Metabolic and functional evaluation of diabetic cardiomyopathy using MR Spectroscopy and MR Imaging

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Chapter 9

Clinical and Metabolic Effects of a 12-week eSupported Lifestyle Intervention in Insulin-dependent Type 2 Diabetes

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Abstract

Background

This study aimed to investigate the effect of a lifestyle intervention on glucose metabolism, ectopic fat and cardiac function in insulin-dependent T2DM patients.

Methods

Eleven T2DM subjects (median diabetes-duration: 11 years) underwent 12-week lifestyle intervention. At baseline, 12 and 50 weeks OGTT was performed to evaluate insulin resistance and beta-cell function. Ectopic fat and cardiac function were assessed with MR. Data are presented as median [IQR]. Statistics were performed using Friedman and Wilcoxon signed-rank tests.

Results

After 12 weeks median weight loss was -9[-11, -8]kg, p=0.003, with reduced insulin requirement (-53[-90, -44]IU/day, p=0.003). Post-intervention, body weight remained stable (+1[1, 3]kg, p=0.05), but HbA1c increased (p=0.01). Insulin resistance improved at week 12 and 50 (+1.0[0.1, 2.9], p=0.04 and +1.1[0.7, 2.4], p=0.005 respectively). Beta-cell function did not change significantly and remained poor. Liver fat reduction at week 12 (-7[-15, -4]%, p=0.008) persisted at week 50. Despite reduction of myocardial fat at week 50 (p=0.003), E/A ratio and ejection fraction did not improve.

Conclusions

Despite long diabetes duration and insulin-dependence, lifestyle intervention improved insulin sensitivity and hepatic and myocardial steatosis. However, beta-cell function and cardiac performance remained unchanged, suggesting irreversible damage. Post-intervention, glycemic control worsened stressing importance of continued vigilant diabetes management.

Introduction

Type 2 diabetes mellitus (T2DM) pathophysiology is hallmarked by two major distinct components: insulin resistance and beta-cell dysfunction[1]. Insulin resistance is commonly the earliest feature leading to impaired glucose tolerance. By the time T2DM is diagnosed, beta-cell function has already deteriorated by 50%[2]. With time, insulin sensitivity and beta-cell function progressively deteriorate, to a point where insulin therapy is needed to restore glycemic control[3]. Because insulin treatment only partly tackles T2DM pathophysiology, less than half of T2DM patients achieve their glycemic target[4]. Since insulin resistance plays a central role in the development of cardiometabolic disease and non-alcoholic fatty liver disease[5], interventions promoting insulin sensitivity should be considered strongly.

The pathophysiology of insulin resistance is complex and involves low-grade inflammation, neurohumoral disturbance and metabolic cross-talk between liver, adipose tissue, pancreas and skeletal muscle[3]. With a positive energy balance, fat accumulates in visceral fat and ectopically in liver, pancreas, skeletal muscle and myocardial tissue[5]. Hepatic steatosis is tightly linked to hepatic insulin resistance, leading to hyperglycemia and atherogenic lipid profile. In turn, increased glucose levels and free fatty acid flux to the pancreas hamper beta-cell function[2]. Magnetic resonance imaging (MRI) and spectroscopy (MRS) have revealed that visceral fat and hepatic steatosis are associated with myocardial steatosis, cardiovascular disease and left ventricular (LV) dysfunction[6]. Fortunately, lifestyle intervention can reverse ectopic fat accumulation, which is associated with improvement of cardiac function[7]. Moreover, reduction of ectopic fat by prolonged use of a very low calorie diet was reported to reverse early stage T2DM after 2 years of follow-up[8].

In light of these observations, it is with good reason that promoting a healthy lifestyle remains the cornerstone of T2DM management[9]. Unfortunately, lifestyle interventions have been limited by poor long term adherence. Continued guidance by health care professionals might positively influence long-term outcome, but it is burdensome to healthcare and costly. Support of lifestyle coaching by continuous online communication and biofeedback through e-health technology could promote

patient adherence. Therefore the aim of the present study was to assess the effects of a 12-week eSupported lifestyle intervention on insulin sensitivity and beta-cell function in relation to ectopic fat accumulation and cardiac performance using MRI and MRS in patients with insulin-dependent T2DM. In addition, the post-intervention durability of any measurable effect was assessed.

Methods

Study design and population

This prospective intervention pilot study took place between September 2015 and September 2016 at the Leiden University Medical Center, The Netherlands. The institutional ethics committee approved the study, and written informed consent was obtained prior to the study in all participants. The study complied with the 1964 Declaration of Helsinki. Recruitment was performed in the institutional outpatient clinic. Insulin-dependent T2DM patients could be included if ≥18 years and if they had basic computer competence. Exclusion criteria were: myocardial infarction within the previous three months, score below 3 out of 5 for motivation to participate[10], blood pressure >170/100mmHg, alcohol consumption > 28 units per week, psychiatric disease, any chronic disease hampering participation, and MRI contraindications.

Study protocol

An extensive hybrid eSupported 12-week lifestyle intervention (Health Coach Program B.V., Delft, The Netherlands) was conducted, aiming to achieve a durable lifestyle change encompassing diet and exercise[11]. Recommendations entailed a very low calorie diet in the first week (500-1000 kcal per day) followed by a diet 500 kcal below estimated required energy consumption based on age, gender, activity level and body composition (Bodystat 1500, Bodystat Ltd., Douglas, UK). Dietary recommendations included the intake of vegetables and fruits low in sugar (each ≥2,5 servings/day) and whole grains, and to limit consumption of refined grains, sugar, red meat, processed meat, trans-saturated fats, and alcohol[12]. Physical exercise recommendations included ≥60 min/day moderate-intensity exercise and ≥30 min intensive-exercise three times weekly. Coaching involved face-to-face group sessions,

supervised exercise training sessions and electronic dashboarding as described previously[10]. Glucose-lowering and anti-hypertensive drug titration was performed by the investigators during the 12-week intervention, and by participant's own physicians between week 12 and 50 while participants were encouraged to keep using the electronic dashboard. At baseline and after 12 and 50 weeks the following study endpoints were assessed, for all participants within two weeks: body weight, waist/hip circumference, blood pressure, drug use (daily insulin dose: average daily dose in previous two weeks), blood and urine samples, body composition, physical fitness tests, oral glucose tolerance tests and MR scans.

Physical fitness tests

Cardiorespiratory fitness was assessed by maximal aerobic capacity ($VO2_{max}$) using a bicycle ergometer test[13]. VO_{2max} was then defined using the Ästrand & Rhyming nomogram[14]. Muscle strength was measured by the 30-s chair-stand test[15].

Diabetic profiling by extended oral glucose tolerance test

75-gram oral glucose tolerance tests were performed after having stopped all glucose-lowering drugs (including insulin) for 24 hours. Blood was drawn via an intravenous catheter at 0, 30, 60, 90 and 120 minutes. Using glucose (mmol/L) and insulin (mU/L) levels, insulin sensitivity and beta-cell function indices were calculated as follows. Whole-body insulin sensitivity was estimated using the Matsuda index: 10.000 / (√(glucose$_{0min}$ x insulin$_{0min}$) x (mean glucose$_{0-120min}$ glucose x mean insulin$_{0-120min}$)[1]. Furthermore, the Homeostatis Model Assessment of Insulin Resistance (HOMA-IR) index was calculated (fasting insulin x fasting glucose / 22.5)[16]. Muscle insulin resistance (MISI) was calculated by (ΔG/Δt ÷ mean plasma insulin concentration (I)), where ΔG/Δt is the rate of decline in plasma glucose concentration and is calculated as the slope of the least square fit to the decline in plasma glucose concentration from peak to nadir[17]. Beta-cell function was assessed using the oral disposition index, which is a measure of insulin secretion corrected for insulin resistance[18]: ($AUC_{0-30min}$ insulin / $AUC_{0-30min}$ glucose) x Matsuda index which is a modified formula to selectively investigate the first phase insulin response19. (AUC_{0-}

$_{120min}$ insulin / $AUC_{0\text{-}120min}$ glucose) x Matsuda index was used to determine total beta-cell function[19].

MR protocol

After at least six hours of fasting, participants underwent MRI and MRS using a 3 Tesla Ingenia system (Philips Medical Systems, Best, the Netherlands). A detailed description of the MR protocol was described previously[7,20]. Abdominal visceral fat imaging was performed using a 2-point DIXON sequence. Using MASS (Medis, Leiden, the Netherlands), average visceral fat area of three consecutive transversal slices at the L4-L5 vertebra level was determined using signal intensity thresholding. Liver and pancreas fat were measured using a 6-point chemical shift GRE sequence (mDIXON Quant, Philips, Best, The Netherlands). Post-processing was performed using MASS software that allowed manual detection of liver and pancreas in consecutive transversal slices. Based on a histogram of signal intensity, the mean value was used to produce the organ's mean fat percentage. Myocardial triglyceride content was assessed using MRS[21]. In short, a voxel was placed in the interventricular septum. Acquisition of 4 signal averages without and 32 signal averages with water suppression was performed during free-breathing with pencil beam navigator-based respiratory triggering and cardiac triggering. Post-processing was performed with the Java-based MR User Interface (version 5.0; Katholieke Universiteit Leuven, Leuven, Belgium). For pericardial fat imaging a 4-chamber view high resolution water suppressed Black-Blood Turbo Spin Echo Sequence was used. Epicardial and paracardial fat were manually separated and quantified, with the atrioventricular plane as basal border. Cardiac morphology and LV function were assessed using MR as described previously[22], with the exception that diastolic function was assessed using 2D transmitral flow imaging using QMass (Medis, Leiden, The Netherlands).

Analytical procedures

HbA1c was measured with ion-exchange high-performance liquid chromatography (Tosoh G8, Sysmex Nederland B.V., Etten-Leur, the Netherlands). The NEFA C kit (Wako Diagnostics; Instruchemie, The Netherlands) was used to assess free fatty acids (FFA). Glucose, insulin, total cholesterol, HDL-c, triglycerides, serum creatinine, urinary creatinine and urinary albumin were measured with a Modular

P800 analyser (Roche Diagnostics, Mannheim, Germany). The Friedewald formula was used to calculate LDL-c.

Statistical analysis

A sample size calculation was not performed since this was a pilot study to explore tolerability and sustainability of the eSupported lifestyle intervention. The study population was limited to eleven participants in order to be able to perform all study measurements within one week, and perform group sessions. The statistical analyses were performed using SPSS (SPPS Statistics 23.0, Chicago, III). Data are expressed as median values with interquartile range [IQR]. To test significance of differences between paired measurements, first the Friedman's two-way analysis of variance by ranks' test was used, and in case of a significant difference, the related-samples Wilcoxon signed rank test was executed. A p-value of <0.05 was significant.

Results

Population and intervention

Of the eleven subjects screened, all met the inclusion criteria and were enrolled in the study. Table 1 shows the baseline characteristics. Diabetes duration was less than five years in one participant, between five and ten years in three participants, over ten years in four, and over twenty years in three participants. Three participants were overweight (BMI: 25-30kg/m^2) and eight were obese (30-40kg/m^2). In addition to peripheral neuropathy in five participants, five suffered from significant musculoskeletal morbidity (low backpain, joint replacement, Ehlers Danlos, rheumatoid arthritis and arthrosis). Nine participants used anti-hypertensive drugs, two had aortic valve stenosis (mild to moderate), one had hypertrophic cardiomyopathy and five others had presence of atherosclerotic disease: stable angina pectoris (n=1), myocardial infarction (n=3), ischemic cerebrovascular accident (n=1), and peripheral artery disease (n=2). All participants completed the 12-week lifestyle intervention, and attended the follow-up visit at week 50 with the exception of one participant that refused the OGTT at week 50. Overall satisfaction with the eSupported lifestyle intervention was high, as described previously10.

Anthropometric measures and blood pressure

Body weight dropped significantly from 99[IQR: 93, 111]kg to 90[87, 102]kg at week 12 (p=0.003) with a BMI decrease from 34[30, 39)]kg/m^2 to 31[27, 35]kg/m^2. As compared to week 12, body weight increased slightly with 1 kg at week 50 (92[87, 102]kg, p=0.05). Waist circumference decreased from 116[111, 133]cm to 110[97, 123]cm at week 12 (p=0.003) and remained stable between week 12 and 50 (110[99, 121]cm, p=0.29). In parallel, hip circumference decreased from 110[103, 123]cm to 104[101, 112]cm at week 12 (p=0.005), and remained stable throughout week 50 (105[100, 108]cm, p=0.76). Bio-impedance measurements estimated a total body fat percentage of respectively 33[29, 36]%, 27 [25, 29] % and 30[25, 35]% at baseline, week 12 and week 50 respectively with significant differences between baseline and week 12 (p=0.004) and 50 (p=0.003), and no significant difference between week 12 and 50 (p=0.16). Systolic and diastolic blood pressure did not change significantly (p=0.81 and p=0.15 respectively), but in four patients, one or more anti-hypertensive drugs were stopped.

Physical fitness tests

Physical performance at the 30-s chair-stand test improved significantly from 14[12, 16] at baseline to 17[15, 21] at week 12 (p=0.005). At week 50, physical performance (21[16, 24]) was significantly better as compared with week 12 (p=0.03) and baseline (p=0.003). $VO2_{max}$ was 2.2[1.8, 2.6]ml/kg/min at baseline, and increased significantly to 2.8[2.6, 3.5]ml/kg/min at week 12 (p=0.005) and remained 2.8[2.5, 3.5]ml/kg/min at week 50 (p=0.005).

Glucose metabolism

The various endpoints reflecting glucose metabolism are shown in table 2 and figure 1. At baseline, all participants used insulin, ten participants were using metformin, and two used a sulfonylurea derivative. Sulfonylurea derivatives were stopped directly at the start of the study. All participants continued using metformin, and one participant was started on metformin. As guided by ambulant blood sugar levels and HbA1c values, two participants on basal insulin could stop using insulin. Four participants could reduce insulin regimen complexity by switching from basal-

bolus (n=2) or premix (n=2) to basal insulin during the intervention period. In the post-intervention period, one participant was started on a dipeptidyl peptidase-4 inhibitor, one participant stopped using metformin, and one switched from basal to basal-bolus insulin regimen. In the other participants, diabetes treatment regimen did not change.

At baseline, HbA1c was 65[54, 79]mmol/mol and above target (53 mmol/mol) in all participants. At week 12, HbA1c decreased non-significantly to 59[53, 67]mmol/mol (p=0.37) with two participants reaching an HbA1c level below target. At week 50, HbA1c increased to 74[60, 86]mmol/mol (p=0.25 for comparison with baseline; p=0.01 for comparison with week 12) and was above target in all participants. Insulin dose dropped significantly from 73[60, 116]IU/day to 20[10, 37]IU/day at week 12 (p=0.003). Whereas insulin dose at week 50 (30[20, 40]IU/day) was still lower than at baseline (p=0.004), it had increased as compared to week 12 (p=0.03). As shown in figure 1, beta-cell function and whole body insulin sensitivity were severely impaired as reflected by a low disposition and Matsuda index, respectively. Disposition index was very low in all participants at baseline (0.21[0.03, 0.68]) and remained so throughout the study (week 12: 0.33[0.15, 0.59]; week 50: 0.35[0.09, 0.60], p=0.84). Matsuda index was within reference value in three participants at baseline (1.0[0.5, 3.2], reference value>3.0[23]), in four at week 12 (2.0[1.4, 5.2]), and three at week 50 (2.8[1.4, 5.2]). HOMA-IR ameliorated significantly from 19[3, 38] at baseline to 8[2, 11] at week 12 and 5[3, 10] at week 50 (reference value ≤ 2.0[24]). Muscle insulin resistance index was normal in five participants at baseline (-0.9[-3.6, -0.1], reference value>-1.0[25]), six at week 12 (-1.1[-7.4, 0.0]) and seven at week 50 (-1.8[-6.8, -0.6]), but did not change significantly during the study (p=0.2).

Renal endpoints and lipids

Serum creatinine was 82[68, 103]µmol/L at baseline and did not change throughout the study (p=0.18). The albumin-creatinine ratio (baseline: 1.9[0.9, 4.8]mg/mmol; week 12: 1.5[0.8, 5.0]mg/mmol; week 50: 1.5[0.7, 3.5]mg/mmol) did not change either (p=0.84). Total cholesterol diminished from 4.7[3.5, 5.4]mmol/L at baseline to 3.7[3.2, 4.9]mmol/L at week 12 (p=0.003) and was still significantly lower at week 50 (4.2[3.3, 5.2]mmol/L, p=0.03). HDL-cholesterol (1.1[1.0, 1.6]mmol/L at

baseline) did not change throughout the study (p=0.22), neither did LDL-cholesterol (2.2[1.6, 2.9]mmol/L at baseline, p=0.15). Serum triglycerides were 2.3[1.3, 3.3]mmol/L at baseline and reduced to 1.4[0.9, 2.3]mmol/L and 1.6[0.9, 2.4] mmol/L at week 12 (p=0.008) and week 50 (p=0.04) respectively. Serum FFA displayed a non-significant increase: baseline: 0.47[0.36, 0.77]mmol/L; week 12: 0.59[0.34, 0.82]mmol/L; week 50: 0.52[0.35, 0.60]mmol/L, p=0.8.

Table 1. Baseline characteristics

Age in years	64 [58, 66]
Gender (n)	8 male; 3 female
Smoking status, n (%)	
Non-smoker	3 (27)
Previous smoker	8 (73)
Current smoker	0
Alcohol consumption in units per week	0 [0, 1]
Diabetes duration in years	11 [9, 20]
Retinopathy, n (%)	4 (36)
Nephropathy, n (%)	8 (73)
Neuropathy, n (%)	5 (45)
Macrovascular disease, n (%)	5 (46)
Metformin, n (%)	10 (91)
Sulfonylurea derivative, n (%)	2 (18)
Insulin users, n (%)	11 (100)
Basal	5 (46)
Premix	2 (18)
Basal-bolus	4 (36)
Insulin dose in IU/day	73 [60, 116]
Antihypertensive drugs, n (%)	9 (82)
Lipid lowering drugs, n (%)	9 (82)
Weight in kg	99 [93, 111]
Body mass index in kg/m^2	34 [30, 39]

Systolic blood pressure (mmHg)	132 [121, 144]
Diastolic blood pressure (mmHg)	76 [68, 82]
HbA1c in mmol/mol	65 [54, 79]
HbA1c in %	8.1 [7.1, 9.4]
Disposition index	0.21 [0.03, 0.68]
Matsuda index	1.0 [0.5, 3.2]
HOMA-IR	19 [3, 38]
Muscle insulin resistance index	-0.9 [-3.6, .01]
Visceral fat in cm^2	265 [158, 342]
Liver fat in %	12 [8, 22]
Pancreas fat in %	20 [14, 26]
Myocardial fat %	1.3 [1.0, 1.6]
Epicardial fat cm^2	6.7 [5.7, 8.4]
Paracardial fat cm^2	16.0 [13.1, 23.7]
Pericardial fat cm^2	24.4 [18.3, 33.5]

Legend: data are presented as median [Interquartile range] unless specified otherwise.

Table 2. Changes in anthropometric, clinical, metabolic and ectopic fat endpoints before and after intervention

	Baseline vs week 12		**Week 12 vs week 50**		**Baseline vs week 50**	
	Change [IQR]	**p**	**Change [IQR]**	**p**	**Change [IQR]**	**p**
Weight (kg)	-9 [-11, -8]	**0.003**	1 [1, 3]	0.05	-7 [-10, -5]	**0.003**
Insulin dose (IU/day)	-53 [-90, -44]	**0.003**	9 [0, 24]	**0.03**	-40 [-88, -28]	**0.004**
HbA1c (mmol/mol)	-5 [-12, 7]	0.37	10 [3, 19]	**0.01**	6 [-3, 18]	0.25
HbA1c (%)	-0.5 [-1.1, 0.6]	0.37	0.9 [0.2, 1.7]	**0.01**	0.6 [-0.2, 1.6]	0.25
Disposition index $AUC_{0\text{-}30min}$	0.03 [-0.14, 0.26]	NS	-0.02 [-0.13, 0.18]	NS	0.04 [-0.20, 0.28]	NS
Disposition index $AUC_{0\text{-}120min}$	0.09 [-0.09, 0.35]	NS	-0.02 [-0.13, 0.18]	NS	0.09 [-0.08, 0.37]	NS
Matsuda index	1.0 [0.1, 2.9]	**0.04**	0.6 [-0.9, 1.7]	0.37	1.1 [0.7, 2.4]	**0.005**
HOMA-IR	-6 [-19, 0]	**0.03**	0 [-3, 1]	0.51	-4 [-17, -1]	**0.02**
Muscle insulin resistance index	-0.2 [-2.5, 0.7]	NS	0.0 [-2.9, 1.1]	NS	-1.4 [-4.1, 0.0]	NS
Visceral fat (cm^2)	-57 [-83, -22]	**0.003**	18 [5, 47]	0.09	-46 [-57, 8]	0.05
Liver fat (%)	-7 [-15, -4]	**0.008**	1 [0, 3]	**0.05**	-6 [-8, -2]	**0.007**
Pancreas fat (%)	-3 [-5, 2]	NS	1 [-1, 2]	NS	-2 [-6, 4]	NS
Myocardial fat (%)	-0.2 [-0.5, -0.1]	**0.02**	-0.2 [-0.3, 0.0]	**0.02**	-0.5 [-0.7, -0.1]	**0.003**
Epicardial fat (cm^2)	0.1 [-0.6, 0.8]	NS	-1.2 [-1.6, 0.1]	NS	-0.5 [-2.2, 0.4]	NS
Paracardial fat (cm^2)	-2.2 [-5.3, 0.9]	NS	0.7 [-1.8, 3.5]	NS	-1.5 [-4.9, 4.4]	NS
Pericardial fat (cm^2)	-2.8 [-5.1, 0.3]	NS	-0.0 [-2.3, 2.1]	NS	-2.0 [-7.5, 3.5]	NS

Legend: median [IQR] changes between different measurements. NS: between group differences were not significant according Friedman's rank test.

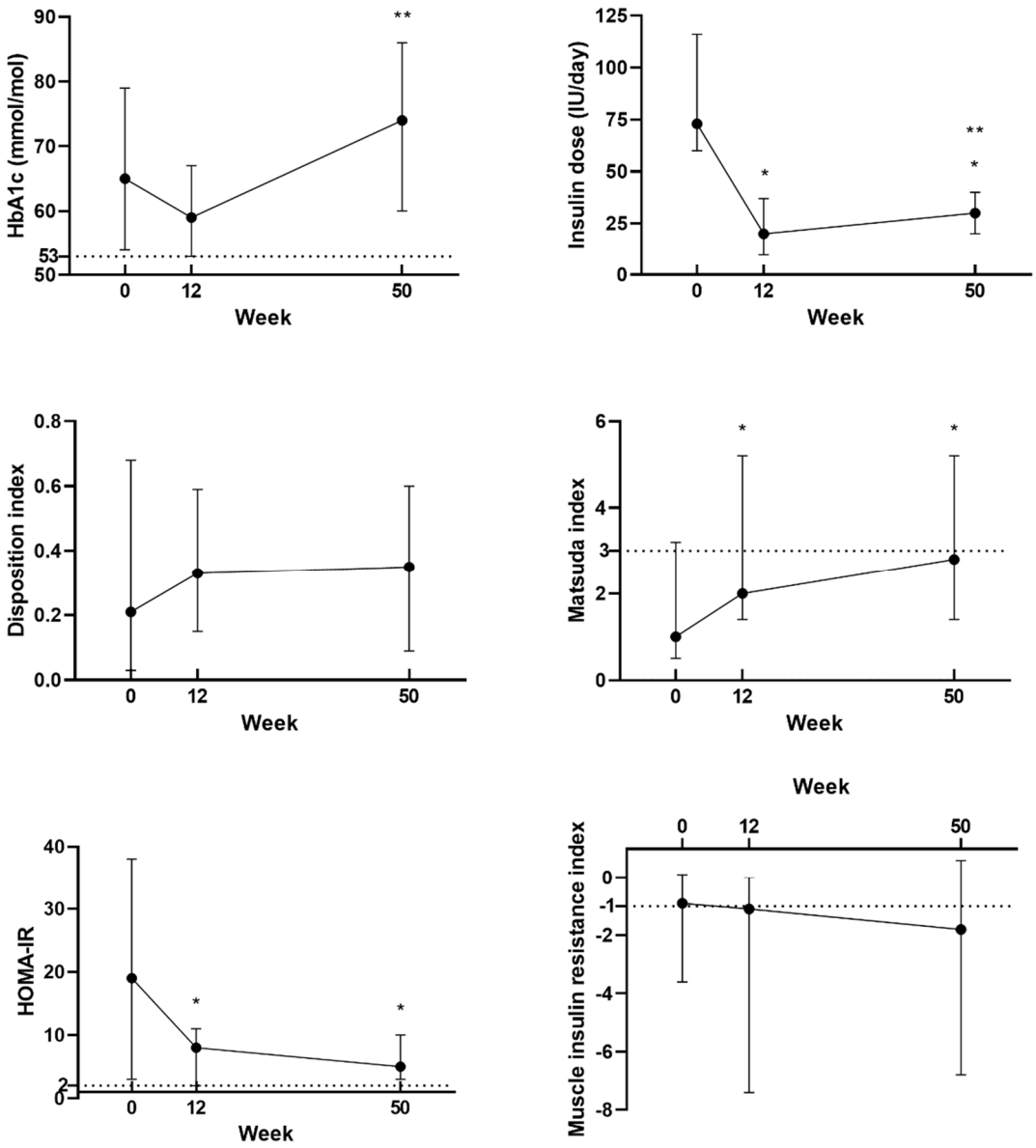

Figure 1. The horizontal dashed lines represent target value (HbA1c) or reference/normal value (Matsuda index > 3.0; Muscle insulin resistance index > -1.0; HOMA-IR ≤ 2.0, see manuscript for references). Disposition index displayed is the AUC0-30min.* denotes a significant difference from baseline; ** denotes a significant difference from week 12. Abbreviations: HOMA-IR: homeostatic model assessment of insulin resistance.

Visceral and ectopic fat accumulation

Changes in ectopic fat accumulation are displayed in table 2 and figure 2. Visceral fat decreased significantly after 12 weeks (p=0.003), but changes between week 12 and 50 and baseline vs week 50 were not significant. On two occasions liver fat could not be assessed due to technical failure. Liver fat reduced significantly at

week 12 compared to baseline (p=0.008), slightly but significantly increased between week 12 and 50 (p=0.05) but remained lower at week 50 as compared to baseline (p=0.007). In nine participants, liver fat fraction was increased (reference value<5.6%[26]) at baseline (12[8, 22]), as opposed to four at week 12 (5[4, 9]) and six at week 50 (6[4, 10]). On one occasion myocardial fat could not be measured due to low signal-to-noise ratio. Myocardial fat reduced significantly at week 12 (p=0.02), and decreased further between week 12 and 50 (p=0.02). At baseline myocardial fat content was normal (reference value<0.6%[27]) in one participant (1.3[1.0, 1.6]), compared to none at week 12 (0.9 [0.8, 1.1]), and four at week 50 (0.7[0.6, 1.0]). Pancreatic fat could not be determined in three participants because the pancreas was (in part) outside the field of view. At baseline, pancreatic fat fraction was 20[14, 26]% and did not change significantly throughout the study (week 12: 16[13, 29]%; week 50 19[14, 29]%, p=0.20). Likewise, ectopic deposition of fat in epicardial, paracardial and pericardial areas did not significantly change (p=0.18, p=0.23, p=0.18 respectively).

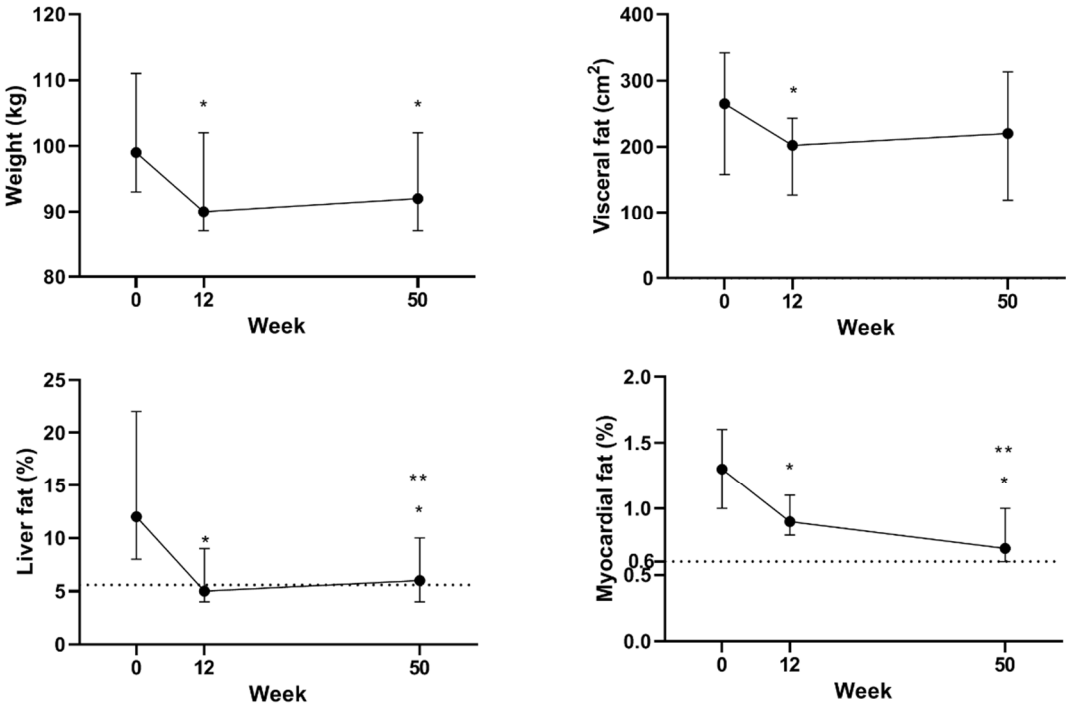

Figure 2. The horizontal dashed lines represent reference values (Liver fat < 5.6%; Myocardial fat < 0.6%, see manuscript for references). * denotes a significant difference from baseline; ** denotes a significant difference from week 12.

Table 3. Indices of cardiac morphology and function

	Baseline	Week 12	Week 50	p
Cardiac morphology				
LVEDV (ml)	158 [151, 177]	162 [145, 186]	151 [142, 180]	0.76
LVESV (ml)	55 [43, 60]	58 [45, 62]	61 [47, 67]	0.08
LVM (g)	109 [93, 121]	102 [89, 121]	100 [88, 123]	0.18
LVMI (g/m^2)	47 [44, 54]	49 [42, 54]	49 [41, 54]	0.70
LVMI/LVEDVI ($g/ml/m^2$)	0.68 [0.65, 0.78]	0.70 [0.55, 0.72]	0.65 [0.59, 0.77]	0.08
LV compliance	19.7 [12.9, 21.0]	16.8 [14.6, 18.6]	17.7 [16.3, 26.3]	0.91
Diastolic function				
E (ml/s)	375 [278, 445]	413 [376, 485]	388 [304, 421]	0.44
A (ml/s)	398 [331, 454]	409 [326, 497]	368 [314, 450]	0.44
E/A ratio	1.0 [0.8, 1.2]	1.1 [0.8, 1.3]	1.0 [0.8, 1.2]	0.53
Edec ($ml/s^2 \times 10^{-3}$)	3.4 [2.9, 3.5]	3.9 [3.0, 4.4]	3.1 [2.3, 4.0]	0.08
Ea (cm/s)	5.0 [3.9, 6.4]	5.2 [4.4, 6.5]	5.5 [5.0, 6.3]	0.70
E/Ea	9.0 [6.1, 11.9]	9.6 [8.8, 11.0]	9.3 [6.8, 10.3]	0.91
Systolic function				
Stroke volume (ml)	98 [81, 114]	100 [85, 117]	100 [89, 114]	0.31
Ejection fraction (%)	63 [61, 72]	63 [59, 70]	64 [59, 71]	0.15
Cardiac output (L/min)	5.7 [5.4, 6.7]	5.5 [5.2, 6.4]	5.8 [5.3, 6.6]	**0.03***
Cardiac index ($L/min/m^2$)	2.5 [2.4, 3.0]	2.6 [2.4, 3.0]	2.9 [2.5, 3.1]	0.18

Data are presented as median [interquartile range]. P values were obtained from Friedman's ranks test. * subsequent Wilcoxon signed rank test p-value for differences between time points were 0.08 for baseline vs week 12 and 0.93 for baseline vs week 50 respectively). *Abbreviations:* E/A: early to late transmitral flow ratio.

Cardiac morphology and function

Cardiac morphology, LV diastolic function, and LV systolic function are displayed in table 3. None of the tested indices displayed a significant difference between measurements. LV mass tended to decline in proportion to body weight throughout the study. Thus, LV mass index remained stable.

Discussion

Despite long diabetes duration and insulin-dependence, overweight and obese T2DM patients can benefit from a lifestyle intervention comprising daily physical activity and calorie restriction guided by blended coaching in terms of insulin dose, whole body insulin resistance, and visceral, myocardial and hepatic ectopic fat accumulation. In contrast, MR and metabolic assessments revealed persistent pancreatic fat deposition and very low beta-cell function respectively, despite significant weight loss. Surprisingly, although fat stores and insulin resistance indices remained significantly reduced throughout the post-intervention period, glycemic control deteriorated in the face of persistently reduced glucose lowering drug doses. This may have been due to increased caloric intake and persistent beta-cell failure. Moreover, despite reduced myocardial steatosis, there was no change in cardiac function or morphology. The data therefore suggest that, while insulin resistance and (ectopic) fat deposition can sustainably improve in response to calorie restriction and physical exercise in people with long-term, insulin dependent T2DM, beta-cell function and cardiac performance may not similarly benefit. Persistent beta-cell failure sustains drug dependence of glycemic control in case food intake returns to pre-intervention habits.

Glucose metabolism in relation to intra-abdominal ectopic fat accumulation

Diabetic profiling allowed us to gain insight into the relative improvement of insulin resistance and beta-cell function. Since insulin resistance is tightly linked to visceral and hepatic fat accumulation[5], and beta-cell dysfunction is associated with pancreatic fat deposition[28], the interaction between glucose metabolism and ectopic fat was studied using quantitative MR. The moderate weight loss during the 12-week intervention period resulted in a considerable reduction of insulin requirement. Diabetic profiling revealed that measures of whole body insulin sensitivity significantly improved during the intervention. This improvement was accompanied by a 20% relative reduction of visceral fat.

The majority of our study population had hepatic steatosis and poor HOMA-IR values at baseline, the latter reflecting hepatic insulin resistance[17]. Both these parameters improved substantially during the intervention, indicating a high degree of reversibility. Improved hepatic steatosis and insulin sensitivity following weight loss have been shown consistently in intervention trials in insulin-independent[7,29] and insulin-dependent T2DM[7]. In contrast to the strongly improved HOMA-IR, reflecting a state of improved hepatic insulin resistance, muscle insulin resistance did not change. The discrepancy between improved hepatic insulin sensitivity and lack of improvement of peripheral insulin sensitivity has previously been demonstrated in T2DM patients[29]. Apparently, the physical exercise portion of the intervention did not affect muscle insulin sensitivity, despite the fact that indices of muscular performance did improve during the study. In accordance with that observation, exercise had no additional beneficial effect on insulin sensitivity when added to a very low calorie diet in insulin-dependent T2DM patients[30]. Moreover, muscle insulin sensitivity was normal in 45% of the study population at baseline and as such perhaps had little potential to improve.

At baseline, the oral disposition index of our population was very low as compared to previous studies in T2DM patients using no medication[31] or only oral glucose-lowering drugs[25,32]. An obvious difference was that our population consisted of insulin-dependent T2DM patients. Throughout the study, beta-cell function did not improve significantly and T2DM remission did not occur in any participant. Recently, a study in T2DM patients with average 13.4 years diabetes duration, a lifestyle intervention resulted in remission of 2 out of 15 subjects[33]. This study population differed from ours because not all participants used insulin, suggesting milder diabetes. In contrast to these low success rates of diabetes remission, previous studies have shown more successful T2DM remission (that requires restoration of both insulin sensitivity and beta-cell function[34]) upon weight loss following a lifestyle intervention[35] or gastric bypass surgery[36]. In the DiRECT study, T2DM patients with less than 6 years of diabetes duration and not receiving insulin underwent a lifestyle intervention during one year. Diabetes remission was achieved in 33.9% in the group that had lost 5-10kg and had a post-intervention BMI of 31kg/m^2 [35]. An important

difference between our study and the DiRECT study are the long diabetes duration (median > 10 years) and insulin-dependence of our participants. In light of the progressive beta-cell dysfunction and apoptosis during the course of T2DM[37], it has previously been hypothesized that reversibility of beta-cell deficiency following weight loss cannot be achieved in diabetes of any duration[2]. In support of this hypothesis, diabetes remission after gastric bypass surgery was shown to be dependent on diabetes duration[36]. The mechanisms responsible for the reversibility of beta-cell function upon weight loss are not fully understood, but a reduction of pancreatic fat accumulation could be a contributing factor[2]. We observed a non-significant decrease of pancreas fat in response to the intervention, but this finding should be cautiously judged, because we could measure pancreas fat in only eight participants. A causal link between fatty pancreas and beta-cell dysfunction has been suggested by preclinical[2,38] and human observational[28] studies. However, intervention studies in humans have shown conflicting results regarding the interaction between weight loss, pancreas fat and beta-cell function. Although pancreas fat has been shown to decrease in response to weight loss in T2DM patients in some[39,40], but not all studies[41,42], a reduction in pancreas fat was not independently associated with metabolic parameters when corrected for visceral fat[40]. Beta-cell mass constitutes a mere 2% of total pancreatic mass. Unfortunately, current MR technology cannot quantify intracellular fat storage, ie. triglycerides in the beta-cell, and so primarily detects fat stored in other than beta-cells, which probably does not contribute to beta-cell failure[38].

The significant increase in HbA1c between week 12 and week 50 - despite stable body weight and sustained improvement of insulin sensitivity - was un unexpected finding. Persistent beta-cell failure and possibly decreased adherence to dietary advice required intensification of drug treatment, which was insufficiently pursued apparently, thereby raising glucose levels which may have further deteriorated beta-cell function[43].

Cardiac fat, morphology and function

Although we did not incorporate a control group in the current study, the cardiac phenotype of our participants can be characterized by comparing myocardial

triglyceride content, cardiac morphology and function with other study populations. Taking distinct age and gender specifications into consideration, our population had myocardial steatosis[25], concentric LV remodeling (increased LV mass), impaired diastolic function (decreased early to late tranmitral flow ratio), and normal global LV systolic function (LV ejection fraction)[27,44]. This cardiac phenotype is congruent with that of T2DM patients with or without hypertension previously established in other studies[45,46]. After the intervention, myocardial fat content decreased to a level approaching that of the healthy control group[27]. However, cardiac morphology and function did not change in parallel in our study. This is in contrast with previous studies performed in obese T2DM patients without cardiovascular disease that have shown a strong positive correlation between myocardial steatosis and diastolic dysfunction[7,47]. However, a very low calorie diet in obese insulin-dependent T2DM patients with established coronary artery disease did not improve diastolic function[7]. We speculate that the presence of underlying cardiovascular disease (hypertension, myocardial infarction, valvular heart disease) may have limited improvements of cardiac morphology and function. Furthermore, moderate weight loss (as opposed to progressive caloric restriction in aforementioned studies), in combination with the small sample size of our study must be taken into account. In contrast to the study performed by Jonker et.al., where a 6-month exercise training intervention (without diet) decreased visceral fat and paracardial fat (but not epicardial fat)[48], our study did not show any changes in epi-or paracardial fat accumulation. Previous studies with a 16-week very low calorie diet[49] and bariatric surgery[50] in T2DM patients have shown that progressive weight loss was accompanied by reduced pericardial fat (both paracardial and epicardial fat).

Limitations

The major limitation was the limited number of participants thereby limiting power to detect less pronounced or consistent effects of lifestyle. In addition, the sample size increased the chance of type 1 errors. Furthermore, we did not use the gold standard for assessment of insulin sensitivity (euglycemic insulin clamp technique[51]). Although OGTT assessment of whole-body insulin sensitivity has been validated against the euglycemic clamp by Matsuda and DeFronzo1, that study did not

include insulin-dependent T2DM patients. OGTT-derived whole-body insulin sensitivity correlates less strongly with euglycemic clamp-derived insulin sensitivity in T2DM patients than in people with normal glucose tolerance, perhaps as a result of beta-cell dysfunction[1].

Conclusions

This study indicates that eSupported blended lifestyle intervention is worthwhile in long-term, insulin-dependent T2DM patients. The co-existence of diabetes complications and significant musculoskeletal morbidity does not preclude significant improvement of physical fitness and insulin requirements. Visceral fat, ectopic fat and insulin resistance appear particularly amenable to calorie restriction and physical exercise, while pancreatic fat, beta-cell failure and cardiac dysfunction seem to be more challenging to combat.

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