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Maastricht University

Faculty of Medicine and Life Sciences
School for Life Sciences
Master of Biomedical Sciences

Masterthesis

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

Dora Colson

Thesis presented in fulfillment of the requirements for the degree of Master of Biomedical Sciences, specialization Clinical Molecular Sciences

SUPERVISOR :

Prof. dr. Virginie BITO

MENTOR :

De heer Maxim VERBOVEN

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www.uhasselt.be
Universiteit Hasselt
Campus Hasselt:
Martelarenlaan 42 | 3500 Hasselt
Campus Diepenbeek:
Agoralaan Gebouw D | 3590 Diepenbeek

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Dora Colson

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List of abbreviations

AGE	advanced glycation and-product
ATP	adenosine triphosphate
AWT	anterior wall thickness
CAD	coronary artery disease
CAF diet	high-sugar cafeteria diet
CICR	Ca^{2+}-induced Ca^{2+} release
CM	cardiomyocyte
CO	cardiac output
CoA	coenzyme A
CS	citrate synthase
CVD	cardiovascular disease
DCM	diabetic cardiomyopathy
DHF	diastolic heart failure
DM	diabetes mellitus
DM	diabetes mellitus
DNA	deoxyribonucleic acid
ECC	excitation-contraction coupling
ECL	enhanced chemiluminescence
ECM	extracellular matrix
EDP	end-diastolic pressure
EDV	end-diastolic volume
EF	ejection fraction
ELISA	enzyme-linked immunosorbent assay
ESD	end-systolic diameter
ESV	end-systolic volume
ETC	electron transport chain
FFA	free fatty acid
FS	fractional shortening
HDLC	high-density lipoprotein cholesterol
HF	heart failure
HIIT	high-intensity interval training
HLA	human leukocyte antigen
HR	heart rate
HRP	horseradishperoxidase
IFG	impaired fasting glucose
IGT	impaired glucose tolerance
IL	interleukin
IRS	insulin receptor substrate
LOX	lipoxygenases

LV	left ventricle/left ventricular
LVEDD	left ventricular end-diastolic diameter
LVESD	left ventricular end-systolic diameter
LVP	left ventricular pressure
MIT	moderate-intensity training
NCX	Na^{+}-Ca^{2+} exchanger
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B-cells
OGTT	oral glucose tolerance test
PAI-1	plasminogen activator inhibitor-1
PLN	phospholamban
PVDF	polyvinylidene fluoride
RAGE	receptor for advanced glycation end-products
ROS	reactive oxygen species
RyR	ryanodine receptor
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
SEM	standard error of mean
SERCA2a	sarco/endoplasmic reticulum Ca^{2+}-ATPase-2a
SHF	systolic heart failure
SR	sarcoplasmic reticulum
T1DM	type 1 diabetes mellitus
T2DM	type 2 diabetes mellitus
TBS-T	tris buffered saline containing 0.1% Tween-20
TNF-α	tumor necrosis factor-α
UCP	uncoupling protein

Abstract

Currently, type 2 diabetes mellitus (T2DM) affects more than 400 million people worldwide, thereby presenting a major epidemic of this time. Apart from leading to comorbidities such as kidney failure and neuropathy, cardiovascular diseases – such as diabetic cardiomyopathy (DCM) – represent the main cause of mortality in T2DM patients. Exercise training is a low cost and safe approach to reduce the risk of cardiovascular disease development. Nevertheless, it is not known which type of exercise training is most potent in reaching this goal. Currently, moderate-intensity training (MIT) is considered as the golden standard during cardiac rehabilitation programs. However, high-intensity interval training (HIIT) is gaining more interest as being as potent as MIT in reducing the adverse cardiac remodeling, while being more time efficient than MIT.

Therefore, a study was set up to verify whether HIIT is efficient as MIT in reducing the adverse cardiac remodeling occurring in DCM. 32 male Sprague-Dawley rats were randomly divided into 4 groups: sedentary control, sedentary diabetic, moderate-intensity training diabetic and high-intensity interval training diabetic. T2DM was induced by a high-sugar diet (referred to as the cafeteria diet, containing 48% sugar) for 18 weeks where after the rats performed treadmill running for 5 days/week, for a period of 12 weeks. Hemodynamic and echocardiographic measurements were used to follow-up the cardiac function. Oral glucose tolerance tests were used to monitor diabetes development. Furthermore, we quantified citrate synthase levels, the level of inflammation by measuring tumor necrosis factor- α, cardiac fibrosis, and hypertrophy.

Our results show that in diabetic animals, glucose and insulin levels were increased, while exercise therapy normalized these parameters to control levels. Plasma triglyceride levels were elevated in diabetic animals, but not in exercised animals. Furthermore, HIIT reduced circulating free fatty acids to normal values. Cardiac inflammation was present in diabetic animals, where MIT, but not HIIT, reduced this to baseline values. End-diastolic pressure was significantly elevated in the diabetic animals, but exercise therapy normalized this pressure. Finally, left ventricle hypertrophy and fibrosis were observed only in sedentary diabetic animals.

Both MIT and HIIT show promising results which could lead to the use of exercise training as a treatment and/or prevention method for DCM, thereby significantly reducing morbidity and mortality in the T2DM patient population. We conclude that only a slight difference in exercise-induced effects was observed between both training modalities. Therefore, more research is needed to further determine the advantages of both training modalities in the treatment and prevention of DCM.

Samenvatting

Type 2 diabetes mellitus (T2DM) treft meer dan 400 miljoen mensen ter wereld en vormt daardoor een epidemie van deze tijd. T2DM leidt tot de ontwikkeling van verschillende comorbiditeiten zoals nierfalen en neuropathie, maar zorgt daarnaast ook voor cardiovasculaire aandoeningen – waaronder diabetische cardiomyopathie (DCM) – die de grootste oorzaak van morbiditeit en mortaliteit binnen de T2DM patiëntenpopulatie vormen. Fysieke activiteit is een goedkope en veilige methode om het risico op cardiovasculaire aandoeningen te verlagen, maar het is niet geweten welk type fysieke activiteit hier het meest efficiënt in is. Momenteel is training met een gemiddelde intensiteit (moderate-intensity training, MIT) de gouden standaard binnen de cardiale revalidatie. Intervaltraining met een hoge intensiteit krijgt echter steeds meer aandacht omdat het even doeltreffend zou zijn als MIT, en tegelijk minder tijd in beslag neemt in vergelijking met MIT.

Bijgevolg werd er een studie opgezet om te achterhalen of HIIT even efficiënt is als MIT in het verminderen van de nadelige effecten die voorkomen bij DCM. 32 mannelijke Sprague-Dawley ratten werden gerandomiseerd in 4 groepen: sedentair controle, sedentair diabeet, diabeet MIT en diabeet HIIT. T2DM werd geïnduceerd met behulp van een dieet met een hoog suikergehalte (48%, ook wel het cafetaria dieet genoemd) voor 18 weken gevolgd door een trainingsperiode van 12 weken bestaande uit training op een loopband gedurende 5 dagen/week. Hemodynamische en echocardiografische metingen werden uitgevoerd om de cardiale functie op te volgen. Gelijktijdig werden er orale glucose tolerantie tests gebruikt om de ontwikkeling van diabetes te monitoren. Bovendien werd er ook gekeken naar de mate van inflammatie (door meting van tumor necrose factor-α), het citraat synthase niveau, cardiale fibrose, en hypertrofie.

Onze resultaten toonden aan dat de niveaus van glucose en insuline verhoogd waren in de diabetische dieren, maar dat fysieke activiteit deze parameters terug kon normaliseren. De triglyceride levels waren enkel verhoogd in de diabetische dieren en niet in de dieren die getraind werden. Verder zorgde enkel HIIT voor een verlaging van de vrije vetzuren tot controle waarden. Cardiale inflammatie was aanwezig in de diabetische dieren, maar werd door MIT gereduceerd tot baseline niveaus. Bovendien was de eind-diastolische druk significant verhoogd in de diabetische dieren, maar zowel MIT als HIIT kon deze druk terug verlagen. Ten slotte werd linker ventrikel hypertrofie en fibrose enkel geobserveerd in de sedentaire diabetische dieren.

Zowel MIT als HIIT tonen veelbelovende resultaten die kunnen leiden tot het gebruik van fysieke activiteit als een behandeling en/of preventiemiddel voor DCM, om zo de morbiditeit en mortaliteit bij T2DM patiënten sterk te verlagen. Uit onze studie kunnen we afleiden dat er enkel een klein verschil is in de effecten van beide trainingsmodaliteiten. Dit toont aan dat er meer onderzoek nodig is naar de voordelen van deze trainingsmodaliteiten in de behandeling en preventie van DCM.

1. Introduction

1.1. Diabetes mellitus

Diabetes mellitus (DM) is a progressive, chronic metabolic disorder originating from an impaired insulin production, resulting in hyperglycemia (1, 2). In physiological conditions, insulin is produced and secreted by the pancreatic β-cells in the islets of Langerhans as a response to an increase in blood glucose level, e.g. after food intake. Insulin then stimulates glucose disposal in peripheral tissues (predominantly in the liver, skeletal muscles, and adipose tissue) thereby normalizing the blood glucose level. In DM, this balance between blood glucose levels and insulin production is disturbed (2, 3).

1.1.1. Types of diabetes mellitus

Two main types of DM are recognized, namely type 1 DM (T1DM) and type 2 DM (T2DM), as shown in figure 1. In T1DM, or insulin dependent DM, an auto-immune induced inflammation of the islets of Langerhans (referred to as insulitis) blocks the insulin production, thereby leading to an absolute insulin deficiency. This cell destruction, induced by inflammatory cells (i.e. T-lymphocytes and macrophages), is associated with variations in the human leukocyte antigen (HLA) genes. These HLA genes are involved in antigen presentation and are thereby able to cause an immune response after the presentation of an autoantigen (1). In T2DM on the other hand, the insulin production is insufficient to compensate for the hyperglycemia induced by peripheral insulin resistance, thereby leading to a relative insulin deficiency (1-3).

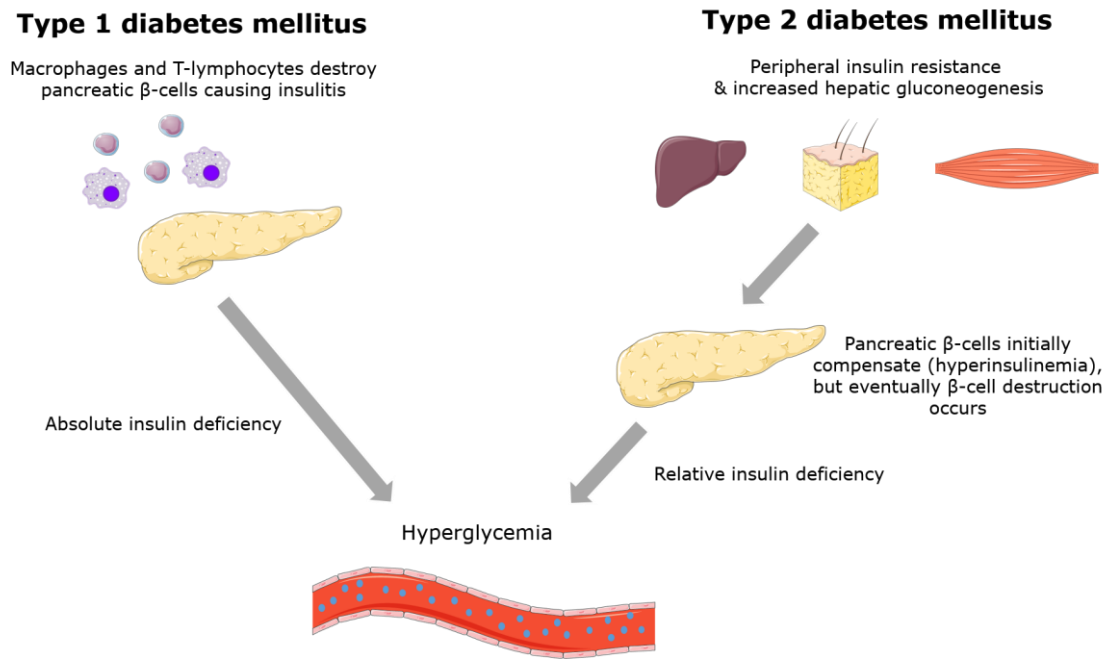

Figure 1 Pathogenesis of type 1 diabetes mellitus and type 2 diabetes mellitus.

Additionally, a third and less common type of DM, called gestational diabetes or maturity-onset diabetes, exists. This disorder consists of a transient period of hyperglycemia that lasts during pregnancy, but normalizes after birth. Nevertheless, this type of diabetes causes an increased maternal risk of developing T2DM and significantly increases the risk of developing complications during both pregnancy and delivery (3, 4).

1.1.2. The rise of diabetes mellitus and its risk factors

Currently, DM affects more than 420 million patients worldwide with 90% of this population suffering from T2DM, thereby causing a significant economic loss (3). Therefore, from here on, mentioning of diabetes will refer to T2DM. It is expected that this prevalence will nearly double by the year 2030, thereby reaching half a billion people worldwide (1, 2, 5). The increased incidence of T2DM will occur worldwide, due to the global spreading of the Western way of life, resulting in a T2DM epidemic in the next one or two decades (1, 2, 5-7). This can be explained by the characteristics of the Western lifestyle (physical inactivity, a sedentary lifestyle and a high-caloric diet) as well-established risk factors for T2DM (1, 2). Nutritional factors such as a diet containing high levels of saturated fatty acids and total fat, as well as an inadequate intake of dietary fibers, cause an increased risk of developing T2DM. At the moment, specifically the highly sugared foods and beverages are recognized as a great risk for the development of T2DM, thereby leading to several campaigns and regulations (e.g. sugar taxes) in order to reduce the intake of dietary sugar. Furthermore, active smoking and overconsumption of alcohol increase the risk of developing T2DM (3, 5).

Yet, T2DM is a multifactorial disorder caused by the interaction between lifestyle factors and genetic risk factors (3, 5). Therefore, other influences such as family history, genetics, and age play an additional role in the development of T2DM. This has been observed in genetic and concordance studies which showed a significantly increased risk of developing T2DM in case of a family history of this disorder, especially in case of first-degree relatives. Previously it has been demonstrated that both risk factors of T2DM, such as obesity, as well as metabolic disease-related processes, such as insulin resistance, can be passed along within a family (5, 6). Furthermore, a number of genes (e.g. TCF7L2, PPARG, FTO, KCNJ11, NOTCH2, WFS1, CDKAL1, IGF2BP2, SLC30A8, JAZF1, HHEX) have been found to influence the development of T2DM. Many of these genes are linked to the development of obesity and play a role in insulin regulation, glucagon regulation or glucose transport (2, 5, 6, 8). Finally, due to progressive age-related β-cell dysfunction, aging has been recognized as a risk factor for T2DM. This was observed in various studies which observed a positive correlation between the incidence of T2DM and the increase in our ageing population (3, 6, 9-13).

1.1.3. Pathophysiology of type 2 diabetes mellitus

A strong association has been established between obesity and T2DM. Obesity, especially visceral obesity, plays a role in the development of T2DM through the accumulation of insulin-resistant adipocytes in skeletal muscle, pancreas, and liver (5, 14). This process, referred to as lipotoxicity, ultimately leads to chronic insulin resistance, which is defined as a decreased insulin-mediated uptake

of glucose in peripheral tissues. Additionally, glucotoxicity occurs, which is defined as the impairment of the pancreatic β-cell function as a consequence of chronic hyperglycemia (1, 2, 6).

Initially, peripheral insulin resistance will cause a compensatory increase in insulin secretion, resulting in an euglycemic and hyperinsulinemic state, as shown in figure 2. Eventually the pancreatic reserve of insulin is unable to compensate for the increased insulin demand, leading to an increase in postprandial blood glucose level and thereafter in fasting glucose levels. Additionally, this hyperglycemic state is enhanced by an increased level of hepatic gluconeogenesis. In this state of impaired glucose tolerance (IGT)[1], already 80% of all pancreatic β-cell function is lost due to the process of glucotoxicity (6). Finally, the increased insulin demand together with pancreatic lipid accumulation will augment the β-cell dysfunction. Ultimately these processes lead to the diagnosis of T2DM, characterized by chronic hyperglycemia and hypoinsulinemia (1, 5, 6, 14).

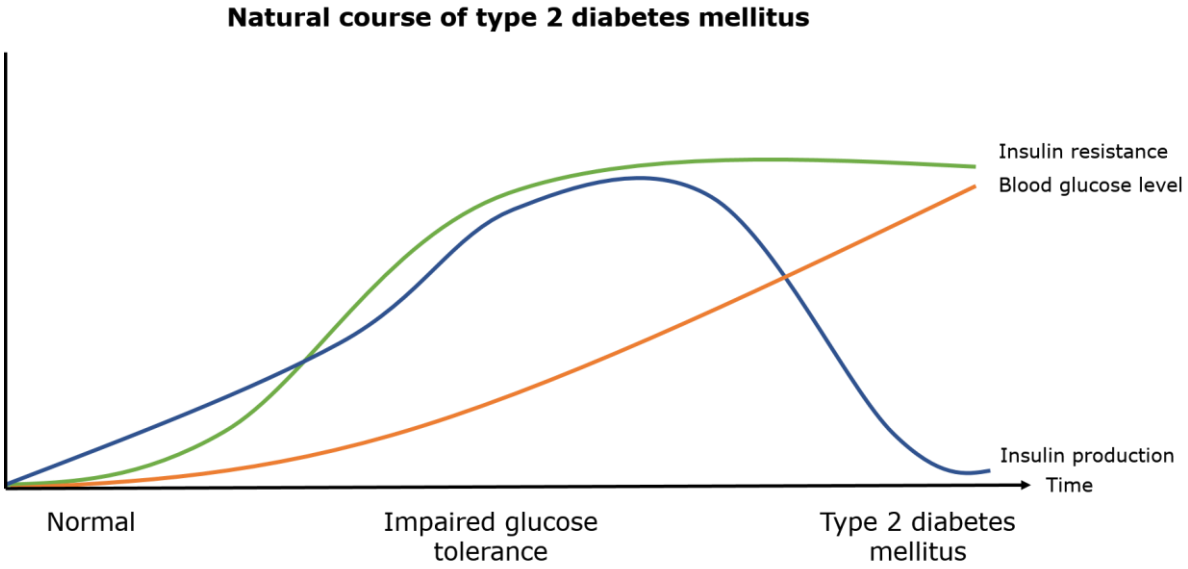

Figure 2 Natural course of type 2 diabetes mellitus. This figure shows the inversed Starling's curve of the pancreas (inverse U-shape in insulin production) formed by an initial increase in insulin secretion as a response to hyperglycemia and peripheral insulin resistance, followed by a decline in insulin production caused by β-cell destruction *(Figure adapted from Henry et al, American journal of medicine, 1998).*

Due to its metabolic nature, T2DM causes many complications, leading to a high level of morbidity and mortality. Examples of these complications are microvascular and macrovascular damage, inflammation, and infections (e.g. non-healing foot ulcers potentially leading to amputation of the foot or limb) (3). Additionally, TD2M increases the risk of cancer, liver disease, nephropathy, retinopathy, and neuropathy (1, 2, 14, 15). Furthermore, diabetic patients have an increased risk of developing cardiovascular diseases (CVDs) such as coronary artery disease (CAD), stroke, atherosclerosis, hypertension, and myocardial infarction, representing the main cause of morbidity and mortality in T2DM patients (1, 14-20).

[1] Impaired glucose tolerance (IGT) as well as impaired fasting glycemia (IFG) are defined as intermediate conditions in the transition from normal glycemic levels to diabetic glycemic levels (World Health Organization, Global report on diabetes, 2016).

1.2. Diabetic cardiomyopathy

Diabetic cardiomyopathy (DCM) is a diabetes-associated type of heart failure (HF) characterized by changes in the physiology, structure, and mechanical function of the heart (15, 21). DCM occurs independently of other established causes of HF such as hypertension, CAD or valvular diseases (15, 16, 20). The diagnosis of DCM is complicated due to the lack of well-recognized criteria and characteristics, nevertheless, the prevalence of DCM is estimated at 17% within the population of T2DM patients (21).

HF is defined as the inability of the heart to provide an adequate cardiac output (CO) to supply all body tissues with a sufficient amount of oxygen and nutrients, both at rest and during physical exercise (22, 23). Two different types of HF can be identified: systolic heart failure and diastolic heart failure (SHF and DHF, respectively). SHF is characterized by a thin and weak left ventricular (LV) muscle that is unable to adequately contract during each heart cycle, or in other words, during systole (24). In DHF on the other hand, the heart fails to relax properly, resulting in inadequate filling of the ventricles during diastole. This insufficient filling is due to stiffening and thickening of the LV muscle caused by fibrosis and hypertrophy, respectively (22, 23).

1.2.1. Hallmarks of diabetic cardiomyopathy

Although the diagnostic criteria of DCM are not well-defined, some papers suggest certain hallmarks for DCM, such as fibrosis, hypertrophy, and diastolic dysfunction (15, 17, 19). In the first stage of the development of DCM the characteristics of DHF appear: a reduced compliance (increased stiffness), slower and longer relaxation of the LV muscle, an increased end-diastolic pressure, decreased end-diastolic volume, and a reduced peak velocity in the early diastolic filling (17, 20). During this phase, the diabetic patient shows no clinical symptoms. In a later phase, SHF will arise leading to clinical symptoms such as dyspnea, edema in lower limbs, exercise intolerance, and fatigue (22). In this stage, a shortened LV ejection time, a reduced LV fractional shortening, and slower systolic peak velocity will lead to a decreased heart rate, an increase in systolic blood pressure, a decreased left ventricular ejection fraction, and an increase in end-diastolic diameter and volume. Eventually, these processes will lead to the occurrence of overt HF (17, 18, 20).

Hypertrophy is observed in T2DM as an increase in LV wall thickness, LV mass and cross-sectional area of the cardiomyocytes (CMs) (18, 25). Furthermore, a correlation has been found between the occurrence of LV hypertrophy and chronic hyperglycemia, insulin resistance, and obesity (20). A second hallmark of DCM is interstitial and perivascular fibrosis (25). This results from an increased deposition of collagen type III and cross-linking of these collagen fibers. The development of fibrosis is caused by the complex interaction between several processed including oxidative stress, myocardial inflammation, and accumulation of advanced glycation end-products (AGEs). Together, hypertrophy and fibrosis decrease the compliance of the LV, thereby acting as a mechanistic cause for DHF (16, 25).

1.2.2. Molecular mechanisms in diabetic cardiomyopathy

The molecular processes responsible for causing DCM are an impaired cardiac excitation-extraction coupling (ECC), alterations in mitochondrial substrate utilization, increased levels of oxidative stress, mitochondrial dysfunction, formation of AGEs, and myocardial inflammation (16-20, 25, 26).

Impaired excitation-contraction coupling

Ca^{2+} is the main ion in the cardiac ECC and is essential for the physiological function of the CMs (17, 20, 25). Ca^{2+} induces the release of the intracellular Ca^{2+} stores from the sarcoplasmic reticulum (SR), leading to the contraction of the CMs, as shown in figure 3 (15, 16, 20, 27).

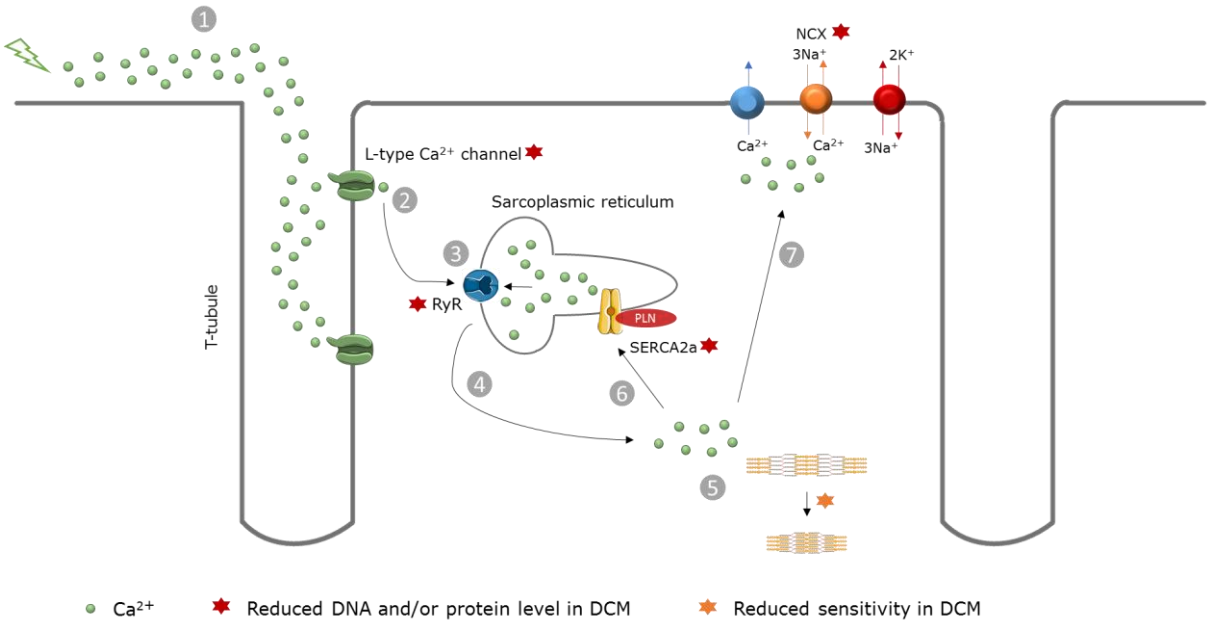

Figure 3 The cardiac extrication-contraction coupling. The process of Ca^{2+}-induced Ca^{2+}-release starts with the depolarization of the cardiomyocytes (1), causing opening of the voltage-dependent L-type Ca^{2+} channels (2) followed by activation of the ryanodine receptors (RyR), causing an efflux of Ca^{2+} from the sarcoplasmic reticulum (3). Subsequently, Ca^{2+} diffuses to the contractile proteins and binds troponin C, thereby releasing inhibition on troponin I (4). This enables cross-bridging of the actin-myosin filaments leading to contraction of the cardiomyocytes (5). During diastole, the intracellular Ca^{2+} concentration is lowered by the sarco/endoplasmic reticulum Ca^{2+}-ATPase-2a (SERCA2a) which collects Ca^{2+} in SR (6). Additionally, the Na^{+}-Ca^{2+} exchanger (NCX) and Ca^{2+}-ATPase cause a Ca^{2+} efflux (maintained by an inward K^{+} current of the Na^{+}-K^{+} exchanger) (7).

In DCM, almost all Ca^{2+} transporters (e.g. Ryanodine receptor, Na^{+}-Ca^{2+} exchanger and sarco/endoplasmic reticulum Ca^{2+}-ATPase-2a) are impaired in their protein and/or DNA level, thereby leading to a decreased activity. This results in a slower and decreased Ca^{2+} efflux during diastole, thereby leading to a prolonged diastole which contributes to the development of DHF, one of the major hallmarks of DCM. The impaired Ca^{2+} currents and reduced SR Ca^{2+} storage will elongate the action potential, ultimately leading to SHF by delaying CM contraction (16-18, 20, 25).

Altered mitochondrial substrate utilization

CMs have a high and continuous demand of ATP synthesis in order to keep up with uninterrupted cycles of contraction and relaxation. Therefore, CMs possess a great number of mitochondria that show a high level of flexibility in the energy source, either free fatty acids (FFAs) or glucose, that is used for generating ATP. This flexibility is essential to adapt to different physiological (e.g. cardiac workload) and pathological (e.g. myocardial infarction) conditions (18, 19). In a normal physiological state, the energy for cardiac contractility is derived primarily (for two-thirds) from the oxidation of FFAs (also referred to as β-oxidation), while only one-third is generated through glucose or lactate oxidation (15). In T2DM, the flexibility in fuel usage is decreased, together with an altered substrate utilization in which almost 90% of ATP synthesis is derived from FFA oxidation (19, 25).

The detrimental consequences of a higher FFA oxidation are linked to the increased need for oxygen, when compared to glucose oxidation. In other words, more oxygen is needed for the oxidation of FFAs when compared to glucose oxidation (15). As a consequence, a lower cardiac efficiency (ratio of energy output and energy input) occurs, which leads to an impaired myocardial function. Furthermore, the lower cardiac efficiency is correlated with insulin resistance, glucose intolerance, and diabetes (16, 19).

Oxidative stress

Reactive oxygen species (ROS) are oxygen-based molecules, consisting of free radicals and chemicals. These molecules cause oxidative stress, which is defined as an increased ROS production that cannot be balanced by antioxidant defenses. Sources of ROS consist of the formation of AGEs and the occurrence of mitochondrial dysfunction. In the diabetic heart, the production of ROS is increased in combination with a decrease in antioxidant defense mechanisms (15, 16, 18, 20, 25, 28). This results in cellular damage through causing DNA damage and oxidation of proteins and lipids. Thereby, ROS can influence many intracellular signaling pathways, together with altering gene expression. Oxidative stress ultimately leads to apoptosis, thereby contributing to myocardial dysfunction by causing abnormal myocardial remodeling (17-19, 25).

Mitochondrial dysfunction

In physiological conditions, the mitochondria are responsible for the production of ATP, homeostasis of ROS and Ca^{2+}, autophagy, apoptosis, and several signaling pathways. In DCM, mitochondrial dysfunction is caused by two processes. The first process is termed lipotoxicity and originates from an enhanced hepatic lipid synthesis and increased lipolysis in the adipose tissue, leading to higher circulating levels of FFAs and triglycerides. The higher supply of FFAs to the mitochondria will increase β-oxidation (18). Additionally, insulin will stimulate the uptake of FFAs into the CMs, thereby further stimulating the FFA oxidation. Eventually the intracellular accumulation of FFAs will exceed the oxidative capacity of the CMs. Simultaneously, the mitochondria show a reduced oxidative capacity and a downregulated expression of proteins of the electron transport chain (ETC) (16, 25). Intermediates of FFAs, such as ceramide, can then potentially stimulate CM apoptosis (15, 25). These processes ultimately lead to the production of ROS and a reduced ATP production which contribute to myocardial dysfunction by causing mitochondrial dysfunction and a reduced myocardial contractility, respectively (15, 16, 28). The second process leading to mitochondrial dysfunction is

mitochondrial uncoupling. During uncoupling of the mitochondria, the transport of electrons will be diverted to other processes (e.g. thermogenesis) instead of ATP synthesis (29, 30). The proteins responsible for causing this imbalance are the uncoupling proteins (UCPs), which are mitochondrial transporters residing in the inner membrane. These proteins are activated by FFAs, ROS, and lipid peroxidation, all present at higher levels in the diabetic myocardium (16, 25, 28).

In short, the mitochondrial function is disturbed by an enhanced ROS production and mitochondrial uncoupling. Both these processes contribute to a decrease in ATP synthesis, which is responsible for impairing myocardial contractility, thereby taking part in the development of DCM.

Advanced glycation end-products

A continuous state of hyperglycemia causes the occurrence of non-enzymatic glycosylation (also referred to as the Amadori reaction) of the amino groups of proteins, lipids, and nucleic acids. Subsequently, a series of chemical rearrangements is responsible for the formation of stable, long-lived AGEs (e.g. glycated hemoglobin, HbA1c), which are found to be increased in various tissues and cell types in diabetes patients. AGEs for example accumulate in heart, kidney, aorta, and liver of diabetic patients and are collected in CMs, fibroblasts, and capillaries (31). Additionally, an increased level of RAGE (receptor for advanced glycation end-products) is observed in DM (32). These increased levels of both AGEs and RAGE play a role in the development of many diabetic complications, including DCM. Firstly, AGEs can cross-link several intracellular proteins (e.g. SERCA2a) and extracellular proteins (e.g. collagen), thereby impairing cell function and increasing vascular and myocardial stiffness, by which they contribute to the development of DHF. On the other hand, AGEs interact with their receptors, thereby influencing intracellular signaling pathways leading to inflammation, oxidative stress, fibrosis, and altered Ca^{2+} handling (31-33).

Myocardial inflammation

A strong association has been observed between chronic low-grade inflammation and the occurrence of obesity and T2DM. High circulating levels of lipids and glucose stimulate inflammation through various signaling pathways such as the nuclear factor-κB pathway (NF-κB pathway). These pathways are responsible for the recruitment of several pro-inflammatory cytokines (e.g. tumor necrosis factor-α, TNF-α), chemokines, adhesion molecules (e.g. vascular cell adhesion molecule-1, intercellular cell adhesions molecule-1), and plasminogen activator inhibitor-1 (PAI-1). Additionally, lipoxygenases (LOX enzymes) contribute to the development of the chronic low-grade inflammation (34). Ultimately myocardial inflammation will contribute to diastolic dysfunction by causing hypertrophy and fibrosis (35-38).

1.3. Exercise training as a therapeutic tool

Currently, there is no effective treatment available specifically for DCM. Therefore, management of DCM occurs through management of diabetes and HF complications. The latter can be performed by angiotensin converting enzyme inhibitors (ACE inhibitors) for reducing hypertension. Additionally, β-blockers are used to reduce the heart rate (HR) in order to further reduce hypertension, thereby reducing the stress on the heart (39).

The current treatment for T2DM consist of a combination of lifestyle modifications and a pharmacological approach for managing the blood glucose levels (2, 14). Examples of these pharmacological agents are biguanides (e.g. Metformin), sulfonylureas (e.g. glipizide, glyburide), meglitinides, and thiazolidinedione. These drugs exert their therapeutic effect on blood glucose levels by reducing peripheral insulin resistance, stimulating pancreatic insulin secretion, and reducing hepatic gluconeogenesis (5, 14).

Apart from the pharmacological approach, lifestyle modifications such as dietary interventions and exercise training are gaining interest as tools for the treatment and/or prevention of T2DM. In Belgium, 60% of the T2DM patients are overweight and more than 20% are diagnosed with obesity. Additionally, almost 40% of Belgian diabetic patients exert a sedentary lifestyle (40). The current Western diet is characterized by high levels of both sugar and fat, and low levels of fibers, which are all linked to the development of T2DM and should therefore be avoided. Especially the high consumption of sugar is presenting a major threat in the development of a T2DM epidemic (3, 5). Additionally, physical inactivity should be halted, since exercise training induces several metabolic and cardiovascular benefits which reduce the risk of developing T2DM. Furthermore, exercise training (e.g. moderate-intensity continuous exercise) is already used for the treatment of several CVDs (41-43). Along with this statement, increasingly more attention is brought to the significance of the mode and intensity of the exercise training, since this is thought to play a role in causing a variety of beneficial effects (26).

1.3.1. Exercise modalities and intensity

In terms of the mode of the exercise training, one can decide between aerobic training (e.g. walking, cycling, swimming) or resistance training (e.g. lifting weights). While aerobic training focusses on cardiovascular improvements, resistance training induces both cardiovascular and muscular-skeletal improvements. Since aerobic fitness is a measure for cardiovascular health and a well-established predictor for all-cause mortality and cardiovascular mortality, the use of an aerobic training program was preferred during this study (44, 45).

Furthermore, exercise can be performed at a moderate intensity or at a high intensity, of which the parameters are described in table 1 (44, 46). Moderate-intensity training (MIT) is considered as a golden standard and is used as part of cardiovascular revalidation programs. It consists of an exercise of a moderate intensity, performed for a continuous period of time. Yet, high-intensity interval exercise (HIIT) is gaining interest as being more effective and time-efficient when compared to MIT (47-51). HIIT includes repeated bouts of short duration (seconds to minutes) high-intensity exercise alternated with recovery exercise and thus comprises a shorter exercise duration (52-54). Since lack

of time is recognized as the leading barrier for performing physical exercise, HIIT is gaining attention as a potential substitute for MIT (54-57). However, molecular mechanisms of HIIT induced health benefits are not yet completely understood and more research is necessary to investigate the advantages of this exercise modality. Therefore, a HIIT program will be compared with a MIT program during this study, in order to determine which training modality is more potent as a treatment for DCM.

Table 1 Overview of cardiometabolic parameters of moderate-intensity training and high-intensity interval training.

	Moderate-intensity training	**High-intensity interval training**
Maximal heart rate	55-70%	≥ 90%
Heart rate reserve	40-60%	85%
Vo_{2max}	40-60%	85%

The heart rate reserve is defined as the heart rate at rest subtracted from the maximal heart rate. Vo_{2max} represents the peak oxygen uptake during exercise.

1.4. Animal models of type 2 diabetes mellitus

The most commonly used animal models for studying T2DM are monogenic mice models possessing a mutation in the leptin gene ($Lep^{ob/ob}$) or in the gene of the leptin receptor ($Lepr^{db/db}$), thereby causing dysphagia and the development of obesity-induced diabetes. These mice are genetically altered and therefore do not fully reflect the human phenotype of T2DM. The Otsuka Long-Evans Tokushima Fat rat (OLETF) is a T2DM rat model that shows hyperglycemia, mild obesity, and fibrotic pancreatic islands. Another example of a T2DM rat model are the Zucker diabetic fatty rats (ZDF) which develop impaired glucose tolerance, obesity, hyperinsulinemia, hyperlipidemia, and hypertension due to a mutation in the leptin receptor (58-61). A third and frequently used option for the induction of diabetes in a rat is the use of streptozotocin. Nonetheless, the streptozotocin-induced rat model does not fully reflect the human phenotype of T2DM, since it does not show obesity and leans towards a T1DM phenotype while simultaneously causing cardiomyocyte atrophy (62-65). Additionally, diabetes can be induced in rats using a diet containing a high level of fat, though, to fully reflect the current Western diet within our animal model, we opted for the use of a high-sugar diet (containing 48% of sugar) to induce T2DM in rats (66). Previous results show the development of hyperglycemia, hyperinsulinemia, hyperlipidemia, obesity, elevated end-diastolic pressure, LV hypertrophy, increased fibrosis, and increased levels of PAI-1 and AGEs after 18 weeks of the high-sugar diet (*Western diet given to healthy rats mimics the human phenotype of diabetic cardiomyopathy*, Verboven et al, data not published).

Gathering all this information, we hypothesize that exercise training can be used as a treatment for DCM. Therefore, we have set up an experiment with the objective to test whether exercise training can act as a treatment for DCM and to determine which training modality (MIT or HIIT) is more efficient as a treatment for DCM. During this experiment, T2DM was induced in 32 male Sprague-Dawley rats using a high-sugar diet. Subsequently, the rats performed treadmill running at a moderate intensity (MIT) or high-intensity (HIIT). The cardiac function was monitored by hemodynamic and echocardiographic measurement, while oral glucose tolerance tests were used to follow the development of T2DM. Additionally, we investigated the level of inflammation, fibrosis, and hypertrophy in the myocardium.

2. Materials and methods

The experiments performed during this study conform to the EU directive 2010/63/EU for animal experiments and are approved by the local ethical committee (Ethical committee for animal experimentation, UHasselt, Belgium, ID 201554).

2.1. Experimental animals

A total of 32 male Sprague-Dawley rats (Charles River laboratories, L' Arbresle, France) arrived at the age of 6 weeks weighing 200 – 225 g. All animals received a one-week acclimatization period after arrival before the start of the study, during which they were fed a standard chow diet (Standard rodent diet, Teklad 18, ENVIGO, Netherlands). A two-week treadmill familiarization period took place in week 16 – 18, before the start of the training period. During this familiarization period, animals were trained 3 days/week at a moderate exercise intensity and duration (ranging from 5 – 15 m/min for 5 – 20 minutes).

Throughout the experiment, animals were kept in a temperature and humidity controlled room and food and water was provided *ad libitum*. Every morning, the rats were provided with freshly prepared food and daily food intake was monitored by weighing the residual food the next morning. All animals were housed per 3 in a cage containing a sufficient amount of bedding material and cage enrichment.

2.2. Study set-up and training protocol

At the start of the 30-week experiment, the animals were randomly assigned (by using excel as a randomization tool) to either the control group or the group receiving the high-sugar diet (referred to as the CAF diet). The control group (CNTL, n = 10) was provided with the standard rodent diet (Teklad 18, ENVIGO, Netherlands), while the animals in the high-sugar diet group received a diet containing 15% protein, 16% fat and 48% sugar (also referred to as the cafeteria diet, CAF, n = 22), as shown in table 2.

The latter group was further divided in 3 experimental groups after 18 weeks of the CAF diet. The first group was kept sedentary and did not receive any type of training (CAF-SED, n = 8). The second group carried out the moderate-intensity training (CAF-MIT, n = 7), which consisted of running on a treadmill at a constant speed of 18 m/min. The third group performed high-intensity interval training (CAF-HIIT, n = 7) including 10 bouts of high-intensity treadmill running alternated with 1-minute active resting periods. The high-sugar diet or control diet was initiated at the beginning of the experiment and lasted throughout the 30 weeks. The training period lasted for 12 weeks until the end of the experiment, as shown in figure 4. Sample size was calculated by a Power analysis based on previous results.

Table 2 Diet composition of the standard diet and the high-sugar diet.

	Standard diet	High-sugar diet (CAF diet)
Total carbohydrates		
Starch	55%	21%
Sugars	8%	48%
Total fat		
Saturated FFAs	3%	8%
Unsaturated FFAs	9%	8%
Total protein	25%	15%

Energy content (% of kcal) of carbohydrates, fat and proteins of the standard diet and the high-sugar diet (CAF diet). *FFAs free fatty acids.*

The training program consisted of a 12-week training period in which the animals were trained 5 days/week. During the first 6 – 8 weeks of the training period, the training protocol of both the MIT and the HIIT groups was further increased in duration and intensity to ensure maximal training capacity. This was assessed at the end of every week through evaluation of the exercise training intensity of the HIIT group by measurements of blood lactate levels directly after exercise, using an Analox apparatus GM7 (Analis SA, Namur, Belgium). Lactate levels above 4 mmol/l were considered HIIT. Subsequently, both training modalities (HIIT and MIT) were adjusted by calculating the net caloric cost (kcal/min) in order to ensure isocaloric exercise interventions in both groups.

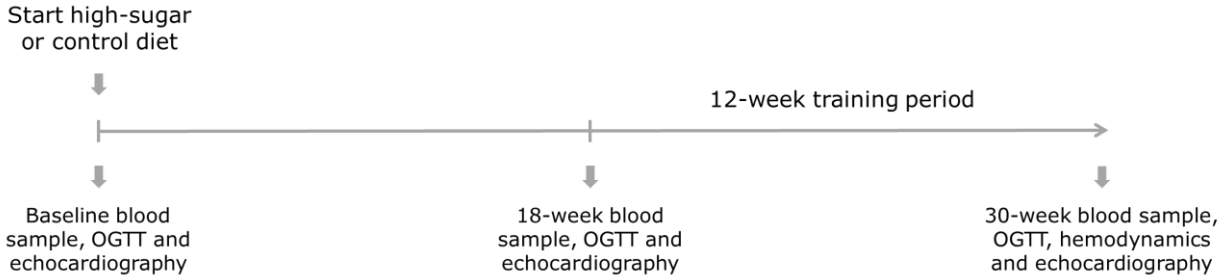

Figure 4 Timeline of the experiment indicating the start of the 12-week training period and performed measurements. The control diet and high-sugar diet (CAF diet) were used throughout the 30-week experiment. The training period consisted of moderate-intensity training or high-intensity interval training and started at 18 weeks lasting until the end of the experiment. *OGTT oral glucose tolerance test.*

Blood samples, echocardiographic measurements and an oral-glucose tolerance test (OGTT) were performed at the start of the experiment (week 0, baseline) and repeated in the middle of the experiment (week 18), as shown in figure 4. These measurements were repeated prior to sacrifice (week 30) together with hemodynamic measurements. 30 weeks after the start of the exercise training and diet, the animals were sacrificed with an overdose of Doletal (200 mg/kg) where after the hearts were perfused. The heart tissue was divided and stored in 4% paraformaldehyde to create microscopic slides, in glutaraldehyde for further electron microscopy analysis, and crushed into powder and frozen at -80°C. Additionally, small parts were stored to generate cryosections.

2.3. Echocardiographic measurements

Transthoracic echocardiography parameters were evaluated at the beginning of the experiment (week 0), halfway the experiment (week 18) and prior to sacrifice (week 30). Measurements were performed under 2% isoflurane anesthesia in all animals, using a Vivid*i* ultrasound machine (GE Vingmed Ultrasound, Little Chalfont United Kingdom) as described previously (67, 68). Briefly, a temporal resolution of approximately 200 frames per second was used to obtain a standard parasternal long-axis image and short-axis views at the mid-ventricular level.

The B-mode images at midpapillary level in the parasternal short-axis view were used to obtain the following conventional echocardiographic parameters: left ventricular end-diastolic diameter (LVEDD), left ventricular end-systolic diameter (LVESD), posterior wall thickness, and anterior wall thickness (AWT). Subsequently, these measurements were used to calculate the left ventricular end-systolic volumes (LVESV) and the left ventricular end-diastolic volumes (LVEDV) by $\pi*D_M^2*B/6$, in which D_M indicates the systolic/diastolic diameter of the left ventricle in mid-ventricular short-axis view and B is the left ventricular length on the parasternal long-axis view. These calculations were then used to determine the ejection fraction (EF, expressed in %) by (LVEDV – LVESV)/(LVEDV). Additionally, images obtained in the M-mode were used to determine the heart rate (HR).

2.4. Hemodynamic measurements

Prior to the sacrifice (at week 30), conductance measurements were performed in all animals. This hemodynamic assessment was performed using the SPR—320 MikroTip high-fidelity pressure transducer (Millar Inc, Texas, USA), which reached the left ventricle through the aorta and aortic valve. The pressure catheter (2F) was connected to a quad-bridge amplifier and a PowerLab 26T module (AD Instruments, Oxford, United Kingdom) was used for the data transfer to LabChart v7.3.7 software (AD Instruments, Oxford, United Kingdom).

2.5. Measurement of fibrosis

The level of fibrosis was evaluated in all animals using the Sirius Red/Fast Green kit (Chondrex, Kampenhout, Belgium). Transversal sections (with a thickness of 10 µm) were obtained at the cardiac midventricular level where after fibrosis was assessed in four randomly chosen sections spread throughout each transversal section. Blood vessels were excluded from these areas due to the presence of high amounts of collagen in the vessel wall. An automated image analysis program (Carl Zeiss, AxoVision 4.6, Zaventem, Belgium) was used to quantify the outlined areas of collagen deposition. Total collagen deposition to the global cardiac area was calculated and expressed as % collagen deposition.

2.6. Western blotting procedure

The BCA protein assay kit (Thermo Fisher, Erembodegem, Belgium) was used to determine the protein concentrations in protein extractions derived from left ventricle tissue samples of all animals. At first, equal amounts of protein (15 µg) were separated on a 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (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 in a 5% milk in Tris-buffered saline solution containing 0,1% Tween-20 (TBS-T). Then, the PVDF membrane was incubated overnight at 4°C in the presence of the primary antibody (as shown in table 3). Eventually the membrane was incubated with a horseradish peroxidase-conjugated secondary antibody (Dako, Belgium) at a dilution of 1/2000 for 1h at room temperature, except for the phospholamban secondary antibody which was incubated for 1,5h at room temperature. All antibodies – both primary and secondary – were diluted in 5% milk-TBS-T. Finally, visualization was performed with the enhanced chemiluminescence (ECL) technique using the Pierce ECL Plus Western Blotting Substrate (Thermo Fisher, Erembodegem, Belgium) as described by the manufacturer. The precision plus dual color protein standard (Bio-rad Laboratories, Temse Belgium) was used as a ladder in all Western blotting procedures.

Subsequently, β-actin was used for the normalization of the protein. First, all antibodies were removed from the membrane using a mild stripping buffer (Abcam, Cambridge, United Kingdom). Then the membrane was blocked in 5% milk-TBS-T for 1h at room temperature. Subsequently the membrane was incubated at room temperature with the β-actin (C4) mouse monoclonal anti-rat IgG (sc-47778, Santa Cruz, USA) for 2 hours and rabbit polyclonal anti-mouse HRP IgG (Dako, Belgium) for 1h, the primary and secondary antibody, respectively. Again, antibodies were diluted in 5% milk-TBS-T. Finally, the ECL procedure using the Pierce ECL Plus Western Blotting Substrate (Thermo Fisher, Erembodegem, Belgium) was used for the visualization of the proteins bands.

Table 3 Overview of the primary and secondary antibodies used in the Western blotting procedure.

Primary antibody → dilution	Secondary antibody (1/2000 dilution)
Goat polyclonal TNF-α (N-19) IgG (sc-1350) → 1/1000	Rabbit anti-goat HRP (Dako P0449)
Goat polyclonal PLN (L-15) IgG (sc-21923) → 1/1000	
Rabbit polyclonal NCX (H-300) IgG (sc-32881) → 1/1000	Swine anti-rabbit HRP (Dako P0217)
Rabbit monoclonal citrate synthetase IgG (Abcam 129095) → 1/5000	
Mouse monoclonal SERCA2a (F-1) IgG (sc-376235) → 1/12000	Rabbit anti-mouse HRP (Dako P0260)

TNF-α tumor necrosis factor-α, PLN phospholamban, SERCA2a sarco/endoplasmic reticulum Ca^{2+} ATPase 2a, NCX Na^{+}-Ca^{2+}-exchanger, HRP horseradish peroxidase.

2.7. Evaluation circulating free fatty acids and advanced glycation end-products

The plasma level of free fatty acids (FFAs) was determined through a FFA quantification kit (ab65341, Abcam, Cambridge, UK) as described by the manufacturer. In short, fatty acids were incubated with an Acyl-CoA Synthetase Reagent to form their coenzyme A derivatives, followed by oxidation through an enzyme mix provided in the kit. Finally, optical density of the samples was measured at 570 nm where after the FFA concentration of the samples was calculated using a standard curve.

Plasma concentrations of advanced glycation end-products (AGEs) were determined using the OxiSelectTM Advanced Glycation End Product Competitive ELISA kit (Cell Biolabs, Inc., San Diego, USA). Briefly, plasma samples were added to the AGE conjugate coated ELISA plate. Subsequently, the wells were incubated with a diluted anti-AGE antibody and a HRP-conjugated secondary antibody was added. Finally, absorbance was measured at 450 nm.

2.8. Measurement of citrate synthase

The mitochondrial function was assessed by measurement of citrate synthase activity in left ventricular cardiac tissue homogenates. This evaluation was performed using a citrate synthase assay kit (CS0720, Sigma-Aldrich, St. Louis, USA) as described by the manufacturer. Briefly, citrate synthase present in the samples will convert CoA and oxaloacetic acid to citric acid. Subsequently, the thioester group of CoA is hydrolyzed forming CoA with a thiol group. The latter will then react with 5,5'-Dithiobis-(2-nitrobenzoic acid) (DNTB) in the reaction mix provided in the kit, thereby producing a yellow byproduct (5-thio-2-nitrobenzoic acid, TNB). This enables measurement of the absorbance of the samples at 412 nm.

2.9. Assessment of glucose tolerance and insulin resistance

A 1h oral glucose tolerance test (OGGT) was performed as described before (69) at baseline, halfway the experiment (18 weeks) and at the end of the experiment (30 weeks). An overnight fasting of 16 hours was followed by capillary blood collection and determination of the blood glucose concentration (i.e. baseline) using the Analox GM7 (Analis SA, Namur, Belgium). Measurement of the blood glucose level was then repeated at 15, 30 and 60 minutes after administration of glucose (2 g/kg) via gastric gavage.

Additionally, serum insulin concentrations were quantified at baseline and 60 minutes after glucose administration. This procedure was performed by electrochemiluminescence (Meso Scale Discovery rat/mouse insulin kit, Gaithersburg, USA) in the university of Maastricht, the Netherlands.

2.10. Statistical analysis

Results were tested for normality prior to statistical tests using the Shapiro-Wilk, Kolmogorov–Smirnov and D'Agostino's-Pearson omnibus test. The measured parameters of the animals receiving either the control or the CAF diet were compared in the middle of the experiment (18 weeks) by a parametric test (t-test) or a non-parametric test (Mann-Whitney U). Subsequently, comparison of the 4 experimental groups (CTRL, CAF-SED, CAF-MIT, and CAF-HIIT) was performed through a one-way ANOVA followed by post-hoc analysis using the Tukey or Dunn test, in case of normality or non-normality, respectively. Data are presented as mean ± SEM or median [75th percentile; 25th percentile] accordingly. Analyses were performed using GraphPad Prism (GraphPad Software, San Diego, CA, USA). A value of $p<0.05$ was considered statistically significant.

3. Results

3.1. MIT and HIIT normalize the high-sugar diet induced increases in body weight and plasma triglycerides

At baseline, no differences were observed between the experimental groups. In the groups receiving the CAF diet (high-sugar cafeteria diet), an increase in body weight and triglycerides was observed at 18 weeks (data not shown). By the end of the experiment (30 weeks), both moderate-intensity training (MIT) and high-intensity interval training (HIIT) were able to reduce body weight and normalized triglycerides, when compared to the sedentary diabetic group (CAF-SED).

Compared to the control group which received a standard diet and no exercise training, a significant increase in body weight, heart weight, heart weight normalized for tibia length, liver weight, and plasma triglycerides was observed after 30 weeks of the CAF diet (as shown in table 4). Furthermore, exercise induced a decrease in high-density lipoprotein cholesterol (HDLC), compared to the control group, yet no significant effect was observed on the total cholesterol level.

Table 4 Animal characteristics at the end of the study (30 weeks).

	CONTROL	CAF-SED	CAF-MIT	CAF-HIIT
Body weight (g)	578,4 ± 18,01	766,3 ± 19,66*	628,6 ± 26,84˜	611,7 ± 18,77˜
Heart weight (g)	1,82 ± 0,08	2,22 ± 0,11*	1,94 ± 0,09	1,96 ± 0,05
Heart weight/tibia length (g/cm)	40,35 ± 1,77	48,05 ± 1,54*	42,67 ± 1,85	42,61 ± 1,30
Lung weight (g)	1,79 ± 0,05	1,73 ± 0,07	1,76 ± 0,02	1,64 ± 0,06
Liver weight (g)	15,27 ± 0,61	19,41 ± 0,63*	17,72 ± 1,18	15,69 ± 0,83˜
Triglycerides (g)	68,7 ± 6,98	132,1 ± 15,94*	65,3 ± 10,11˜	60,4 ± 3,66˜
Total cholesterol (mg/dl)	73,5 ± 4,13	69,1 ± 3,98	58,3 ± 3,43	59,4 ± 3,34
HDLC (mg/dl)	63,9 ± 3,81	56,0 ± 3,12	48,7 ± 3,39*	49,3 ± 3,77*

Measurements performed at 30 weeks (i.e. prior to sacrifice) in the control group (sedentary and standard diet, n = 10), CAF-SED (sedentary and cafeteria diet, n = 8), MIT moderate-intensity training (n = 7) and HIIT high-intensity interval training (n = 7). *HDLC high-density lipoprotein cholesterol*. * denotes $p < 0,05$ compared to the control group, ~ denotes $p < 0,05$ compared to CAF-SED, data are shown as mean ± SEM.

The animals receiving the CAF diet showed a significant increase in circulating free fatty acid (FFA) levels ($p = 0.0367$), when compared to the control group. Furthermore, only HIIT, but not MIT, was able to reduce these FFA levels (CAF vs HIIT $p = 0.0239$), as shown in figure 5.

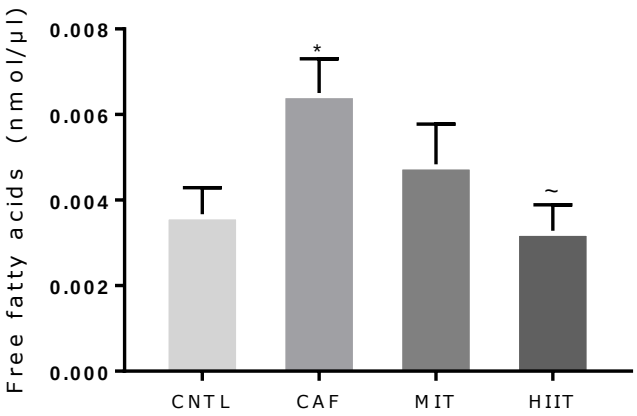

Figure 5 Effect of exercise on circulating fatty acid levels. Plasma levels of free fatty acids quantified in the control group (sedentary and standard diet, n = 10), CAF (sedentary and cafeteria diet, n = 8), MIT moderate-intensity training (n = 7) and HIIT high-intensity interval training (n = 7). * denotes $p < 0,05$ compared to the control group, ~ denotes $p < 0.05$ compared to the CAF group, data are shown as mean ± SEM.

3.2. High-sugar diet alters the glucose-induced insulin response

No significant differences in glucose or insulin values were observed between the experimental groups at the beginning of the study. Glucose and insulin levels were measured halfway the study (18 weeks) and at the end (30 weeks). At 18 weeks, we compared the control group with the animals receiving the high-sugar diet. Here, glucose levels were significantly increased in a fasted state as well as 60' after glucose administration ($p > 0.05$), as shown in figure 6A and 6B. A similar increase in insulin levels was observed, indicating a compensatory rise in insulin as a response to the high glucose levels ($p > 0.05$).

Approaching the end of the experiment (at 30 weeks) glucose and insulin levels were normalized, showing no significant difference between the control group or the groups receiving the CAF diet, as shown in figure 6C – 6F.

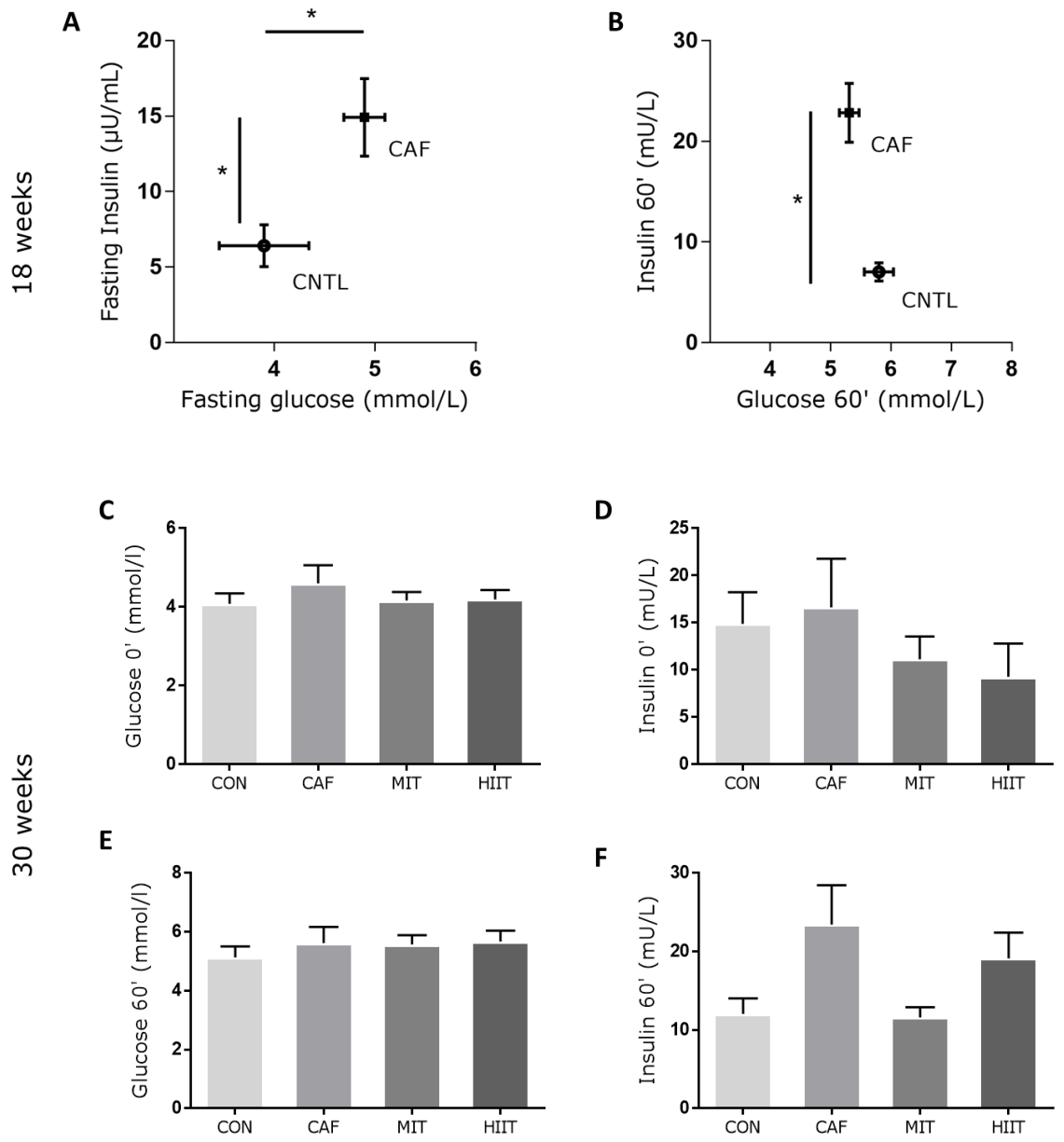

Figure 6 Glucose and insulin levels measured at 18 weeks (A and B) and 30 weeks (C – F). Representation of plasma glucose and insulin levels at 18 weeks (A, B) in the control group (CNTL, n = 10) and the group receiving the high-sugar diet (CAF, n = 22) and at 30 weeks (prior to sacrifice, C – F) in the control group (sedentary and standard diet, n = 10), CAF (sedentary and cafeteria diet, n = 8), MIT moderate-intensity training (n = 7) and HIIT high-intensity interval training (n = 7). * denotes $p < 0,05$ compared to the control group, data are shown as mean ± SEM.

3.3. Both MIT and HIIT improve cardiac function and myocardial remodeling

At the start of the study, no differences in cardiac function or architecture were observed between the experimental groups. The CAF diet caused a significant increase in left ventricular pressure (LVP) and end-diastolic pressure (EDP) when compared to the control group, as shown in table 5. EDP, one of the hallmarks of DCM, was significantly increased in the CAF-SED group, but not in the trained animals. Furthermore, HIIT caused a significant decrease in the minimal dp/dt, which is a marker for LV relaxation. Finally, no significant effect was observed on dp/dt_{max} (marker for LV contractility), Tau (marker for LV diastolic function) or diastolic duration.

Table 5 Hemodynamic measurements at the end of the study (30 weeks).

	CONTROL	CAF-SED	CAF-MIT	CAF-HIIT
LVP (mmHg)	85,1 ± 2,49	99,0 ± 3,15*	96,5 ± 2,77	103,6 ± 4,50*
EDP (mmHg)	5,4 ± 1,32	14,05 ± 3,06*	5,6 ± 1,21~	6,7 ± 0,54
Tau (s)	0,014 ± 0,00095	0,028 ± 0,015	0,015 ± 0,0017	0,035 ± 0,020
dp/dtmax	5319 ± 467	6776 ± 698,6	5831 ± 382	6975 ± 927
dp/dtmin	-4130 ± 488	-5941 ± 474	-5673 ± 542	-6747 ± 831*
Diastolic duration (s)	0,11 ± 0,0060	0,10 ± 0,0110	0,12 ± 0,0092	0,09 ± 0,0094

Results of hemodynamic measurements performed at 30 weeks (i.e. prior to sacrifice) in the control group (sedentary and standard diet, n = 10), CAF-SED (sedentary and cafeteria diet, n = 8), MIT moderate-intensity training (n = 7) and HIIT high-intensity interval training (n = 7). *LVP left ventricular pressure, EDP end-diastolic pressure*, * denotes $p < 0,05$ compared to the control group, ~ denotes $p < 0,05$ compared to CAF-SED, data are shown as mean ± SEM.

After 18 weeks, a significant increase ($p < 0.001$) in anterior wall thickness (AWT) was observed in the animals receiving the CAF diet, as shown in table 6. Towards the end of the study (30 weeks), the CAF diet caused a significant increase in both the AWT and in the end-systolic volume (ESV) ($p < 0.05$), when compared to the control group, as shown in table 7. Additionally, the same diet reduced the ejection fraction (EF, $p < 0.05$) and fractional shortening (FS, $p < 0,001$). Furthermore, only MIT, and not HIIT, was able to prevent the FS to decrease in diabetic animals while both training modalities prevented worsening of EF. In addition, both training modalities were able to normalize AWT values to baseline by the end of the study.

Table 6 Echocardiographic measurements after 18 weeks of the CAF diet.

	CONTROL	CAF-SED
Heart rate (beats/min)	354,2 ± 17,95	339,5 ± 8,08
Anterior wall thickness (mm)	1,488 ± 0,0189	1,682 ± 0,03133***
End-diastolic volume (µl)	325,3 ± 27,69	330,2 ± 17,99
End-systolic volume (µl)	85,64 ± 10,97	96,2 ± 6,703
Cardiac output (ml/min)	85,63 ± 8,537	79,86 ± 4,907
Ejection fraction (%)	73,85 ± 2,156	70,85 ± 1,351
Fractional shortening (%)	42,88 ± 2,735	39,31 ± 1,55

Results of echocardiographic measurements performed at 18 weeks (halfway the experiment) in the control group (sedentary and standard diet, n = 10) and the animals receiving the CAF diet (n = 22). *** denotes $p < 0,001$ compared to the control group, data are shown as mean ± SEM.

Table 7 Echocardiographic measurements at the end of the study (30 weeks).

	CONTROL	CAF-SED	CAF-MIT	CAF-HIIT
Heart rate (beats/min)	337,8 ± 12,32	333,9 ± 8,34	326,9 ± 18,84	319,9 ± 16,26
Anterior wall thickness (mm)	1,494 ± 0,02	1,841 ± 0,03*	1,634 ± 0,04	1,659 ± 0,05
End-diastolic volume (µl)	328,5 ± 26,6	391,5 32,7	412,7 ± 41,3	370,6 ± 30,5
End-systolic volume (µl)	74,86 ± 10,1	136,3 ± 12,4*	107,8 ± 17,6	110 ± 10,5
Cardiac output (ml/min)	82,5 ± 7	86,2 ± 10	99,4 ± 12	85,1 ± 11
Ejection fraction (%)	77,6 ± 1,9	64,9 ± 1,8*	75 ± 2,3	69,7 ± 2,5
Fractional shortening (%)	47,15 ± 1,9	35,12 ± 1,4***	43,56 ± 2,5~	38,01 ± 2,4*

Results of echocardiographic measurements performed at 30 weeks (i.e. prior to sacrifice) in the control group (sedentary and standard diet, n = 10), CAF-SED (sedentary and cafeteria diet, n = 8), MIT moderate-intensity training (n = 7) and HIIT high-intensity interval training (n = 7). * denotes $p < 0.05$ compared to the control group, *** denotes $p < 0,001$ compared to the control group, ~ denotes $p < 0,05$ compared to CAF-SED, data are shown as mean ± SEM.

Fibrosis, another hallmark of diabetic cardiomyopathy, was significantly increased in animals receiving the CAF diet, when compared to the control group ($p < 0.0001$). Furthermore, both MIT and HIIT significantly lowered the level of fibrosis ($p < 0.001$), when compared to the sedentary group receiving the CAF diet.

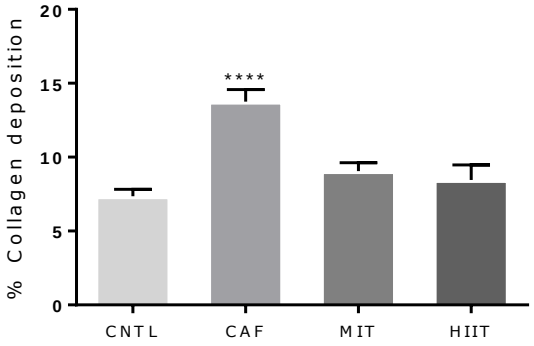

Figure 7 Level of left ventricular fibrosis. Total interstitial collagen quantification in left ventricle of the heart performed at 30 weeks in the control group (sedentary and standard diet, n = 10), CAF (sedentary and cafeteria diet, n = 8), MIT moderate-intensity training (n = 7) and HIIT high-intensity interval training (n = 7). *** denotes $p < 0.001$ when compared to the control group, data are shown as mean ± SEM.

3.4. Effects of exercise on cardiomyocyte metabolism

Moderate-intensity training reduces myocardial inflammation

When compared to the control group, a significant increase ($p = 0.0007$) was observed in the level of inflammation – measured by quantifying tumor necrosis factor-α (TNF-α) – in the animals receiving the CAF diet. After the 12-week training period, only MIT but not HIIT was able to significantly decrease TNF-α levels in the diabetic heart.

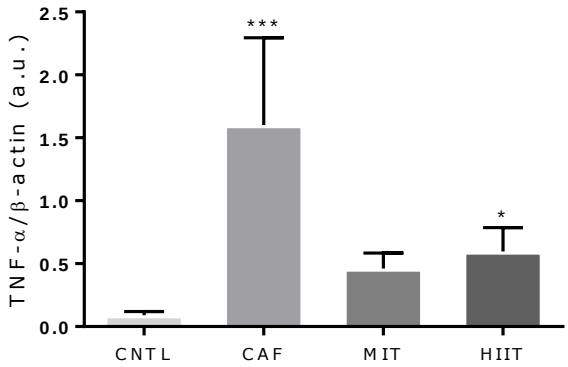

Figure 9 Effect of exercise on the level of myocardial inflammation. Protein level of TNF-α in cardiac tissue normalized for β-actin in the control group (sedentary and standard diet, n = 10), CAF (sedentary and cafeteria diet, n = 8), MIT moderate-intensity training (n = 7) and HIIT high-intensity interval training (n = 7). *TNF-α tumor necrosis factor-α*. *** denotes $p < 0,001$ compared to the control group, * denotes $p < 0,05$ compared to the control group, data are shown as mean ± SEM.

Exercise training does not significantly affect Ca^{+2} cycling proteins

No changes in calcium handling were observed between different groups. Both sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA2a), Na^{+}-Ca^{2+} exchanger (NCX) and phospholamban (PLN) remained the same. Additionally, no significant difference could be observed in the Ca^{2+} cycling proteins after a 12-week training period consisting of either MIT or HIIT.

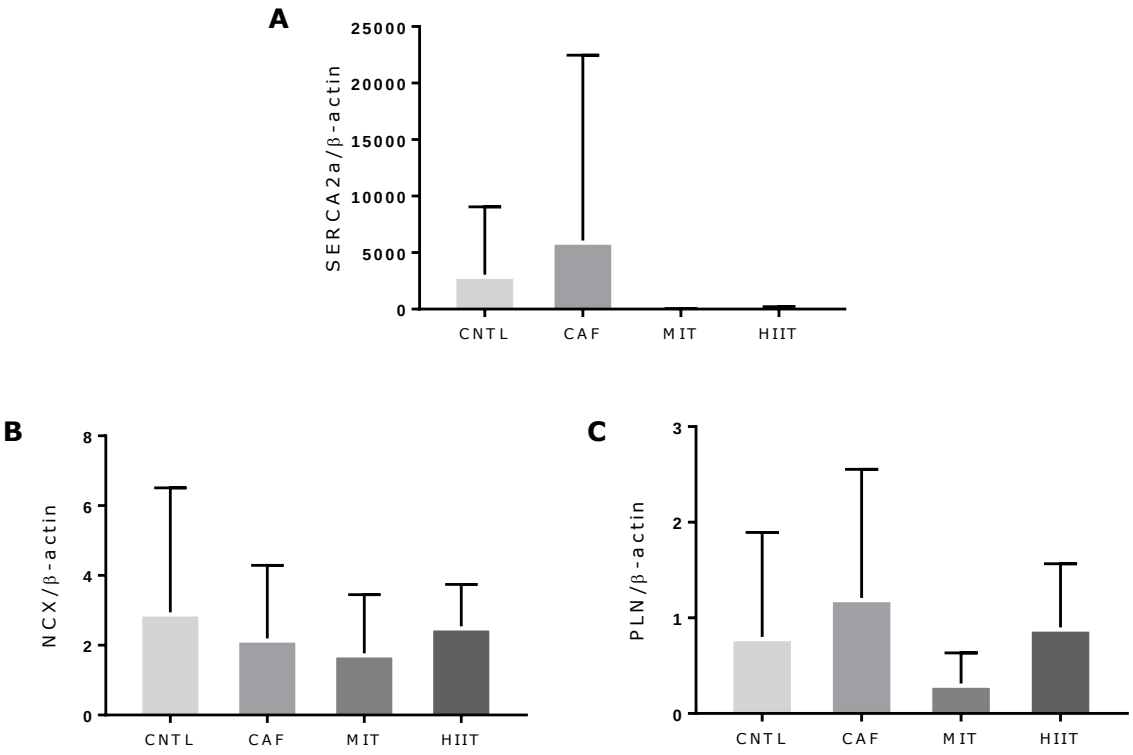

Figure 8 Effect of exercise on myocardial Ca^{2+} cycling proteins. Protein levels of SERCA2a (A), NCX (B) and PLN (C) in left ventricle tissue homogenates normalized for β-actin in the control group (sedentary and standard diet, n = 10), CAF (sedentary and cafeteria diet, n = 8), MIT moderate-intensity training (n = 7) and HIIT high-intensity interval training (n = 7). *Sarco/endoplasmic reticulum Ca^{2+}-ATPase SERCA2a, Na^{+}-Ca^{2+} exchanger NCX and phospholamban PLN.* Data are shown as mean ± SEM.

Exercise training does not significantly affect mitochondrial function and level of glycation end-products

Neither the CAF diet nor the exercise training significantly affected the activity of citrate synthase (CS) measured in left ventricle tissue samples. Furthermore, no significant difference was observed in the plasma level of advanced glycation end-products (AGEs).

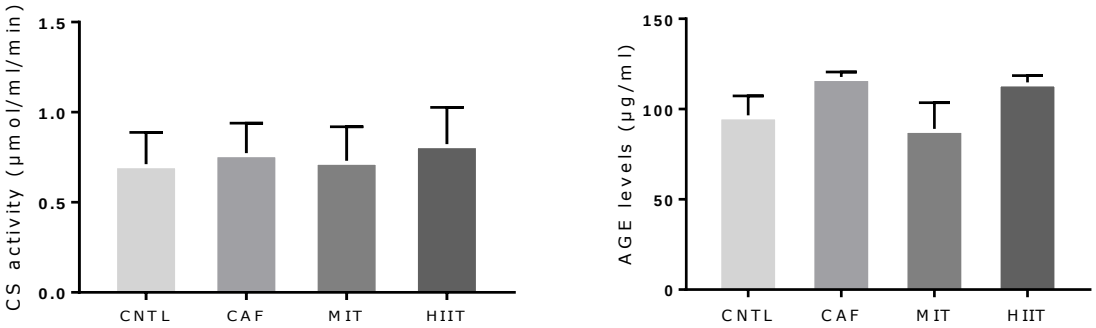

Figure 10 Effect of exercise on citrate synthase activity and circulatory advanced glycation end-products. Level of citrate synthase activity in left ventricular homogenates (A) and plasma levels of advanced glycation end-products (B) in the control group (sedentary and standard diet, n = 10), CAF (sedentary and cafeteria diet, n = 8), MIT moderate-intensity training (n = 7) and HIIT high-intensity interval training (n = 7). *CS citrate synthase, AGEs advanced glycation end-products.* Data are shown as mean ± SEM.

4. Discussion

Previous research showed that exercise training reduces the chronic low-grade inflammation that characterizes metabolic diseases such as type 2 diabetes mellitus (T2DM) (41). Furthermore, physical activity improves mitochondrial efficiency, altered substrate utilization, and antioxidant capacity while reducing oxidative stress. Additionally, it enhances the Ca^{2+} homeostasis, thereby stimulating myocardial contractility and reducing mitochondrial dysfunction. Finally, exercise training has proven to be effective in gaining control of blood glucose levels by directly reducing hyperglycemia. Therefore, physical activity can aid in the treatment of diabetic cardiomyopathy (DCM) (26, 41, 70, 71). In the current study, we show that the induction of T2DM by a high-sugar diet (high sugar cafeteria diet, CAF diet) followed by a 12-week training period leads to a reduction in the level of left ventricular (LV) fibrosis and hypertrophy, inflammation, end-diastolic pressure, body weight, plasma triglycerides, and free fatty acids (FFAs).

Both moderate-intensity training (MIT) and high-intensity interval training (HIIT) were able to decrease the body weight and level of triglycerides after a 12-week training period. Yet, only HIIT was able to reduce the increased liver weight. The increase in the liver weight after the CAF diet is most likely to be caused by the accumulation of FFAs causing hepatic steatosis, a common co-morbidity of T2DM and obesity (72, 73). The observed increase in heart weight (same holds for heart weight/tibia length) is due to LV hypertrophy.

Hemodynamic measurements showed an increase in both the end-diastolic pressure (EDP) and left ventricular pressure (LVP), indicating the occurrence of diastolic heart failure (DHF). While HIIT caused a further increase in the LVP, both MIT and HIIT resulted in a decreased EDP, thereby ameliorating the DHF. Additionally, an increase in dp/dt_{min} was observed after performing HIIT, indicating an improvement in the diastolic relaxation after this training modality. From these results we conclude that both MIT and HIIT are potentially able to recover the diastolic dysfunction after a training period of 12 weeks, with a slight advantage in HIIT trained animals. These results are in correspondence with previous literature. Brassard et al observed a normalization of the diastolic function in humans with well-controlled T2DM after a 3-month training period consisting of moderate intensity cycling (74). Additionally, Epp et al found an increase in the diastolic function, observed in an increased dp/dt_{min}, in a streptozotocin and high-fat diet induced T2DM rat model after 12 weeks of voluntary wheel running (75). Furthermore, other authors suggest HIIT to be more potent in restoring cardiac function, including diastolic function, when compared to MIT (76), while several studies observed no effect of exercise training on the myocardial function (77, 78).

4.1. Exercise training improves LV fibrosis and hypertrophy by reducing myocardial inflammation and AGE production

Chronic low-grade inflammation is a characteristic occurring in several chronic disorders, including obesity, cardiovascular diseases (CVDs), and T2DM (41, 79, 80). This systemic inflammation is characterized by high levels of circulating cytokines (e.g. tumor necrosis factor-α, TNF-α), chemokines, and peptides secreted by visceral adipose tissue. Additionally, this secretion is upregulated by high levels of plasma triglycerides and glucose, both of which are observed in our animal model after the CAF diet. These circulatory inflammatory mediators are involved in causing insulin resistance, β-cell destruction, and several inflammation-induced T2DM comorbidities. One of these comorbidities is the development of DCM, since the systemic inflammation causes myocardial inflammation through the activation of several signaling pathways that converge in the activation of the NF-κB pathway. Not only is the latter overexpressed in diabetic hearts and vasculature, activation of this pathway is associated with cardiovascular damage (81, 82). Since it triggers the secretion of inflammatory mediators from fibroblasts, cardiomyocytes (CMs), and endothelial cells, it eventually causes chronic myocardial inflammation, resulting in cardiac failure (83, 84). The cytokines involved in causing the inflammatory state can cause cardiomyocyte hypertrophy through stimulating protein synthesis, ROS production, and cardiomyocyte growth, while simultaneously reducing protein degradation (85, 86). Due to the chronic myocardial inflammation, multiple signaling pathways will lead to fibroblast proliferation and collagen production. These pathways are activated by several cytokines (e.g. TNF-α, IL-6, IL-1β) secreted by CMs and inflammatory cells as a response to the inflammatory state (38, 87-89). Additionally, the accumulation of advanced glycation end-products (AGEs) contributes to the development of myocardial inflammation and oxidative stress. Subsequently, the myocardial inflammation will lead to the development of two DCM hallmarks, namely LV hypertrophy and fibrosis. Both these processes result in a stiffer and less flexible myocardium, thereby causing it to be unable to properly relax during diastole. Therefore, fibrosis and hypertrophy represent mechanistic causes for the development of DHF (90).

In the current study, an increase in the anterior wall thickness (AWT) was observed in the animals receiving the CAF diet, indicating the development of LV hypertrophy in the diabetic animals. Towards the end of the study (30 weeks), hypertrophy remained and occurred together with an increased end-systolic volume (ESV) in the diabetic animals, but not after exercise intervention. At this point, a decreased ejection fraction (EF) was observed in the diabetic animals, but not in the animals that performed exercise. Furthermore, only MIT was able to normalize the reduced fractional shortening (FS) that was observed in diabetic animals. Additionally, only sedentary diabetic animals showed LV fibrosis, but not those who underwent exercise training. Hypertrophy and fibrosis will cause the LV to reduce in size and compliance, respectively, thereby resulting in a lower filling volume during diastole. Subsequently, a lower volume of blood is pumped out of the LV during systole, causing a decrease in the EF and FS. The increase in ESV could be explained by the amount of fibrosis present in the LV wall, causing it to fail to fully contract during systole. These results correspond with findings of many other authors (28, 42, 71, 91, 92). The exercise-induced improvements in cardiac function are attributed to the decrease in fibrosis and hypertrophy observed after MIT and HIIT, thereby increasing the EF.

A potential mechanism by which exercise training causes a decrease in LV fibrosis and hypertrophy is a reduction in myocardial inflammation through a lower level of systemic inflammation and circulatory FFAs levels. Additionally, increased mitochondrial function (leading to lower oxidative stress levels) and a lower production of AGEs present possible mechanisms that decrease LV fibrosis and hypertrophy, thereby ameliorating cardiac function.

Physical exercise induces an anti-inflammatory effect through the production of muscle-derived myokines, which are cytokines and peptides that exert an anti-inflammatory effect (e.g. IL-10, IL-4) or that counteract the effect of pro-inflammatory cytokines such as TNF-α, IL-1β. An example of the latter is IL-6 which is thought to cause an anti-inflammatory effect through the inhibition of IL-1β signaling (by stimulating IL-1 receptor antagonist) and TNF-α, yet the role of IL-6 remains controversial (93). While IL-1β is believed to play a role in causing β-cell destruction, TNF-α is involved in the development of insulin resistance (94, 95). TNF-α and other inflammatory cytokines (including IL-1 and IL-8) contribute to insulin resistance through several mechanisms that inhibit the insulin signaling. In physiological conditions, insulin binds its receptor, thereby causing both autophosphorylation and phosphorylation of the tyrosine kinase residues on the insulin receptor substrates 1 and 2 (IRS-1 and IRS-2). This process is then followed by the activation of multiple intracellular signaling pathways including the phosphatidyl-inositol 3-kinase (PI3K) pathway and the mitogen activation protein kinase (MAPK) isoforms of extracellular signal-regulated kinases 1 and 2 (ERK1 and ERK2). The mechanisms by which TNF-α causes insulin resistance include the phosphorylation of IRS-1 at the serine residues (instead of tyrosine residues) and the reduced transcription of both IRS-1 and GLUT-4, a glucose transporter (96, 97)

Since exercise is known to induce an anti-inflammatory effect, one expects the levels of TNF-α and other inflammatory cytokines to decline after exercise training. This observation appeared in the current study, which showed decreased levels of TNF-α in LV tissue after MIT, but not after HIIT. Literature shows no consensus on the effect of exercise on the levels of inflammatory markers, including TNF-α. While some studies show no significant effect of exercise on the levels of inflammatory markers (98-100), others observe a significant decline in TNF-α levels after exercise training (101, 102). A possible explanation for this discrepancy is proposed by Hopps et al and states that the anti-inflammatory effects of exercise are dependent on the exercise modality (e.g. swimming, running, walking), intensity (e.g. moderate-intensity, high-intensity, high-intensity interval training), and duration (e.g. weeks to months) (103). This theory potentially explains why the decrease in TNF-α was observed only after MIT, and not after HIIT. Additionally, the influence of the different subjects (e.g. humans, genetic T2DM animal models, Zucker diabetic fatty rats) used in researching the effect of exercise on inflammatory parameters, should be taken into account. Finally, most studies examined circulating TNF-α levels, while we used LV tissue homogenates to measure the myocardial protein level of TNF-α. This could be the final reason behind the heterogenous results in the effect of exercise on TNF-α levels.

Another cause for the development of LV fibrosis is the production of AGEs. These molecules are formed from the glycation and oxidation of proteins, lipids, and nucleic acids as a result of chronic hyperglycemia. During aging, a natural increase in AGE levels occurs. In diabetic patients on the

other hand, circulatory AGE levels show an early increase and accumulate in various tissues (e.g. myocardium, kidneys and liver) thereby causing several diabetic comorbidities. Additionally, the expression of the AGE receptor (RAGE) is increased in the diabetic heart. Thereby, AGEs can lead to the development of myocardial fibrosis through various processes. Firstly, they can bind the RAGE through which they influence various cellular processes including signaling pathways. Activation of RAGE leads to secretion of the fibrosis growth factor and increases the deposition of extracellular matrix proteins, resulting in fibrosis. Additionally, RAGE stimulation is associated with activation of the NF-κB pathway, thereby leading to inflammation, oxidative stress, and atherosclerosis. Secondly, AGEs can directly cause fibrosis through crosslinking of proteins of the extracellular matrix (ECM) and increasing the expression of these proteins (e.g. collagen). This process results in a stiffer ECM, making it resistant to hydrolysis. Eventually, an increased deposition of ECM will lead the development of both vascular and myocardial fibrosis (33, 104-107).

Physical exercise is known to reduce the AGE levels through a direct reduction of hyperglycemia. Our study surprisingly showed no significant effects of either MIT or HIIT on the level of AGEs after the 12-week training period. In contrast, other authors found lowered amounts of circulatory AGEs or their precursors (α-oxoaldehyde) following varying training modalities (108, 109). These contrasting results could be explained by the observation of the normalized glucose and insulin levels by the end of the study. At 30 weeks glucose and insulin values showed no significant difference between the trained and the sedentary animals, thereby explaining the absence of a decreased AGE level in the trained animals.

Another process by which AGEs can cause heart failure is through the glycation and oxidation of intracellular proteins resulting in an impaired cell function. Thereby, AGEs can affect the Ca^{2+} cycling through the glycation of the Ca^{2+} proteins including sarco/endoplasmic Ca^{2+}-ATPase-2a (SERCA2a) and the ryanodine receptor (RyR), thereby altering their structure and function. This process is in part responsible for the impaired Ca^{2+} handling occurring in the diabetic heart. In physiological conditions, depolarization of the CMs causes opening of the voltage-dependent L-type Ca^{2+} channels, thereby leading to Ca^{2+} influx. Subsequently, Ca^{2+}-induced Ca^{2+} release (CICR) occurs: the influx of Ca^{2+} activates the ryanodine receptor, which in turn elicits the release of Ca^{2+} from the sarcoplasmic reticulum (SR), the intracellular Ca^{2+} store. The resulting rise in intracellular Ca^{2+} concentration will cause diffusion of Ca^{2+} to the contractile proteins, leading to the binding of Ca^{2+} to troponin C. This will release the inhibition by troponin I, resulting in the cross-bridging of the actin-myosin filaments, leading to CM contraction. Conversely, the intracellular Ca^{2+} concentration will decrease during diastole by a Ca^{2+} efflux caused by the Na^{+}-Ca^{2+} exchanger (NCX) and sarcolemmal Ca^{2+}-ATPase. Additionally, the intracellular Ca^{2+} is pumped into the SR by SERCA2a, leading to relaxation of the CMs (15, 16, 20, 27). Phospholamban (PLN) serves as a potent regulator of SR Ca^{2+} release through inhibition of SERCA2a. After phosphorylation of PLN by β-adrenergic stimulation, SERCA2a function is inhibited (110).

In DCM, the function and activity of these Ca^{2+} cycling proteins is reduced; therefore, one expects to observe a normalization (i.e. increase) in the level of these proteins after exercise training. However, we found no significant effect of exercise training (either MIT or HIIT) on the protein levels of SERCA2a, PLN or NCX in the heart tissue. In contrast, Epp et al observed an increase in the protein

level of these Ca^{2+} cycling proteins after a 12-week training program in a rat model of T2DM (75). Additionally, a 13-week training period was able to restore the CICR process, shown by Stolen et al (111). An explanation for the different outcomes compared to our study could possibly be the use of different animal models. Additionally, the lower levels of AGEs that were observed could have resulted in an insignificant effect on the Ca^{2+} cycling proteins, since these levels were not affected.

1.1. Effect of exercise on circulatory lipid profile

Lipolysis of triglycerides derived from the adipose tissue represent the main source of FFA production. These circulating FFAs in turn serve as an energy substrate for our body. Higher plasma levels of FFAs are associated with several disorders including insulin resistance, CVDs and myocardial dysfunction (112, 113). Hence, an increased level of plasma FFAs is observed in T2DM and heart failure patients. FFAs exert several harmful effects on the cardiac function, predominantly through causing mitochondrial dysfunction. An increase plasma level of FFAs increases the mitochondrial β-oxidation which requires a larger amount of oxygen, when compared to glycolysis. In physiological conditions, 70% of the energy production is derived from FFA oxidation while glucose or lactate oxidation is responsible for only 30% (114, 115). The increase in FFA oxidation (up to 90% of energy production) occurring in the diabetic heart causes an increase in oxygen demand, referred to as oxygen wasting. Additionally, FFAs cause mitochondrial dysfunction through upregulation of the uncoupling proteins (UCPs) (112). These proteins cause a dysregulated mitochondrial membrane potential, thereby impairing ATP production and further increasing oxygen demand.

Additionally, FFAs contribute to the development of T2DM through causing insulin resistance and enhancing hepatic glucose production (116). Firstly, FFAs and their metabolites (e.g. ceramide) accumulate in skeletal muscle and liver, thereby causing insulin resistance in these tissues (117). Additionally, chronic exposure of pancreatic cells to FFAs causes β-cell toxicity, resulting in a reduced insulin secretion. The enhanced β-oxidation is responsible for inhibiting IRS-1 and phosphoinositol-3-kinase, which results in reduced intracellular effects of insulin, and increases hepatic gluconeogenesis. The latter is caused by the increased acetyl coenzyme A production (derived from FFA oxidation) and takes place through stimulation of several enzymes (e.g. pyruvate carboxylase) involved in the hepatic glucose production and by activating glucose-6-phosphatase, the rate-limiting enzyme in the process of glucose secretion (116, 118-122).

In the current study, triglyceride levels were increased after 18 weeks of the CAF diet, where after triglycerides were normalized to baseline values by both MIT an HIIT. In contrast, only HIIT was able to reduce the CAF diet-induced increase in FFAs. These results correspond with comparable experiments performed by Madsen et al, in which HIIT caused a decrease in circulating FFA levels in T2DM patients (123). During low or moderate-intensity training, carbohydrates (e.g. glycogen stored in liver and skeletal muscles) are used as energy source for muscle activity during the onset of exercise training. After a few minutes of continued low or moderate-intensity training, fuel for the muscle will switches from carbohydrates to FFAs, causing lipolysis to occur in the adipose tissue. During HIIT on the other hand, muscles use carbohydrates as a fuel since it is easier and faster to oxidize when compared to using FFAs as a fuel, thereby providing fast energy production to the

muscles. Therefore, one would expect the FFA levels to be increased after HIIT when compared to post-MIT levels. However, prolonged HIIT causes depletion of the carbohydrate stores, thereby causing the need to use FFAs as a fuel, explaining the decreased FFAs levels observed during the current study (124, 125).

1.2. Exercise training improves the mitochondrial function, thereby ameliorating myocardial contractility

Mitochondrial dysfunction represents one of the main causes for HF in the diabetic heart. Inflammation, accumulation of AGEs, and ROS are responsible for reducing the ATP production while simultaneously further increasing oxidative stress, eventually leading to the development of myocardial dysfunction. Exercise is known to normalize mitochondrial function, thereby diminishing myocardial dysfunction in diabetic patients (19, 28). Therefore, a marker for mitochondrial function was measured. Citrate synthase (CS) is the first enzyme in Krebs cycle and performs the conversion of acetyl coenzyme A (originating from glucose molecules) and oxaloacetic acid to citric acid. The activity of this enzyme is considered as a validated biomarker for the mitochondrial content, thereby reflecting the oxidative and respiratory capacity when measured in muscle tissue (126-128). In T2DM however, the activity of this enzyme is significantly decreased (129). Literature shows that both endurance and acute bouts of exercise can induce an increase in the activity level of several mitochondrial enzymes, including CS (130-134). Nevertheless, the mechanisms by which exercise causes an increase in the CS activity level has not yet been fully elucidated. A potential mechanism involved is an increase in the protein synthesis of several mitochondrial enzymes, including CS (135, 136). In the current study, we observed no exercise-induced elevation in cardiomyocyte CS activity levels. This can be explained by several studies that state that the elevation in the mitochondrial enzymes is noticeable only after measurements in skeletal muscle, and not in cardiac muscle (137-142). Still, some studies report an increased myocardial SC activity after exercise training (143, 144). From this information we can conclude that the absence of a significant difference in CS activity is presumably due to the fact that this parameter was measured in cardiac tissue, and not in skeletal muscle.

1.3. Limitations and future perspectives

The main limitation of the current study lies in the non-voluntary base of the exercise that was performed, thereby potentially leading to a high amount of stress. This increased level of stress could thereby influence several parameters that were measured during the study.

Future studies of our research group will focus on the role of exercise training, either MIT or HIIT, as a tool for the prevention of DCM. To investigate this subject, a study will be set up in which the induction of T2DM (through the high-sugar diet) is started simultaneously with the exercise training. To our knowledge, using the high-sugar diet to induce T2DM is the only method to study the preventive effect of exercise training. During this study, additional measurements will be performed on the level of myocardial apoptosis (via TUNEL assay or Bax/Bcl2 ratio), oxidative stress (through

measurement of NOX2 and NOX4 protein levels), adipokines (e.g. leptin), and glucose transporters (e.g. GLUT4). Furthermore, we will focus on the altered mitochondrial substrate utilization (by measuring pyruvate dehydrogenase and citrate synthase activity or measurement of lipid uptake through PPARα) and endothelial function and damage (through measurement of endothelial nitric oxide synthase or endothelin-1). In addition, measurement of the inflammatory and fibrosis marker plasminogen activator inhibitor-1 will be performed together with an expanded array of both circulatory and myocardial inflammatory markers (e.g. IL-6 and IL-1β) Lastly, further investigations will focus on specific types of AGEs instead of total AGEs. Most frequently low-molecular AGEs are measured which are therefore associated with cardiovascular diseases. However, lately more attention is brought to the high-molecular weight AGEs as they are thought to play a part in the development of LV fibrosis and cardiac dysfunction.

Furthermore, future research will be performed on the role of the detraining effect in prescribing exercise training as a therapy. The detraining effect is defined as the partial or complete loss of the training-induced adaptions on metabolic, ventilatory, and cardiovascular level. Compliance of patients to an exercise regimen is often very low, therefore our attention will be aimed at the effect of physical inactivity after a training period.

Finally, further research will help us to understand the underlying pathways involved in the development of both T2DM and DCM. More research is needed on the pathways responsible for the altered substrate utilization, myocardial inflammation, and oxidative stress. This thorough knowledge will possibly lead to the development of a new genetic animal model that gives a complete representation of the human T2DM and/or DCM phenotype.

1.4. Conclusion

Both MIT and HIIT show promising results that could lead to the use of physical activity as a tool for the prevention and/or treatment of DCM, thereby significantly reducing morbidity and mortality in the T2DM patient population. We conclude that only a slight difference in exercise-induced effects was observed between both training modalities. However, more research is needed to further examine the molecular effects of exercise training on the diabetic heart, as well as to determine the use of HIIT as a preventive tool and/or treatment for DCM.

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Ik/wij verlenen het wereldwijde auteursrecht voor de ingediende eindverhandeling:
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**

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