physiological stimulus and adaptations greater than those observed with moderate intensity exercise (373). As lack of time is considered one of the barriers of adherence to physical activity, HIIT could be a potential option of short duration to encourage exercise and thus reduce the risks of developing chronic cardiovascular and metabolic diseases (366, 374, 375). As HIIT is perceived as more enjoyable than MIT, long-term adherence to HIIT possibly is higher compared to MIT (376). Typically, a HIIT program has a ratio of 1 min high-intensity exercise followed by 1 min of low-intensity exercise (377). Epidemiological and experimental data suggest that HIIT might provide additional benefits and be more potent than the classical MIT modality (378). In healthy subjects, sparse data suggest that the potential of HIIT in comparison to MIT to improve cardiac function is most likely to be attributed to a higher mitochondrial fatty acid oxidation and metabolism (379). A meta-analysis performed by Weston et al. concluded that in patients with lifestyle-induced cardiometabolic diseases, HIIT was more effective in improving the VO_2max compared with MIT trained patients (380), with other studies confirming these data (381-383). HIIT also improves systolic blood pressure (SBP), HbA_{1C}, HDL-C, malondialdehyde (MDA) levels, NO and von Willebrand factor (vWF) in T2DM patients (384). Furthermore, HIIT improves skeletal muscle oxidative capacity, glycemic control and insulin sensitivity in T2DM patients (385, 386). Additionally, HIIT seems to be more potent than MIT in reducing cardiovascular risk factors (377, 387, 388).

Overall, HIIT seems superior in improving vascular functions compared with MIT and a good time-efficient alternative approach for T2DM patients.

2

Objectives and general outline

2 Objectives and general outline

This PhD project includes three original research papers, combined with two systematic reviews, presented schematically in Figure 3. All studies presented in this manuscript are published, submitted or are ready for submission in peer-reviewed international journals. All the performed research fit in the research objectives and the main topic of this PhD, namely discovering the effect of two different exercise modalities (MIT vs HIIT) on diabetic cardiomyopathy. First, I developed an animal model that mimics the phenotype of the development of T2DM and DCM. Simultaneously, the second objective was to identify the nature of remodelling in the heart induced by the two exercise modalities in healthy animals. Hereafter, I combined my findings and investigated the effect of training modalities in the clinically diabetic rat model developed previously. Lastly, I report the known effects of detraining in diabetes in a systematic review.

The first objective (**Chapter 3**) of this PhD-project was to validate an animal model of T2DM and DCM. To date, many genetic models (e.g. ob/ob, db/db mice, Zucker diabetic fatty, Goto-Kakizaki and Otsuka Long Evans Tokushima fatty rats) have been used to understand the underlying mechanisms involved in disturbed energy homeostasis in DCM (389). However, despite shared common traits with human DCM features, none of these models reflects the human phenotype entirely. To avoid potential problems related to altered leptin signalling observed in the genetically modified rodents, many researchers have used diet-induced obesity and diabetes (389). Traditionally, these diets consist of an exchange of carbohydrate-derived calories with fat-derived calories (i.e. high fat diet) and have been extensively used to study DCM. The current obesity pandemic in Western countries however is associated with an increased sugar intake rather than an increase in fat intake (390). Therefore, using a diet based on high sugar rather than high fat, a so-called "cafeteria diet" or "Western diet" provides a highly relevant model to study DCM. It has been shown that rats undergoing the "cafeteria diet" display many features of DCM, including elevated glucose, insulin and non-esterified fatty acids accompanied with decreased glucose and insulin tolerance (166, 391). In addition, in this model, chronic inflammation of the liver and adipose tissues, typically seen in patients suffering from the syndrome, were observed (391, 392). Such an alternative model was used to mimic T2DM and

DCM in this project. Validation and characterisation of this model was the first objective of this PhD.

The second objective of this project (**Chapter 4**) was to identify the nature of the changes induced by physiological remodelling after 2 types of exercise on the healthy heart. MIT or HIIT was performed in healthy rats to investigate the similarities and differences between both exercise interventions on cardiac function and vascularity and to identify which of the two training modalities could be an added value later on in an unhealthy situation. While some studies demonstrate that MIT improves cardiac contractility, mainly due to improved myocardial perfusion and improved nitric oxide sensitivity, others report minimal changes. At the cellular level, studies with rodents undergoing MIT demonstrate an increase in LV cardiomyocyte length and cell surface, not accompanied by myocardial collagen accumulation. Data suggest that HIIT is overall better in improving micro- and macrovascular functions compared with MIT.

In **Chapter 6**, I combined the knowledge and results obtained in the previous studies in order to investigate the effect of MIT and HIIT in our animal model of DCM. Beforehand, a systematic literature search was performed to identify and analyse published data on the exercise effect on cardiac function in T2DM and DCM (**Chapter 5**).

At last, a review was written to report – in a systematic manner - the effects of detraining in diabetes (**Chapter 7**). Detraining is defined as the partial or complete loss of training-induced metabolic, respiratory and cardiovascular adaptations, in response to an insufficient training stimulus. The majority of patients will gradually or suddenly stop exercising, showing the urge to discover the possible jeopardies of detraining in diabetes and paving the road for future studies regarding the subject.

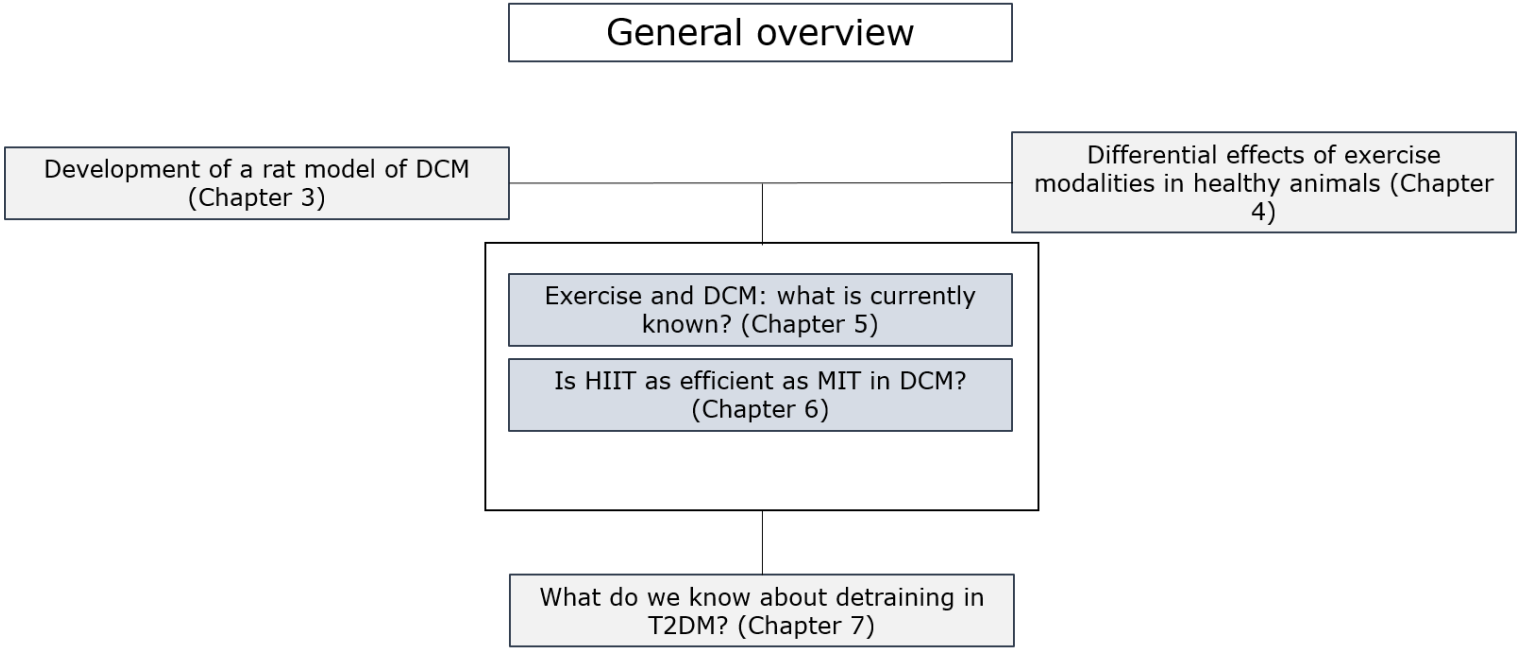

Figure 3: Schematic presentation of my PhD project.

3

Western diet given to healthy rats mimics the human phenotype of diabetic cardiomyopathy

Based on:

Western diet given to healthy rats mimics the human phenotype of diabetic cardiomyopathy

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3 Western diet given to healthy rats mimics the human phenotype of diabetic cardiomyopathy

3.1 Abstract

Diabetes mellitus (DM) is a major problem worldwide. Within this patient group, cardiovascular diseases are the biggest cause of morbidity and mortality. Diabetic cardiomyopathy (DCM) is defined as diabetes-associated structural and functional changes in the myocardium, not directly attributable to other confounding factors such as coronary artery disease or hypertension. Pathophysiology of DCM remains unclear due to a lack of adequate animal models reflecting the current pandemic of diabetes, associated with a high increased sugar intake and the 'Western' lifestyle. The aim of this study was to develop an animal model mimicking this 'Western' lifestyle causing a human-like phenotype of DCM. Twenty-four Sprague–Dawley rats were randomly assigned into a normal or a 'Western' diet group for 18 weeks. Glucose and insulin levels were measured with an OGTT. Heart function was assessed by echocardiography and hemodynamic measurements in vivo. Cardiac fibrosis and inflammation were investigated in vitro. 'Western' diet given to healthy rats for 18 weeks induced hyperglycemia together with increased AGEs levels, insulin levels and hypertriglyceridemia. Heart function was altered with increased end-diastolic pressure, left ventricle hypertrophy. Changes in vivo were associated with increased collagen deposition and increased PAI-1 levels in the heart. High-sugar diet or 'Western' diet causes T2DM and the hallmarks of DCM in rats, reflecting the phenotype of the disease seen in patients. Using this new model of T2DM with DCM might open new insight in understanding the pathophysiology of DCM and on a long term, test targeted therapies for T2DM with DCM patients.

3.2 Background

Diabetes mellitus (DM) is a major chronic disease directly resulting from the so-called 'Western diet/lifestyle'. It affects approximately 300 million people worldwide, with an alarming increasing incidence. Estimations indicate that in 2030 about 450 million people will suffer from DM (93), which is an important risk factor for developing heart failure (393). In diabetes, progressive structural and functional remodelling leads to gradual cardiac function impairment and ultimately compromises life expectancy. Diabetic cardiomyopathy (DCM) is defined as diabetes-associated structural and functional changes in the myocardium, not directly attributable to other confounding factors such as coronary artery disease or hypertension (188, 394). In many cases, diabetes can lead to DCM, with left ventricular (LV) hypertrophy and diastolic dysfunction as early markers of cardiac dysfunction (187, 395) characterised in the clinic by an impaired relaxation, a decreased diastolic chamber compliance and increased end-diastolic pressure (396). DCM is characterised by concentric hypertrophy, extracellular fibrosis, alterations at the cardiomyocyte level and changes in metabolism, leading to impaired cardiac output (94). Despite a lot of progress in understanding DCM aetiology during the last few decades, molecular mechanisms involved are complex and multifactorial and remain incompletely understood (95, 394). It is currently speculated that oxidative stress, fibrosis, apoptosis, impaired autophagy, inflammation and impaired calcium handling are contributing factors to the development of DCM (91, 95, 106, 397). However, underlying pathophysiological mechanisms remain unclear due to a lack of adequate animal models mimicking the 'Western lifestyle' and human phenotype.

Currently, a variety of animal models examining underlying mechanisms of type 2 diabetes mellitus (T2DM) and heart failure (HF) are available (389, 398, 399). Despite some common traits, none of the available animal models fully mimic the human phenotype of DCM, comprising obesity, polyphagia, altered lipid profile, altered cardiac function and inflammation. These are however essential as they are all consequences of the Western diet containing high sugar and high fat. The urge to develop a representative model reflecting this phenotype is then mandatory to further unravel mechanisms, treat and prevent developing stages of diabetes and DCM.

A high intake of saturated fatty acids, high total fat intake and insufficient consumption of dietary fibres are risk factors for developing obesity and T2DM (400-402). In addition, recently, it has been demonstrated that sugar-sweetened beverages, common in our Western diet, are associated with an increased risk of developing T2DM and increase the likelihood for obesity (73, 78, 400, 403).

The aim of this study was therefore to develop an animal model that combines features observed in the human phenotype of T2DM with DCM using a high-sugar diet, or so-called Western diet.

3.3 Research design and methods

This investigation conformed to the EU Directive 2010/63/EU for animal experiments and was approved by the local ethical committee (Ethical committee for animal experimentation, UHasselt, Belgium, ID 201554).

3.3.1 Study protocol

Twenty-four male Sprague–Dawley rats (Charles River Laboratories, L'Arbresle, France), weighing 200–225 g, were randomly assigned in two experimental groups by using a randomisation tool (excel). Group 1 (n=12) received a normal rodent diet (ENVIGO, The Netherlands, Control diet) throughout the study and served as a control group. Group 2 (n=12) was fed a high-sugar and high-fat diet (Western diet) with underlying distribution (total fat=20%, sugars=61%, proteins=19%) comparable with a fast-food meal including sugar-sweetened beverage and dessert (391). All animals were housed per 3 in a cage and maintained in a controlled environmental condition and had water and food available ad libitum. Every morning, rats were provided freshly prepared food (Western or control diet). Daily food intake was calculated by weighing the residual food the next morning. After 18 weeks and an overnight fast, an OGTT was performed. Blood samples, hemodynamic and echocardiographic measurements were executed prior to sacrifice of the animals, 18 weeks after the start of the diet. A power analysis using G*Power was performed to determine sample size.

3.3.2 Conventional echocardiographic measurements

As described previously (133), transthoracic echocardiography parameters were assessed 18 weeks after the start of the diet with a Vividi ultrasound machine (GE Vingmed Ultrasound) using a 10 MHz linear array transducer under 2% isoflurane anaesthesia. Briefly, a standard parasternal long axis image and short axis views at the mid-ventricular level were obtained at a temporal resolution of approximately 200 frames per second. Conventional echocardiographic parameters (e.g., LV end-diastolic diameter (LVEDD), LV end-systolic diameter (LVESD), anterior wall thicknesses (AWT)) were obtained from the B-mode images at midpapillary level in the parasternal short-axis view. Left-ventricular end-

systolic volumes (LVESV) and end-diastolic volumes (LVEDV) were calculated by π*DM2*B/6, where DM indicates the systolic/diastolic diameter of the ventricle in mid-ventricular short-axis view and B is LV length on parasternal long-axis image. Subsequently, ejection fraction (EF) was measured as (EDV–ESV)/EDV, and expressed in %. Analysis was done single blinded by coding of the animals.

3.3.3 Hemodynamic measurements

Pressure measurements were performed in all animals with the use of an SPR-320 MikroTip high-fidelity pressure transducer (Millar) that was advanced into the left ventricle via the right carotid artery. The pressure catheter (2F) was connected to a quad-bridge amplifier and PowerLab 26 T module (AD Instruments, United Kingdom) was used to transfer the data to LabChart v7.3.7 software (AD Instruments, United Kingdom).

3.3.4 Oral glucose tolerance test and insulin resistance assessment

Glucose tolerance was assessed at baseline, 6 weeks, 12 weeks and 18 weeks (i.e., the time of sacrifice), with a 1 h OGTT as described by Stevens et al (404). After an overnight fasting, glucose (2 g/kg) was administered via gastric gavage. Prior to glucose administration, blood glucose concentration was determined from capillary tail blood collection with Analox GM7 (Analis SA, Namur, Belgium), and repeated 15, 30 and 60, minutes after administration. Glucose response was expressed as total area under the curve (AUC). At baseline and after 60', serum insulin concentrations were measured by electrochemiluminescence (Meso Scale, Gaithersburg, MD).

3.3.5 Lipid profile determination

Triglycerides, total cholesterol and HDL cholesterol (HDLC) were determined in the Ziekenhuis Oost-Limburg (Genk, Belgium) using Roche/Hitachi cobas c systems (Rotkreuz, Switzerland). Quantification of the NEFA was assessed by colourimetry using a NEFA quantification assay kit (Abcam, ab65341, Cambridge, United Kingdom).

3.3.6 Fibrosis measurement

Transversal sections of 7 µm thick were obtained at the midventricular level of the left ventricle and stained using the Sirius Red/Fast Green kit (Chondrex). Fibrosis was assessed in all animals in 3–4 randomly chosen fields per section. The area of collagen deposition indicated by red staining was outlined and quantified using an automated image analysis program (Carl Zeiss, AxoVision 4.6, Zaventem, Belgium). Blood vessels were excluded. Total collagen deposition to the global cardiac area was calculated, normalised to total surface area and expressed as %.

3.3.7 Western blot

Protein concentrations of the LV tissues were determined by the BCA protein assay kit (Thermo Fisher, Erembodegem, Belgium). Western blot was performed as previously described (133). Briefly, equal amounts of proteins (15 µg) were separated on a 12% SDS-PAGE gel with a mini protean 3 electrophoresis system (Bio-rad Laboratories, Temse, Belgium), transferred to a polyvinylidene fluoride (PVDF) membrane and subsequently, blocked for 2 h with 5% milk in Tris-buffered solution containing 0.1% Tween-20 (TBS-T) followed by incubation overnight at 4 °C in the presence of a specific lysyl oxidase (LOX) antibody (1/1000, rabbit polyclonal IgG, Abcam, ab 31,238, Cambridge, United Kingdom), plasminogen activator inhibitor-1 (PAI-1) antibody (1/200, Abcam, ab66705, Cambridge, United Kingdom) or TNF-α antibody (1/1000, goat polyclonal IgG, Santa Cruz, N-19, Heidelberg, Germany). Horseradish peroxidase-conjugated secondary antibodies (DAKO, Belgium) at a dilution of 1/2000 were used. Both primary and secondary antibodies were diluted in 5% milk-TBS-T, except for PAI-1 which was in 5% BSA. Visualisation was performed with the enhanced chemiluminescence (ECL) technique using the Pierce ECL Plus Western Blotting Substrate (Thermo Fisher, Erembodegem, Belgium).

3.3.8 Real-time PCR

Total RNA was extracted using RNeasy fibrous tissue kit (Qiagen, Antwerpen, Belgium) following the manufacturer's guidelines. The concentration and purity of the RNA were assessed using a NanoDrop 2000 spectrophotometer (Isogen Life Science, Temse, Belgium). Synthetisation of cDNA was done by using a QuantiNova Reverse Transcription kit (Qiagen, Antwerpen, Belgium). The expression of TNF-α (forward primer: GTC-TGT-CC-TCA-GCC-TCT-TC, reverse

primer: CCC-ATT-TGG-GAA-CTT-CTC-CT) was evaluated. Primers were designed in the coding sequence of the mRNA. Real-time PCR was carried out in an optical 96-well plate using the StepOnePlus (Applied Biosystems, Belgium). SYBR Green (Invitrogen, Merelbeke, Belgium) chemistry-based qPCR was performed. Gene expression data were analysed with MIQE guidelines taken into account. Normalisation of the data with reference genes was performed using qBase software (Biogazelle, Zwijnaarde, Belgium).

3.3.9 AGEs determination

Plasma advanced glycated end-products (AGEs) were determined with an OxiSelect Advanced Glycation End Product (AGE) Competitive ELISA kit (Cell Biolabs, San Diego, CA, USA). Samples were added to the AGE conjugate pre-absorbed ELISA plate. Hereafter, wells were incubated with a diluted anti-AGE antibody and an HRP-conjugated secondary antibody was added. Absorbance was measured at 450 nm.

3.3.10 Statistical analysis

Results were tested for normality prior to statistical tests. Accordingly, parametric test (t-test) or non-parametric test (Mann–Whitney U test) were performed comparing results of both groups. Data are presented as mean±S.E.M. or median [75th percentile; 25th percentile] accordingly. Simple linear regression model was applied to assess a possible correlation. Analyses were performed using GraphPad Prism (GraphPad Software, San Diego, CA, USA). A value of $P<.05$ was considered statistically significant.

3.4 Results

3.4.1 Effect of Western diet on animal characteristics

At baseline, no difference in global parameters was found between animals (data not shown). Eighteen weeks of Western diet (i.e., a diet based on high-sugar content) did not lead to any mortality. As shown in Table 2 and compared to control diet, 18 weeks of Western diet increased body weight significantly (+12.5%). Food intake, used as a surrogate for polyphagia, was significantly increased in animals undergoing Western diet. Global animal characteristics were accompanied with alterations in blood lipid profiles with augmented blood triglycerides and reduced HDLC levels with Western diet. Total cholesterol levels and NEFAs remained comparable.

Table 2: Animal characteristics following 18 weeks Control or Western diet.

	Control Diet	Western Diet
BW increase (%)	232 ± 6	261 ± 8*
Food intake (g/day)	35± 1	46± 1 *
Triglycerides (mg/dl)	211 ± 11	242 ± 4 *
Total Cholesterol (mg/dl)	148 ± 5	152 ± 4
HDLC (mg/dl)	22 ± 2	15± 1 *
NEFA (µM)	0.4 ± 0.1	0.6 ± 0.1
AWT (mm)	1.46 ± 0.02	1.71 ± 0.04 *
EF (%)	74 ± 2	69 ± 3
LVP (mmHg)	99 ± 3	105 ± 2

EDP (mmHg)	6.46 ± 0.61	10.65 ± 1.90 *

BW, body weight; HDLC, High-density-lipoprotein cholesterol; NEFA, non-esterified fatty acids; AWT, anterior wall thickness; EF, ejection fraction; LVP, left ventricle pressure; EDP, left ventricular end diastolic pressure. Data are presented as mean ± SEM. * denotes $p < 0.05$ vs. control diet.

3.4.2 Glucose tolerance is progressively altered with Western diet

Fig. 4A summarises the evolution of glucose tolerance parameters over time in both groups. Total glucose levels, as measured as AUC, were progressively and significantly increased in animals undergoing Western diet already after 12 weeks diet (left panel). Similarly, fasting insulin increased with time in both groups but this increase was more pronounced in the Western diet (right panel).

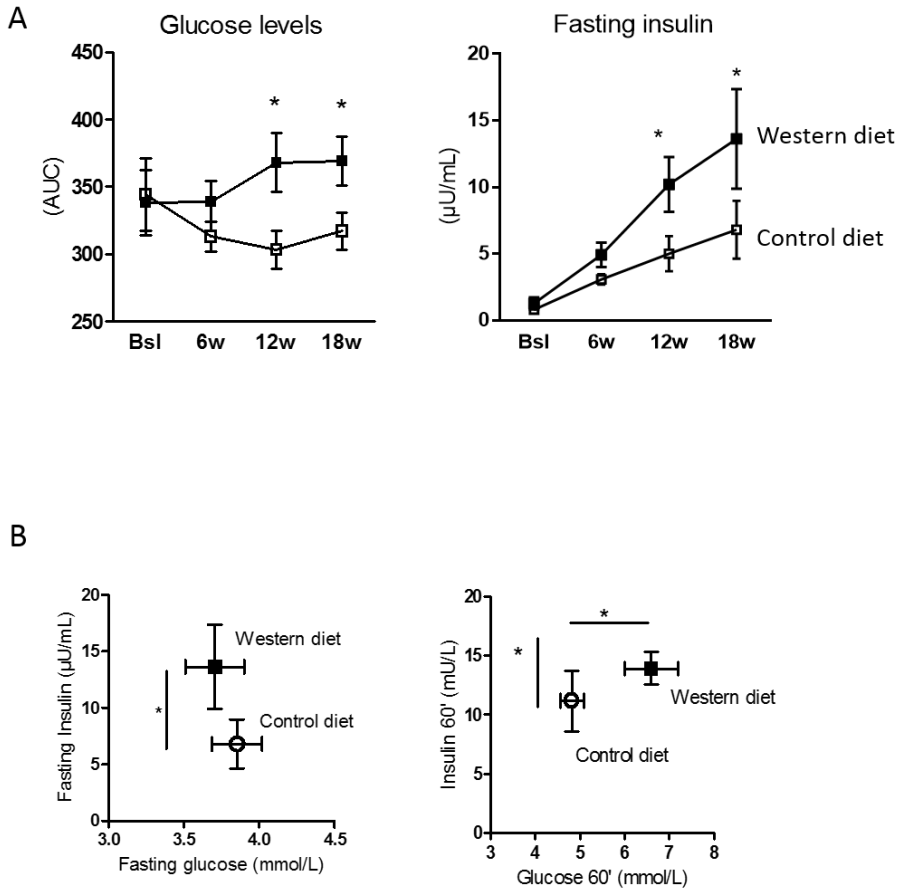

Figure 4: Western diet leads to T2DM. (A) Fasting glucose and insulin levels over time in both groups. (B) Fasting and 60’ post-glucose insulin and glucose levels after 18 weeks of control or Western diet. Left panel shows the fasting levels, right panel display s levels 60’ after start of the OGTT. (n=12/group). Data are shown as mean ± S.E.M. * denotes P<.05.

After 18 weeks Western diet, animals displayed a significantly increased fasting insulin levels to maintain comparable fasting glucose levels (Fig. 4B, left panel). After 60’ glucose administration, the higher insulin level observed in Western diet was unable to maintain comparable glucose levels. Indeed, in the Western diet group, glucose levels after 60’ glucose administration remained significantly higher than in control diet (Fig. 4B, right panel).

3.4.3 Western diet promotes the development of early signs of cardiac alteration

After 18 weeks of Western diet, heart rate, LV volumes, cardiac output and ejection fraction remained comparable between the groups (data not shown). However, despite unchanged global cardiac parameters, anterior wall thickness (AWT), an early marker of cardiac hypertrophy and remodelling, was significantly increased in animals fed with Western diet as seen in Table 2. Heart weight to tibia length ratio was comparable between control (39±3 mg/cm) and Western diet (37±2 mg/cm) fed animals.

This change in cardiac morphology was associated with changes in hemodynamic parameters. After 18 weeks Western diet, LV pressure was comparable between the groups (Table 2). EDP, an early marker of diastolic dysfunction, was however significantly increased in Western diet (Table 2). Parameters such as relaxation time constant (i.e., Tau), peak rate of pressure rise (i.e., dP/dtmax) and decline (i.e., dP/dtmin) remained comparable between the groups (data not shown).

Fig. 5A is a representative image of LV sections after 18 weeks diet from both groups, stained with Sirius red. As summarised in Fig. 5B (left panel), total interstitial collagen was significantly increased in animals undergoing 18 weeks Western diet. This increase was associated with an increased LOX levels (Fig. 5B, right panel).

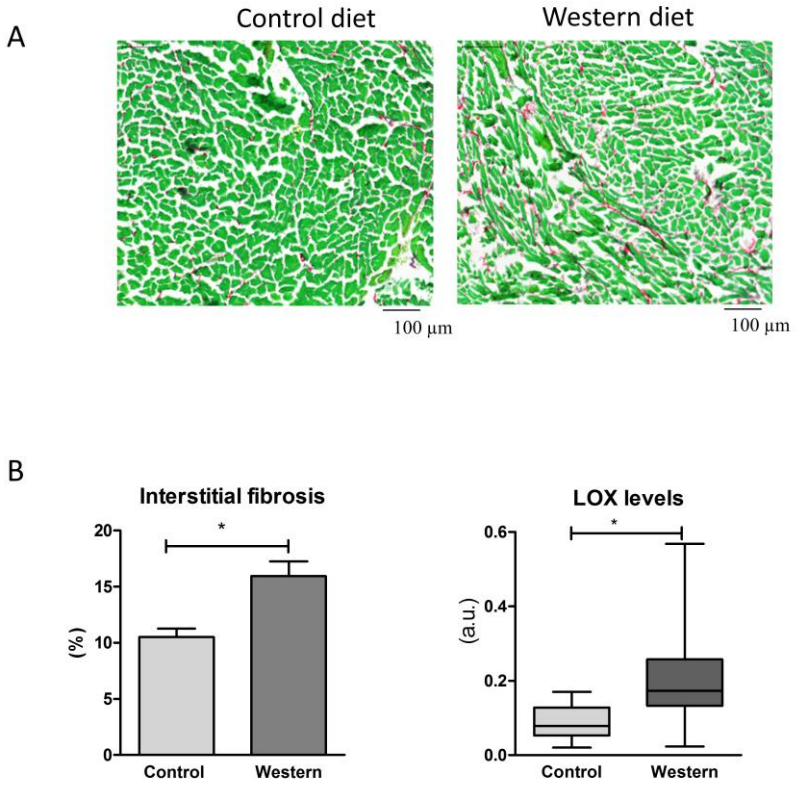

Figure 5: Western diet increases cardiac fibrosis through an increase in LOX expression. (A) Transversal sections of the cardiac left ventricle of a rat undergoing control diet (left panel) and Western diet (right panel), 20 times magnified. Left panel is a normal rat heart, right panel is a rat heart after 18 weeks of Western diet. (B) Total interstitial collagen quantification in the left ventricle of the rat heart (left panel). Protein LOX levels normalized to β-actin (right panel) (n=12/group). Data are shown as mean±S.E.M. or median [75th percentile; 25th percentile], * denotes P<.05.

3.4.4 Other associated hallmarks of DCM are present after 18 weeks Western diet

Western diet given for 18 weeks resulted in a four times increase in circulating plasma AGEs levels (Fig. 6A).

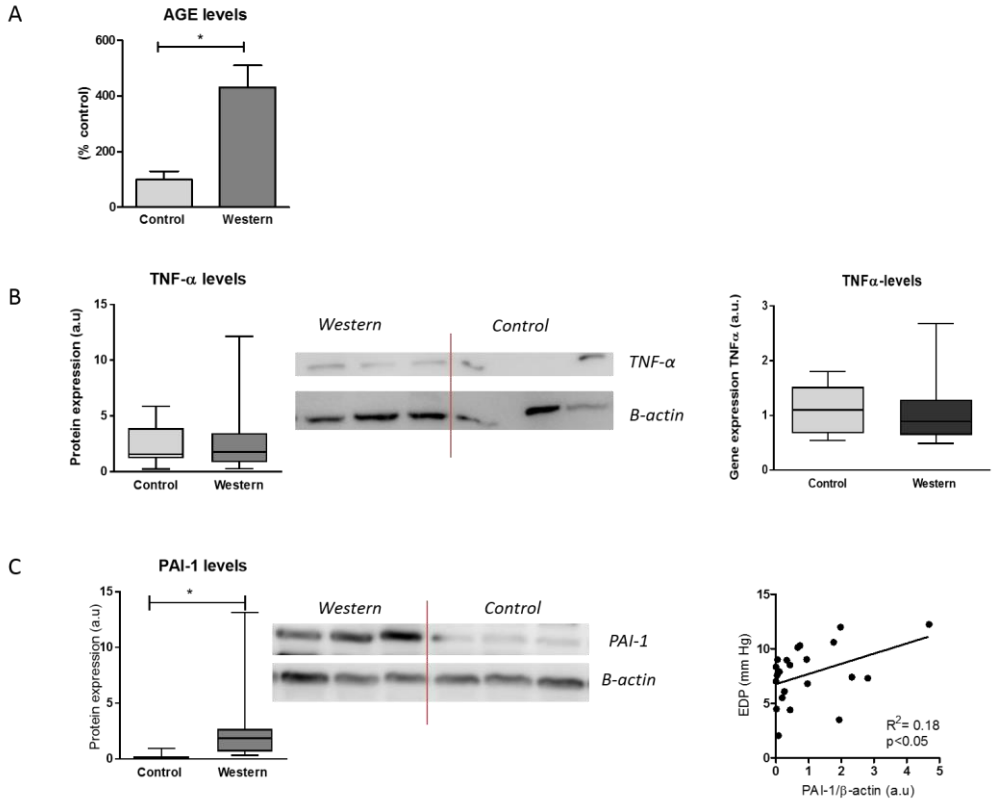

Figure 6: Other hallmarks of DCM are also present after 18 weeks Western diet. (A) Plasma AGEs in both groups measured 18 weeks after the diet. (B) Protein level of TNF-α normalised to β-actin, including Western blot image. Gene expression level of TNF-α (right panel) (C) Quantitative analysis of PAI-1 protein expression normalised to β-actin (left panel), including Western blot image. Correlation between end-diastolic pressure (i.e., EDP) and PAI-1 levels (right panel). (n=12/group, except for PAI-1 where n=11 for the Western diet group due to an outlier). Data are shown as mean±S.E.M. or median [75th percentile; 25th percentile], * denotes P<.05.

TNF-α protein level was not altered neither at gene level, nor at protein level (Fig. 6B). Protein levels of PAI-1 were significantly increased after 18 weeks Western diet (Fig. 6C, left panel) and positively correlated with the increased EDP (P<.05, R2=0.18) (Fig. 6C, right panel).

3.5 Discussion

In our study, we show that healthy rats undergoing 18 weeks of a Western-style diet comprising high sugar content display features of T2DM with DCM, such as hyperglycemia, hyperinsulinemia, hypertriglyceridemia, elevated EDP, LV hypertrophy and increased fibrosis. The present study clearly shows that Western-style diet given to rats results in a T2DM with DCM, with a phenotype similar to humans.

3.5.1 Relevance of the animal model used

To date, many genetic animal models (e.g., ob/ob, db/db mice, Zucker diabetic fatty, Goto-Kakizaki and Otsuka Long Evans Tokushima fatty rats) have been used to unravel the underlying mechanisms involved in disturbed energy homeostasis in T2DM with DCM (389, 405). However, despite shared common traits with human phenotype, models display either absence (406) or presence of obesity, hyperlipidemia and/or inflammation (407). Zucker diabetic fatty rats are an excellent model to observe T2DM. However, next to the fact that this model does not completely mimic the human phenotype as the phenotype occurs in rather young animals (8 weeks), altered leptin signalling observed in genetically modified rodents may also interfere with the interpretation of the data. To avoid potential misinterpretation resulting from phenotypes not directly attributed to the pathophysiology of the disease mechanisms, many researchers have used diet-induced diabetes as new alternatives (389). Traditionally, those diets consist of an exchange of carbohydrate-derived calories with fat-derived calories (i.e., high fat diet), mostly combined with a small dose of streptozotocin (HFD/STZ). In most models, animals exhibit hyperglycemia and hyperlipidemia associated with hyperinsulinemia. However, with the injection of STZ, the direct toxic effect of STZ on its own could likely be a cause for the observed phenotype (398, 408-412). In addition, in humans, pancreatic β-cell failure is multi-causal, including insulin resistance, hyperinsulinemia, stress, inflammation, lipotoxicity and glucolipotoxicity (413). Altogether, the relevance of such models for T2DM and DCM in particular remain questionable.

Furthermore, the current obesity pandemic in Western countries is associated with an increased sugar intake rather than an increase in fat intake (390). Therefore in our study, we used a diet based on high sugar rather than high fat, a so-called "cafeteria diet" or "Western diet" providing a progressive and more relevant model to study T2DM with DCM. In fact, diet composition given to our model was 20% total fat, 61% sugars and 19% proteins. As a comparison, composition of a classical fast food menu (comprising a hamburger, fries, beverage and dessert) is 25% total fat, 58% sugars and 16% proteins, further emphasising the relevance of the diet used in our study.

Regarding the phenotype obtained following such a diet, our data are in accordance with previous data showing that rats undergoing the "Western diet" display many features of T2DM (166, 391). Overweight and obesity are the strongest risk factors, accounting for a large proportion of T2DM (414-417). In our study, animals given a Western diet displayed a significantly increased body weight associated with polyphagia, reflecting the human situation. Another characteristic of T2DM is the altered lipid profile and hyperlipidaemia, with increased circulating free fatty acids and triglycerides (418). Lipid profile observed in our model also confirmed those data, showing an increase in circulatory triglycerides, lower HDLC levels and the slightly increased NEFA.

Very interestingly in our study, animals undergoing Western diet did not only develop T2DM but also developed early signs of cardiac dysfunction as shown by LV hypertrophy, altered hemodynamic pressures and increased interstitial fibrosis. To our knowledge, our study is the first that combines changes in the metabolic profile with early structural and functional changes in cardiac function in the setting of a Western diet given to healthy rats, making our model unique of its kind.

Finally, T2DM with DCM is characterised by chronic inflammation of the liver and adipose tissues, typically seen in patients (391, 392). In our model, we did not specifically examine liver inflammation but examined inflammation directly in cardiac tissue. In our model, tissue PAI-1 levels were significantly increased in animals undergoing Western diet. In one hand, it has been shown that PAI-1 has an important role in adipocyte hypertrophy and insulin resistance (419-421). Whether this increase results in a downregulation of peroxisome proliferator-

activated receptor (PPAR-γ) is likely to occur but remains to be confirmed in our model. In the other hand, PAI-1 is known to play an important role in fibrinolytic process and inflammation resulting in fibroblast activation and increased cardiac fibrosis content (422, 423). Together with the increased circulating AGEs levels known to be involved in cardiac diastolic dysfunction through increased fibrosis (129, 133), the increased PAI-1 might play an important role in the pathophysiological mechanism of the disease development as levels of PAI-1 correlated with the increased EDP, being a hallmark of early cardiac remodelling. Finally, serum AGE compounds are closely related with poorly controlled diabetes, as reactive carbonyl compounds (such as glucose) are increased in diabetic patients. AGEs are known to affect myocardial function through binding with their receptor (RAGE) and initiating signalling pathways resulting in the release of growth factors, formation of reactive oxygen species (ROS), endothelial dysfunction and angiogenesis (424, 425).

3.5.2 Unanswered questions and future research

In our study, we did not examine HbA1c. Changes in HbA1c levels is currently used as a diagnostic tool for diabetes (426). However, examining total AGEs rather than HbA1c might be a better marker for diabetes and pre-diabetes status as changes in circulating AGEs have been shown to occur even before changes in glycemia occur (133). TNF-α, a classical marker of inflammation was not changed in our model. However, we examined TNF-α levels in cardiac tissue specifically. Whether circulating levels of this inflammatory marker are changed remain to be determined. Finally, underlying pathophysiological mechanisms involved in the disease progression, including changes at the cardiomyocyte level and the role of oxidative stress in that process were not examined but would deserve further investigation.

3.6 Conclusion

In conclusion, the minimal clinical criteria to define DCM are not yet described in the clinic, but specific hallmarks could include diastolic dysfunction, fibrosis and LV hypertrophy, associated with hyperglycemia, hyperinsulinemia, disturbed lipid profile and signs of inflammation. Our animal model displays those features, indicating that Western diet is a cause not only for T2DM but also for DCM.

4

High intensity training improves cardiac function in healthy rats

Based on:

High intensity training improves cardiac function in healthy rats

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4 High-intensity training improves cardiac function in healthy rats

4.1 Abstract

Exercise training is a low cost and safe approach for reducing the risk of cardiovascular disease development. Currently, moderate-intensity training (MIT) is the most preferred exercise type. However, high-intensity interval training (HIIT) is gaining interest especially among athletes and healthy individuals. In this study, we examined cardiac remodelling resulting from MIT and HIIT in healthy rats. Healthy male Sprague-Dawley rats were randomly assigned to MIT or HIIT for 13 weeks. Animals kept sedentary (SED) were used as control. Cardiac function was evaluated with echocardiography and hemodynamic measurements. Heart tissue was stained for capillary density and fibrosis. After 13 weeks of training, only HIIT induced beneficial cardiac hypertrophy. Overall global cardiac parameters (such as ejection fraction, cardiac output and volumes) were improved similarly between both training modalities. At tissue level, collagen content was significantly and similarly reduced in both exercise groups. Finally, only HIIT increased significantly capillary density. Our data indicate that even if very different in design, HIIT and MIT appear to be equally effective in improving cardiac function in healthy rats. Furthermore, HIIT provides additional benefits through improved capillary density and should therefore be considered as a preferred training modality for athletes and patients.

4.2 Background

Exercise training intervention is recognised as an important and low-cost and safe strategy to prevent and treat cardiovascular diseases (427, 428). Exercising on a regular basis is indeed associated with a decreased risk for coronary artery diseases and heart failure through a reduced blood pressure, increased myocardial perfusion capacity and improved cellular metabolism in the healthy heart (429, 430). Overall, physiological left ventricular (LV) remodeling, as generated by repeated bouts of endurance exercise, has been shown to improve cardiac performance in healthy subjects and increase tolerance for ischemia and reperfusion injury, resulting in a beneficial effect on disease progression and survival in patients with LV dysfunction (255, 431-433).

In that context, many studies have demonstrated that moderate-intensity endurance training (MIT) is beneficial for cardiac function, both in healthy and pathological situations (255, 434). Recently, high-intensity interval exercise training (HIIT) has raised much attention, not only to assist coaches in adjusting training programs for elite and recreational athletes (435), but also as a new approach to handle heart failure patients (369, 380). Indeed, HIIT is a widely used and effective training method in various sports, including endurance and sprint events, combining respectively glycolytic and oxidative metabolism (436). Recent data indicate that HIIT provides indeed additional benefits and could be even more potent than MIT in improving cardiorespiratory function, metabolic response and cardiac function in healthy adults (253, 378, 382, 437, 438). A number of studies attribute this potential to a higher cardiomyocyte mitochondrial fatty acid oxidation and metabolism (253, 378, 379, 437, 438). Data also suggest that HIIT might provide additive benefits compared with the classical MIT modality (379). In cardiac rehabilitation in T2DM patients, HIIT provides improved systolic blood pressure (SBP), HbA1C, high-density lipoprotein cholesterol (HDL-C), malondialdehyde (MDA) levels, Nitric Oxide (NO) and von Willebrand factor (vWF). Overall, HIIT seems superior compared with MIT in improving vascular functions (384). Importantly, molecular pathways and effects of these different exercise modalities on cardiovascular effects are however still equivocal and remain to be elucidated.

In this study, we hypothesised that HIIT could provide higher cardiovascular gain in physiological remodelling, potentially through improved mitochondrial metabolism and tissue vascularisation. The aim of the current study was therefore to perform a thorough comparison on the beneficial effects induced by either MIT or HIIT on cardiac function in healthy rats. This could lead to optimisation of exercise programming for healthy individuals, athletes and heart failure patients seeking for strategies to enhance cardiac function.

4.3 Methods

This investigation conforms to the EU directive 2010/63/EU for animal experiments and was approved by a local ethical committee (Ethische Commissie Dierproeven, UHasselt, Diepenbeek, Belgium). All experiments and methods were performed according to the relevant guidelines.

4.3.1 Study protocol and exercise intervention

Twenty-six male Sprague-Dawley rats (Charles River Laboratories, L'Arbresle, France), weighing 200-225 g were used throughout the study. All animals were familiarised with the treadmill (IITC, California, United States of America) prior the experiment (for 3 days/week) and were randomly assigned (by randomisation program) to one of the three experimental groups after two weeks familiarisation. Group 1 did not undergo exercise training throughout the study (SED, N=10). Group 2 was subjected to moderate intense endurance training schedule, consisting in running on a treadmill, 18m/min, 5° inclination, 1h/day, 5days/week (MIT, N=8). Group 3 consisted in 10 bouts of high-intensity treadmill running (18m/min, 30° inclination), separated by 1 min of active rest, 5 days/week (HIIT, N=8). Sample size was calculated by a Power analysis based on previous results. The intensity of exercise training was assessed by measuring blood lactate levels directly after exercise with an Analox apparatus (Analis, Namur, Belgium). Levels >4 mmol/l lactate were considered HIIT (439). Training modalities were adjusted to lead to an equal energy expenditure between interventions by calculating the net caloric cost (kcal/min) using following calculations:

- VO_2 max = S_a * 0.2 + (S * G_b) * 0.9 (S_a = speed (m/min), S = speed (m/min), G_b = inclination);
- Net caloric cost (kcal/min) = VO_2 max * 3.5 * body mass (kg) / 200

All animals were maintained in a controlled environmental condition of temperature and humidity and had water and food (normal rodent diet, ENVIGO, The Netherlands) available *ad libidum.* Blood samples, hemodynamic measurements and echocardiographic measurements were executed 13 weeks after the start of the training program.

4.3.2 Conventional echocardiographic measurements

Prior to sacrifice, transthoracic echocardiography was performed under 2% isoflurane in all animals with a Vivid I ultrasound machine (GE Vingmed Ultrasound) using a 10 MHz linear array transducer. The protocol used is as described previously (133, 440). Briefly, a standard parasternal long axis image and short axis views at the mid-ventricular level were obtained at a temporal resolution of approximately 200 frames per second. Conventional echocardiographic parameters (e.g. LV end-diastolic diameter (LVEDD), LV end-systolic diameter (LVESD), posterior wall thicknesses (PWT) and anterior wall thicknesses (AWT)) were obtained from the B-mode images at midpapillary level in the parasternal short-axis view. End-systolic volumes (ESV) and end-diastolic volumes (EDV) were calculated by π*DM2*B/6, where DM indicates the systolic/diastolic diameter of the ventricle in mid-ventricular short-axis view and B is LV length on parasternal long-axis image. Subsequently, ejection fraction (EF) was measured as (EDV–ESV)/EDV, and expressed in %.

4.3.3 Hemodynamic measurements

Pressure measurements were performed in all animals with the use of an SPR-320 MikroTip high-fidelity pressure transducer (Millar Inc) that was advanced into the left ventricle via the right carotid artery, as described previously (440). The pressure catheter (2F) was connected to a quad-bridge amplifier and PowerLab 26T module (AD Instruments, United Kingdom) was used to transfer the data to LabChart v7.3.7 software (AD Instruments, United Kingdom).

4.3.4 Citrate synthase activity in cardiac homogenates

Citrate synthase activity was determined in LV cardiac homogenates using a citrate synthase assay kit (CS0720; Sigma-Aldrich, St. Louis, MO) (441).

4.3.5 Fibrosis and capillary density measurements in tissue sections

Transversal sections of 7 μm thick were obtained at cardiac midventricular level and stained using the Sirius Red/Fast Green kit (Chondrex). Fibrosis was assessed in all animals in four randomly chosen fields per section. The area of collagen deposition indicated by red staining was outlined and quantified using an automated image analysis program (Carl Zeiss, AxoVision 4.6, Zaventem,

Belgium). Blood vessels were excluded. Total collagen deposition to the global cardiac area was calculated and expressed as percent collagen deposit.

Capillary density was quantified from histological sections by immunohistochemical staining for CD31 (SC-1506, Santa Cruz, 1/100). Capillaries were visualised by 3-3-diaminobenzidine (DAB) and counterstained using hematoxylin. The amount of blood vessels were counted in 10 different fields per section and averaged. Data are expressed as amount of capillaries per µm2.

4.3.6 Endothelin-1, OXPHOS and NOX2 protein levels

Protein concentrations of the LV tissues were determined by the BCA protein assay kit (Thermo Fisher, Erembodegem, Belgium). Western blot was performed as previously described (133). Briefly, equal amounts of proteins (15 µg) were separated on a 12% SDS-PAGE gel with a mini protean 3 electrophoresis system (Bio-rad Laboratories, Temse, Belgium), transferred to a polyvinylidene fluoride (PVDF) membrane and subsequently, blocked for 2h with 5% milk in Tris-buffered solution containing 0.1% Tween-20 (TBS-T) followed by incubation overnight at 4°C in the presence of a specific endothelin-1 antibody (1/2500, Abcam, ab117757, Cambridge, United Kingdom), NOX2 antibody (1/2500, Abcam, ab31092, Cambridge, United Kingdom) or an OXPHOS antibody (1/1000 , Abcam, ab110411, Cambridge, United Kingdom). Horseradish peroxidase-conjugated secondary antibodies (DAKO, Belgium) at a dilution of 1/2000 were used. Both primary and secondary antibodies were diluted in 5% milk-TBS-T. Visualisation was performed with the enhanced chemiluminescence (ECL) technique using the Pierce ECL Plus western Blotting Substrate (Thermo Fisher, Erembodegem, Belgium). Data were normalised to β-actin protein levels.

4.3.7 Complex II enzyme activity

Complex II enzyme activity in cardiac tissue was determined using a Complex II Enzyme Activity Microplate Assay Kit (Abcam, ab109908, Cambridge, United Kingdom). Samples were prepared as described in the protocol, and added to an anti-Complex II monoclonal antibody-coated 96-well plate. Absorbance was measured at OD600 nm for 60 minutes, measuring every 20 seconds.

4.3.8 Statistical analysis

Results were tested for normality prior to statistical tests. Subsequently, one-way ANOVA test was performed combined with a post- hoc test, dependent of normality. If data were normally distributed, Tukey's range test was performed. Elsewise, the Dunn's test was used. Analyses were performed using GraphPad Prism (GraphPad Software, San Diego, CA, USA). A value of $p<0.05$ was considered statistically significant.

4.4 Results

4.4.1 Different exercise types improve cardiac function to the same extent

At baseline, no differences were observed neither in body weight nor in cardiac function assessed by conventional echocardiography between the three groups (data not shown). Thirteen weeks after the start of exercise intervention, sedentary animals gained significantly more weight than trained animals (body weight SED: 596 ± 16 g; MIT: 483 ± 18 g; HIIT: 472 ± 13 g, $p<0.05$). Body weight change was comparable between the two training groups.

Conventional echocardiographic characteristics of the animals are summarized in Table 3. Ejection fraction, a parameter for global cardiac function, was significantly improved after 13 weeks of exercise training ($p < 0.05$). Furthermore, animals undergoing HIIT specifically displayed changes in LV morphology with wall hypertrophy (increased of 24% for AWT and 17% for PWT compared to SED, $p<0.05$) while MIT did not induce wall hypertrophy. Finally, EDV was not different between groups, while ESV significantly and progressively decreased with MIT and HIIT intervention ($p< 0.05$). These changes resulted in a significant increase in SV of 27% in both training groups ($p< 0.05$), while cardiac output (CO) was preserved.

Table 3: Conventional echocardiographic parameters after 13 weeks of exercise training

	SED (N=10)	**MIT (N=8)**	**HIIT (N=8)**
HW (g)	1.6 ± 0.06	1.4 ± 0.04	1.5 ± 0.05
HR (bpm)	347 ± 7	325 ± 10	314 ± 13
AWT (mm)	1.4 ± 0.1	1.5 ± 0.1	1.7 ± 0.4*
PWT (mm)	1.4 ± 0.1	1.4 ± 0.1	1.6 ± 0.1*
EDV (µL)	413 ± 24	436 ± 27	426 ± 12

ESV (µL)	154 ± 10	107 ± 10*	95 ± 7*
SV (µL)	259 ± 17	329 ± 22*	331 ± 7*
CO (ml/min)	89.4 ± 5.5	106.7 ± 7.6	104.0 ± 5.7
EF	63 ± 1	76 ± 2*	78 ±1*

HR, heart rate; AWT, anterior wall thickness; PWT, posterior wall thickness; EDV, end-diastolic volume; ESV, end-systolic volume; SV, stroke volume; CO, cardiac output; EF, ejection fraction. Data are shown as mean ± SEM. * denotes $p<0.05$ *vs.* SED

Hemodynamic measurements are summarised in Table 4. Both exercise modalities were equally able to decrease LVP as compared to SED animals ($p< 0.05$). Contractility, as evaluated with dP/dtmax, as well as relaxation parameters, i.e. dP/dtmin and tau, were comparable in all groups ($p< 0.05$).

Table 4: Hemodynamic parameters after 13 weeks of exercise training

	SED (N=10)	**MIT (N=8)**	**HIIT (N=8)**
LVP (mmHg)	108 ± 3	96 ± 2*	96 ± 2*
EDP (mmHg)	5.2 ± 0.4	3.9 ± 0.4	3.7 ± 0.6
Tau (ms)	0.01 ± 0.001	0.01 ± 0.001	0.008 ± 0.002
dP/dt_{max} (mmHg/s)	7354 ± 274	7382 ± 277	7340 ± 258
dP/dt_{min} (mmHg/s)	-7530 ± 211	-7489 ± 215	-7190 ± 203

LVP, left ventricular pressure; EDP, end diastolic pressure; Tau, relaxation time constant; dP/dt_{max}, peak rate of pressure rise; dP/dt_{min}, peak rate of pressure decline. Data are shown as mean ± SEM. * denotes $p<0.05$ *vs.* SED

Overall, data indicate beneficial cardiac remodelling following exercise training, most global improvements are independent of the exercise modality.

4.4.2 As opposed to MIT, HIIT training is able to increase capillary density

Figure 7A are representative images of interstitial collagen obtained with Sirius red/Fast Green in heart sections after 13 weeks of exercise training. As summarised in Figure 7B, total interstitial collagen was significantly and equally decreased in animals subjected to exercise training. Citrate synthase activity, a marker of aerobic capacity and mitochondrial mass, improved equally in both groups undergoing a training program ($p < 0.05$) (Figure 8A). Additionally, capillary density, evaluated by a CD31 staining in cardiac tissue sections, was unchanged with MIT ($p > 0.05$) but was significantly increased with HIIT ($p < 0.05$) (Figure 8B). Finally, no difference was observed in Endothelin-1 (Figure 8D), NADPH Oxidase 2 (NOX2) nor oxidative phosphorylation (OXPHOS) protein levels (Figure 8E). Complex II enzyme activity levels were not altered after exercise, however, a positive trend was observed in HIIT animals (Figure 8F).

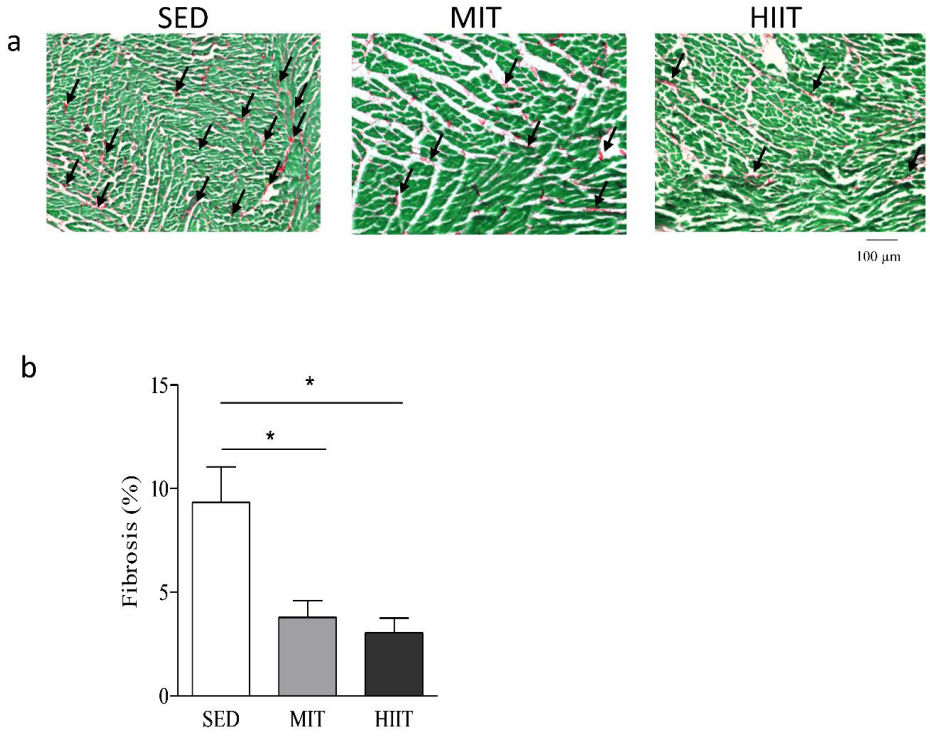

Figure 7: Exercise intervention reduces cardiac collagen content.

Representative images of interstitial collagen, as indicated by the arrows, in LV sections obtained with Sirius red/Fast Green in SED, MIT and HIIT groups. B. Total interstitial collagen quantification in SED (N=10), MIT (N=8) and HIIT (N=8). Data are shown as mean ± SEM. * denotes p<0.05 *vs.* SED.

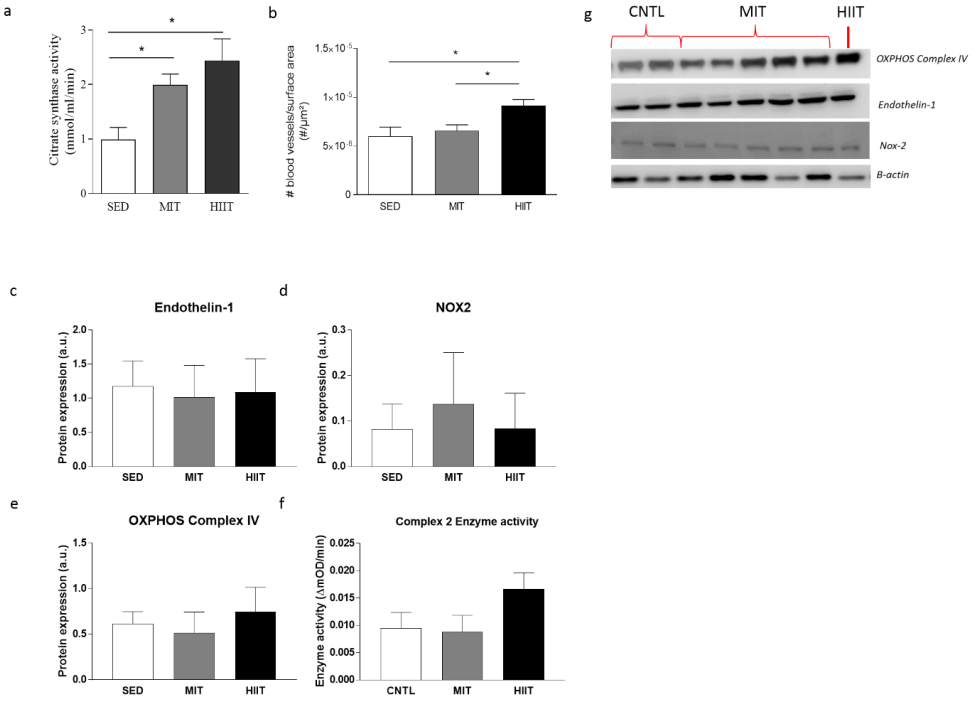

Figure 8: Exercise intervention increases citrate synthase activity and capillary density.

A. Citrate synthase activity, measured in LV homogenates, in all groups. B. Capillary density expressed as blood vessels per surface area, in the three groups. C. Protein level of endothelin-1, normalised to β-actin. D. Protein level of NADPH Oxidase 2 (NOX2), normalised to β-actin. E. Protein level of oxidative phosphorylation (OXPHOS) complex IV, normalised to β- actin. F. Enzyme activity of complex II in cardiac tissue. G. Results of representative Western blots. Cropped images of Western blot. All blots were performed individually. Data are shown as mean ± SEM from SED (N=10), MIT (N=8) and HIIT (N=8) for graphs, blots represent a partial blot. * denotes p<0.05 *vs.* SED.

4.5 Discussion

In this study, we demonstrate that both MIT and HIIT intervention, although very different training types, lead to beneficial effects on cardiac function in healthy animals. Importantly, HIIT might provide additional benefits as opposed to MIT as HIIT is able to increase blood capillary density, an important feature for optimal cardiac muscle metabolism.

4.5.1 Both HIIT and MIT lead to beneficial cardiac remodelling, resulting in an improved cardiac function through reduced afterload

While some studies demonstrate that MIT improves cardiac function, others report very little changes (442). The beneficial effects of exercise training are believed to be mainly attributed to improved neurohumoral, inflammatory, metabolic and central hemodynamic responses as well as on endothelial, skeletal and cardiovascular function, leading to an overall cardiac improvement and an improved tolerance for ischemia and reperfusion injury (432, 433). One major hallmark of LV remodelling related to endurance exercise training, independent of the applied training modality, is the increase in LV mass. Cardiac hypertrophy is commonly accepted to be physiological and not associated with adverse effects, as opposed to pathological hypertrophy which involved substantially different signalling pathways (443-446). Pathological hypertrophy is related with an increase in collagen accumulation and wall thickness, causing reduced cardiac function (427) which was not observed here. In our study, we have shown that unlike MIT, HIIT increases AWT and PWT, markers of LV wall hypertrophy, as also confirmed in the study of Hafstad et al (427, 447). In the study of Kemi et al. a HIIT modality was more efficient in inducing LV hypertrophy compared when moderate-intensity training was applied (448). These data suggest that HIIT might be more efficient than MIT in physiological cardiac remodelling. However, whether this is related to activation of different/specific signaling pathways remains to be elucidated. Endothelin-1 is known to promote hypertrophic remodeling, but no change was observed in the different groups in our study, indicating alternative pathways for remodeling after exercise.

Physiological adaptation of cardiac function to exercise training involves structural and metabolic remodeling, resulting in a lower resting HR, improved CO and changed volumes (449). In our study, independent of the applied training modality, HR and EDV were not statistically different from sedentary animals. Noteworthy, EF, a marker of global cardiac function, was significantly improved with both exercise training interventions. As also shown by Dawes et al (449), the improved global cardiac function was associated with an increased SV in MIT and HIIT groups. The increase in SV, together with unchanged EDV but reduced ESV, indicates an increased cardiac contractility and/or reduced afterload in trained animals (450). The effect of endurance training on cardiac contractility remains however controversial. While some studies demonstrate an increased contractility attributed to an improved oxygen delivery, angiogenesis and nitric oxide (NO) sensitivity after endurance exercise training (444, 451), others report very little changes (442). In our study, dP/dtmax, a parameter reflecting cardiac contractility, was not changed, indicating that the beneficial cardiac changes observed are likely to be attributed to a decreased afterload, rather than a major change in contractility. This is further confirmed by the decreased LVP, which, combined with a CO that tended to be higher, indicates a decrease in vascular resistance, resulting in a reduced afterload. In that context, the effect of exercise training on endothelial function and, as a result, on vasodilatation, was already well described by others in humans and experimental models (369, 452-454). Indeed, exercise training has been shown to lead to vascular adaptation and improved vascular function through increased NO levels (455, 456). Our in vivo data indicate that an improved cardiac function is likely attributed to vascular adaptations, leading to a reduced afterload. However, the exact mechanisms and further study on endothelial function, examining the effect of the different training interventions on NO levels remain to be performed.

It has been shown in rodents that exercise training is not necessarily accompanied by myocardial collagen change (444). In our study, we show that both exercise interventions were able to substantially decrease interstitial collagen content in cardiac muscle. This decrease in fibrosis was however not strongly associated with improvements of diastolic function as previously described (457-460). Indeed, despite the unchanged EDV and unchanged CO, EDP only tended to be decreased in both training modalities ($p=0.07$). Whether an improved diastolic function

related to a decreased fibrosis content following exercise training can be completely ruled out remains to be further confirmed as it has been shown that diastolic function (i.e. EDP) is directly associated with cardiac fibrosis level (133, 440). However, most experiments examining fibrosis and diastolic function have been performed under pathological settings (457, 461). In our study, we examined physiological remodelling in healthy rats. Therefore, the effect of exercise on cardiac fibrosis level and its impact on diastolic function is likely to be substantially more important in pathological situations as levels of fibrosis in pathological settings are at least two times larger than what is reported in healthy situations (133, 404).

4.5.2 What is the added value of HIIT?

It is known that an increase in cardiac capillary density is important for the development of physiological hypertrophy (462, 463). In that context, the link between angiogenesis and hypertrophy has been previously emphasised (462) where the seen effects were shown to be mediated through paracrine effects and secretion of NO and growth factors (such as VEGF, NRG-1). In our study, only HIIT was able to increase capillary density, indicating a somehow higher potential for HIIT as compared to MIT. Very recently, further confirming our results, the study of Machado et al. demonstrated that exercise training was able to prevent capillary rarefaction in obese rats with the metabolic syndrome (464). An adequate capillary density allows for greater oxygen transport to the cardiac muscle and improves ultimately cardiac metabolism. In our study, citrate synthase activity, a measure of mitochondrial mass, was significantly improved after MIT and HIIT. Together with this, complex II enzyme activity seemed to be increased in HIIT compared with sedentary animals, however not significantly. Our data therefore suggest that HIIT might provide additional benefits compared to MIT, through increased capillary density and the resulting improved oxidative metabolism, not limited to the skeletal muscle as shown by Takada et al. (465) but also in the heart, as suggested in the current study and by others (466). Cardiac metabolism requires aerobic production of ATP. In our study, HIIT provides increased cardiac vascularity to counter high oxygen requirements during exercise, as in HIIT exercise is performed at almost maximal oxygen consumption. Increased mitochondrial activity and mass will result in an increased ATP

production, necessary to keep up with the cardiac energy demand (467). Since blood supply is essential to improve cardiac function, particularly in pathological situations where blood vessels are dramatically reduced (468) or when angiogenesis needs to be restored to optimise cardiac repair through stem-cell therapy for instance (469), HIIT might be a great advantage compared to MIT. However, further investigation is required to draw conclusions as underlying changes related to the different training interventions on endothelial function, including mitochondrial morphology and mitochondrial function, are so far unknown. Exercise and oxidative stress have a complicated relationship, as exercise can have both a positive and negative effect on oxidative stress (470). NOX-2 plays a role in hypertrophy and interstitial fibrosis, and is a superoxide generating enzyme playing a role in oxidative stress and reactive oxygen species (ROS) (471). However, in our study, no effect on oxidative stress levels was observed after exercise. This can be due to multiple causes, as mode, intensity and duration of the exercise are known to play an important role in the observed result.

Finally, because training modalities used in HIIT are short in time, motivation and adherence of patients to HIIT training as compared to MIT, could be potentially higher. This could be a serious advantage in the clinical setting since both trainings offer beneficial cardiac remodelling with an added value to HIIT at the molecular level. Last but not least, not limited to a pathological setting, one could speculate that HIIT could be a way to improve in a more efficient and faster way, cardiovascular function in athletes. However, translation of results from bench to bed-side should be done with caution taking into account the differences between animal and human studies.

4.6 Conclusion

Our data indicate that even if very different in design, HIIT and MIT appear to be equally effective in improving cardiac function in healthy rats. Furthermore, HIIT shows some promising results and might provide additional benefits through improved capillary density. Therefore, it could be considered as a preferred training modality for athletes and for patients for whom improvements in cardiac function are aimed at.

5

Effect of Exercise Intervention on Cardiac Function in Type 2 Diabetes Mellitus: A Systematic Review

Based on:

Effect of Exercise Intervention on Cardiac Function in Type 2 Diabetes Mellitus: A Systematic Review

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5 Effect of Exercise Intervention on Cardiac Function in Type 2 Diabetes Mellitus: A Systematic Review

5.1 Abstract

Background: The effect of exercise on cardiac function/structure in type 2 diabetes mellitus (T2DM) with or without diabetic cardiomyopathy (DCM) is not yet completely understood. To date, results of studies have been controversial with variable outcomes due to the variety of exercise modalities.

Objective: The aim of the present review was to examine the impact of exercise intervention, and different types of exercise, on cardiac function and structure in T2DM through a systematic literature review, combining both pre-clinical and clinical studies.

Methods: A systematic literature search was performed on PubMed, Web of Science, and PEDro to identify studies up to 2 April 2018. Articles were included when well-defined exercise protocols were provided, and cardiac function in T2DM patients or validated animal models was examined.

Results: In diabetic animals, improvements in both diastolic and systolic function through exercise therapy were mainly attributed to reduced collagen deposition. In T2DM patients, improvements were observed in diastolic function, but not consistently in systolic function, after endurance (and combined resistance) exercise training. Different exercise intervention modalities and exercise types seemed equally effective in improving cardiac structure and function.

Conclusion: Exercise training elicits significant improvements in diastolic function and beneficial remodelling in T2DM and DCM animal models, but not necessarily improvements in systolic function and left ventricular structure, regardless of exercise type. Therefore, exercise intervention should be a cornerstone in the treatment of T2DM patients not only to improve glycemic control but also to specifically enhance cardiac function.

5.2 Introduction

Diabetes is a major global health problem, affecting>400 million individuals worldwide, where the majority have type 2 diabetes mellitus (T2DM) (3). In T2DM patients, cardiovascular diseases are the major cause of mortality and morbidity. The risk of developing heart failure in T2DM is closely related to an increase in blood haemoglobin A_{1c} (HbA_{1C}) levels, and is closely associated with insulin resistance and/or hyperglycemia (109). In addition, diastolic dysfunction and left ventricular (LV) hypertrophy, early markers of cardiac failure, occur in T2DM patients, respectively, in 19.6–54.4% and 21% of the cases (395, 472). Diabetic cardiomyopathy (DCM) is defined as diabetes-associated structural and functional changes in the myocardium, not directly attributable to other confounding factors such as coronary artery disease (CAD), valve disease, or hypertension (188, 394). The prevalence of DCM is not yet clear due to the lack of large cohort studies on different diabetic populations and the discrepancy in outcomes (473). DCM is characterised by extracellular and cardiomyocyte remodelling, both contributing to the observed impaired cardiac output (94) and resulting in impaired diastolic and/or systolic function. These molecular changes are also known to be partially driven by inflammation, hyperglycemia, hyperinsulinemia, and lipotoxicity (474). Patients diagnosed with T2DM and DCM generally receive medication that is aimed at better glucose control and/or lipid-lowering therapies, but for these patients, specific cardiac remodelling is often neglected.

Exercise intervention is an effective, low-cost, and safe strategy for the prevention and treatment of cardiovascular diseases (CVD) (427). Indeed, repeated bouts of endurance exercise lead to advantageous LV remodeling, improvements in cardiac performance, and greater tolerance for ischemia and reperfusion injury (432, 433), resulting in a slower myocardial disease progression and greater survival and/or lower hospital admission rates (255, 431, 475). In addition, exercise is known to exert anti-inflammatory effects, reduce blood HbA_{1c} concentrations, oxidative stress and hyperglycemia, and improve insulin action and lipid profile in T2DM (260, 347, 476-479). In this context, supplementing current approaches with exercise interventions could substantially improve cardiac outcome for T2DM with DCM, which would be of great advantage for the patient and society. However, it remains unclear which type of exercise intervention (with regard to

intensity, type, duration, etc.) can improve cardiac function and reverse adverse remodelling. In this review, we therefore systematically evaluated the impact of different exercise training modalities on cardiac function and structure in T2DM with or without DCM. Because underlying mechanisms can only be unravelled in animal models, it was deliberately decided to examine and combine outcomes from pre-clinical and clinical studies in the present systematic review, which represents a further novelty of our approach.

5.3 Methods

5.3.1 Literature Search and Selection Criteria

The primary objective of this systematic review was to assess the impact of exercise intervention on cardiac function in animals and patients with T2DM [PICO: P=patients (with T2DM), I=intervention (exercise), C=comparison (of different exercise types), O=outcome (cardiac function)]. The secondary objective was to assess the impact of different types of exercise on cardiac function. In this systematic review, databases were searched for articles published from inception until 2 April 2018, for both animal and patient studies. For animal studies, the electronic databases PubMed and Web of Science were used with the following Mesh Terms: 'diabetic cardiomyopathy,' 'exercise,' and 'training'. For human studies, the electronic databases PubMed and PEDro were used. In PubMed, the following keywords were used: (training OR exercise) AND (type 2 diabetes OR diabetic cardiomyopathy OR diabetic*) AND (diastolic OR systolic OR cardiac OR heart). In PEDro, the search strategy was adjusted to Diabet*, exercis*, and train* to find all relevant articles. Literature searches for animal and patient studies were independently performed by two reviewers (LVR and MV). In case of disagreement, a consensus-based decision was made by the two reviewers (LVR and MV) and the last authors (DH and VB) to include/ exclude an article. Results of the search are shown in Fig. 9 for the patient studies and in Fig. 10 for the animal studies. Quality assessment was based on the table of Kilkenny et al. for animal studies (480), examining 20 criteria. For the patient studies, methodological quality was evaluated via manually calculated PEDro scores including 11 criteria, and PEDro scores≥6 were considered as "good quality" (481). Outcome parameters (relating to cardiac function and structure) for animal

and patient studies were based on echocardiographic assessments, hemodynamic measurements, or measurements of myocardial fibrosis.

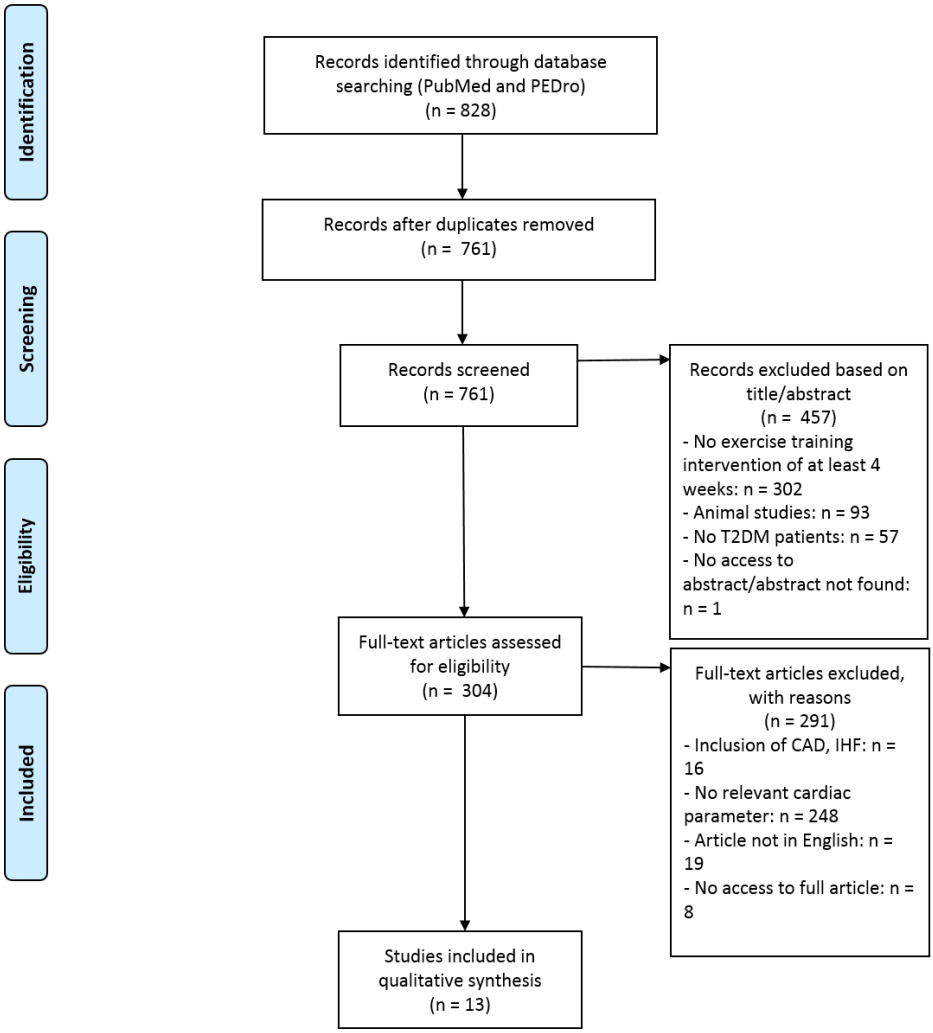

Figure 9: Flowchart summary of the search for and selection of the human studies.
IHF = ischemic heart failure, T2DM = type diabetes mellitus, CAD = coronary artery disease

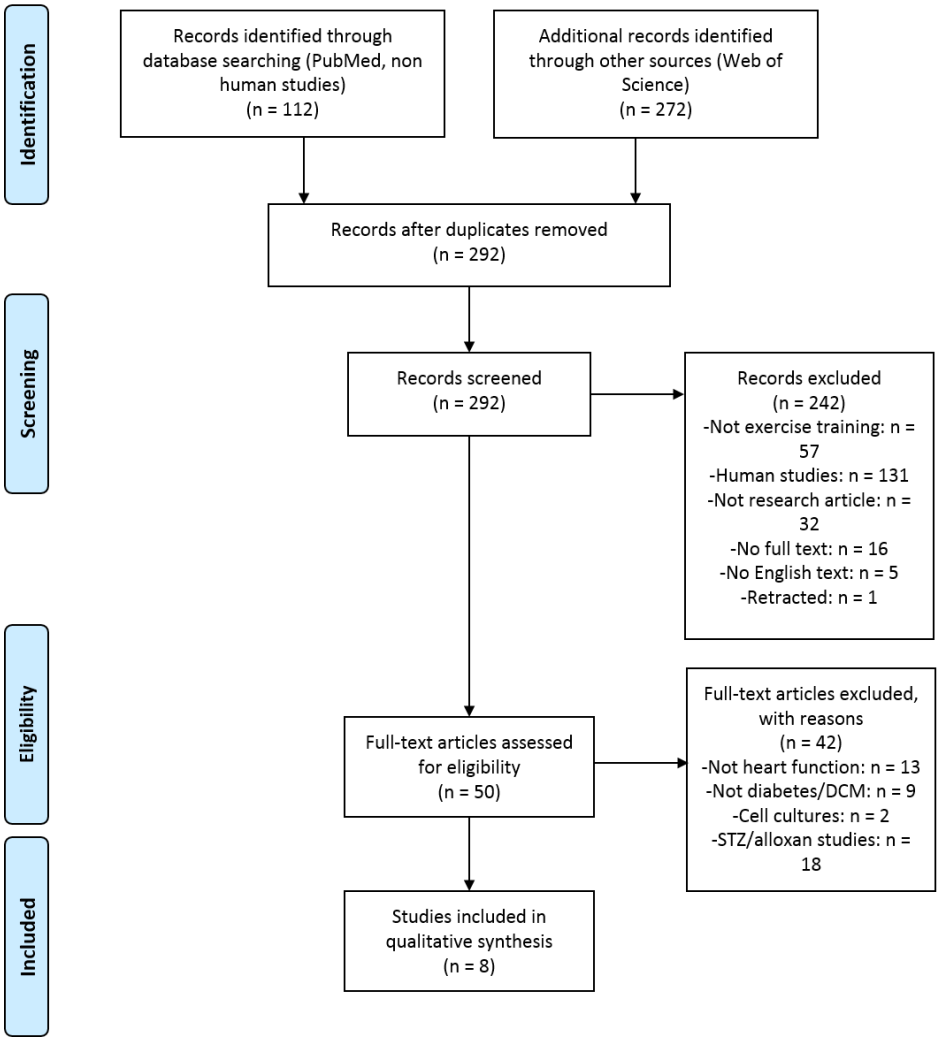

Figure 10: Flowchart summary of the search for and selection of the animal studies. DCM = diabetic cardiomyopathy, STZ = streptozotocin

5.4 Results

5.4.1 Quality Control of Included Studies

All patient studies consisted of clinical trials, of which nine were randomised (RCT) and four were non-randomized (non-RCT). In all of them exercise therapy was used as an intervention for T2DM patients. Five out of nine RCTs displayed a score of≥6 for PEDro and were therefore considered as "good quality." The lack of blinding of subjects and therapists were the main limitations. The use of control groups was only applied in the RCTs. In all animal studies, exercise therapy was used to treat DCM in rats or mice models of T2DM. According to the Kilkenny et al. table, all animal studies were considered to be "good quality" (480).

5.4.2 Human studies

5.4.2.1 Diastolic Function

As shown in Table 5, primary read-out parameters and manner of comparison (within- or between-group analyses) differed substantially between studies. In order to specifically investigate the isolated effect of exercise training, only studies reporting between-group analyses are discussed; other comparisons can be found in Table 5. Between-group analyses were reported for the following diastolic parameters: early diastolic filling E (n=3 out of four studies), atrial filling (A) (n=1 out of two studies), LV filling pressure (E/e') (n=3 out of five studies), E/A (n=3 out of five studies), early diastolic tissue velocity (e') (n=3 out of four studies), and deceleration time (Dt) (n=2 out of three studies). Exercise training was not able to exert superior effects on E or A compared with the control group or alternative exercise training (e.g., high-intensity interval training (HIIT) compared with moderate intensity training (MIT)) (482-484). However, it should be noted that one study reported an increase in E after HIIT training, which did not occur in the MIT group (482). Results for E/A are inconsistent. Two studies reported a between-group interaction; however, one of these studies reported an increase in E/A in the exercise group (485), whereas the other study reported an increase in the control group (484). Comparable to the effect on E, Hollekim-Strand et al. (482) reported an increase for E/A after HIIT training, which was not the case for MIT training. Further, two studies reported superior effects of exercise training in

terms of increases in e' (482, 485), although another study failed to confirm this (431). Again, HIIT seemed to be superior compared to MIT (482). Lastly, one study reported superior effects of exercise training with respect to reductions in E/e' and Dt (485), although other studies failed to confirm these effects (482, 484). Again, Hollekim-Strand et al. (482) reported reductions in E/e' in the HIIT group and not in the MIT group. Overall, these studies indicate that endurance (and/or resistance) exercise training can to a certain extent ameliorate or at least positively influence different aspects (E/A, E/e', e', Dt) of diastolic function. The influence of type and modality of exercise intervention remains unclear. Although the study by Hollekim-Strand et al. (482) justifies the current interest in HIIT training.

Table 5: Summary of studies in humans included in the review, showing training type and intensity, and effects on diastolic and systolic function and left ventricle morphology

	Training			**Method**	**Effect on cardiac function**		
Study	*Type of training*	*Intensity*	*Duration and frequency*	*Outcome*	*Diastolic function*	*Systolic function*	*LV structure and other measurements*
Brassard et al. (2008)	Endurance exercise training Cycling	60-70% VO2max	12 weeks (3/week)	TTE	Normalisation of LVDD ↗ E/A ↘ A wave ↔ Dt, E wave	↔ LVEF	↔ LV dimensions, LV mass, LA volume, posterior wall thickness ↗ IVRT (control group)
Cassidy et al. (2016)	HIIT Cycling	Borg Rating of Perceived Exertion (16-17)	12 weeks (3/week)	Cardiac MRI	↗ Early filling rate, early diastolic filling rate ↔ Late diastolic filling rate Significant between-group	↗ SV and LVEF ↔ Cardiac output and longitudinal shortening Significant between-group comparisons of changes:	↗ LV wall mass, EDV ↔ ESV ↗ Wall thickness during systole and diastole (control group) Significant between-group

					comparisons of changes: ↗ Early filling rate (control group < exercise group)	↗ SV and LVEF (control group < exercise group)	comparisons of changes: ↗ LV wall mass, EDV (control group < exercise group)
Cugusi et al. (2014)	Endurance exercise training Aquatic exercises	50-75% VO2max	12 weeks (3/week)	TTE	↘ E/e'	↔ LVEF, S wave, strain rate	↔ EDV and ESV
Hollekim-Strand et al. (2016)	HIIT vs. MIT HIIT: walking/running MIT: home-based exercise	HIIT: 90-95% HRmax MIT: guidelines	12 weeks (3/week)	TTE			↘ Time to peak basal UTR, time to peak apical UTR and time to peak UTR (both groups) ↘ Apical UTR (MIT group) ↔ Peak apical and basal rotation, basal twist and untwist rate (TR and UTR), apical TR, peak twist, peak TR and UTR

							Significant between-group comparisons of changes: See Electronic Supplementary Table 2.
Hollekim-Strand et al. (2014)	HIIT vs. MIT Home-based exercise	HIIT: 90-95% HRmax MIT: unknown	12 weeks (3/week)	TTE	↗ e' (both groups) ↗ E, E/A ↔ E/e' Significant between-group comparisons of changes: ↗ e' (HIIT group > MIT group)	↗ S wave ↘ Global strain rate Significant between-group comparisons of changes: ↗ S wave (HIIT group > MIT group) ↘ Global strain rate (HIIT group > MIT group)	

Hordern et al. (2009)	Endurance exercise training Gym sessions and home-based exercise	Moderate (Borg scale 12-13)	1 year (3/week)	TTE	↗ e' (both groups)	↗ S wave, myocardial strain* (both groups) ↔ LVEF and myocardial strain rate	↔ LV mass, LV mass index, LV dimensions
Howorka et al. (1997)	Endurance exercise training Cycling	60-70% HRmax	12 weeks (2/week)	TTE	Improvements in relaxation disturbances		↔ Intraventricular wall thickness and LVEDD
Jonker et al. (2013)	Endurance/ resistance exercise training Individualized training program	Moderate intensity	24 weeks (4/week)	MRI, magnetic resonance spectroscopy	↔ E/A and E/e'	↔ LVEF, SV and cardiac index	↔ LV mass, LV mass index and LV dimensions (EDV and ESV) ↔ Epicardial fat and myocardial triglycerides content ↘ Pericardial fat

Loimaala et al. (2007)	Endurance/ resistance exercise training Running/walking	Running/ walking: 65-75% VO2max Resistance exercise: 70-80% MVC	12 months (2/week)	TTE	↔ E, A, mean peak mitral annular early diastolic velocity and mean peak mitral annular late diastolic velocity	↔ Mean peak mitral annular systolic velocity	
Sacre et al. (2014)	Endurance/ resistance training Gym sessions and home-based exercise	Moderate-vigorous	24 weeks (2/week)	TTE	↗ E/A (control group) ↗ e' (both groups) ↔ Dt ↗ E/e' Significant between-group comparisons of changes: ↗ E/A (control group > exercise group)	↗ Strain* and strain rate* (both groups) ↔ S wave and LVEF	↔ LV mass index

Schmidt et al. (2013)	Endurance exercise training Soccer training	Unknown	24 weeks (2/week)	TTE	↗ E/A, E' and e' ↘ Dt, E/e' ↔ A' Significant between-group comparisons of changes: ↗ E/A, E' and e' (control group < exercise group) ↘ E/e' (control group < exercise group)	↗ TAPSE ↗ Global strain and LV displacement (both groups) ↔ LVEF, S wave Significant between-group comparisons of changes: ↗ TAPSE (control group < exercise group) ↘ Global strain and LV displacement (control group > exercise group)	↗ LVEDD, EDV and LV mass index Significant between-group comparisons of changes: ↗ LVEDD, EDV and LV mass index (control group < exercise group)
Schrauwen-Hinderling et al. (2011)	Endurance/ resistance exercise training	55% of predetermined maximal workload	12 weeks (3/week)	MRI, magnetic resonance spectroscopy		↗ LVEF, cardiac index, cardiac output	↘ ESV ↔ EDV and cardiac lipid content

	Unknown	(aerobic exercise) 55-75% of MVC (resistance exercise)					
Schultz et al. (2011)	Endurance/ resistance exercise training Gym sessions and home-based exercise	Moderate intensity (Borg scale 12-13)	12 months (4 weeks: 2x/week, thereafter: home-based)	TTE			↔ LV mass index, LV RWT ratio

Changes refer to exercise group, unless stated otherwise A atrial mitral inflow velocity, A' peak late diastolic velocity measured at the annulus, Dt deceleration time, E early mitral inflow velocity, E' peak early diastolic velocity measured at the mitral annulus, e' peak early diastolic tissue Doppler velocity, E/e' left ventricle filling pressure, E/A ratio of early (E) and atrial (A) inflow velocity, EDV end-diastolic volume, ESV end-systolic volume, HIIT High-intensity interval training, HRmax maximal heart rate, LV left ventricle, LVDD left-ventricular diastolic dysfunction, LVEDD LV end-diastolic diameter, LVEF left ventricular ejection fraction, IVRT Isovolumic relaxation time, MIT moderate-intensity training, MR magnetic resonance, MRI magnetic resonance imaging, MVC maximum voluntary contraction, RWT relative wall thickness, S wave systolic tissue velocity, SV stroke volume, TAPSE tricuspid annular plane systolic excursion, TR twist rate, TTE transthoracic

echocardiography, UTR untwist rate, VO2max maximal oxygen uptake, ↗ increase, ↘ decrease, ↔ no changes *:Strain rate analyses positively expressed

5.4.2.2 Systolic Function

Between-group analyses were reported for the following parameters: left ventricular ejection fraction (LVEF) (n=3 out of seven studies), strain rate (n=4 out of five studies), stroke volume (n=1 out of one study), cardiac output (n=1 out of one study), systolic tissue velocity (n=5 out of six studies), and tricuspid annular plane systolic excursion (TAPSE) (n=1 out of one study). HIIT training was able to improve LVEF and SV compared to the control group (427), although endurance exercise-training studies failed to exert these effects on LVEF (484, 485). Regarding strain rate and systolic velocity, only one study reported improvements with HIIT training compared to MIT training (482), whereas these effects were not confirmed in the study by Cassidy et al (427). The same applies to endurance exercise-training studies, as only the study by Schmidt et al. (485) reported improvements in strain rate compared to the control group, whereas this could not be confirmed in other studies (431, 483, 484). Schmidt et al. (485) also reported superior effects on TAPSE compared to the control group (485). However, it is worth noting that LVEF was normal at baseline in the majority of the studies, which may suggest that LVEF was not suitable as a surrogate for impaired systolic cardiac function. Lastly, one study reporting twist (systolic function) and untwist (diastolic function) rates found contradictory results, as different results were observed in regional domains (486). Overall, only three studies indicated a superior effect on one of the systolic parameters. Given these heterogeneous results, it is objectively impossible to draw unambiguous conclusions regarding the effect and the type of exercise training on systolic function, even if the general perception of exercise training and systolic function is different

5.4.2.3 LV Dimensions and Structure

Between-group analyses were reported for the following parameters: LV mass (n=2 out of four studies), LV mass index (n=3 out of four studies), LV dimensions (n=1 out of three studies), end-diastolic volume (EDV) (n=2 out of five studies), end-systolic volume (ESV) (n=1 out of four studies), LV end-diastolic diameter (LVEDD) (n=1 out of one study). HIIT training superiorly increased LV mass (427) whereas MIT was not able to exert this effect (431). For LV mass index, only one

study reported a superior effect of exercise training (485). Regarding EDV, both endurance training and HIIT training were able to increase this parameter (427, 485). However, exercise training was not able to exert superior effects on ESV or LV dimensions (427, 431).

5.4.3 Animal Studies

As shown in Table 6, the majority of animal studies were performed in genetically modified mice or rats mimicking T2DM (6/8), and only one study used female mice.

5.4.3.1 Diastolic Function

Despite the fact that T2DM is characterised by important changes in cardiac diastolic function, only 3/8 studies specifically examined the effect of exercise intervention on cardiac diastolic parameters (Table 6). In these studies, exercise intervention was able to improve cardiac diastolic function, as assessed by hemodynamic parameters (e.g., −dP/dt) (447, 487) and time to 50% relengthening (488). In the articles included, only the studies of Hafstad et al. and Boardman et al. compared the effect of exercise type on diastolic function (447, 487). In their studies, MIT and HIIT were equally effective in improving diastolic function.

5.4.3.2 Systolic Function

Changes in systolic function as a result of exercise intervention were assessed in 7/8 studies. All studies reported improved systolic function, by examining either LVEF, fractional shortening (FS), or arterial pressure after exercise training (447, 487-492). Both MIT and HIIT training appeared to be equally effective in improving systolic function.

5.4.3.3 Cardiac Remodelling

Interstitial fibrosis is also a common feature observed in the diabetic heart and is partially responsible for the impaired diastolic function. In all studies examining collagen content (4/8 studies), exercise intervention was able to reduce myocardial fibrosis (447, 489-491). However, only MIT, but not HIIT, was able to exert such an effect in the study by Hafstad et al (447). Regarding LV hypertrophy,

one study reported a less pronounced hypertrophy after exercise training (493) and another decreased cell volume after exercise intervention (488), while in the studies by Hafstad et al. (447) and Boardman et al. (487) only HIIT was able to induce physiological hypertrophy.

Table 6: Summary of studies in animals included in the review, showing training type and intensity, and effects on diastolic and systolic function and left ventricle morphology

Study	Animal model	Training			Outcome assessment method	Effect on cardiac function		
		Type	Intensity	Duration		Diastolic function	Systolic function	Cardiac remodelling
Ko et al. (2018)	Male OLETF rats (28 weeks)	Resistance exercise (ladder climbing)	Moderate	12	Echocardiography	/	↗ Systolic function	/
Boardman et al. (2017)	Male C57BL/6J mice + high-fat diet	Running	High and moderate	10 or 3	Hemodynamic measurements	↗ Diastolic function	↗ Systolic function	↗ Hypertrophy in HIIT
Veeranki et al. (2016)	Male db/db mice	Running	Low to moderate	5	Echocardiographic analyses, blood pressure recording, Sirius Red staining	/	↗ FS ↗ EF ↗ SV ↗ Mean arterial pressure	↘ Fibrosis

Wang et al. (2015)	Male db/db mice	Running	Moderate	15	Echocardiographic analyses, Masson's trichrome staining	/	↗ EF ↗ FS	↘ Fibrosis
Kesherwani et al. (2015)	Female C57BL/6J + high-fat diet	Swimming	Low	8	Echocardiographic analyses, Masson's trichrome staining	/	↗ %EF ↗ %FS	↘ Fibrosis
Hafstad et al. (2013)	DIO mice, high-fat diet initially, after 9 weeks Western diet	Running	High and moderate	8 to 10	Hemodynamic measurements, Sirius Red staining	↗ Early and late diastolic function	↗ Systolic function	↘ Fibrosis in MIT ↗ Hypertrophy in HIIT
VanHoose et al. (2010)	Male Zucker Diabetic Fatty rats	Running	Low to moderate	7	Electrocardiogram	/	/	↘ Hypertrophy
Stolen et al.	Male db/db mice	Interval running	High	13	Echocardiographic analyses	↘ Time to 50% relengthening	↗ SV ↗ FS	/

(2009)								

OLETF Otsuka Long-Evans Tokushima Fatty, db diabetes, DIO diet-induced obesity, FS fractional shortening, EF ejection fraction, SV stroke volume, HIIT high-intensity interval training, MIT moderate intensity training, / not applicable, ↗ increased, ↘ decreased

5.5 Discussion

According to this systematic literature review, exercise training elicits significant improvements in diastolic function in T2DM with or without DCM and a reduction in myocardial fibrosis, but not necessarily improvements in systolic function. The impact of different exercise modalities on these outcomes remains uncertain.

5.5.1 Exercise Improves Cardiac Function

In both T2DM patients and animal models for T2DM with or without DCM, cardiac function improves significantly following exercise intervention. Effective improvements were observed in diastolic function after only 12 (human studies) and 3 (animal studies) weeks of exercise training (mainly endurance). These data thus indicate that significant improvements in diastolic function can be achieved by exercise training in a relatively short timeframe of 12 weeks in T2DM patients. However, it should be noted that studies examining the effects of shorter-term exercise interventions (<12 weeks) in the T2DM population have not thus far been performed. Targeting diastolic function with exercise intervention is important as impaired diastolic function is an early marker of DCM, and can eventually lead to heart failure.

Systolic function in animal models shows substantial improvements after just 3 weeks of exercise training associated with a decrease in myocardial collagen deposition (after 5 weeks). Conversely, effects of exercise on systolic function in T2DM patients remain controversial. An explanation for this apparent discrepancy could possibly rely on the fact that LVEF was preserved in the T2DM patient groups included in this review. It is therefore likely that the potential positive effects of exercise intervention on systolic function were underestimated and/or could not be detected in the patient studies due to non-impaired LVEF at baseline. The impact of exercise intervention thus remains to be studied in T2DM patients with impaired systolic function.

Currently, it is recommended that diabetes patients perform exercise at least 150 min/week at moderate to vigorous intensity and for at least 3 days/week (266, 363). Regarding HIIT, the American Diabetes Association (ADA) states that vigorous-intensity or interval training for at least 75 min/week may be sufficient

for younger adults and more physically fit individuals. For four out of the 13 studies, it was doubtful whether the prescribed exercise training volume was sufficient to comply with current guidelines (427, 483, 485, 494). However, the outcomes from these studies seemed comparable with those of the other studies. Nevertheless, these are guidelines aiming to specifically improve glycemic control, rather than cardiac function. For a patient with diabetes and impaired cardiac function, the European Association of Preventive Cardiology Exercise Prescription in Everyday Practice and Rehabilitative Training (EXPERT) tool could be useful (495). To improve cardiac function and glycemic control, the EXPERT tool recommends daily activity of at least 30 min/session at moderate intensity for at least 8 weeks. Therefore, it appears to be appropriate to use the ADA recommendations.

5.5.2 Type of Exercise Does not Seem to Affect Outcome

Surprisingly, the variance in applied training modalities was small and different parameters were used to describe cardiac function/structure, thus impeding the detection of a clear relation between exercise modalities and changes in cardiac function/structure. Recently, the impact of HIIT has attracted greater interest in cardiovascular rehabilitation. Epidemiological and experimental data suggest that HIIT might provide additional benefits and be more potent than the classical MIT modality (378). In healthy subjects, sparse data suggest that the potential of HIIT in comparison to MIT to improve cardiac function is most likely attributable to a higher mitochondrial fatty acid oxidation and metabolism (379). HIIT, but not MIT, also lowers systolic blood pressure (SBP), and reduces HbA1C, high-density lipoprotein cholesterol (HDLC), malondialdehyde (MDA) levels, nitric oxide (NO), and von Willebrand factor (vWF) in T2DM patients (384). Overall, HIIT seems superior in improving vascular functions compared with MIT. In animal studies, three studies focused on HIIT, while all others examined MIT. We observed no difference between diastolic and systolic improvements after both exercise modalities. However, only MIT and not HIIT was able to reduce cardiac fibrosis, while only HIIT induced physiological hypertrophy. Physiological hypertrophy is a result of developmental growth and exercise with normal cardiac structure without dilated cardiomyopathy, while in diabetes pathological hypertrophy can occur through pathological stimuli (pressure or volume overload) creating a

compensating response (443, 496). For clinical studies, three studies investigated the effect of HIIT in diabetic patients. Two of these studies investigated mitral inflow pattern as a marker of diastolic function and reported beneficial results. However, only one study used echocardiography, whereas the other used magnetic resonance imaging (MRI), further complicating the comparison with the MIT studies and making it unable to draw firm conclusions. In addition to exercise mode, exercise frequency (from two up to four sessions/week) and applied continuous intensity [50–75% of maximal oxygen uptake (VO2max)] were comparable between studies and did not display different beneficial patterns. Finally, it is important to mention that beneficial changes in cardiac function (mainly diastolic) were already noticed after a relatively short exercise intervention (12 weeks) in most studies (Table 5). These data may thus contradict the widely held belief that prolonged exercise interventions are mandatory to enhance cardiac function.

5.5.3 Different Underlying Mechanisms of Exercise Training Modalities in T2DM with DCM Remain Unclear

Underlying mechanisms involved in T2DM and DCM from animal studies and the potential beneficial effects of exercise intervention are shown in Fig. 11. Altered contractile and electrophysiological cardiomyocyte function combined with an increased fibrosis are known to contribute to impaired cardiac function in T2DM, especially in the presence of inflammation, hyperglycemia, hyperinsulinemia, and elevated blood free fatty acid (FFA) concentrations (94). Although HbA_{1C} decreased significantly (up to 0.7%) after exercise intervention in most human studies, this was not always accompanied by decreased fasting blood glucose concentrations. In addition, exercise training intervention did not consistently modulate blood insulin concentrations or indicators of insulin resistance (431, 483, 497). However, diastolic function was enhanced. As a result, it appears that improvements in blood glucose and insulin concentrations are not prerequisites to enhancement of cardiac function in T2DM patients, but probably other blood parameters may be (such as FFAs or inflammatory factors). This is further suggested in animal studies where blood glucose concentrations do not consistently improve after exercise intervention, despite improvements in cardiac function (447, 488-490). Indeed, as shown in the studies by Stolen et al. (488),

Ko et al. (492), and Hafstad et al. (447), exercise did reduce FFAs and triglycerides in the plasma of diabetic animals (487). In addition, the balance between inflammation markers, i.e., interleukin 10 (IL-10) and tumor necrosis factor alpha (TNF-α), was positively affected after exercise in heart tissue itself (489). Taken together, data indicate that changes in lipids rather than changes in glucose levels play a role in the improved cardiac function. However, the importance of improved inflammation parameters in enhancing cardiac function in T2DM with or without DCM and the underlying mechanisms involved remain to be determined. It is well described that an excess of lipid accumulation in cardiac tissue results in cardiac lipotoxicity, leading to myocyte apoptosis and contractile dysfunction (498, 499). Alterations in the lipid metabolism at the cardiac level contribute to the pathogenesis of diabetic cardiomyopathy by increasing myocardial triacylglycerol (TAG) and ceramide content (500, 501).

Extracellular remodelling and reduced fibrosis are known to be associated with improved cardiac function (459, 502). All studies that were included showed a reduction of fibrotic tissue in the heart, together with less apoptosis after exercise intervention. However, whether exercise modality and duration play differential roles in the beneficial remodelling remains unclear.

In addition, adequate mitochondrial function to ensure proper metabolism is mandatory to induce improved cardiac function, given the high energetic demand of the heart. In that context, it is well known that mitochondrial function is decreased in DCM (103). Energy availability and demand in the diabetic heart are imbalanced, resulting in a heart with reduced working capacity. In the studies included, exercise training was able to restore impairments in mitochondrial DNA (mtDNA) transcription and replication and increase mtDNA. Dynamin-related protein 1 (Drp-1) levels were normalised after exercise, balancing the excessive mitochondrial fission in DCM and repairing contractile defects in the myocytes. In addition, mitochondrial transmembrane depolarisation improved mitochondrial cytochrome c levels contributing to improved myocyte function, and exercise increased mitochondria levels and restored their appearance. Effects of exercise also reduced myocardial reactive oxygen species (ROS) content and increased mitochondrial superoxide dismutase (SOD), further emphasizing the beneficial effect of exercise on mitochondrial function (447, 492). After exercise

intervention, mitochondrial respiratory capacity and efficiency will be improved, resulting in enhanced cardiac function due to improved ATP synthesis and energy handling (107).

Peroxisome proliferator-activated receptor gamma coactivator 1-α (PGC-1α) plays a key role in the regulation of myocardial energy metabolism and is important in mitochondrial biogenesis. After diabetic animals were subjected to exercise, messenger RNA (mRNA) and protein levels of PGC-1α and other components of the pathway were upregulated, while inhibition of Akt phosphorylation in the heart was reversed (491, 492). However, another study showed no difference between diabetic animals and wildtype animals in PGC-1α (488). Finally, Hafstad et al. showed an increased myocardial glucose oxidation metabolism and lower fatty acid oxidation rates, resulting in a mild change in substrate utilisation after exercise (447). The altered substrate metabolism was confirmed by Ko et al., where exercise upregulated protein expression levels of glucose transporter type 4 (GLUT4), while peroxisome proliferator-activated receptor alpha (PPARα) levels were lowered (492). Changes in [Ca2+] homeostasis are disrupted in DCM animals and were reversed after exercise (107). Indeed, diastolic and systolic Ca2+ levels were normalised with HIIT training (488). These changes were associated with improved SR Ca2+ load, sarcoplasmic reticulum (SR) Ca2+ leak, phospholamban (PLB) phosphorylation, sarcoplasmic/endoplasmic reticulum Ca(2+) ATPase 2a (SERCA2a), and sodium-calcium exchanger (NCX) protein expression and function, all contributing to improved cardiomyocyte function with exercise (488). However, changes in SERCA2a or Ryr2 mRNA expression were not confirmed by others (447).

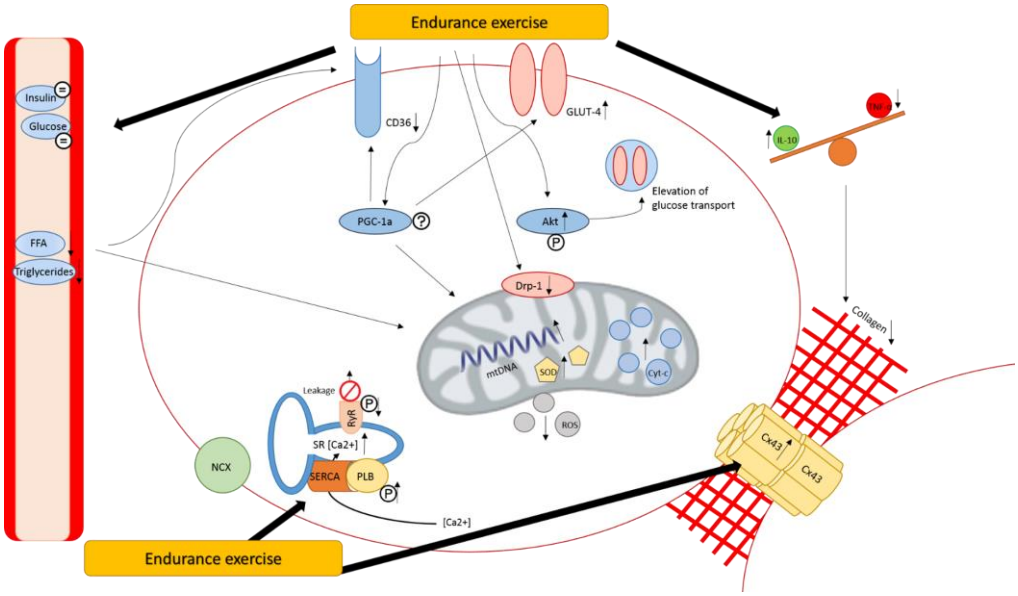

Figure 11: Exercise affects both cardiomyocyte function itself and circulating factors in DCM. The circulatory concentrations in pro-inflammatory cytokines, as well as FFAs, decrease during exercise intervention, which relates to less fibrotic tissue in the heart. Balance in substrate metabolism between FFA and glucose oxidation is restored after exercise intervention. Mitochondrial function is improved, with less mitochondrial fission and less oxidative stress. Calcium handling in the cardiomyocyte is improved and communication between cells through increased gap junctions is significantly altered after exercise intervention. DCM diabetic cardiomyopathy, FFAs free fatty acids, TNF-α tumor necrosis factor alpha, PLB phospholamban, SERCA sarcoplasmic/endoplasmic reticulum Ca (2+) ATPase 2a, SR sarcoplasmic reticulum, NCX sodium-calcium exchanger, GLUT-4 glucose transporter type 4, PGC-α peroxisome proliferator-activated receptor gamma coactivator 1-α, Drp-1 dynamin-related protein 1, mtDNA mitochondrial DNA, SOD superoxide dismutase, Cyt-c cytochrome-c, ROS reactive oxygen species, IL-10 interleukin-10, P phosphorylated, ? No disclosure about effect between groups

5.5.4 Limitations

The main limitation of our review was that conducting a meta-analysis was very difficult, mostly due to the substantial heterogeneity of outcome parameters, the different techniques/methods used (e.g., some researchers included both septal and lateral early diastolic velocity to calculate E/e', whereas others used only one of these) and the lack of explanation in the methodology sections; these

considerations impeded appropriate comparisons and the performance of a meta-analysis. This emphasises the need for the standardised use of echocardiographic parameters as per official guidelines (e.g., guidelines for the evaluation of diastolic dysfunction (503) or speckle tracking echocardiography for deformation imaging (504)) and detailed explanations of methodologies, which would enable appropriate comparisons to be made. In addition, as very few human studies have explored the molecular pathways leading to improvements in cardiac function/structure, conclusions on underlying mechanisms involved are solely based on results obtained from animal studies. Therefore, translation of the observed mechanisms in the clinical context remains speculative. Finally, in patient studies, an accurate PEDro score could only be assessed in 9/13 studies due to study design. Quality scores were lower than expected (due to lack of blinding subjects, therapist administering the exercise therapy, and assessors who evaluated the tests). Indeed, 4/9 studies had a score < 6 and were considered "poor quality" studies. However, the outcomes for low- and high-quality studies were similar.

5.6 Conclusion

Our systematic literature review shows that exercise intervention in patients with T2DM results not only in improved metabolic parameters but also in molecular adaptations Effect of Exercise Intervention in Type 2 Diabetes Mellitus (e.g., extracellular remodelling and reductions in fibrosis) that enhance cardiac function in most cases. More specifically, exercise training ameliorates (or at least positively influences) different aspects (E/A, E/e', e', Dt) of diastolic function and leads to beneficial remodelling in T2DM and DCM animal models. However, exercise training is not necessarily accompanied by improvements in systolic function and LV structure, and more extensive studies are therefore required to elucidate these issues, particularly in terms of unravelling the roles of specific types of exercise. Nevertheless, our review strongly suggests that exercise training should be a cornerstone in the treatment of T2DM, not only to improve glycemic control but also to specifically enhance cardiac function.

6

Is high-intensity interval training as efficient as moderate intensity training in reversing the adverse effects of diabetic cardiomyopathy?

Based on:

Is high-intensity interval training as efficient as moderate intensity training in reversing the adverse effects of diabetic cardiomyopathy?

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6 Is high-intensity interval training as efficient as moderate intensity training in reversing the adverse effects of diabetic cardiomyopathy?

6.1 Abstract

Introduction: Type 2 Diabetes Mellitus (T2DM) is affecting more than 400 million people worldwide and leads to diabetic cardiomyopathy. Exercise intervention is a low cost and safe approach in reducing the adverse effects of diabetic cardiomyopathy. Moderate intensity training (MIT) has been so far the most preferred exercise therapy in the clinic, however high-intensity interval training (HIIT) is gaining interest as a potentially more efficient therapy. In this study, we examined the effect of both training modalities on the cardiac function in a rat model of T2DM.

Methods: Rats were randomly assigned into control (chow diet, N=10) or high sugar diet (Western diet, N=22) for 18 weeks to induce T2DM. After 18 weeks, diabetic rats were assigned to the 3 different training modalities, i.e. sedentary (SED, N=8), MIT (N=7) or HIIT (N=7) for 12 additional weeks. Animals undergoing chow diet were kept sedentary and used as controls. Blood samples, echocardiography and hemodynamic measurements were evaluated to assess cardiac function and lipid profile. Inflammation, fibrosis and other mechanisms were measured from LV tissue homogenates and tissue sections respectively.

Results: After 30 weeks, diabetic animals displayed hypertrophy and altered lipid homeostasis. Both exercise modalities were able to significantly improve all these parameters. However, only HIIT was capable to normalise circulating free fatty acid levels. End-diastolic pressure was increased in sedentary diabetic animals, but normalised after exercise training. In addition, both training modalities were able to reduce the increased collagen deposition observed in diabetic animals, as well as pathological LV wall hypertrophy. Finally, fractional shortening and inflammatory status of the cardiac tissue were improved only with MIT.

Conclusion: Despite very different training modalities, both exercise interventions reversed to the same extent adverse remodelling in diabetic cardiomyopathy. MIT seems however to additionally reduce cardiac inflammation.

6.2 Introduction

Diabetes mellitus (DM) is a disease on the rise, already affecting > 420 million individuals worldwide, with 90% type 2 diabetes mellitus (T2DM) patients. Predicting models estimate that in 2040, these numbers could rise to > 640 million diabetic patients worldwide (3). Globally, DM is ranked as seventh cause of death since 2016 according to the World Health Organization (WHO) (505), making it one of the largest health care challenges of the 21st century. In diabetic patients, the major cause of mortality and morbidity are cardiovascular diseases (109). Diastolic dysfunction, together with increased fibrosis and left ventricle hypertrophy, a hallmark of diabetic cardiomyopathy (DCM), is present in 19.6 % to 54.4 % of the T2DM patients (395, 472). DCM is defined as diabetes-associated structural and functional changes in the myocardium, not directly attributable to other confounding factors such as coronary artery disease or hypertension (91). It is characterised by extracellular and cardiomyocyte remodelling, resulting in impaired cardiac output, diastolic and systolic function (94). Despite a lot of progress in understanding its aetiology during the last few decades, molecular mechanisms remain incompletely understood (95). Risk factors in the development of T2DM and DCM are a sedentary lifestyle, a lack of physical activity and an unhealthy diet (417). Exercise intervention is an effective, low-cost and safe strategy for the prevention and treatment of cardiovascular diseases (CVD). Exercise decreases the risk of developing T2DM and obesity. Moreover, an active lifestyle and a healthy diet affects both cardiovascular health and metabolic control. While obesity and hypertension occur frequently in T2DM patients and are well-known risk factors, studies have shown that cardiorespiratory fitness is a more powerful predictor of CVD. Prevention of CVD in T2DM related to exercise is thought to be attributed to improved vascular function and hereby related tissue perfusion.

The beneficial effects of moderate intensity training (MIT) in normal as well as in pathological situations are often described. However, high-intensity interval training (HIIT) has gained much attention recently. HIIT is characterised by session consisting of successive bouts of short duration at a relative high-intensity workload, alternated with small periods of active rest. Epidemiological and experimental data suggest that HIIT might provide additional benefits and be

more potent than the classical MIT modality. There is growing evidence showing that the beneficial effects of HIIT over MIT in both healthy and chronic diseased populations. In healthy adults, HIIT provides greater improvements in cardiorespiratory fitness compared with MIT (382, 383). Research in patients with coronary heart disease and cardio-metabolic disorders confirmed these findings (506, 507). In T2DM, patients are showing a good adherence to dietetic and pharmacologic interventions, but not to exercise (385, 508). This is mostly due to barriers of physical activity, such as lack of time and a difficulty to participate in exercise (375, 509). In that context, HIIT might be more interesting as a training modality of short duration in the diabetic population to encourage physical activity and thus reduce their chances of chronic complications (374). Previous studies reported that HIIT improves patient adherence to physical activity (510, 511), glycemic control (512, 513) and mitochondrial function (514).

In this study, we compared the effects of two exercise modalities, namely HIIT and MIT, on cardiac function in a rat model of DCM and identified the different underlying mechanisms.

6.3 Methods

This investigation conforms to the EU directive 2010/63/EU for animal experiments and was approved by a local ethical committee (Ethical Committee for Animal Experiments, UHasselt, Diepenbeek, Belgium). All experiments and methods were performed according the relevant guidelines.

6.3.1 Experimental set-up

Thirty-two male Sprague-Dawley rats (Charles River Laboratories, L'Arbresle, France), weighing 200-225 g were used throughout the study. Rats were randomly assigned in two experimental groups by using a randomisation tool (Excel). Group 1 (n=10) received a normal rodent diet (ENVIGO, The Netherlands, Control diet) throughout the full experiment and served as control group. Group 2 (n=22) was fed a high-sugar and high-fat diet (Western diet) as described previously by our group (515). Animals were housed per 3 in a cage, were maintained in a controlled environmental condition and had water and food available *ad libitum.* Every morning, rats were provided freshly prepared food

(Western or control diet). After 18 weeks of diet, animals of group 2 were randomly divided to one of the three experimental training groups during 12 additional weeks, as described previously by our research group (516). Group 2A (n=8) did not undergo exercise training throughout the study. Group 2B (n=7) was subjected to moderate intensity training (MIT), consisting in running on a treadmill, 18m/min, 5° inclination, 5 days/week, time-depending on energy expenditure of the comparable modality (High-Intensity Interval Training; HIIT). Group 2C underwent 10 bouts of high-intensity treadmill running (18m/min, 30° inclination), separated by one minute of active rest, 5 days/week (HIIT). Sample size was calculated by a power analysis based on previous results in our research group (516).

The intensity of exercise training was assessed by measuring blood lactate levels directly after exercise with an Analox apparatus (Analis, Namur, Belgium). Levels >4 mmol/l lactate were considered HIIT (439, 516). Training modalities were adjusted to lead to an equal energy expenditure between interventions by calculating the net caloric cost (kcal/min) as previously described (516). Blood samples, echocardiographic measurements and an oral glucose tolerance test (OGTT) were executed at baseline, 18 weeks and 30 weeks after the start of the diet. Hemodynamic measurements were performed at sacrifice, 30 weeks after start of the experiment.

6.3.2 Conventional echocardiographic measurements

Prior to sacrifice, transthoracic echocardiography was performed under 2% isoflurane in all animals with a Vivid I ultrasound machine (GE Vingmed Ultrasound) using a 10 MHz linear array transducer. The protocol used is as described previously (515). Briefly, a standard parasternal long axis image and short axis views at the mid-ventricular level were obtained at a temporal resolution of approximately 200 frames per second. Conventional echocardiographic parameters (e.g. LV end-diastolic diameter (LVEDD), LV end-systolic diameter (LVESD), posterior wall thicknesses (PWT) and anterior wall thicknesses (AWT)) were obtained from the B-mode images at midpapillary level in the parasternal short-axis view. End-systolic volumes (ESV) and end-diastolic volumes (EDV) were calculated by π*DM2*B/6, where DM indicates the systolic/diastolic diameter of the ventricle in mid-ventricular short-axis view and

B is LV length on parasternal long-axis image. Subsequently, ejection fraction (EF) was measured as (EDV–ESV)/EDV, and expressed in %.

6.3.3 Hemodynamic measurements

Pressure measurements were performed in all animals with the use of an SPR-320 MikroTip high-fidelity pressure transducer (Millar Inc) that was advanced into the left ventricle via the right carotid artery, as described previously (515). The pressure catheter (2F) was connected to a quad-bridge amplifier and PowerLab 26T module (AD Instruments, United Kingdom) was used to transfer the data to LabChart v7.3.7 software (AD Instruments, United Kingdom).

6.3.4 Oral glucose tolerance test and insulin resistance assessment

Glucose tolerance was assessed at baseline, 18 weeks and 30 weeks after the start of the diet, with a 1 h OGTT as previously described (404, 515). After an overnight fasting, glucose (2 g/kg) was administered via gastric gavage. Prior to glucose administration, blood glucose concentration was determined from capillary tail blood collection with Analox GM7 (Analis SA, Namur, Belgium), and repeated 15, 30 and 60, minutes after administration. Glucose response was expressed as total area under the curve (AUC). At baseline and after 60', serum insulin concentrations were measured by electrochemiluminescence (Meso Scale, Gaithersburg, MD) (515).

6.3.5 Lipid profile determination

Triglycerides, total cholesterol and HDL cholesterol (HDLC) were determined in the Ziekenhuis Oost-Limburg (Genk, Belgium) using Roche/Hitachi cobas c systems (Rotkreuz, Switzerland). Quantification of the NEFA was assessed by colourimetry using a NEFA quantification assay kit (Abcam, ab65341, Cambridge, United Kingdom) (515).

6.3.6 Fibrosis measurement

Transversal sections of 7µm thick were obtained at the midventricular level of the left ventricle and stained using the Sirius Red/Fast Green kit (Chondrex), as previously described (515). Fibrosis was assessed in all animals in 3–4 randomly chosen fields per section. The area of collagen deposition indicated by red staining

was outlined and quantified using an automated image analysis program (Carl Zeiss, AxoVision 4.6, Zaventem, Belgium). Blood vessels were excluded. Total collagen deposition to the global cardiac area was calculated, normalised to total surface area and expressed as %.

6.3.7 Western blot

Protein concentrations of the LV tissues were determined by the BCA protein assay kit (Thermo Fisher, Erembodegem, Belgium). Western blot was performed as previously described (133). Briefly, equal amounts of proteins (15 µg) were separated on a 12% SDS-PAGE gel with a mini protean 3 electrophoresis system (Bio-rad Laboratories, Temse, Belgium), transferred to a polyvinylidene fluoride (PVDF) membrane and subsequently, blocked for 2h with 5% milk in Tris-buffered solution containing 0.1% Tween-20 (TBS-T) followed by incubation overnight at 4°C in the presence of a NOX2 antibody (1/2500, Abcam, ab31092, Cambridge, United Kingdom), TNF-α antibody (1/1000, goat polyclonal IgG, Santa Cruz, N-19, Heidelberg, Germany) or an PPAR antibody (1/1000, Abcam, ab110411, Cambridge, United Kingdom). Horseradish peroxidase-conjugated secondary antibodies (DAKO, Belgium) at a dilution of 1/2000 were used. Both primary and secondary antibodies were diluted in 5% milk-TBS-T. Visualisation was performed with the enhanced chemiluminescence (ECL) technique using the Pierce ECL Plus western Blotting Substrate (Thermo Fisher, Erembodegem, Belgium). Data were normalised to β-actin protein levels.

6.3.8 Citrate synthase activity in cardiac homogenates

Citrate synthase activity was determined in LV cardiac homogenates using a citrate synthase assay kit (CS0720; Sigma-Aldrich, St. Louis, MO) (516).

6.3.9 Statistical analysis

Results were tested for normality prior to statistical tests. Subsequently, one-way ANOVA test was performed combined with a post- hoc test, dependent of normality. If data were normally distributed, Tukey's range test was performed. Elsewise, Dunn's test was used. Analyses were performed using GraphPad Prism (GraphPad Software, San Diego, CA, USA). A value of $p<0.05$ was considered statistically significant.

6.4 Results

6.4.1 Both exercise modalities are equally efficient on general animal characteristics

No differences were observed between animals at baseline. Hallmarks of T2DM were observed in cafeteria-fed animals after 18 weeks by OGTT examining glucose and insulin levels. Echocardiographic analysis showed left ventricle hypertrophy, as already previously described (515). An altered lipid profile was observed in CAF animals compared with CNTL animals (515). After 12 weeks of exercise, clear differences were found between sedentary and exercised diabetic animals. Cafeteria-fed sedentary animals were significantly heavier than their counterparts that did receive normal diet, while both exercise trainings were able to decrease body weight. Both heart weight and heart weight/tibia length were elevated in diabetic animals. Exercise training, independent of the training modality, prevented the increase in these parameters. A cafeteria diet induced an increase in liver weight, which was reduced by HIIT. Lipid profiles were altered in diabetic animals after 30 weeks compared with control animals, with increased triglycerides and free fatty acids (FFAs). Both exercise modalities were capable of reducing circulating triglycerides, however only HIIT was able to normalise circulating FFAs. All data are summarised in table 7.

Table 7: Animal characteristics 30 weeks after the start of the study.

	CNTL (N = 10)	CAF-SED (N = 8)	CAF-MIT (N = 7)	CAF-HIIT (N = 7)
BW (g)	578 ± 18	766 ± 20*	629 ± 27~	612 ± 19~
HW (g)	1.8 ± 0.1	2.2 ± 0.1*	1.9 ± 0.1	2.0 ± 0.1
HW/BW (mg/g)	3.0 ± 0.1	2.9 ± 0.3	3.1 ± 0.0	3.2 ± 0.1

HW/TL (g/cm)	40.4 ± 1.8	48.0 ± 1.5*	42.7 ± 1.8	42.6 ± 1.3
Lung weight (g)	1.8 ± 0.1	1.7 ± 0.1	1.8 ± 0.02	1.6 ± 0.1
Liver weight (g)	15.3 ± 0.6	19.4 ± 0.6*	17.7 ± 1.2	15.7 ± 0.8~
Triglycerides (mg/dl)	68.7 ± 7.0	132.1 ± 16.0*	65.3 ± 10.1~	60.4 ± 3.7~
Total cholesterol (mg/dl)	73.5 ± 4.1	69.1 ± 4.0	58.3 ± 3.4	59.4 ± 3.3
HDLC (mg/dl)	63.9 ± 3.8	56.0 ± 3.1	48.7 ± 3.4*	49.3 ± 3.8*
FFAs (µM)	2.4 ± 0.1	2.9 ± 0.1*	2.7 ± 0.1	2.4 ± 0.1~

BW, body weight; HW, heart weight; TL, tibia length; HDLC, high-density-lipoprotein cholesterol; FFA, free fatty acids. Data are shown as mean ± S.E.M. * denotes $p<0.05$ vs. CNTL, ~ denotes $p<0.05$ vs. CAF-SED.

6.4.2 Cardiac function is restored after both exercise modalities in diabetic animals

Echocardiographic analysis revealed an increase in anterior wall thickness (AWT) after 18 weeks in animals subjected to the cafeteria diet (data not shown). Thirty weeks after the start of the experiment, this observation was confirmed by echocardiography in sedentary diabetic animals, where an increased AWT was shown. However, both MIT and HIIT were capable of reversing left ventricle hypertrophy (LVH) in diabetic animals. As shown previously (515), end-diastolic pressure (EDP) was increased after 18 weeks of cafeteria diet, together with an increase in fibrotic tissue in cardiac tissue. After 30 weeks diet and 12 weeks exercise training, end-systolic volume (ESV) was elevated in diabetic non-trained animals but not in trained diabetic animals. Both ejection fraction (EF) and fractional shortening (FS) were reduced in diabetic sedentary animals. Both HIIT

and MIT improved EF, but only MIT was capable of restoring FS. EDP remained significantly increased in diabetic animals after 30 weeks of diet, but not in those who underwent either exercise modality. Data are displayed in table 8.

Table 8: Echocardiographic analyses and hemodynamic measurements at sacrifice.

	CNTL (N = 10)	CAF-SED (N = 8)	CAF-MIT (N = 7)	CAF-HIIT (N = 7)
HR (beats/min)	338 ± 12	334 ± 8	327 ± 19	320 ± 16
AWT (mm)	1.5 ± 0.02	1.8 ± 0.03*	1.6 ± 0.04	1.7 ± 0.05
EDV (µl)	329 ± 27	392 ± 33	413 ± 41	371 ± 31
ESV (µl)	75 ± 10	136 ± 12*	108 ± 18	110 ±10
CO (ml/min)	83 ± 7	86 ± 10	99 ± 12	85 ± 11
EF (%)	78 ± 2	65 ± 2*	75 ± 2	70 ± 3
FS (%)	47 ± 2	35 ± 1***	44 ± 3~	38 ± 2*
LVP (mmHg)	85 ± 2	99 ± 3*	97 ± 3	104 ± 5*
EDP (mmHg)	5.4 ± 1.3	14.1 ± 3.1*	5.6 ± 1.2~	6.7 ± 0.5
Tau (ms)	14 ± 0.95	28 ± 15	15 ± 0.17	35 ± 20

HR, heart rate; AWT, anterior wall thickness; EDV, end-diastolic volume; ESV, end-systolic volume; CO, cardiac output; EF, ejection fraction; FS, fractional shortening; LVP, left ventricle pressure; EDP, end-diastolic pressure. Data are shown as mean ± S.E.M. * denotes $p<0.05$ vs. CNTL, ~ denotes $p<0.05$ vs. CAF-SED.

After 18 weeks of high-sugar and high-fat diet, fasting glucose and insulin were significantly higher in diabetic animals compared with chow-fed animals. Moreover, 60 minutes after administering a glucose solution, insulin levels remained higher in diabetic animals. After 30 weeks of diet, no significant differences were observed between groups. However, a trend (p-value = 0.0591) was observed between control animals and sedentary diabetic animals in 60' post-glucose insulin levels. Furthermore, MIT but not HIIT training tended to reduce the insulin levels (p-value = 0.0773).

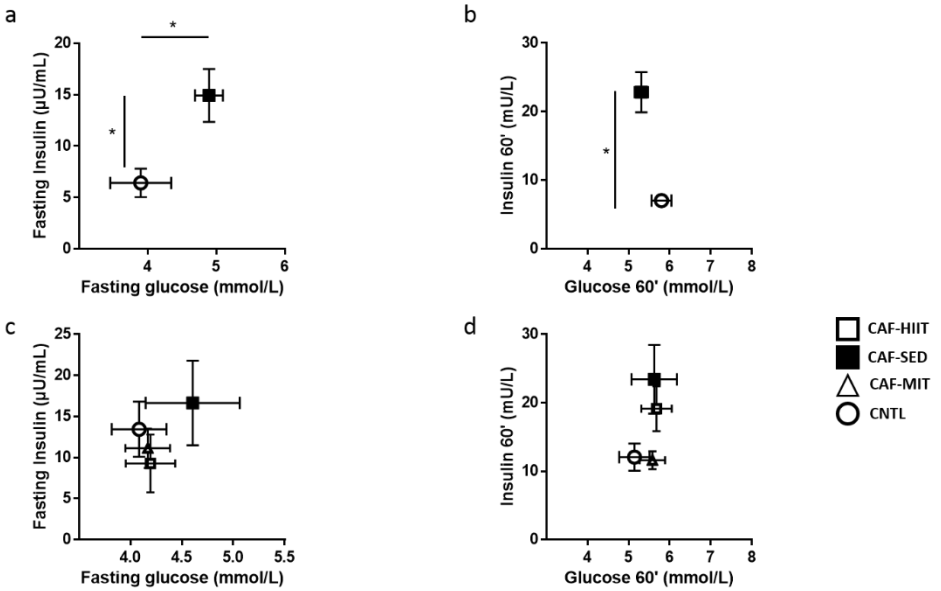

Figure 12: The effect of a combined Western diet with exercise intervention on insulin and glucose levels in T2DM. (A) Fasting glucose and insulin levels after 18 weeks of control or Western diet. (B) 60' post-glucose insulin and glucose levels after 18 weeks of diet. (C) Fasting glucose and insulin levels after 30 weeks of diet, combined with exercise intervention (MIT or HIIT). (D) 60' post-glucose insulin and glucose levels after 30 weeks of diet and exercise intervention on different groups. Data are shown as mean ± S.E.M. in N = 10 in CNTL, N = 8 in CAF-SED, N = 7 in CAF-MIT and N = 7 in CAF-HIIT. * denotes p<0.05 vs. CNTL.

An increased collagen deposition in the heart, one of the hallmarks of DCM, is shown in figure 13. Diabetic animals kept sedentary displayed significantly more fibrotic tissue in the heart than control animals and diabetic exercised animals.

The increased collagen deposition was not associated with changes in LOX levels in this study.

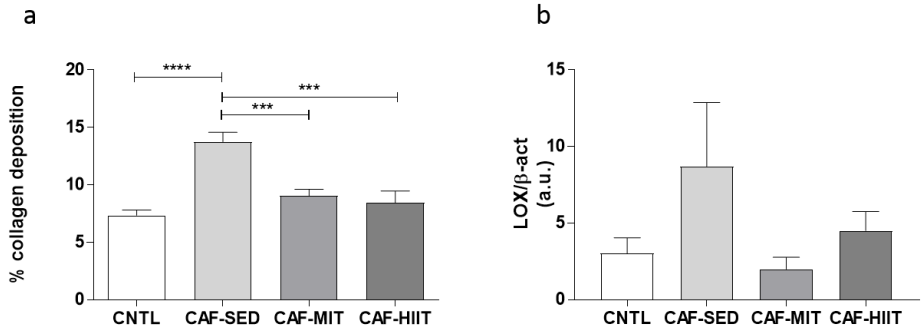

Figure 13: Effect of exercise in interstitial cardiac fibrosis. (A) Total interstitial collagen quantification in the left ventricle of the rat heart. (B) Protein LOX levels normalised to β-actin. Data are shown as mean ± S.E.M. N = 10 in CNTL, N = 8 in CAF-SED, N = 7 in CAF-MIT and N = 7 in CAF-HIIT * denotes p<0.05.

6.4.3 Molecular mechanisms in the development of diabetic cardiomyopathy

After 30 weeks of Western diet, protein levels of cardiac TNF-α levels were significantly increased compared with chow-fed animals. As shown is figure 14A, MIT could prevent the increase in this marker of cardiac inflammation, whereas HIIT could not. However, this result was not seen at the gene level, suggesting post-translational changes. In addition, no difference in IL-6 gene levels were seen in the different groups.

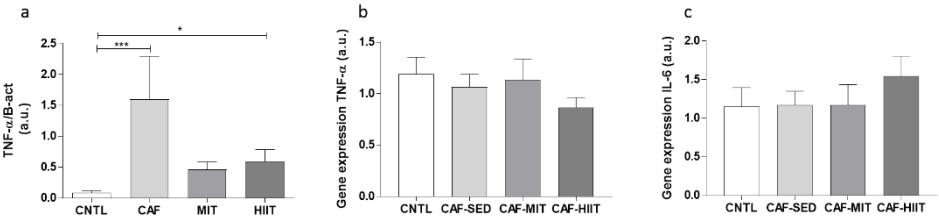

Figure 14: Cardiac inflammation in trained and untrained diabetic animals. (A) Protein TNF-α levels normalised to β-actin. (B) Gene expression level of TNF-α. (C) Gene expression of IL-6. Data are shown as mean ± S.E.M. N = 10 in CNTL, N = 8 in CAF-SED, N = 7 in CAF-MIT and N = 7 in CAF-HIIT. * denotes p<0.05 vs. SED.

Both substrate metabolism and mitochondrial function are altered in diabetic animals (106). As shown in figure 15, PPAR-α – a regulator of fatty acid uptake in cardiomyocytes – attended to be increased in the CAF-SED group (p-value = 0.07). Exercise training prevented the increase in PPAR-α. NOX2 protein expression, known to be involved in generating ROS, was not different between groups.

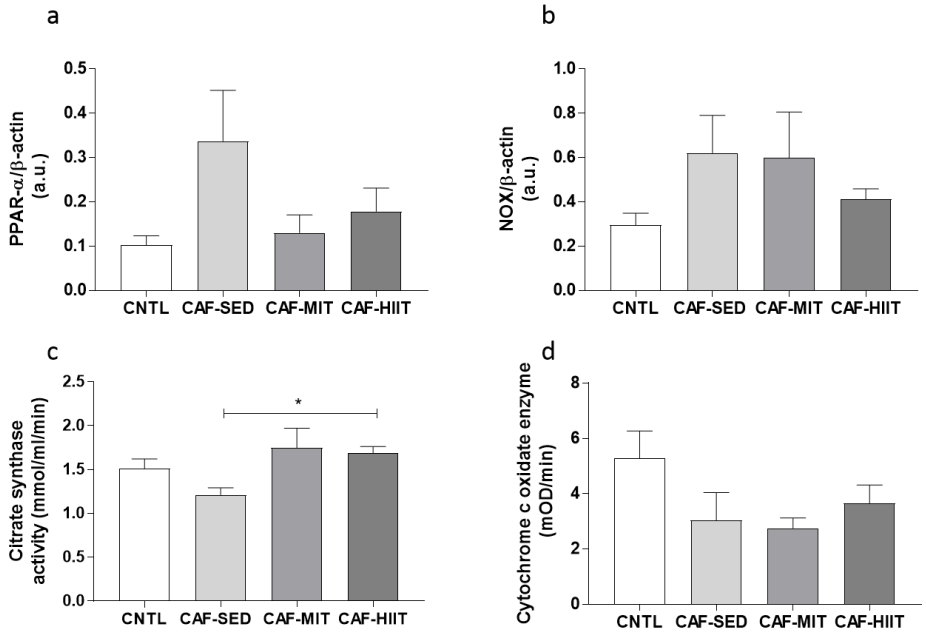

Figure 15: Effect of exercise training on markers of mitochondrial mass and function in DCM (A) PPAR-α protein levels normalised to β-actin. (B) NOX protein levels normalised to β-actin. (C) Citrate synthase activity. (D) Cytochrome c oxidase enzyme activity. Data are shown as mean ± S.E.M. N = 10 in CNTL, N = 8 in CAF-SED, N = 7 in CAF-MIT and N = 7 in CAF-HIIT. * denotes $p<0.05$ vs. SED.

Citrate synthase activity, a marker of aerobic capacity and mitochondrial mass, improved significantly in HIIT trained animals compared with CAF-SED. Moreover, a trend was observed in MIT trained animals compared with sedentary animals (p-value = 0.07). As shown in figure 15D, cytochrome c oxidase activity, used as a surrogate for oxidative phosphorylation capacity, was not different between groups.

6.5 Discussion

Diabetes is becoming one of the challenges of this time, already affecting more than 400 million persons (93). DM is associated with an unhealthy diet, sedentary lifestyle and physical inactivity (517). In this study, we assigned rats to a high-sugar and high-fat diet to induce T2DM and DCM, as previously shown by our research group (515). Diabetic rats were then subjected to a training intervention (MIT or HIIT) (516). Here, we show that both exercise modalities (MIT and HIIT) are effective in improving and reversing the negative phenotype of DCM. We found that outcome was comparable between the two different training modalities, demonstrating the efficiency of HIIT in this setting and further suggesting a potential role for HIIT as a therapy in T2DM patients. Physical activity plays a key role in the therapy and prevention of T2DM and DCM. Yet exercise adherence remains low in the diabetic population (518). As the lack of time is one of the major barriers for adherence to exercise training (509), HIIT is interesting due to its modality as being short in duration. There are concerns about the feasibility of implementing high-intensity exercise in the T2DM population, especially in older, sedentary and overweight participants. Primary barriers here are concerns over the risk of injury, poor adherence and low self-efficacy in implementing exercise (519-521). By administering high-intensity interval exercise, recovery periods are implemented in the training schedule, to lower these barriers of physical activity. Indeed, a study by Terada et al. has shown that adherence to HIIT was similar to MIT (522). Moreover, HIIT is more effective in improving aerobic fitness compared with MIT (369-372). Low aerobic fitness is associated with higher all-cause and cardiovascular mortality (523, 524). The feasibility of HIIT in a T2DM population is demonstrated recently by Hwang et al., showing completion rate for HIIT of 81% (368). Altogether, these data suggest HIIT as a preferable therapy for T2DM patients, with similar effects to the cardiac phenotype, in shorter exercise programs.

We already showed previously that after 18 weeks of high-sugar and high-fat diet, rats display a T2DM phenotype together with hallmarks of DCM (515). These hallmarks are increased fibrotic tissue, hypertrophy and diastolic dysfunction (187, 395). Again, after 18 weeks of diet, left ventricle hypertrophy was observed characterised by a significant increase in wall thickness (CNTL = 1.49 ± 0.019;

CAF = 1.69 ± 0.031). This hypertrophy remained present and further worsened after 30 weeks of diet (table 8). Exercise was able to reverse hypertrophy and further prevent its worsening. The beneficial effect of exercise on cardiac hypertrophy was also demonstrated by others (525). Furthermore, after 18 weeks of diet, only increased fibrosis and an elevated end-diastolic pressure were present, as described previously (515), indicating that DCM occurs early in the development of the disease. Indeed, impaired diastolic function combined with systemic insulin resistance are indicators of early DCM (111, 526, 527). When DCM progresses to the late stage, systolic function and metabolism will be affected (189, 528). Inflammation and ROS both promote the development of interstitial collagen deposition and crosslinking, both associated with increased fibrosis and impaired relaxation (109). In our model, we observed both increased fibrosis and cardiac inflammation after 30 weeks of Western diet, together with an impaired cardiac function. Systolic function was significantly altered, with diminished ejection fraction and fractional shortening, both indicators of systolic dysfunction. These data confirm the development of severe HF in diabetic patients, as also shown in the DIABHYCAR study (529). In this study, patients with DM and HF had a 12-fold higher annual mortality compared with patients that only had DM (530). By changing lifestyle – weight loss, physical activity, limitation of fat and total energy intake – positive effects were observed on metabolic, systemic and cardiac levels. In our study, we see that systolic function improves significantly with both MIT and HIIT. Moreover, both MIT and HIIT were able to reduce the increased end-diastolic pressure observed in CAF-SED animals. Together with a reduction in cardiac fibrosis, our data indicate that exercise improves functional hallmarks of DCM. Between exercise modalities, no differences were observed on cardiac function, suggesting that HIIT could be an interesting alternative therapy in diabetic patients that lack motivation to exercise due to its time-consuming nature.

6.5.1 Mechanisms behind diabetic cardiomyopathy and the effect of exercise

Both exercise modalities improve cardiac function in diabetic animals to the same extent. However, due to the nature of the training modality, underlying mechanisms and activated differential signalling pathways could differ between

both training interventions. DCM is a complex disease and the mechanisms behind the development and progress are yet to be established. Important contributors to DCM are hyperglycemia, hyperinsulinemia and lipotoxicity. In this study, an increase in circulating triglycerides and free fatty acids was observed in diabetic animals. In the diabetic heart, despite hyperglycemia, FFA oxidation is the primary source of energy with glucose oxidation reducing (98-100). Increased circulating FFAs are associated with cardiac mitochondrial uncoupling protein (UCP) 3 (531-535). Peroxisome proliferator-activated receptor (PPAR)α regulate the uptake and oxidation of myocardial FFA. Transcriptional induction of enzymes involved in FA β-oxidation and transport enhance FA uptake, and consequently lead to suppressed glucose oxidation (107). Moreover, PPARα activation can also lead to accumulation of triacylglycerol, leading to toxic intermediates as diacylglycerol and ceramides in the cardiomyocyte resulting in reduced cell viability (101, 107, 108, 536). In exercised animals, both MIT and HIIT, triglycerides and FFAs were reduced or even normalised to CNTL levels. Our data suggest that both MIT and HIIT are able to partially restore the lipid profile in T2DM with DCM. An hypothetical explanation could be related to reduced circulating lipids (triglycerides and FFAs), leading to a diminished FA uptake and more glucose oxidation in the heart. Indeed, in our study, a trend to an increased PPARα expression was observed (p-value = 0.07) in CAF-SED, but not in trained animals. As shown by others, increased PPARα expression lead to higher FA oxidation and reduced glucose utilisation and triacylglycerol (TAG) accumulation (537), typically seen in T2DM and DCM. Insulin signalling will then be impaired by ceramides and diacylglycerol, leading to even further development of DCM (106). Eventually, excessive lipid accumulation contributes to inflammation, fibrosis, and diastolic dysfunction, all observed in this study. Moreover, an excess in fatty acid uptake could exert detrimental consequences on mitochondria (103).

Hyperglycemia, hyperlipidemia and insulin resistance are all improved after exercise therapy. Insulin resistance is associated with obesity, present in our diabetic animals (538). Animals subjected to a high-sugar and high-fat diet were significantly heavier as their counterparts that were fed a normal diet. Exercise training, independent of the modality, reduced body weight in Western diet-fed animals. Before training and as shown previously (515), insulin and glucose levels were significantly altered in diabetic animals. However, after exercise

intervention, no significant differences were observed. Insulin handling in sedentary diabetic animals seemed to be affected compared with control animals. Exercise is known to improve insulin resistance in diabetic patients (258), with higher intensities exert greater effects on glycemic control and insulin sensitivity (370, 380, 447). However, this was not observed in this study, as only MIT but not HIIT seemed to improve insulin levels in diabetic animals.

Furthermore, obesity – specifically an excess in visceral fat – is associated with chronic low-grade inflammation and metabolic disorders (539). Importantly, a correlation has been found between inflammation and adverse effects of HF (540). One of the main inflammatory cytokines is TNF-α, which is increased in cardiac tissue in sedentary diabetic animals after 30 weeks. Previous studies have shown that increased levels of TNF-α are related to cardiac fibrosis, together with dilation and increased mortality (541-543). Cardiac inflammation is also partially responsible of fibrosis in the heart and therefore cardiac remodelling (307). Inflammatory signalling in the cardiomyocyte can indicate myocardial injury and is often coexisting with elevated mitochondrial ROS levels (181). Insulin resistance and TNF-α are also associated, as TNF-α activate intracellular kinases that phosphorylate the serine on insulin receptor substrate (IRS)-1, resulting in insulin resistance (544). This is confirmed in our study, as HIIT trained animals have both increased TNF-α levels while insulin levels were not reduced compared to CAF-SED.

In the diabetic heart, increased ROS levels and mitochondrial dysfunction play a crucial role in the development of cardiac tissue damage (188, 194-196). Most intracellular ROS in diabetes is originating from aged or injured mitochondria (197-200). In our study, citrate synthase activity, a measure of mitochondrial mass, was significantly increased in HIIT trained animals. The same trend was observed with MIT. These data suggest improved oxidative metabolism in trained animals compared with sedentary animals. Impaired mitochondrial function in diabetes can lead to an increase in ROS production and inflammation, causing eventually heart failure (106). However, no difference was observed in groups in cytochrome c oxidase enzyme activity. This could be explained by increased mitochondrial uncoupling, as partial uncoupling while maintaining sufficient ATP production could be a potential protective mechanism against increased ROS

(545). Indeed, in diet-induced DCM, increased uncoupling was observed as shown by an increased activity of uncoupling proteins (546-548). The increased PPARα expression in diabetic hearts is associated with a reduction in PGC-1α, a co-activator of transcription factors regulating mitochondrial numbers (103), further supporting our data on mitochondrial mass. Summarised, our study demonstrate that both inflammation and mitochondrial mass are improved by exercise training.

6.6 Conclusion

In this study, we report the positive effect of exercise training on the cardiac function in diabetic rats with DCM. Differences in modality, namely MIT or HIIT, does not seem to play a crucial role as both training modalities are able to improve cardiac function to the same extent. On a molecular basis, MIT and HIIT are beneficial regarding inflammation and mitochondria markers, both important factors in the development of DCM. For the first time, we show that exercise should be considered as a full-fledged therapy option in diabetic patients, with HIIT as a preferable option to conquer the lack of time barrier and still obtain the best cardiac outcome.

7

Detraining in Type 2 Diabetes Mellitus and underlying mechanisms: a systematic review

Based on:

Detraining in Type 2 Diabetes Mellitus and underlying mechanisms: a systematic review

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In preparation

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7 Detraining in Type 2 Diabetes Mellitus and underlying mechanisms: a systematic review

7.1 Abstract

Type 2 diabetes mellitus (T2DM) is becoming a global pandemic, with over 400 million patients worldwide. Currently, exercise is advised as an adjuvant therapy in diabetic patients to help them in controlling their diabetes and metabolic disturbances. Even if beneficial effects of exercise are well-documented, the effects of detraining after an exercise period are however not yet completely understood. The aim of this review was to examine the impact of exercise intervention and importantly, the consequences and underlying mechanisms of a detraining period after exercise intervention. A systematic literature search was performed on PubMed and Web of Science to identify studies up to 15 April 2019. Articles were included when exercise and detraining protocols were well-defined and diabetes was present. In both diabetic patients and animals, improvements in both metabolic and cardiac health were observed after exercise training, while detraining blunted the positive changes after exercise. Here, we show that to prolong the effects of an exercise intervention, a combination of aerobic and resistance training should be implemented as this seems to delay detraining effects in the diabetic population. However, more research is necessary to determine the optimal exercise intervention and frequency to further delay deleterious detraining effects.

7.2 Introduction

Nowadays, the growing pandemic of diabetes mellitus (DM) poses a significant threat to public health across the globe, affecting more than 400 million individuals and being a 'global emergency' (3). Type 2 diabetes mellitus (T2DM) is the sixth leading cause of death worldwide. Nearly 70% of T2DM patients will develop cardiovascular diseases (CVD), being the major cause of mortality and morbidity in this patient population (434). It has been demonstrated that the risk of developing heart failure in diabetes is closely associated with levels of circulating HbA_{1C} levels, hyperglycemia and insulin resistance (549). Exercise intervention as such is known to be as efficient, if not more, than classical medication targeting glycemic and metabolic control. While obesity and hypertension frequently occur in T2DM patients and are well-known risk factors, studies have shown that cardiorespiratory fitness is a more powerful predictor of CVD (550, 551). Exercise intervention is an effective, low-cost and safe strategy for the prevention and treatment of CVD (427), as it improves T2DM and obesity, diseases associated with an unbalanced diet and a sedentary lifestyle. Exercise training improves both aspects, namely cardiovascular and metabolic health (271, 552, 553). Insulin sensitivity, blood glucose and HbA_{1C} are parameters reflecting the effect of exercise on metabolic control. As previously shown, HbA_{1C} reductions related to exercise are generally fairly modest (average HbA_{1C} reduction = -0.8%). However, it is demonstrated that even a small decrease in HbA_{1C} levels exerts a clinically significant effect on macrovascular, microvascular and nonvascular events, comparable to the effects of pharmaceutical intervention (554). In addition, clinical trials examining exercise intervention have shown a reduction in body and fat mass, improved blood pressures, blood lipid profile and body composition in T2DM patients (273, 555, 556). Beneficial effects of exercise on the prevention of CVD in diabetes by exercise is thought to be mediated through improving vascular function and hereby related tissue perfusion (254). Exercise reduces TNF-α and IL-1β circulating levels (557, 558), inflammation markers typically upregulated in T2DM and CVD, while data on exercise and oxidative stress markers are less consistent.

Overall, the effects of exercise on DM are positive (559), leading to its inclusion in adjuvant treatment guidelines for DM. Currently, exercise is indeed

recommended as a therapy in diabetic patients by the American Diabetes Association (ADA) (560). However, taken into account the positive effects training has on blood glucose and insulin levels, cholesterol and lipid profile, blood circulation, pressure and cardiovascular complications, exercise should be seen as a complete part of therapy and not only recommended. Guidelines for physical activity in T1DM and T2DM adult patients are 150 min or more of moderate-to-vigorous intensity aerobic activity per week, spread over at least 3 days/week. More than 2 consecutive days without exercise should be avoided. This should be combined by 2-3 sessions/week of resistance training on nonconsecutive days. This resistance training is indeed recommended for improving strength, balance and glycemic control (266).

What remains so far underexplored is to what extent the beneficial effects of exercise intervention on cardiac function in T2DM are maintained once the supervised exercise intervention is stopped. Detraining (or de-conditioning) is defined as the partial or complete loss of training-induced metabolic, respiratory and cardiovascular adaptions, in response to an insufficient training stimulus (561, 562). This insufficient training stimulus can be caused by injury, immobilisation or fatigue. Although the current guidelines prescribe exercise and strongly advise DM patients to stay physically active throughout their entire lifetime in order to prevent CVD (563), a large majority of patients gradually or suddenly stop exercising (564-566). Indeed, as reported previously in CVD patients, only 30 – 60% of patients are still regularly exercising after 6 months (567-569).

In this review, we systematically evaluated the impact of detraining on metabolic status and cardiac function in T2DM. Because underlying mechanisms can only be unrevealed in animal models, we combined outcomes from pre-clinical and clinical studies in this review.

7.3 Methods

7.3.1 Literature search, selection criteria and quality control

In this systematic review, both animal and patient studies were included. For these studies, a literature search was performed on PubMed and Web of Science for articles published until 15/04/2019. The following Mesh Terms were used to

include articles: 'Diabetes' and 'Detraining'. Literature searches were performed by MV. Results of the search are shown in Figure 16. When no clear training and detraining protocol was present, studies were excluded out of our review.

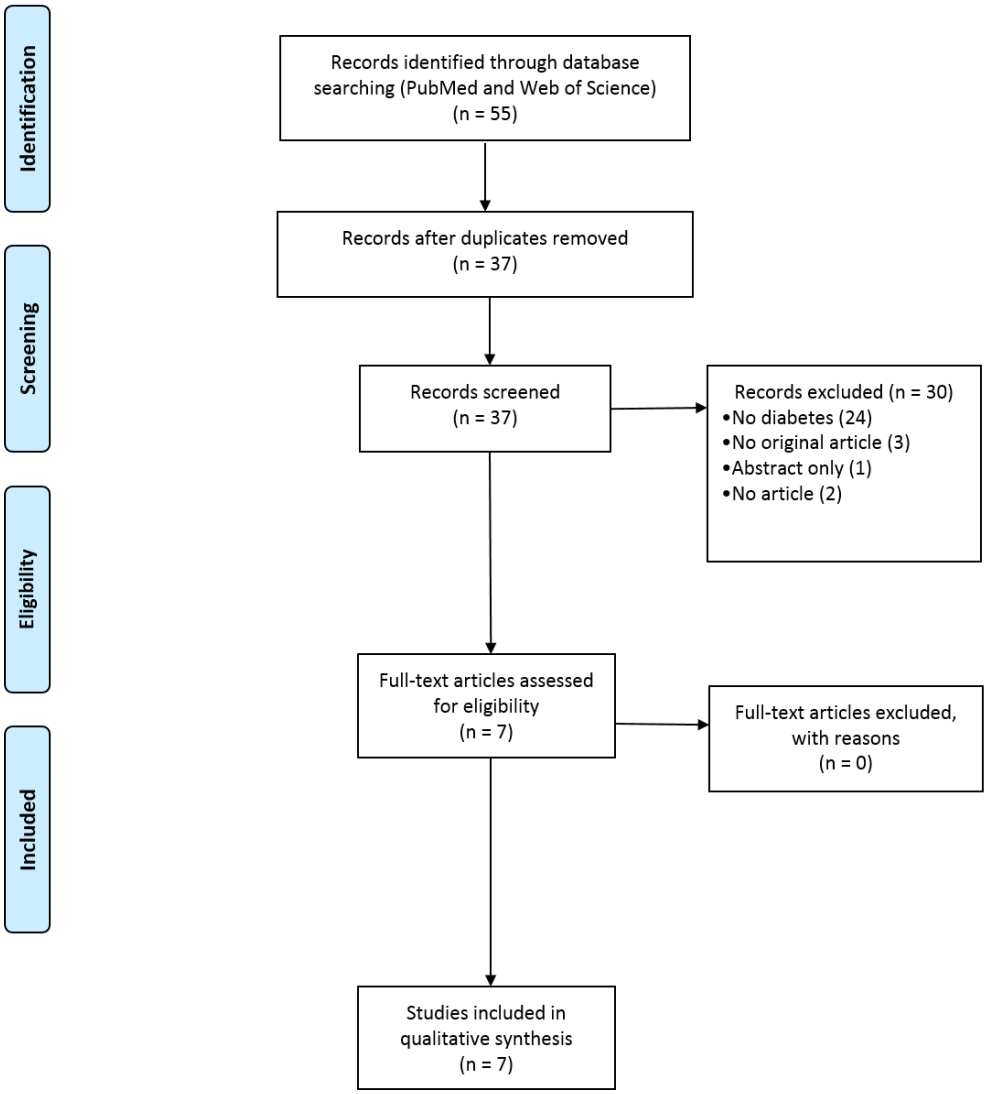


Figure 16: Prism flowchart of search on Web of Science and PubMed.

7.3.2 Quality control of the selected articles

Quality assessment for the included animal studies was based on the Kilkenny et al (2010) table, examining 20 criteria (570). Patient studies were assessed by using manually calculated PEDro scores including 11 criteria (481).

In total, seven studies (including animal or human data) were included in this review.

7.4 Results

7.4.1 Quality assessment of the included studies

Three out of the five included patient studies were non-randomized while two of them were randomised. Only one study had a PEDro score of ≥ 6, considered "good quality". For the others, the PEDro score ranged from 3 to 5. According to the criteria defined by Kilkenny et al, the two animal studies were considered of good quality (570).

7.4.2 Human studies

As reported in Table 9, five out of seven included articles were conducted in patients. In those, exercise training consisted of aerobic training and/or resistance training, at low-to-moderate intensity. When performed without resistance training, 6 weeks of aerobic training were able to reduce weight, body mass index (BMI), cholesterol levels and glycaemia (571). In this study, an equivalent detraining period of 6 weeks significantly worsened all parameters (571). In contrast, Kubota et al. showed that 12 weeks of mechanic horseback riding did not affect body weight, BMI, body fat, nor fasting glucose levels, HbA1C or insulin concentrations. Lipid levels (plasma cholesterol, HDL and triglycerides) remained unaltered after 12 weeks training (572). Only glucose infusion rate (GIR) increased significantly after 12 weeks training indicating an improved glucose uptake. After a detraining period of 12 weeks, a worsening e in GIR levels was observed (572). The lack of worsening in parameters could be attributed to the type of exercise program (mechanic horseback riding), being a balancing training exercise compared with running or weight lifting. As some patients are not able

to perform recommended exercises due to diabetic complications and numbness in the lower extremities, horseback riding could be seen as an alternative.

In the study of Farias et al., resistance training was capable of reducing cholesterol and triglyceride levels, together with glycaemia and HbA_{1C} levels. LDL levels remained altered after a period of 6 weeks detraining and the increase of HbA_{1C} seen in the aerobic training program was not shown after resistance training (571). Combining aerobic training with resistance training resulted in longer lasting effects, even after a six weeks detraining period. In the studies of Tokmakidis et al. and Park et al. however, training resulted in reduced body weight, BMI, waist circumference, postprandial glucose, LDL, cholesterol and an increase in strength (573, 574). Reductions on HbA_{1C} levels, although others reported to be improved with exercise training, were only seen in the study of Park et al. while Tokmakidis et al. did not observe any significant change. Three months detraining resulted in a reduced total strength that remained still significantly higher than the pre-training levels (574). After eight weeks of detraining, lipid profile returned to baseline values after this period of detraining, while the reduced HbA_{1C} observed with exercise in the study of Park, remained (573). Unlike controversial results on lipid profile, deleterious effects on insulin-stimulated glucose clearance seem to occur very fast as they were already visible after six days detraining (575).

Overall, only aerobic or resistance training was insufficient to retain positive exercise effects after a relatively short period of detraining. Combining both exercise modalities delayed but did not prevent the occurrence of deleterious detraining effects on cardiometabolic profile.

7.4.3 Animal studies

To further investigate the underlying mechanisms, studies examining effect of detraining in animal models of diabetes were included in the search. Aerobic exercise intervention for 10 weeks significantly improved glycaemia while a detraining period of 3 weeks normalised these values to the baseline level (576). Systolic arterial pressure (AP) and heart rate (HR) were significantly increased in trained diabetic animals and were maintained after a period of 3 weeks detraining (576). Tachycardic and bradycardic responses were both improved after 10 weeks

of training. However, only tachycardic response remained improved after three weeks of detraining (576). In addition, exercise training increased intrinsic heart rate (IHR) and vagal tonus in diabetic rats with no detraining effect during a period of 3 weeks, while the positive training effects on pulse interval (PI) variance ceased after detraining (576). Finally, skin blood flow and endothelial function improved with exercise training. This effect was blunted after detraining in diabetic rats, likely related to the altered L-ARG/Nitric oxide (NO) pathway (577).

Altogether, data indicate that detraining blunted multiple positive effects of training in diabetic animals, including microvascular improvements in skin blood flow induced by exercise intervention.

Table 9: Overview of the included studies with effects of training and detraining in the diabetic population.

Author	Patient/animal group	Type of exercise	Training duration	Effects after training period	Detraining duration	Effect after detraining period compared with training period
Human studies						
Farias et al. 2015	21 women and 9 men, aged 48.1 +- 1.7 year, BMI = 26.0 ± 2.1 kg/m²	Resistance or aerobic training (65 % of total strength or maximum aerobic capacity)	3 sessions/ week for 6 weeks	**Aerobic training:** Weight, BMI, total cholesterol, LDL, glycaemia ↓ HDL ↑ **Resistance training:** Total cholesterol, LDL, triglycerides,	6 weeks	**Aerobic training:** LDL, triglycerides, glycaemia, HbA_{1C} ↑ HDL ↓ **Resistance training:** Weight, BMI, total cholesterol, triglycerides, glycaemia, HbA_{1C} ↑ HDL ↓

				glycaemia, HbA_{1C} ↓ HDL ↑		
Park et al. 2015	20 women and 17 men, aged over 65 year, BMI = 25.5 ± 2.5 and 23.6 ± 2.2 kg/m^2	Circuit training (combination of aerobic (9 – 14 RPE scale) and resistance training (45 – 75 % of 1 RM))	3 sessions/ week for 12 weeks	Strength ↑ HbA_{1C} , LDL, total cholesterol ↓	8 weeks	LDL, total cholesterol ↑

Tokmakidis et al. 2014	13 women, aged 55.8 +- 5.1 year, BMI = 34.0 ± 3.9 kg/m^2	Aerobic (60 – 85 % HR) and strength training program (50 – 80 1 RM method)	4 sessions/ week for 9 months	Weight, BMI, waist circumference, postprandial glucose ↓ Total strength ↑	3 months	Total strength ↓
Kubota et al. 2006	6 women, age 65 +- 3 years, BMI = 22.8 ± 1.1 kg/m^2	Riding using the Joba apparatus at movement intensity of 1.2 Hz	4 days/week for 12 weeks	GIR (glucose infusion rate) ↑	12 weeks	GIR ↓
Dela et al. 1995	7 men, aged 58 +- 3 years, BMI = 29.1 ± 0.4 kg/m^2	One-legged ergometer bicycle, 6 days/ week for 10 weeks (70 % VO2	6 days/ week for 10 weeks	Fasting plasma insulin ↓ Insulin-stimulated	6 days	Insulin-stimulated glucose clearance ↓

		max), 6 days of detraining		glucose clearance ↑		
Animal studies						
Heidarianpour et al. 2010	Male Wistar rats (8 weeks old), STZ injection (60 mg/kg)	Low-intensity exercise training, twice a day, 5 days a week.	5 days/week for 10 weeks	No baseline data. L-ARG and Ach-induced cutaneous ↑	5 weeks	Microvascular function (L-ARG and Ach) ↓
Mostarda et al. 2009	Male Wistar rats (250 g), STZ injection (50 mg/kg)	Moderate-intensity training, twice a day	5 days/week for 10 weeks	Glycaemia ↓ Bradycardic and tachycardic responses, IHR and vagal tonus ↑	3 weeks	Glycaemia ↑ Bradycardic response ↓

BMI, body mass index; LDL, low-density lipoproteins; HDL, high-density lipoproteins; HbA_{1C}, glycated haemoglobin; RPE, rate of perceived exertion; HR, heart rate; RM, repetition maximum; GIR, glucose infusion rate; STZ, streptozotocin; Ach, acetylcholine; L-ARG, L-arginine.

7.5 Discussion

According to this systematic literature review and as previously shown, exercise training elicits significant improvements in metabolic profile and cardiac function in diabetic patients and animals. However, most of these positive effects are lost, completely or partially, after a relatively short period of detraining. We show here that to maintain and delay the loss of exercise effects in the diabetic patient, both aerobic and resistance training should be combined.

7.5.1 Effects of different exercise modalities on the occurrence of detraining effects

In this systematic review, multiple exercise modalities were performed as an adjuvant treatment in T2DM patients. Moderate aerobic and resistance training were used either combined or separately. When used separately, both exercise modalities were shown to exert equal beneficial effects in T2DM patients (571). Weight and BMI are not reduced after resistance training but this is likely to be due to an increase in muscle mass (578). In studies where only aerobic training was performed, HbA_{1C} levels increased even more after a period of 6 weeks detraining (571). As the risk of developing heart failure in diabetic patients is closely associated with an increase in HbA_{1C} levels, independent of other risk factors (106), controlling HbA_{1C} levels in the T2DM patient population is crucial. Clearly, only aerobic or resistance training alone is not sufficient to keep these levels reduced after a period of detraining. However, when both modalities are combined, Park et al. observed a long-lasting decrease in HbA_{1C} levels (573). These results were however not confirmed in the study of Tokmakidis et al. where HbA_{1C} levels were not altered after training nor after detraining (574). Same results were found on body weight and waist circumference which remained after a period of detraining (574), while the effect on BMI was not consistent (573, 574). The latter can be related to the different patient population used in both studies, with a patient group of normal BMI (BMI = 25) in the study of Park et al., while a patient group of a larger BMI was present at start (BMI = 34) in the study of Tokmakidis et al. (573, 574).

Obesity is related with multiple risk factors for T2DM as insulin resistance, hyperglycemia, dyslipidemia and hypertension (579). Generally pro-inflammatory

markers are known to be elevated in obese and T2DM patients. However, when a more active lifestyle is conducted these levels are reduced, together with weight loss (580-585). In the study of Tokmakidis et al., a decrease in waist circumference, a predictor of intra-abdominal fat, was observed after training which is maintained after detraining (574). Reducing waist circumference in T2DM patients, who are often overweight or obese, is essential as intra-abdominal fat is a predictor of morbidity and mortality (586-590). This can be attributed to inflammation and its association with cardiovascular events as coronary heart disease (591-593). Finally, combining resistance training and aerobic training seems to be the best exercise modality in T2DM to reduce body weight in overweight patients and retain these beneficial effects after a period of detraining (574). Resistance training or aerobic training however was not sufficient enough to induce long-lasting effects in this patient population (571).

7.5.2 Underlying mechanisms

As molecular mechanisms and pathways are limited in patient studies, animal studies were included in this systematic review. Lipid profile, glycaemia and $Hb_{A1}C$ were all improved after exercise. However, detraining reversed the obtained beneficial effects after a relative short detraining period.

In T2DM, circulation is often reduced combined with poor vascularity and micro- and macrovascular complications, being a major cause of mortality in these patients (594, 595). Heidarianpour et al. emphasised the beneficial effects of chronic exercise on the vasodilatation of vessels in diabetic rats (577). They showed that both L-arginine (L-ARG) and acetylcholine (Ach)-induced cutaneous perfusions were increased, both being associated with NO production. Data indicate that this increased cutaneous blood flow during exercise could possibly alter endothelial function and increase sensitivity of stimulated endothelium-derived relaxing factor (EDRF)/NO release in skin vasculature, improving blood flow in diabetic rats (577). However, after five weeks of detraining, these beneficial effects obtained with exercise training were abolished. Where training did not affect endothelium-independent vasodilatation, detraining decreased it significantly (577). Detraining altered beneficial vascular exercise effects by increasing peripheral vascular resistance and reduced blood flow during exercise,

with the latter one leading to smaller blood vessel diameters. These change could be attributed to lower levels of NO after a period of detraining (577).

In diabetic patients, the major cause of death is attributed to the development of cardiovascular diseases. In diabetic rats, systolic arterial pressure (AP), heart rate (HR), baroreflex sensitivity (BRS), intrinsic heart rate (IHR) and heart rate variability (HRV) were reduced. (576). Exercise improves these markers and even after a period of three weeks of detraining, diabetic animals display an improved autonomic control of circulation compared with sedentary rats (576). Additionally, exercise improved glycemic control in exercised animals. This could be attributed to an increased blood flow to skeletal muscle and the heart. An increased glycemic control is indeed known to improve AP, BRS, HR and HRV and thus suggesting exercise-induced an improvement in autonomic function (596).

7.5.3 Limitations

Diabetes is one of the major pandemics worldwide. When used in PubMed, the Mesh Term 'diabetes' gave 647701 results, showing that diabetic research is pending. Exercise proved to be an effective adjuvant therapy in diabetes patients and research in the effects of exercise in diabetes is performed widely, as shown by the resulting 23673 hits on PubMed with Mesh Terms 'diabetes' and 'exercise'. However, when combining 'diabetes' and 'detraining', only 29 results appeared. This result demonstrates the huge gap in knowledge about the effects of detraining in diabetic patients. As a consequence, it was only possible to add seven research articles in this systematic review from which only two are on animal studies, examining detraining in a relatively short period. In addition, specific changes in cardiac function, although being a major complication of diabetes and being influenced by exercise intervention (559), were not systematically evaluated and reported. Conclusion on the mentioned studies should be then taken with caution as extensive consistent knowledge on the subject is lacking.

7.6 Conclusion

The beneficial effects of exercise in diabetic patients are well described, showing the importance of exercise as an adjuvant efficient therapy. However, adherence to training programs in heart failure patient groups is low. Here, we show that

detraining has clear negative effects in the diabetes population, meaning in other words that beneficial effects on cardiac function and metabolic profile are lost after a period of detraining. Even short periods of detraining are sufficient to negate most of the positive effects of exercise, with some parameters being worse than pre-training levels. A period of inactivity should thus be avoided by diabetic patients. However, training modality, intensity, load and duration should be identified and are currently lacking. More research is necessary to identify how maintenance of the beneficial effects of exercise can be guaranteed in T2DM patients, even after a period of detraining.

8

Summary and general conclusions

8 Summary and general conclusions

Diabetes is becoming one of the greatest challenges of this time, with currently more than 400 million patients worldwide and this number is expected to rise above 640 million adults by 2040 (597). Moreover, diabetes is closely associated with heart failure, with cardiovascular diseases responsible for up to 80% of diabetic mortality (598). In well-controlled diabetic patients, approximately 60% cardiac stiffness and diastolic dysfunction are observed (599, 600). The current diabetes pandemic is closely associated with the global obesity epidemic (52). Research suggests that both a sedentary lifestyle and an energy-dense Western diet are the major risk factors for the development of T2DM (51). One of the lifestyle and dietary risk factors that are related to the development of T2DM are the consumption of sugar-sweetened beverages, increasing the risk by 20 to 30% compared with non-consumers (65-67). A sedentary lifestyle and lack of physical activity promote even more the chances of developing T2DM by 40% (79, 601). The aim of this PhD was to study the effects of different types of exercise intensities on the development of T2DM and DCM. In that context, an animal model combining the risk factors of developing T2DM with the diabetic phenotype observed in the diabetic population was necessary and currently not available. In **Chapter 3,** we validated our animal model developing T2DM and DCM. To observe the effect of our chosen exercise modality on cardiac function, a first experiment was performed on healthy animals, as described in **Chapter 4.** To investigate the effect of exercise training on DCM and T2DM and to summarise the already known literature, a systematic review was written (**Chapter 5)** describing the known facts of exercise effects on T2DM and the cardiac function in diabetic patients. This review clearly demonstrated that there was a lack of knowledge of the effects of different kinds of exercise on cardiac function in T2DM. Here, we filled the gap by investigating the effect of different kind of exercise modalities (HIIT and MIT) on T2DM and DCM (**Chapter 6)**. At last, in **Chapter 7,** we discussed what is currently known about detraining and diabetes.

8.1 An animal model representing T2DM and DCM

To investigate properly the complexity of T2DM and DCM, preclinical animal models reflecting the phenotype and pathophysiology of the disease are critical in

research. Most models used for induction of diabetes use toxic chemicals, as alloxan or streptozotocin (STZ) (602). However, by inducing diabetes using a toxin, tissue toxicity can be increased (603). Moreover, STZ will lead to β-cell toxicity and necrosis resulting in insulin deficiency (604). There is a close correlation between obesity and type 2 diabetes, and excessive weight is an established risk factor for the development of T2DM. However, in animal models using an STZ injection, diabetic animals often weigh less than the control groups. Thereby the obesity-associated effects in T2DM could not attribute to the development of DCM or T2DM in these animal models. Other models often used are genetically modified obese models as ob/ob and db/db mice. These models have a defect in the leptin synthesis or action while in humans a monogenic mutation is rarely observed in T2DM (605). Therefore we used a model based on a high-sugar and a high-fat diet representing a Western diet. In this model, we observed hyperglycemia, hyperinsulinemia and hyperlipidemia, three major hallmarks in diabetes. On cardiac function, we observed the hallmarks of DCM namely increased cardiac collagen deposition, diastolic dysfunction and left ventricle hypertrophy. These data demonstrate that indeed our model reflect the phenotype observed in patients as it is able to induce T2DM with cardiac failure after 18 weeks of diet.

8.2 The effects of exercise on healthy and diabetic animals

Exercise and physical activity are known to exert beneficial effects on both diabetes, metabolic disorders and cardiovascular diseases (606). In healthy rats, we showed that both exercise modalities had similar effects on cardiac function (**Chapter 4**). Moreover, HIIT seemed to improve vascularity in the heart significantly compared with MIT trained animals. It is well known that in diabetes, macrovascular and microvascular problems occur, resulting in visual impairment, diabetic cardiomyopathy, kidney failure, stroke and lower extremity dysfunction (607). Exercise will increase number and size of arterial blood vessels in both skeletal muscles and myocardium (608). These data demonstrate that exercise is an efficient adjuvant therapy in diabetes mellitus and other cardiovascular diseases. In healthy subjects, sparse data suggest that the potential of HIIT in comparison to MIT to improve cardiac function is most likely to be attributed to a

higher mitochondrial fatty acid oxidation and metabolism (379). We did not observe significant differences in citrate synthase activity – a marker for mitochondrial mass – between the two different exercise modalities. On the other hand, complex II enzyme activity tended to be increased compared with the control group, but only in HIIT trained animals. Furthermore, HIIT but not MIT was able to induce physiological hypertrophy in healthy trained animals. Functionally, both exercise modalities were equally efficient in improving cardiac function in T2DM and DCM. Altogether, our data suggest that HIIT could be a useful alternative to MIT for patients with cardiovascular disorders.

Our review in **Chapter 5** showed the importance of exercise on cardiac function in type 2 diabetes patients. Exercise improved metabolic and molecular parameters important in cardiac function, and improves diastolic function in humans and animals. The role of different kinds of exercise in the improvement of diabetic control and cardiac function remained however unknown, as insufficient research was performed to unravel the role of exercise types. This gap of knowledge was filled in with **Chapter 6**, as we used two different kinds of exercise modalities in our validated animal model of T2DM and DCM. No differences were observed in the functional parameters of cardiac function, making both MIT and HIIT equally in improving cardiac function in diabetic patients. On the molecular level, some differences, likely related to differential underlying mechanisms involved, could be observed. Indeed, HIIT but not MIT, was able to reduce circulating FFAs in diabetic rats while only MIT reduced cardiac inflammation. However, differences between exercise groups were small and not significant. Only HIIT increased citrate synthase, showing and confirming that HIIT is able to improve mitochondrial function and mass compared with MIT. Altogether, these data suggest that HIIT could be introduced as a therapy in diabetic patients, as in this group lack of time is one of the major barriers to exercise (264). One of the concerns of HIIT in a diabetic population, characterised by high cardiovascular mortality, could be the safety of implementing HIIT is this population. Multiple studies investigated the safety of HIIT in heart failure patients. In patients with coronary heart diseases no adverse effects were observed (609, 610). In chronic heart failure patients, safety parameters remained within acceptable values (611). A large study by Rognmo et al. showed no only two non-fatal cardiac events in 46.364 hours of HIIT and one fatal cardiac event in 129.456 hours of MIT,

executed by 4846 cardiac rehabilitation patients (612). It has to be noted that all these patients underwent full medical screening and cardiopulmonary test before exercise. In addition, all exercise was supervised. These data suggest HIIT as a safe exercise strategy in diabetic patients, at least equally efficient as MIT. As important as safety of the exercise protocol is adherence to the exercise modality. HIIT was observed to be more pleasant by obese women, some T2DM patients and by coronary heart patients (610, 613). Moreover, patients preferred HIIT protocols with shorter intervals (614). Adherence to a HIIT protocol in a non-supervised environment has been demonstrated in multiple studies (427, 510, 615-617). These data of good adherence and safety of HIIT in the diabetic population, combined with the results of our study in animals on cardiac function, prove the potential of HIIT as a patient-tailored therapy in the diabetic population.

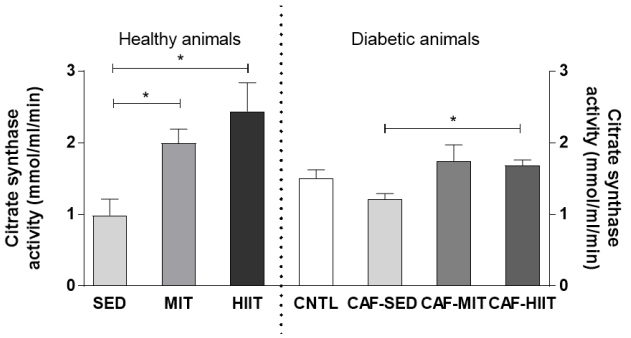
The effects of exercise differ partially between healthy subjects and diabetic rats, as shown in our research. In both diabetic and healthy animals, exercise reduced significantly collagen deposition in the heart. Indeed, it is known that exercise is recommended to both prevent and treat collagen deposition and heart disease (618). On the other hand, exercise increased hypertrophy in healthy animals but reversed left ventricle hypertrophy in diabetic rats with a reduction in AWT. Exercise training is however able to counteract structural and functional changes in cardiovascular diseases changing pathological to physiological hypertrophy, as described before (619-621). Furthermore, another cardiac parameter differing between healthy and diabetic animals is left ventricle pressure. In healthy animals both exercise modalities were capable of reducing LVP, while this effect was not observed in diabetic rats. Moreover, LVP was significantly increased after HIIT training, or HIIT was not capable of reducing systolic blood pressure in diabetic animals. End-diastolic pressure however was reduced by both exercise modalities. At last, differences in citrate synthase activity were observed between healthy and diabetic rats. Both exercise modalities increased citrate synthase or mitochondrial mass in healthy subjects, whereas in diabetic rats only HIIT slightly improved this parameter. It has been suggested before that mitochondrial dysfunction play a crucial role in diabetic cardiomyopathy (107). High-intensity interval training is shown to improve cardiac mitochondrial content in healthy and obese animal models, while a positive effect of MIT was not observed in these studies (447, 622).

The differences between exercise in healthy and diabetic animals are summarized in figure 17.

a

	Healthy animals			Diabetic animals			
	CNTL	MIT	HIIT	CNTL	CAF	CAF-MIT	CAF-HIIT
Bodyweight (g)	596 ± 16	483 ± 18*	472 ± 13*	578 ± 18	766 ± 20*	629 ± 27~	612 ± 19~
AWT (mm)	1.4 ± 0.1	1.5 ± 0.1	1.7 ± 0.4*	1.5 ± 0.02	1.8 ± 0.03*	1.6 ± 0.04	1.7 ± 0.05
EF (%)	63 ± 1	76 ± 2*	78 ±1*	78 ± 2	65 ± 2*	75 ± 2	70 ± 3
LVP (mmHg)	108 ± 3	96 ± 2*	96 ± 2*	85 ± 2	99 ± 3*	97 ± 3	104 ± 5*
EDP (mmHg)	5.2 ± 0.4	3.9 ± 0.4	3.7 ± 0.6	5.4 ± 1.3	14.1 ± 3.1*	5.6 ± 1.2~	6.7 ± 0.5

b



c

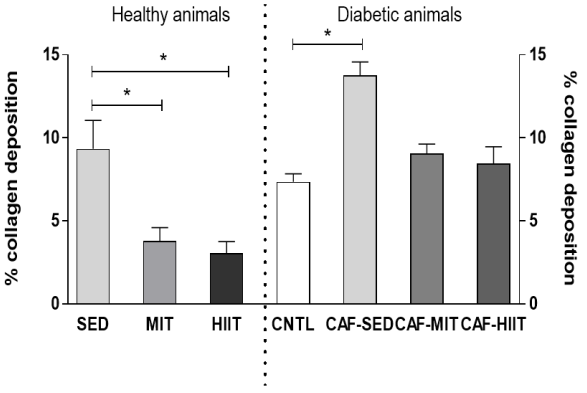


Figure 17: A comparison of the effects of exercise in both healthy and diabetic animals. AWT, anterior wall thickness; EF, ejection fraction; LVP, left ventricle pressure;

EDP, end-diastolic pressure. Data are shown as mean ± S.E.M. * denotes p<0.05 vs. CNTL, ~ denotes p<0.05 vs. CAF-SED.

8.3 What after revalidation?

As described in **Chapter 7**, detraining is a problem in all cardiovascular patients. Detraining (or de-conditioning) is defined as the partial or complete loss of training-induced metabolic, ventilator and cardiovascular adaptions, in response to an insufficient training stimulus. This insufficient training stimulus can be caused by injury, immobilization or fatigue. Although the current guidelines prescribe exercise to DM patients, many patients does not remain regularly active. It is already shown that long-term exercise adherence is necessary to sustain the health benefits of exercise (623). In a systematic review, performed by our research group, we identified indeed the dangers of non-adherence to an exercise program. Most positive effects were at least deteriorating after periods of detraining rather rapidly. However, a lot more research needs to be done to define the negative effects of detraining in the diabetes population, as we could only include seven original research articles in our review. T2DM is a chronic disease and thus exercise adherence is critical for the welfare of the patient. As lack of time is one of the main parameters of non-adherence to an exercise program, HIIT could also be interesting to counter the effects of detraining and increase training adherence. Finally, as being very different in modalities, MIT and HIIT are likely to be triggered by differential underlying mechanisms. One could then speculate that HIIT, unlike MIT, could delay the occurrence of the deleterious detraining effects seen once exercise is stopped.

8.4 Limitations

Like all research, studies included in this doctoral thesis have some limitations. The main limitation is based on the exercise protocols executed in the studies performed. As we performed forced treadmill exercise running, our animals were subjected to a stress response which can result in counteracting some of the positive exercise effects like reduced inflammation (624). Animals forced to treadmill running also show higher levels of stress and anxiety (624). This could

affect the results obtained in the exercise studies. However, no alternative is available to compare two different kinds of exercise modalities. To show the effect of exercise, without comparing different kinds of exercise modalities, the optimal situation would be to give animals freedom of choice for running on a voluntary wheel-running rad. However, using this option was not possible for comparing MIT and HIIT.

Other limitations in these studies were related to the treadmill itself, as it was not possible to measure VO_{2max} to determine the high-intensity and the moderate intensity protocols. In optimal conditions, HIIT is corresponding to 85-90% of the VO_{2max} measured in a metabolic chamber equipped with a treadmill (447, 622). For MIT trained animals, VO_2max would be 60-70%. In our case, intensity of the training protocol was determined by lactate measurement and were approximated using calculations used for humans and not rodents, as not available.

8.5 Future perspectives

The research in this field is not finished with this doctoral thesis and future experiments should be added to strengthen the results obtained and move a step further.

8.5.1 Is exercise able to prevent the development of T2DM and DCM?

It is well-known that an unhealthy diet and a sedentary lifestyle are major risk factors for the development of T2DM and DCM. Multiple studies investigating the effects of exercise in T2DM use genetic models or toxin-induced diabetes. These models are not suitable to look to the preventive effect of exercise on the development of T2DM in animals. In our model, T2DM and DCM are induced by diet, making our model perfect for investigating the optimal modality to prevent T2DM in high-risk groups. The same approach could be used as in **Chapter 6**, with the difference that exercise would start at the onset of the diet. In this way, it would be possible to determine whether exercise is efficient enough in preventing T2DM and which exercise modality is better in that setting.

8.5.2 Is HIIT more efficient in sustaining the exercise effects after a period of detraining in DCM?

Here, the objective would be to identify whether HIIT could be more efficient to prevent or delay detraining effects. Generally, beneficial effects of exercise training are mainly attributed to improved neurohumoral, inflammatory, metabolic and central hemodynamic responses as well as on endothelial, skeletal and cardiovascular function, leading to an overall cardiac improvement and an improved tolerance for ischemia and reperfusion injury. However, to date, little is known of the specific effects of detraining and the role of different training modalities. DCM is defined as ventricular dysfunction that occurs in diabetic patients independent of a recognised cause such as hypertension or coronary artery disease. The structural effects of DCM are related to adverse remodelling, the most prominent changes being myocardial hypertrophy and fibrosis, increased wall thickness to chamber ratio and elevated left-ventricular (LV) mass relative to chamber volume. Diastolic dysfunction is recognised as the earliest functional effect of these structural changes, and is characterised by increased LV diastolic stiffness and relaxation disturbances. The hallmark of systolic dysfunction, the later stage of the dysfunction, is a depressed LV ejection fraction and an upregulation of the renin-angiotensin system.

Current exercise guidelines by the American Diabetes Association for people with diabetes suggest that adults should engage in 'minimal 150 minutes of moderate-to-vigorous intensity activity weekly, spread over at least 3 days/week, with no more than 2 consecutive days without training '. However, when detraining occurs, the underlying mechanisms and early markers of detraining, including inflammatory markers, are currently unknown. As lack of time is one of the major barriers in non-adherence to exercise therapy in the T2DM population, HIIT can be an interesting alternative training modality. We showed that in healthy animals, no difference was observed between MIT and HIIT on cardiac function. Moreover, we observed increased vascularity in healthy hearts of HIIT-trained animals. This increases the potential of HIIT in the diabetic population, as vascularity and cardiovascular diseases are common. In the diabetic population, accelerated development of atherosclerotic lesions is present. Atherosclerosis is an inflammatory disease typically present at sites of endothelial injury in arteries. Endothelial cells will respond to the injury by overexpressing cell surface proteins as vascular cell adhesion molecule-1 and P-selectin, mediating the attachment and infiltration of circulating monocytes and T cells. Chronic inflammation is also

involved in the pathophysiology of both T2DM and insulin resistance, tying up the different parts of this study. Exercise has proven to lower inflammation and atherosclerosis in diabetic patients.

8.5.3 Mechanisms and evolution of T2DM and DCM.

It would be interesting to go deeper into molecular and cellular mechanisms of DCM, and what goes wrong in this complex disease. As shown in **Chapter 1**, DCM is a complex and multifactorial disorder. It would be interesting to follow-up animals not only for 18 or 30 weeks, but longer to see the evolution of T2DM and DCM in a high-sugar fed animal. When comparing 18 weeks and 30 weeks fed animals, we can already observe a change from only diastolic failure (early phase) to diastolic and systolic failure (late phase). Therefore, to follow animals for a longer period of time such as 12 and 24 months and evaluate them at fixed time points, could give more insights on how T2DM and DCM are induced and are evolving in animals. Furthermore, it would be interesting to see at what point DCM becomes a life-threatening disease and how the molecular pathways change during progress of the disease, and what are the crucial parameters involved in the disease progression.

9

Nederlandse samenvatting

9 Nederlandse samenvatting

Diabetes mellitus (DM) is één van de grootste pandemieën van onze tijd, waarbij de meeste diabetes patiënten lijden aan type 2 DM (T2DM). Op dit moment zijn er wereldwijd meer dan 400 miljoen personen die lijden aan diabetes, en er wordt verwacht dat dit aantal de komende jaren zal blijven stijgen. De grootste doodsoorzaak bij deze patiënten zijn cardiovasculaire aandoeningen. In diabetes is er een specifiek soort van hartfalen, genaamd diabetisch cardiomyopathie (DCM). DCM wordt gedefinieerd als structurele en functionele veranderingen in de cardiomyocyten, onafhankelijk van ischemisch hartfalen of hypertensie. Diastolische dysfunctie – samen met een verhoging aan bindweefsel en hypertrofie van het linker ventrikel één van de kenmerken van DCM – is aanwezig in 19.6 % tot 54.4 % van de diabetes patiënten. Hierdoor zal de vulling van het hart vermindert zijn. De laatste jaren is er ontzettend veel onderzoek gevoerd naar de pathofysiology van DCM, maar nog steeds zijn de moleculaire mechanismen nog niet volledig geweten. Er zijn verschillende risicofactoren die een rol spelen in de ontwikkeling van T2DM, zoals een sedentaire levensstijl, een gebrek aan fysieke activiteit en een ongezond dieet. Het is reeds bewezen dat sport en fysieke training de fysiologische en psychologische gezondheid verbeterd in zowel diabetes patiënten als gezonde personen. Fysieke training zal onder meer insulinesensitiviteit, glycemische controle en vetoxidatie verbeteren in diabetes patiënten. Verder zijn een verbeterde cardiorespiratoire functie en fysieke activiteit geassocieerd met een verminderde cardiovasculaire mortaliteit in diabetes patiënten.

In deze doctoraatsdissertatie wordt er dieper ingegaan op de risicofactoren (een ongezond dieet en een sedentaire levensstijl) in T2DM, alsook het effect van fysieke training bij de diabetes patiënten. Voor dit laatste worden er twee soorten fysieke training toegepast, namelijk moderate-intensiteit training (MIT) en hoge-intensiteit interval training (HIIT). Verschillende onderzoeksvragen worden er gesteld in dit manuscript. Wat is het effect van fysieke training bij gezonde dieren? Alsook, is het mogelijk om diabetes cardiomyopathie te induceren bij dieren door middel van een specifiek dieet? Is fysieke training ook in staat om diabetes cardiomyopathie te verbeteren of eventueel zelfs te genezen?

In **hoofdstuk 3** wordt er gekeken of een hoog-suiker en hoog-vet dieet in staat is om diabetes cardiomyopathie te induceren in gezonde dieren. Hiervoor zijn de dieren gedurende 18 weken onderworpen aan dit dieet. De hartfunctie werd onderzocht door middel van echocardiografie en hemodynamische drukmetingen, terwijl de glucose en insuline waarden bekeken werden via een orale glucose tolerantie test (OGTT). Na analyse van de resultaten bleek de cardiale functie inderdaad aangetast te zijn na een hoog-suiker en hoog-vet dieet, en toonden de dieren tekenen van T2DM, zoals insulineresistentie en hyperglycemie. De kenmerken van DCM waren ook aanwezig, met een verdikking van de linkerventrikelwand, een verhoogde eind-diastolische druk en een verhoogde aanwezigheid van fibrose in het cardiale weefsel.

Wanneer gezonde dieren werden aangezet tot het verrichten van fysieke training (MIT of HIIT), konden er verscheidene effecten worden geobserveerd op de hartfunctie en de algemene gezondheid. Zo was het duidelijk dat de bloeddrukken verminderd waren en de ejectiefractie (EF) verbeterd was. Ook kon training er voor zorgen – onafhankelijk van welk soort training (MIT of HIIT) – dat het bindweefsel in het hart daalde. Verder zorgde HIIT, maar niet MIT, voor meer vascularisatie in het hart ten opzichte van sedentaire dieren. In gezonde dieren zorgt training – zowel MIT als HIIT – voor een verbetering van de hartfunctie, gewichtsverlies en verbeterde mitochondriale activiteit, én heeft HIIT het voordeel om ook nog eens de cardiale vascullariteit te verbeteren, zoals aangetoond in **hoofdstuk 4**.

Hoofdstuk 6 combineert de twee voorgaande studies, waarbij er wordt gekeken naar het effect van deze twee soorten training op diabetes dieren na 18 weken dieet. Alvorens deze studie te starten, is er een literatuurstudie uitgevoerd (**hoofdstuk 5**) om de reeds gekende literatuur te onderzoeken naar wat geweten is over training in diabetes of diabetes cardiomyopathie. Hierbij werd duidelijk dat DCM op verschillende manieren beïnvloed wordt door training, en dat voornamelijk de diastolische functie verbeterd na training. Dit maakt onze studie interessant, aangezien er een verhoging van de eind-diastolische druk aanwezig was in dieren met diabetes. In **hoofdstuk 6** is het effect van training op diabetes dieren duidelijk zichtbaar. Het lipidenprofiel (cholesterol, vrije vetzuren en triglyceriden) is na training duidelijk verbeterd, met verminderde circulerende

lipiden ten opzichte van sedentaire dieren. Ook de cardiale functie was sterk verbeterd na training wanneer vergeleken met sedentaire diabetes dieren. Zowel de diastolische als systolische functie toonden vooruitgang na training, tekenend voor het belang van fysieke activiteit bij diabetes. De onderliggende mechanismen werden ook bekeken, waarbij inflammatie een rol leek te spelen. Ook de mitochondriale functie en het substraatmetabolisme van het hart leken aangetast bij diabetes dieren, terwijl training deze negatieve aanpassingen terugkeerden. De verschillen tussen MIT en HIIT in het genezen van DCM waren minimaal, en tonen het belang van training aan in de diabetes populatie. Ook bieden deze resultaten potentieel voor HIIT, aangezien dit trainingschema van kortere duur is, waarbij een gebrek aan tijd vaak wordt aangehaald door T2DM patiënten om niet te sporten.

Als laatste is er een literatuurstudie uitgevoerd om het effect van detraining na te gaan bij diabetes (**hoofdstuk 7**). Detraining is gedefinieerd als het gedeeltelijk of volledig verlies van de door training-geïnduceerde metabole, respiratoire en cardiovasculaire veranderingen, door een onvoldoende trainingstimulus. Hier is allereerst opnieuw aangetoond dat fysieke activiteit verschillende positieve effecten uitoefent bij diabetes patiënten. Na een korte periode van detraining, zijn de effecten van detraining echter reeds zichtbaar. Het is dus van belang dat een periode van inactiviteit bij diabetes patiënten vermeden wordt.

De algemene conclusies uit deze doctoraatsdissertatie zijn als volgend: (1) Een ongezond of Westers dieet kan leiden tot T2DM en DCM. (2) Zowel MIT als HIIT zijn in staat om positieve cardiale remodeling door te voeren, en de hartfunctie te verbeteren bij gezonde dieren. (3) Beide trainingsmodaliteiten hebben therapeutische mogelijkheden in T2DM, zonder verschil in effectiviteit op het verbeteren van cardiale functie tussen beidde modaliteiten. (4) De positieve effecten van een trainingsinterventie kunnen snel te niet gedaan worden door een periode van detraining, wat moet vermeden worden bij de diabetes patiënten.

10

Other

10 Other

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Curriculum Vitae

Maxim Verboven was born on the 27th of August 1990 in Geel, Belgium. In 2008, he graduated from Pius X College Tessenderlo, science-mathematics. In 2012, he obtained a bachelors degree at the Provenciale Hogeschool Limburg, Hasselt, in Biotechnology. In 2015, he obtained a Master's degree at Hasselt University/translational University Limburg in Biomedical Sciences, option Clinical Molecular Sciences. His Master dissertation was performed at the Ziekenhuis Oost-Limburg and entitled 'In vitro simulations of valvular heart diseases' under supervision of Dr. Philippe Bertrand and Prof. Dr. Pieter Vandervoort.

In November 2015, he started his PhD at the Biomedical research institute in the research group of Prof. dr. Virginie Bito. During his PhD, Maxim followed several courses and workshops, such as effective imaging skills, diabetes educator, introduction to JMP, and organized a symposium 'Obesitas: inzichten en behandeling in de 21ste eeuw'.

The results obtained during this PhD were partially published in international peer reviewed journals and were presented at several (inter)national conferences, such as the American Heart Association and the European Society of Cardiology.

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"The most exciting phrase to hear in science, the one that heralds new discoveries, is not 'Eureka!' but 'That's funny…' "

Isaac Asimov