EFFECTS OF ACUTE HIGH INTENSITY TRAINING ON CARDIAC FUNCTION

A CLINICAL PILOT STUDY COMPARING SUBJECTS WITH TYPE 2 DIABETES AND HEALTHY CONTROLS

RANDI HANSGÅRD GJELSVIK

PROJECT THESIS

FACULTY OF MEDICINE

UNIVERSITY OF OSLO

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Abstract

Background. Regular endurance exercise is an important non-pharmacological preventive and therapeutic approach in subjects with type II diabetes (T2D). Several reports indicate, however, that healthy individuals participating in prolonged exhaustive endurance competitions display cardiac dysfunction after completion, with the right ventricle appearing more affected than the left. T2D subjects have reduced aerobic capacity and increased risk of developing heart disease and may therefore be more prone to cardiac alterations following exhaustive exercise than healthy subjects. The purpose of this study was to evaluate the acute cardiac alterations after one session of exhaustive interval exercise training in subjects with T2D compared to matched healthy controls.

Methods. Seven participants with T2D and seven controls performed 4 x 4 minutes interval exercise training (90-95% of maximal heart rate), followed by a fifth bout consisting of a short ramp protocol with increments in speed to measure peak oxygen uptake (VO_{2peak}). All participants underwent echocardiography immediately before and one hour after the single session of exhaustive exercise. Blood samples of troponin I (TnI), glucose and creatin kinase-MB were measured before, immediately after, and 24 hours after workout. Insulin C peptide, LDL cholesterol and total cholesterol was measured prior to workout.

Results. VO_{2peak} was lower in T2D patients (33 ± 6 vs. 44 ± 7 ml/kg/min, $p < 0.01$). Compared to controls, T2D patients had smaller left ventricular (LV) end-diastolic volume (106 ± 18 ml vs. 125 ± 14 ml) and right ventricular (RV) basal diameter (36 ± 6 mm vs. 46 ± 4 mm), both $p < 0.05$, respectively. When combining both groups, we found that acute exhaustive exercise caused a decrease in LV end-diastolic volume and left atrial end-systolic volume (mean difference for both 11 ml, both $p \leq 0.02$). LV ejection fraction ($p \leq 0.06$), tricuspid annular plane systolic excursion ($p \leq 0.02$), LV and RV peak annular early diastolic tissue Doppler velocities decreased after the exhaustive exercise session ($p < 0.01$). However, the cardiac response to exhaustive exercise was not significantly different between groups. TnT was elevated after exercise in 3 T2D patients and 2 controls, with maximal value 20 ng/L.

Conclusion. One single session of exhaustive exercise induced acute cardiac alterations in both left- and right-sided cardiac chambers with reduction in volumes and indices of systolic and diastolic function in both T2D patients and healthy controls. The results indicate a modest

dysfunction of both left and right ventricle following a single session of exhaustive exercise with no significant difference between patients with T2D and healthy controls.

Preface

A great thank you to Morten Høydal, for your patience and for giving the best introduction to medical research possible. I have learned so much through this process. Thank you, Håvard Dalen, for your great contributions to this paper.

Thank you Øystein for correcting all my spelling errors, and thank you Morfar, for showing the importance of learning more about exercise training and cardiovascular disease.

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1.Introduction

1.1 Exercise training as preventive medicine.

Exercise science has been a growing field of research over the last decades. It has led to discoveries regarding the body`s response to acute stress and shed light on mechanisms in the body that are still not fully understood. The research has also strengthened the role of exercise as a non-pharmacological contribution to treatment in a number of conditions such as cancer, chronic pain disorders and lifestyle related diseases like ischemic heart disease (1). In general, it is well documented that regular physical activity is a beneficial public health strategy (2, 3) through its effect on prevention, risk factor modification, treatment and rehabilitation of numerous cardiovascular diseases (4-7). High intensity training has been the focus of a significant part of the research. However, questions remain regarding the myocardium and its acute response to exercise training at such high intensity.

1.2 The myocardial response in exercise

The myocardial metabolism differs from that of skeletal muscle in several ways. One of them is in how they handle oxygen. Myocardium extracts about 70-80% of available oxygen from the blood (8). In comparison, other tissue in the body extracts about 25% when at rest. During exercise, myocardial cells therefore depend on increased blood flow to meet their need for oxygen. In a healthy heart, 4 to 6 liters of blood are pumped per minute when at rest. Depending on training status, the cardiac output can increase 4- to 7-fold during intense exercise (9). Several mechanisms contribute to this increase. The sympathetic nervous system in combination with inhibition of the parasympathetic nervous system ensures dilatation of the coronary arteries, and increased contractility of the myocardium. Large veins are contracted, i.e., venous return is increased. Simultaneously, heart rate and stroke volume is also increased during exercise (9).

In addition to the acute neurologic changes, there will be physiological adaptions in the heart of individuals who perform regular endurance exercise (9, 10). A myocardial hypertrophy with increased myocardial mass will result in increased contractile strength, and a heart that is

able to pump a greater volume of blood with each heartbeat. Enlarged chambers will contribute further to an increased cardiac output.

1.3 Right and left ventricle during exercise

The main function of the right ventricle is to pump through the pulmonary circulation. This is a low pressure system which requires little work compared to that required from the left ventricle, which pumps blood into the systemic circulation (11). During exercise, there is an increase in pulmonary artery pressure which is probably caused by increased pressure in the left atrium, and limited reserve capacity in the pulmonary system (11-13). In a previous study, it was shown that exercise resulted in increased afterload on the right ventricle, and thus, a higher workload on the ventricle. Furthermore, the increased demand of ejection of blood into the circulation will contribute to a further increase in workload, which is disproportional compared to the workload in the left ventricle (12). To illustrate, the latter study found that end-systolic wall stress in the left ventricle increased by 14% during exercise, when the increase in the right ventricle measured 125% (12).

Meta-analysis of the acute effects of prolonged endurance exercise has shown a more or less unaffected left ventricle, whereas significant impairment was found in the right ventricle (14). A possible explanation to the RV fatigue may be the disproportionate workload (12, 15, 16). The exact cellular mechanisms causing the predilection for the RV is however unknown.

1.4 Type 2 Diabetes and heart disease

Type 2 diabetes (T2D) has become an increasingly prevalent condition worldwide and is likely to reach pandemic levels within the coming decades (17). The condition is characterized by high blood glucose and altered insulin regulation and is strongly linked to obesity and inactivity. A number of complications are associated with the disease, such as kidney failure, retinopathy and cardiovascular diseases (18). The study of Zhuo et.al. suggests that macrovascular diseases account for 57% of the complication costs (18). If some of these complications could be prevented through exercise, both the societal as well as individual costs of T2D could potentially be dramatically reduced.

Diabetic cardiomyopathy is defined as functional and structural changes in the myocardium independent of diseases such as hypertension and coronary artery disease. This assertion is

supported by studies in diabetic animals and humans showing diastolic dysfunction without symptoms of known heart disease (17). Furthermore, animal studies have proven high intensity exercise training to be more efficient than training at moderate intensity, especially regarding improved glucose tolerance (19). More importantly, both high- and moderate intensity gave positive effects regarding cardiovascular health and reduction in remodeling of the left ventricle. The importance of physical activity is recognized in the guidelines for this patient group (20, 21).

The myocardium has a limited anaerobic capacity and is vulnerable to reduced oxygenation. Each muscle fiber is supplied with oxygen from a single capillary. It is a vulnerable system in individuals that for various reasons are not able to provide these muscle fibers with oxygen. One concern is that the increased oxygen demand caused by high intensity exercise training may induce ischemic injury in individuals where some of the compensating mechanisms are not functioning properly (8).

1.5 Type 2 Diabetes and exercise

The myocardial metabolism depends on sufficient energy substrate. Depending on the workload there is a dynamic variation in need for energy, which mainly is provided by fatty acids and glucose (22). Individuals with diabetes will have a reduced glucose uptake due to insufficient insulin function. This leaves diabetic hearts with increased reliance on oxidation of fatty acids and reduced substrate flexibility, which will reduce cardiac efficiency (23-28). However, diabetic hearts have demonstrated a further reduction in efficiency which cannot be explained only by the reduced substrate flexibility (27, 29). This reduction is to some extent explained by increased oxygen consumption by non-contractile processes such as increased mitochondrial uncoupling and production of reactive oxygen species. Studies suggests that impaired excitation-contraction coupling also contributes to this increased consumption (30, 31). These changes can be prevented by exercise training (32-35).

Of cellular changes causing less efficient work on the cardiac muscle, we also have disturbances in Ca^{2+} handling. Several studies have found that this is a possible important factor for the development of dilated cardiomyopathy (DCM) (36-39). Still, there is limited data when it comes to the acute effect on these processes following exercise.

1.6 Possible negative effects of the exercise training

Despite numbers of studies showing beneficial effects by exercise, there are studies showing impairment of the cardiac muscle after vigorous prolonged exercise. A study from the North See Race showed elevations of troponin after prolonged vigorous exercise among well trained athletes (40). The response was more prominent, and could also persist, in individuals with asymptomatic heart disease. Other studies show a reduction in contractility, increase in biomarkers for cardiac cell injury and reduced ejection fraction (EF) after long periods training at high intensity (12, 14, 16, 41-55). The same findings have been made in animal studies and suggests that high workload is not exclusively beneficial for cardiac muscle (51). The effects are in most cases transient, but persistent changes such as development of fibrosis have been observed in cases where the patient have been exposed of extreme amounts of training in combination with having a predisposition for cardiac dysfunction (45).

Already in 1987 Douglas et al. (43) introduced the expression of "cardiac fatigue", after finding both systolic and diastolic dysfunction in the left ventricle of athletes participating in a Hawaii Ironman triathlon. All the echocardiographic changes were transient and had returned to baseline after 24 hours post race. Their conclusion was however that there was no significant cardiac injury present in the contestants.

It is important to underline the well-established fact that exercise training has documented positive effects on health, such as reduced risk of cardiovascular disease and increased life expectancy(4, 56-63). However, there are observational studies suggesting that an increased activity level may have its cost when reaching a certain level. There have been developed models for the health effects of exercise training shaped as a reversed J-shaped curve between exercise dose and the beneficial effects in cardiovascular health, which argues that increased activity gives beneficial health effects only to a certain level (62, 63). How this applies to patients of T2D, is still uncertain.

1.7 Aim of the study

There is still a lot we do not know regarding cardiac response to exercise and restitution in individuals with T2D and other predispositions for ischemic heart disease. The present study aimed to investigate the response to a single high intensity (exhaustive) workout session in patients with T2D and compare this to healthy individuals. Our hypothesis was that acute

exercise training could provoke greater indications of cardiac injury in patients with T2D when compared to healthy individuals. For both groups we expected to find elevated levels of TnT and some degree of diastolic dysfunction that could be more prominent in the right ventricle.

2. Methods

2.1 Subjects

Subjects with T2D (n=7) and the healthy controls (n=7) were recruited via advertisement and from our database from earlier T2D projects. In total, fifteen men volunteered for the study. One person was excluded in the T2D group due to lack of parameters confirming T2D. Mean age was 56 in both groups, all male, and there was no significant difference in their physical characteristics (table 1). Written informed consent was obtained in all and the protocol was approved by the regional committee for medical research ethics, REK (2016/1596).

2.2 Test procedure and protocols

2.2.1 Participant registration and timeline

Day 1. The participants signed written consent prior to data collection. First, all participants completed a questionnaire from a medical doctor for evaluation of health status and medical consent for training and cardiopulmonary exercise testing. Blood pressure was measured following 10 minutes of rest in a chair. Three tests were performed and the average of the two last testes were used in the analysis. Thereafter the height was measured, followed by an Inbody scan (Inbody, AU). After this, an Holter EKG was placed on the participants in order to monitor the electrical activity 24 hours prior, during and after exercise. Lastly, a blood sample was collected from the participants at the end of day 1.

Day 2. Echocardiography was first performed before the single bout of exercise consisting of 4x4 minutes interval exercise + one last bout performed as a ramp protocol to asses peak oxygen uptake (VO_{2PEAK}). After the exercise all participants rested for 30 minutes before a second session of echocardiography. At the end of day 2, 30 minutes after the echocardiography, a second blood sample was taken.

Day 3. Participants delivered Holter EKG and completed one last blood sample 24 hours after the exercise test.

2.2.2 Interval training and maximal oxygen uptake

The exercise was performed as a 4x4 minutes high intensity interval training. The intervals were conducted at about 90% of VO_{2PEAK}, and were followed by 2 minute breaks with active recovery at 60%. We then added a fifth interval performed as a ramp protocol with increments in speed and/or incline measure VO_{2PEAK}.

Cardiopulmonary exercise testing (CPET) to measure VO_{2PEAK} was performed at the NeXt Move core facility at NTNU - Norwegian University of Science and Technology. Because diabetic patients sometimes exhibit physical limitations, experienced personnel determined the best individual CPET regimen during a 6-minute warm-up on a treadmill (Woodway PPS55, USA Inc., Waukesha, WI, USA), by detecting functional walking or running speed and inclination, as well as subjective moderate aerobic intensity based on rated perceived exertion (RPE Borg scale 6-20). Subjects were then fitted with a heart rate monitor (H7, Polar Electro, Kempele, Finland) and facemask (7450 Series V2 CPET mask, Hans Rudolph Inc., Shawnee, KS, USA). During an initial period of 4 minutes at fixed submaximal workload serving as an extended warm-up, work economy measurements were made.

Maximal oxygen uptake (VO_{2max}) was defined using the following criteria: 1) VO2 levelling off (<2 mL/(kg × min)) despite increase in workload and 2) Respiratory exchange ratio ≥1.05. If these criteria were not met, the term VO_{2PEAK} was used. A subject's VO_{2PEAK} was defined as the mean of the three successive highest VO2 registrations achieved during the CPET. For simplicity, the term VO_{2PEAK} is used for all patients.

An individualized ramp protocol was used, until either exhaustion or fulfilment of the criteria for VO_{2max} or VO_{2PEAK} . Workload was gradually increased, and gas measurements were recorded every tenth second using a mixing chamber ergospirometry system (Metalyzer II, Cortex Biophysik Gmbh, Leipzig, Germany).

2.2.3 Echocardiography

Participants were assessed with a resting transthoracic echocardiography (TTE). Echocardiographic recordings and analyses of the different chambers follows the recommendation by the American Society of Echocardiography (64).

All examinations were conducted by one cardiologist (H.D.) experienced in echocardiography. All participants were examined in the left-lateral decubitus position. A

Vivid E95 scanner with a phased-array transducer (M5S) (GE Ultrasound, Horten, Norway) was used for the echocardiographic scanning. Echocardiographic data were stored digitally and analyzed subsequently by the same physician echocardiographer. All analyses were performed with the operator blinded to group assignment and whether the echocardiogram was recorded before or after the exhaustive exercise session.

Grey-scale two-dimensional (2D) views were recorded from the parasternal border in short- and long-axis, and the apical position in 4-chamber, 2-chamber and long-axis views. For volumetric measurements special attention was made to enhance the endocardial border between the lumen and the myocardial wall, and similarly care was taken to avoid foreshortening of the specific chambers. Separate recordings were made to optimize the volumetric measurements of the left atrium (LA), left ventricle (LV) right atrium (RA) and right ventricle (RV). LA and LV volume was measured by the summation of discs method in 4- and 2-chamber view. The estimated volumes from biplane analyses are used in the analyses. Right-sided volumes were estimated from dedicated 4-chamber views. Similarly, recordings by colour coded tissue Doppler volumes were optimized for the cardiac chamber included in the specific view. Frame rate was set to minimum of 50 fps. Colour coded Doppler recordings were recorded through all valvular orifices and vessels to identify pathology as regurgitations and stenoses.

Three-dimensional (3D) volumes were obtained be narrowing the volume to include the whole cardiac chamber throughout the cardiac cycle into the volume. 2-4 cardiac cycles were merged into a full volume to achieve the optimal spatial and temporal resolution. Breath-hold was used to reduce the movement of the cardiac chamber due to respiration. Frame rate was set to 30+.

Spectral Doppler recordings of blood-flow were recorded with sample volume; a) 0.5-1 cm in to the pulmonary veins, b) at tip of the mitral leaflet and in the presence of mitral regurgitation along the regurgitant jet, c) in the distal LV outflow tract, d) aligned with the blood-flow through the aortic valve, e) at tip of the tricuspid valve and in presence of tricuspid regurgitation aligned with the regurgitant jet, f) in the RV outflow tract and aligned to the blood flow direction in the pulmonary artery.

Pulsed-wave tissue Doppler velocity curves were recorded from the basal part of the left and right ventricle, at the septal and lateral points (near the insertion of the mitral valve) and from the RV free wall (near the insertion of the tricuspid valve). Colour coded tissue Doppler cine-

loops were recorded in the apical 4-chamber, 2-chamber and long-axis views, and a dedicated view aligned to the RV free wall were also recorded. Target frame-rate for the colour tissue Doppler recordings was 100 fps.

All measurements reflect the average of three cardiac cycles, as recommended for patients in sinus rhythm. The measurements are reported as absolute values and not indexed to body surface area.

All measurements were made according to Lang et.al. 2015, which provide the latest instructions for echocardiographic imaging. Shortly, linear measurements of the LV myocardium and dimensions were done in parasternal long-axis at end-diastole and end-systole immediately below the level of the mitral valve leaflet tips. The fractional shortening was calculated by the change in LV dimension divided by the end-diastolic dimension. The LV volume was measured from apical four- and two chamber views by tracing of the endocardial border at end-diastole and end-systole. Ejection fraction was calculated as the percentage ejected blood volume during systole using biplane method of disc summation (Simpson's method). Peak systolic (S') and early diastolic (e') mitral annular velocities were measured at the base of the six myocardial walls by colour tissue doppler, and the average values are used as measurements of the LV myocardial velocities. In addition, e' was measured at the base of the septal and anterolateral wall by pulsed-wave tissue Doppler for the calculation of E/e' ratio. The mitral inflow peak early (E) and late (A) diastolic velocities and the early diastolic deceleration time was measured by pulsed-wave Doppler, and the ratios of E/e' and E/A were calculated.

The dimension of the right ventricle was measured in gray scale dedicated 4-chamber views at the basal and mid level. Tricuspid annular plane systolic excursion was measured by reconstructed motion mode aligned to the movement of the basal right ventricular free wall. The tricuspid early inflow velocity (TV_E_Vel) was measured in pulsed-wave Doppler recordings, and similarly the timing of tricuspid valve closure to opening (Time_TCO) was measured in the same spectrum. Timing and velocities of the tricuspid regurgitant jet was measured in continuous wave Doppler. Myocardial performance index was calculated as the sum of isovolumetric relaxation and contraction times, divided by the ejection time. The tricuspid annular peak systolic and early diastolic velocities were measured by colour tissue Doppler, as well as pulsed-wave Doppler, in the basal part of the right ventricular free wall.

The volumes of the left and right atrium were measured in dedicated views aligned to the maximal length of the atria. For the left atrium the endocardial border was traced in 4- and 2-chamber views at end-systole and the volume was calculated by the summation of discs method. The right atrium volume was calculated by the tracing and the length of the endocardial border in 4-chamber view by the area-length method.

2.2.4 Biochemical analysis

Blood samples were analyzed following standard operating procedures at St. Olavs Hospital. Glucose, Hemoglobin A1c (HbA1c), total cholesterol, Low density lipoprotein (LDL) cholesterol, Creatine Kinase-MB (CK-MB), TnI and insulin C peptide were all obtained before the training. Glucose, CK-MB and TnI were also obtained straight after training and 24 hours post workout.

2.2.5 Body composition and weight: Body composition was measured using bioelectrical impedance (Inbody 720, BIOSPACE, Seoul, Korea). In this machine, four pairs of electrodes are implanted into the handles and floor scale of the analyzer. Before testing, subjects had fasted for minimum two hours. They were encouraged to go to the toilet right before entering the scale. The subjects stood five minutes in upright position before entering the scale. They were barefoot. Due to the electrical impulse, people with pacemaker were not tested. Height, age and gender were plotted om the scale-display. After two minutes, weight (kg), body mass index (BMI), muscle mass (kg), fat % and visceral fat (cm^2) was measured by the scale. The device was auto- calibrated every time the machine is turned off. In this set up it happened once a week. Validation studies have shown that Inbody 720 is an appropriate alternative to dual-energy x-ray absorptiometry to measure body composition (65), (66).

2.3 Statistical analysis

Pearson *t*-test was applied to compare the groups on the different parameters. Coupled tests were used to compare changes within each group before and after exercise. We also used coupled test to compare overall change in the two groups considered as one. Independent samples were used when comparing means between the two groups. Significant t-tests were taken into consideration and are presented in the results.

3.Results

3.1 Baseline characteristics

We found no significant differences in central variables between the groups at baseline. Age, height and body weight were similar and suitable for comparison Characteristics of the subjects are listed in table 1.

Table 1: Physical characteristics presented as mean ± standard deviation.

	T2D group	**Control group**	**p-value**
Age (years)	55.9±10.9	56.1±10.9	0.96
Height (cm)	177.7±8.2	183.1±6.0	0.18
Body weight (kg)	87.9±19.3	90.7±9.5	0.74
Skeletal muscle mass (kg)	37.7±7.3	28.8±4.6	0.72
Body fat (%)	25.2±5.3	22.9±8.2	0.55
Body fat (kg)	22.1±7.9	21.9±8.7	0.96
Visceral fat area	103.3±29.6	101.3±38.3	0.92
In body health score	77.1±5.6	76.4±10.7	0.88
BMI	28.0±5.0	27.0±2.8	0.67

3.1.1 Baseline blood samples

HbA1c, glucose and insulin C peptide, LDL and total cholesterol were measured before the exercise training, as well as TnI and CK-MB. We found several differences between the groups at baseline. HbA1c was significantly higher in diabetics (33%). The glucose levels were 57% higher amongst the diabetics, and the insulin C peptide was also higher by 50%. No other parameters were different between the groups at baseline. The results are presented in table 2.

Table 2: Baseline blood samples presented as mean ± standard deviation

Blood sample prior to testing	T2D group	Control group	P -value
HbA1c (mmol/mol)	6.9±1.1	5.2±0.2	0.001
Glucose (mmol/L)	8.5±2.9	5.4±0.3	0.01
Insulin C peptide (pmol/L)	0.9±0.2	0.6±0.2	0.05
LDL cholesterol (mmol/L)	2.9±0.9	3.7±0.5	0.07
Total cholesterol (mmol/L)	4.7±1.0	5.5±0.7	0.13
CK-MB (U/L)	1.9±0.8	3.2±3.6	0.37

TnI measurements under 10 ng/L does not give an exact value and cannot be presented with SD and p-value. All participants in the control group had values under 10 ng/L before the exercise training. In the T2D group, two participants had higher values, 13 ng/L and 11 ng/L respectively, whereas the remaining participants in the group had had values under 10 ng/L.

3.1.2 Baseline VO_{2PEAK} and heart rate

We found that VO_{2PEAK} was significantly different between the groups (Fig.1A), as well as the minute ventilation (V/E')(Fig.1B) and the ventilation of CO_2 pr minute (Fig. 1C). The diabetes group had a reduced fitness level compared to the control group. The average VO2 peak in the diabetes group was 33.07±5.8 ml/min/kg. In comparison the control group had 44.02±6.6 ml/min/kg, ($p<0.01$). The peak heart rate was similar in the two groups with 181±11.9 in the T2D group and 175±9.9 in the control group (Fig.1D).

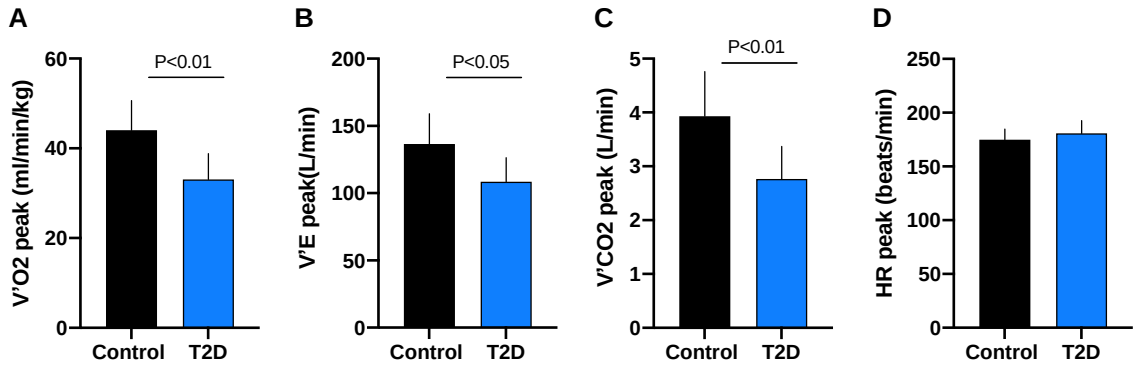


Figure 1: Baseline data from cardiopulmonary exercise tests between control individuals and individuals with type 2 diabetes (T2D. A, peak oxygen uptake (V'O2 peak) displayed by ml/min/kg; B, Minute ventilation (V'E) displayed as L/min; O2; C, Ventilation of CO2 exhaled (V'CO2) displayed as L/min; D, peak heart rate (HR) displayed as beat/min. Data presented as mean ± SD.

3.1.3 Baseline echocardiography

Baseline echocardiographic examination revealed smaller LV end-diastolic volume and smaller RV basal diameter in patients with T2D compared to the control group (Figure 2). In other words, the right and left ventricle of the subjects in the control group were larger than those in the T2D group. The peak tricuspid annular early diastolic velocity in those with T2D was lower than that in the control group. The ejection fraction was similar in the two groups, 61.7 ml in the T2D group and 59.6 in the control group ($p<0.49$). There were no other differences between the two groups at baseline.

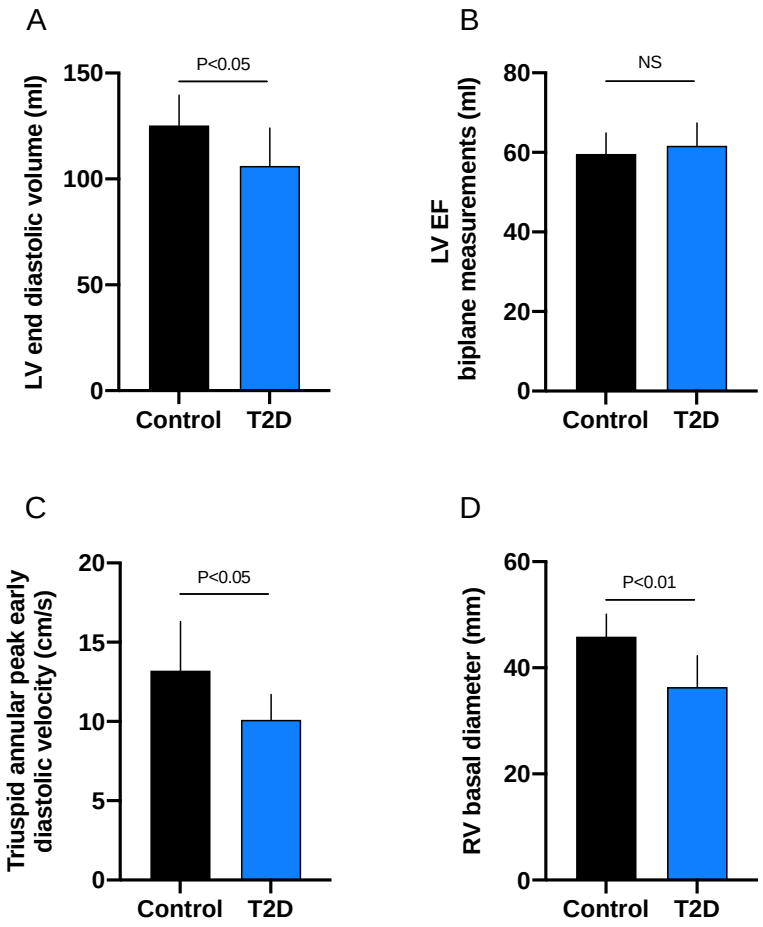


Figure 2: Selection of variables from baseline echocardiography. A) Left ventricle (LV) end diastolic volume (ml); B) LV ejection fraction (EF) biplane measurements (ml); C) Tricuspid annular peak early diastolic velocity (cm/s); D) Right ventricle (RV) basal diameter (mm). Data presented as mean ± SD.

3.2 Training response and group differences

We did not find any difference in the response to acute exercise between the two groups. When comparing the response to acute exercise within each group independently we did, however, observe a significant change from pre- to post-test on several parameter. When analyzing the total population together, by merging both groups to one, we also observed significant changes induced by acute exercise training. The data from individuals with TnI increase above 10 ng/L were also further analyzed as a separate post hoc analyze population, ie. TnI increase group.

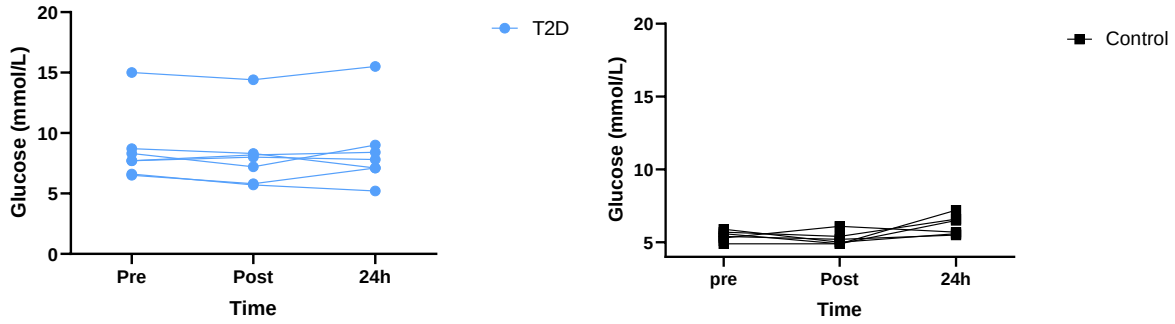
3.2.1 Change in blood samples after exercise

The exercise training resulted in changes in the different blood parameters. There was no difference between the groups, but individual changes were detected. The individual changes in each group are presented in the figures below.

Glucose

Glucose levels were expected to decrease in diabetics during workout. It remained close to constant in the control group where average glucose level was 5.41 mmol/L pre workout, 5.25 mmol/L one hour after workout and 6.18 mmol/L 24 hours after workout. In the control group, one subject had a serum glucose level considerably higher than the rest of the group. Overall, there was a small reduction in average serum glucose in the T2D group after training (8.64 mmol/L before and 8.23 mmol/L after). 24 hours post training the serum glucose was 8.50 mmol/L. There was no statistical significance between different time points.

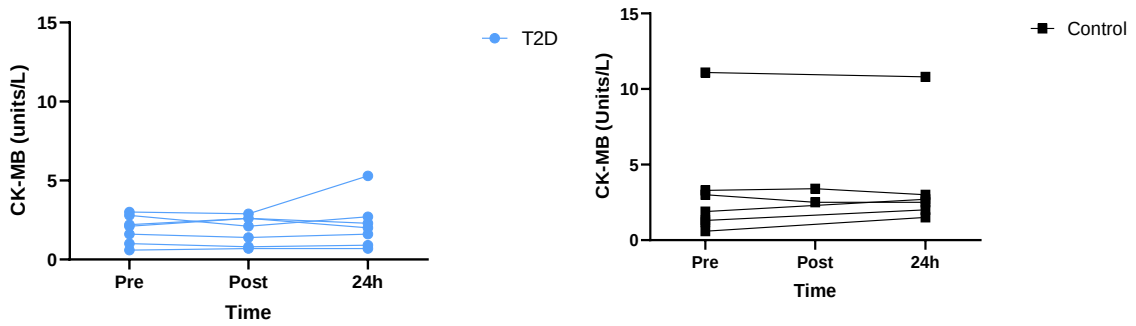
Figrue 3: glucose concentrations



Creatine Kinase -MB

In most subjects, the CK-MB concentrations remained stable post workout. One individual in the control group had serum CK-MB over reference level. The average levels in the control group were 3.2 units/L before exercise training, 2.95 units/L post workout and 3.75 units/L 24 hours after exercise training. In the T2D-group there was a lower average with 1.9 units/L before training, 1.9 one hour post workout and 2.2 units/L 24 hours after workout. The differences were not numerically significant.

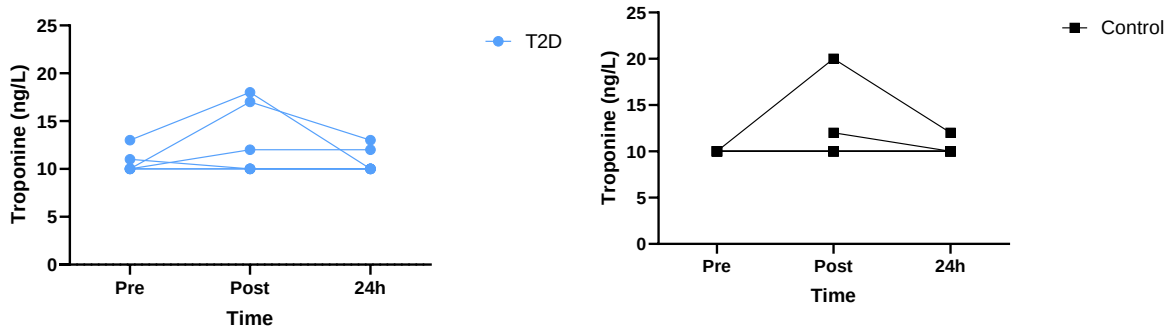
Figure 4: CK-MB concentrations



Troponin I

Three subjects in the T2D group had TnI increase above 12 ng/L after workout. Two subjects reached 17 and 18 ng/L. In the same two subjects the serum levels of TnI were still elevated after 24 hours. In the control group two subjects had TnI elevation exceeding 10 ng/L post workout, where the subject with the highest measured TnI level (20ng/L) still had elevated values 24 hours post workout.

Figrue 5: Troponine concentrations



3.2.2 Change in echocardiography following exercise

When looking at the change in cardiac measurements before and after exercise, there was not detected any significant difference between the two groups. However, each group displayed changes in systolic and diastolic function, in the right and left ventricle, as well as in the left atrium.

These results are presented in table 4, where the difference pre to post exercise are calculated within each group. We found a reduction in both systolic and diastolic function that was present post workout in analyzes in both groups.

Table 4.

Parameters	Control (n=7)				Diabetes (n=7)			
	Pre	**Post**	**Diff (post -pre)**	**P**	**Pre**	**Post**	**Diff (post -pre)**	**P**
Left ventricle (LV)								
Intraventricular septum thickness, end-diastolic (mm)	8.8±1.0	10.2±0.9	1.5	0.05	10.3±2.1	10.0±1.5	-0.3	0.64
LV internal dimension, end-diastolic (mm)	49.1±4.1	48.4±4.1	-0.7	0.48	45.7±4.7	43.7±4.4	-1.9	0.27
LV thickness, end-diastolic (mm)	9.2±1.3	10.1±0.9	0.9	0.01	9.7±1.7	9.5±2.0	-0.2	0.81
LV fractional shortening (%)	27.1±4.0	29.6±5.5	2.6	0.41	26.1±4.4	27.1±5.1	1.0	0.71
LV ejection fraction, biplane measurements (%)	59.6±5.3	56.2±3.9	-3.4	0.22	61.7±5.7	58.2±6.7	-3.4	0.18
LV end-diastolic volume, biplane (ml)	125.3±14.3	113.9±18.6	-11.4	0.10	106.2±17.2	96.0±16.2	-10.1	0.13
Peak systolic mitral annular velocity, mean six walls (colour tissue doppler) (cm/s)	7.4±1.0	7.1±1.2	-0.3	0.29	7.2±1.8	7.5±1.5	0.2	0.55
Peak early diastolic mitral annular velocity, mean six walls (colour tissue doppler) (cm/s)	7.7±2.0	6.3±1.9	-1.4	0.00	6.8±1.4	6.0±1.7	-0.8	0.03
Ratio of mitral early diastolic inflow (E) to mitral annular early diastolic (e') velocity	7.5±2.6	7.0±3.0	-0.5	0.46	8.4±2.3	8.7±3.4	0.3	0.75
Mitral inflow peak early diastolic (E) velocity (cm/s)	66.2±13.6	52.6±12.7	-13.6	0.02	69.0±11.8	56.8±6.8	-12.2	0.06
Mitral inflow early diastolic deceleration time (ms)	217.1±37.8	337.5±129	120.4	0.09	231.7±55.4	281.3±108	49.5	0.20
Mitral inflow peak early to late diastolic velocity (E/A ratio)	1.4±0.3	1.0±0.2	-0.4	0.03	1.2±0.5	0.8±0.2	-0.4	0.03
Right ventricle (RV)								
Tricuspid early inflow velocity (TV_E_Vel) (cm/s)	50.3±9.4	46.7±11.1	-3.7	0.92	57.8±4.0	49.5±8.5	-8.3	0.04
RV basal diameter at end-diastole (RV recorded at maximum area, 4Ch-view)(mm)	45.9±4.2	43.5±3.4	-2.4	0.10	36.4±5.9	34.9±6.5	-1.5	0.46
RV mid diameter at end-diastole (RV recorded at maximum area, 4Ch-view)(mm)	30.1±3.4	28.2±3.0	-1.9	0.25	30.9±1.3	26.5±4.0	-4.4	0.04
Tricuspud annular plane systolic excursion (mm)	28.0±4.2	24.7±3.0	-3.3	0.14	23.4±4.3	21.3±1.8	-2.2	0.07
Tricuspid annular peak early diastolic velocity, colour tissue Doppler tissue Doppler ((cm/s)	9.9±2.3	8.6±1.3	-1.3	0.04	8.3±1.9	6.5±1.9	-1.8	0.00
Time tricuspid valve closure to opening, pulsed wave Doppler (Time_TCO)	416.4±106.0	422.6±31.4	6.2	0,31	393,0±58,9	368,9±45,4	-24,1	0,15
Time tricuspid regurgitation, continuous wave Doppler (Time_TR)	339.3±29.6	304.3±27.1	-34.9	0,05	328,7±31,4	288,4±18,7	-40,4	0,29
Left atrium (LA)								

LA end-systolic volume, biplane, (summation of discs method) (ml)	66.5±13.0	54.5±15.5	-12.0	0.04	60.3±20.2	50.1±17.7	-10.2	0.06
Right atrium (RA)								
RA end-systolic volume based on 4-chamber view (area-length method) (ml)	58.1±19.2	61.5±17.9	3.4	0.66	41.8±17.3	37.3±14.7	-4.5	0.12

The alterations in echocardiographic indices in subjects with TnI above 10 ng/L are presented in table 5 (significant data only).

Table 5. Selection of echocardiographic values according to TnI levels

	TnI> 10			
Parameters	Pre	Post	Diff (mean PO-mean pre)	P-value
Tricuspud annular plane systolic excursion (mm)	25.7±5.0	21.2±1.6	-4.46	0.05
Tricuspid annular peak early diastolic velocity, colour tissue Doppler (cm/s)	9.3±1.7	7.5±1.9	-1.75	0.00
Left atrium end-systolic volume, biplane, (summation of discs method) (ml)	63.1±24.6	47.6±16.4	-15.53	0.01
Left ventricular ejection fraction, biplane measurements (Simpsons method) (%)	62.2±5.3	55.5±3.0	-6.72	0.01
Left ventricular end-diastolic volume, biplane(ml)	114.6±21.6	94.8±16.0	-19.84	0.00
Tricuspid annular peak early diastolic velocity (mean six walls) (cm/s)	6.6±1.8	5.7±1.8	-0.97	0.00
Mitral inflow peak early diastolic velocity (cm/s)	72.6±10.4	54.3±11.0	-18.28	0.01

Left ventricle

There was a general LV volume reduction post workout. The end-diastolic volume had an average reduction of 11.4 ml compared to baseline levels in the control group ($p < 0.10$) and 10.1 ml in the diabetes group ($p < 0.13$). In patients with elevated TnI there was a reduction of 19.8 ml ($p<0.001$) in the end-diastolic volume. The same was found when performing analyses in three-dimensional recordings (values not shown). Correspondingly, the internal dimension also displayed a small reduction in both groups.

There was a significant reduction in the peak mitral annular velocity in the early diastole. The reduced velocity was at 1.4 cm/s in the control group ($p<0.00$) and 0.8 cm/s in the T2D group ($p<0.03$). In patients with elevated TnI the velocity had an average reduction of 0.97 cm/s ($p<0.00$). The peak annular velocity in the systole did not show consistency in our results: the control group had a reduced velocity but the T2D group had an increase in velocity. However, a change in mitral inflow peak early diastolic velocity was present in both groups. The velocity was reduced, by 13.6 cm/s in the control group ($p<0.02$), and 12.2 in the T2D group ($p<0.06$). The pulsed-wave tissue Doppler measurements of the mitral annular velocities were in line with the presented (data not shown).

The intraventricular septum thickness at end-diastole and ventricular thickness at end-diastole both increased in the control group and decreased in the T2D group. In the control group the increase was at 1.5 mm and 0.9 mm, respectively ($p< 0.05$ and 0.01).

There were no significant changes in systolic fractional shortening in either group after exercise (control group 2.6% ($p<0.41$), T2D group 1% ($p<0.71$).

There was a non-significant reduction of ejection fraction (EF) by 3.4 % in both the control group and the T2D group ($p=0.22$ and $p=0.18$, respectively). In patients with elevated TnI the EF was reduced by 6.7 % ($p<0.01$).

The E/e' ratio decreased in the control group and increased in the T2D group. None of the changes were statistically significant. However, the ratio of the mitral inflow peak early to late diastolic velocity was significantly reduced by 0.4 in both groups ($p<0.03$ in both groups). In patients with elevated TnI the ratio was reduced by 0.5 ($p<0.02$).

Right ventricle

In the right ventricle (RV), we found that tricuspid inflow early velocity decreased in both groups, by 3.7 cm/s in the control group ($p<0.92$) and 8.3 cm/s in the T2D group ($p<0.04$). No other significant blood stream measurements across the tricuspid valve was found. The tricuspid annular peak early diastolic velocity was reduced by 1.3 cm/s ($p<0.04$) in the control group and 1.8 cm/s ($p<0.001$) in the T2D group. In patients with elevated TnI the reduction was 1.75 cm/s ($p<0.001$). The peak tricuspid annular systolic velocities did not differ between groups, but there was non-significant reduction following exercise (mean 0.7 cm/s, $p=0.11$). The pulsed-wave tissue Doppler measurements of the tricuspid annular velocities were in line with the presented (data not shown).

RV end-diastolic diameter (both basal and mid-ventricular) decreased post workout. The measurements of the RV outflow tract at end-diastole and the RV outflow tract proximal diameter showed similar findings (values not shown). Tricuspid annular plane systolic excursion was reduced in all groups after workout. In patients with elevated TnI the reduction was at 4.5 mm ($p<0.05$).

Myocardial performance index increased in both groups post workout. In the T2D group we found a change from 0.20 to 0.28 and in the control group from 0.23 to 0.39. The changes were not statistically significant.

Left atrium

In the left atrium we found a volume reduction post workout which was consistent independently of type of measurement used (4 chamber and 2-chamber view). According to biplane measurements we found a 12 ml reduction in the control group ($p<0.04$) and 10.2 ml reduction in the T2D group ($p<0.06$). In patients with elevated TnI there was an average volume reduction at 15.5 ml ($p<0.01$).

Right atrium

In the right atrium, the echocardiography revealed no clear change in volume or function in the two groups, nor in patients with elevated TnI. The end-systolic volume was increased by 3.4 ml in the control group ($p<0.66$) and reduced by 4.5 ml in the T2D group ($p<0.12$). The measurements of RA length and area gave similar results (values not shown).

4.Discussion

This study aimed to investigate potential injury to the cardiac muscle following high intensity exercise in T2D patients. The results demonstrate small differences between the control- and diabetic group in their cardiac response to exercise training. Although statistically significant, several of the differences lack the effect size to have great physiological impact. Still, the findings indicate that one single session of exercise training at high intensity may induce a transient impairment of cardiac function in both healthy subjects and patients with T2D.

Before committing the exercise, we expected to find that diabetics were more prone to alterations in cardiac function. As expressed in the hypothesis, we expected that this would result in a greater increase in TnI post workout in the T2D group compared to the control group. However, an acute increase of TnI was expected in both groups. Several studies have made the same observation after exercise training of short and long durations (55, 67-69). The mentioned studies have detected increments far above 14 ng/L which is a criterion for myocardial infarction (70). It should however be noted that it is currently no certain method of distinguishing a physiological response induced by exercise from pathology. One possible finding is that individuals with asymptomatic cardiovascular disease have a greater TnI

increase that persist for a longer duration of time. A study from the university of Stavanger have shown increase in TnT immediately after workout as presented in our results (71).

Myocardial infarction (MI) causes an increase of complex bound TnI that in today's analyzes cannot be distinguished from free TnI that is excreted from myocytes during exercise. The study argues that the TnI increase may be considered physiological, but we still lack data to conclude if there is TnI increase because of myocyte necrosis or because of myocyte work. However, in the Stavanger study the duration of TnI increase was associated with occult obstructive coronary artery disease. Nevertheless, TnI has limitations in its clinical use. Copeptin has been investigated as a new potential biomarker without success (72). However, the present study demonstrates the need for new and more precise biomarkers in the future.

As presented in our results, five subjects had a marked increase in TnI whom of which three were in the T2D group and two in the control group. There is a broad agreement that cardiac impairment following exercise training is greatest in the least trained. With this taken into consideration, one could expect a greater increase of TnI in the T2D group since their VO_{2PEAK} was significantly lower than in the control group, indicating that T2D subjects had a lower fitness level, with $VO_{2\ peak}$ at 33.07 ml/min/kg vs 44.02 ml/min/kg in the control group ($p<0.01$). However, this is one of few parameters separating the two groups and may not be of great significance. Interestingly, we found the greatest TnI increase in a subject in the control group (20 ng/L). This subject was also the one having CK-MB levels above reference level. There may be reason to believe this subject was not representative for the control group. In that case, the tendency of more TnI increase in the T2D group would have been more prominent. Nevertheless, subjects with the most prominent increase of TnI had clearer indications of dysfunction, independently of their original group. Interestingly, the subjects with increase in TnI displayed impairment in both ventricles as well as reduced contractility.

We hypothesized that we would a greater impairment of the right ventricle compared to the left, after the exercise training. However, our results had no such indication. This may be explained by the relatively short duration of the exercise training. Although conducted at high intensity, the duration is short compared to earlier reports (12, 14, 16, 41).In fact, duration of the exercise training has been linked to degree of impairment of the right ventricle, where longer durations seem to cause greater impairment (16). The same study discovered RV dysfunction in endurance athletes, suggesting that long term processes like fibrosis might play a central part in the development of the dysfunction. Repeating the experiment with varying durations would be an interesting next step.

In the present study, the subjects went through only one high intensity workout, that interestingly resulted in detectable findings. Over the years there have been written many articles about exercise and its effect on cardiovascular health. However, few studies have been conducted at such low training volume. It is reasonable to believe that athletes conducting regular physical activity would display more prominent changes. Although T2D patients are prone to cardiovascular disease, the typical patient will be inactive, as obesity and inactivity are factors that are linked to the debut of the insulin resistance. Patients with T2D in Norway are routinely treated with statins in order to prevent the further cardiac damage from high levels of cholesterol(73). One could ask if they really are as prone to cardiac alterations compared to athletes that exercise regularly. In any case they will have great health effects from exercise training.

In the left ventricle we found a general volume reduction suggesting reduced blood volume as due to dehydration. The changes in volume and ejection fraction of the LV indicate that some LV dysfunction was present after exhaustive exercise, and could not be easily explained by hypovolemia (dehydration) alone. The E/e' ratio reflects the filling pressure which in increased under diastolic dysfunction. Furthermore, our findings imply a reduced contractility of the myocardium post workout. Still, we found that septum thickness and LV thickness increased. This may be due to edema or increased volume in the myocardium itself. It could also be a potential measuring error. Since it is present in several parameters, it may be induced by change of size of the myocardium, and contrast in the transition between blood and muscle. However, this is unclear.

We believe the acute changes observed after exercise training at high intensity are transient. There is still a lot we do not know regarding the heart's restitution. In skeletal muscle, mechanical stimuli induce minor injuries which again stimulates regeneration and improved function, as an adaption to the muscle work. We do not fully know by which mechanisms the heart operates, but maybe there are similar things happening: excessive stimuli, a degeneration before regeneration and adaption is induced. Of course, these are only speculations, but it may support the findings of reduced contractility and ejection fraction post workout.

An apparent weakness of this study is the small sample size. With the small number of 14 participants it is hard to discover clear differences between the groups as individual variation in the echocardiographic examination will affect the results. However, a large number of parameters were measured, and findings were detected across several measuring methods.

The examination was conducted by an experienced operator, who was blinded and unfamiliar with the group of the subjects that were examined, as well as their clinical information. The findings point in the same direction regardless of measuring methods. To avoid dehydration following exercise to impact on the results, all individuals where hydrated immediately after exercise. We can, however, not rule out the hydration status of the individuals as a potential confounding factor. All analyses were performed following standard operating procedures by experienced users.

This study found significant effects on cardiac function, presented as reduced contractility and some degree of impairment in both ventricles following just one bout of high intensity training. Questions remain regarding the consequences of regular exercise at such intensity, the importance of restitution, and comorbidity with other diseases. Increased knowledge about TD2 and its consequences is of great importance, and these questions should be the focus of future studies.

5.Conclusion

In the present study we report that the response to acute exercise between individuals with T2D and healthy controls have similar cardiac response. However, we did observe that both the control group and the T2D group displayed an acute reduction in cardiac function following exercise training. Individual subjects in both groups had TnI increase above reference level. In these individual with increased TnI we observed echocardiographic changes that suggested a more severe impairment of the cardiac function.

There were more individuals in the T2D group with TnI increase than in the control group. However, the most severe TnI increase was present in the control group, and thus there is no indication of saying the diagnose of T2D in itself gives an increased risk for TnI increase in exercise training based on our results.

We expected the T2D group to be more prone to cardiac dysfunction after conducting exercise training at high intensity. Our results do not support this hypothesis. Although the study is of small sample size, thorough echocardiographic examination gives valuable information regarding the acute cardiac response in high intensity training, and the subject should be further investigated in future research.

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