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CLINICAL RESEARCH

Long- but not short-term multifactorial intervention with focus on exercise training improves coronary endothelial dysfunction in diabetes mellitus type 2 and coronary artery disease

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Aims Patients with type 2 diabetes mellitus (T2DM) suffer from accelerated coronary artery disease. We assessed the effects of a multifactorial intervention with focus on exercise training on coronary endothelial function, vascular structure, and inflammation in serum and skeletal muscle biopsies, including mRNA expression of diabetes candidate genes.

Methods and results Twenty-three patients were randomized to either 4 weeks in-hospital exercise training (6 × 15 min bicycle/day, 5 days/week) and a hypocaloric diet, followed by a 5 months ambulatory program (30 min ergometer/day, 5 days/week, plus 1 h group exercise/week), or a control group. At the beginning of the study, at 4 weeks, and after 6 months changes in diameter of coronary arteries in response to acetylcholine and mean peak flow velocity were invasively measured; intramural plaques were assessed by intravascular ultrasound. Six months of intervention led to significant improvement of coronary endothelial function, whereas intramural plaque burden remained unchanged. After 4 weeks, endothelial function remained unchanged, however, lowest values for fasting glucose, HbA1c, high-sensitive C-reactive protein, total and LDL-cholesterol, and highest values for mRNA expression in skeletal muscle of p22, gp91, haem oxygenase 1, peroxisome proliferator activator receptor (PPAR) α and γ were observed. There was a continuous increase for AdipoR1, AdipoR2, Glut4, interleukin-6, endothelial nitric oxide synthase, and PPARγ-coactivator-1α mRNA expression in skeletal muscle.

Conclusion This is the first study to demonstrate improvement in coronary endothelial function by a multifactorial intervention which focused on exercise training in patients with T2DM. This coincided with improved markers of hyperglycaemia, insulin sensitivity, and inflammation both in serum and skeletal muscle biopsies.

Keywords Exercise training • Coronary endothelial function • Diabetes mellitus • Skeletal muscle • Intracoronary ultrasound

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Introduction

Type 2 diabetes mellitus (T2DM) is associated with endothelial dysfunction and accelerated atherosclerosis. Whereas physical activity is associated with improved endothelium-dependent vasodilatation in normoglycaemic patients with coronary artery disease,[1] it remains unknown whether exercise training also improves coronary endothelial function in T2DM and if such effects are mediated by improved markers of inflammation-like soluble intercellular adhesion molecule (ICAM-) 1, soluble vascular adhesion molecule (VCAM-) 1, interleukin (IL-) 6, C-reactive protein, or adiponectin. Furthermore, we chose a candidate gene association study approach to test the hypothesis that alterations in skeletal muscle mRNA expression contribute to the beneficial effects of exercise on endothelial dysfunction. The extent to which multiple genes and the environment impact on T2DM predisposition and progression is an ongoing challenge for researchers.[2] Selection of diabetes candidate genes in this study was based on own previous published data.[3,4]

It was thus the objective of this study to determine whether a multifactorial intervention which focuses on exercise training exerts beneficial changes on the above mentioned markers.

Methods

Subjects and study design

Twenty-three patients with coronary artery disease and T2DM (*Tables 1* and *2*) were randomized to an intervention program ($n = 11$) or a control group ($n = 12$).

Inclusion criteria were left ventricular function $\geq$50%, physical work capacity $\geq$50 W, and at least one significant coronary stenosis $\geq$50%, whereas either the left anterior descending (LAD) or circumflex coronary artery (RCX) had to be free from disease or stenoses $\geq$25% for intracoronary flow measurements.

Exclusion criteria comprised diseases affecting endothelial function, untreated hypertension (systolic blood pressure >160 mmHg or a diastolic blood pressure of >90 mmHg), cigarette smoking during the previous 6 months, LDL-cholesterol >4.3 mmol/L, ventricular tachyarrhythmias, chronic obstructive pulmonary disease, valvular heart disease, and myocardial infarction within the previous 4 weeks. The protocol was approved by the Ethics Committee of the University of Leipzig, and written informed consent was obtained from all patients before enrolment into the study.

Exercise training program

In the intervention group, patients were hospitalized for 4 weeks and enrolled into an exercise training program, which consisted of cycle ergometer training six times a day on 5 days a week: 5 min of warming up, 15 min at of 80% of patients' individual maximal heart rate previously obtained during maximal ergometry, and 5 min of cooling down. On weekends, cycle ergometer training was continued at home for 30 min each day. While in hospital, patients received a hypocaloric heart healthy diet of 1500 kcal/d, and their medication was optimized. After discharge, home-based cycle ergometer training was continued for 30 min each day in addition to supervised swimming or endurance training for 1 h once a week. Patients in the control group were discharged from hospital on average 2 days after invasive measurement of endothelial function. Muscle biopsies from the vastus lateralis were obtained from all patients.[5]

In vivo measurement of endothelial function

In vivo endothelial function was assessed in the LAD or RCX, which had to be free from disease or stenoses of >25%.[1] An 8 F guiding catheter was used to cannulate the target vessel and a 2.5 F infusion

Table 1 Biochemical characteristics

Parameter	Intervention group (n = 11, 10 males, 1 female)			Control group (n = 12, 8 males, 4 females)		
	Baseline	4 Weeks	6 Months	Baseline	4 Weeks	6 Months
Body weight (kg)	98.3 ± 17.6	92.3 ± 16.7[a]	95.4 ± 15.4	90.7 ± 16.4	92.0 ± 16.3[a]	92.5 ± 15.4[a]
Ergometer exercise duration (min)	15.4 ± 3.6	19.1 ± 4.5[a]	18.9 ± 4.9[a]	12.6 ± 4.0	13.3 ± 4.4	12.8 ± 4.0
Maximal exercise capacity (W)	131.8 ± 31.8	167.5 ± 39.2[ab]	162.5 ± 42.9[ab]	112.5 ± 37.7	116.7 ± 37.4	112.5 ± 36.1
Fasting plasma glucose (mmol/L)	8.2 ± 4.0	6.0 ± 0.8[a]	7.0 ± 2.0	8.2 ± 4.0	7.8 ± 1.5	7.9 ± 1.8
Triglycerides (mmol/L)	2.02 ± 1.37	1.44 ± 0.44	1.46 ± 0.79	2.02 ± 1.37	1.73 ± 0.76	1.66 ± 0.64
Total cholesterol (mmol/L)	4.46 ± 0.90	3.51 ± 0.64[a]	4.22 ± 0.63	4.46 ± 0.90	4.62 ± 1.07	4.88 ± 1.62
HDL-cholesterol (mmol/L)	1.21 ± 0.42	1.10 ± 0.29	1.43 ± 0.531	1.21 ± 0.42	1.16 ± 0.25	1.20 ± 0.27
LDL-cholesterol (mmol/L)	2.52 ± 0.77	1.85 ± 0.50[a]	2.26 ± 0.58	2.52 ± 0.77	2.92 ± 0.97	3.14 ± 1.54
High-sensitive C-reactive protein (mg/L)	4.0 ± 4.8	1.4 ± 1.5[a]	3.6 ± 2.8	4.0 ± 4.8	5.3 ± 4.7	5.4 ± 3.9
Fasting plasma insulin (pmol/L) in OAD treated patients	869 ± 24	610 ± 31[a]	449 ± 27[a]	905 ± 54	928 ± 33	902 ± 48
Adiponectin (mg/L)	24.7 ± 23.9	13.7 ± 76.8[a]	22.4 ± 13.5	24.0 ± 23.7	20.2 ± 12.3	18.9 ± 82.5
VCAM-1 (pg/mL)	409.8 ± 64.5	391.5 ± 61.4	409.6 ± 84.7	447.9 ± 61.0	445.6 ± 101.0	429.3 ± 67.3
ICAM-1 (ng/mL)	154.6 ± 46.4	134.0 ± 40.5	155.4 ± 60.8	101.0 ± 51.9	181.6 ± 82.4	168.2 ± 47.0
HbA1c (%)	6.6 ± 1.3	6.1 ± 0.7[a]	6.4 ± 0.7	7.1 ± 0.9	6.8 ± 1.0	7.2 ± 1.3

OAD, oral antidiabetic medication.
[a]Significant difference (at least $P < 0.05$) when compared with baseline values.
[b]Significant difference (at least $P < 0.05$) when compared with control group.

Table 2 **Coronary vasodilation**

Parameter	Training group (n = 11, 10 males, 1 female)			Control group (n = 12, 8 males, 4 females)		
	Baseline	4 Weeks	6 Months	Baseline	4 Weeks	6 Months
Coronary blood flow (baseline NaCl) (mL/min)	56.5 ± 22.0	63.1 ± 19.6	41.5 ± 23.4	54.2 ± 26.8	46.4 ± 20.1	41.5 ± 17.1
Coronary blood flow (acetylcholine 7.2 μg/min) (mL/min)	83.8 ± 40.0	92.8 ± 46.4	82.5 ± 50.4	82.3 ± 38.7	80.4 ± 50.1	60.7 ± 39.9
Coronary blood flow (adenosine 2.4 mg/min) (mL/min)	244.4 ± 125.9	255.2 ± 96.0	233.3 ± 78.3	179.7 ± 56.7	164.7 ± 52.8	142.2 ± 67.6
Coronary blood flow (nitroglycerin 200 μg) (mL/min)	107.8 ± 42.4	127.4 ± 41.7	104.0 ± 46.1	119.4 ± 45.3	113.9 ± 50.2	111.8 ± 59.0
Coronary artery diameter (baseline NaCL) (mm)	2.8 ± 0.9	3.0 ± 0.8	2.4 ± 0.7	3.0 ± 0.8	2.9 ± 0.7	2.7 ± 0.6
Coronary artery diameter (acetylcholine 7.2 μg/min) (mm)	2.5 ± 0.9	2.7 ± 0.8	2.4 ± 0.7	2.8 ± 0.7	2.7 ± 0.7	2.5 ± 0.6
Coronary artery diameter (adenosine 2.4 mg/min) (mm)	3.0 ± 0.9	3.2 ± 0.9	2.6 ± 0.7	3.1 ± 0.8	3.0 ± 0.8	2.9 ± 0.6
Coronary artery diameter (nitroglycerin 200 μg) (mm)	3.0 ± 0.9	3.2 ± 0.8	2.7 ± 0.6	3.2 ± 0.8	3.1 ± 0.7	3.0 ± 0.6
Δ Coronary blood flow (acetylcholine 7.2 μg/min) (%)	46.8 ± 45.4	49.2 ± 46.9	111.7 ± 79.5[ab]	57.98 ± 41.57	78.7 ± 68.7	44.7 ± 59.7
Δ Coronary blood flow (adenosine 2.4 mg/min) (%)	340.9 ± 208.7	344.2 ± 241.4	546.4 ± 321.9[ab]	315.6 ± 252.8	336.5 ± 266.1	265.0 ± 171.4
Δ Coronary blood flow (nitroglycerin 200 μg) (%)	139.0 ± 73.1	111.9 ± 69.4	165.6 ± 69.5	163.4 ± 81.3	157.7 ± 64.7	164.6 ± 50.3
Δ Coronary artery diameter (acetylcholine 7.2 μg/min) (%)	−11.9 ± 10.7	−8.3 ± 12.7	−3.3 ± 5.7[a]	−6.7 ± 7.4	−7.7 ± 7.4	−10.2 ± 9.0
Δ Coronary artery diameter (adenosine 2.4 mg/min) (%)	5.8 ± 6.3	6.3 ± 4.5	8.2 ± 3.3	4.2 ± 4.1	5.3 ± 2.9	6.3 ± 4.7
Δ Coronary artery diameter (nitroglycerin 200 μg) (%)	6.8 ± 5.1	6.3 ± 5.5	11.1 ± 5.3	5.1 ± 5.3	5.7 ± 3.5	8.5 ± 7.1

[a]Significant difference (at least $P < 0.05$) when compared with baseline values.
[b]Significant difference (at least $P < 0.05$) when compared with control group.

catheter (Transit Infusion Catheter, Cordis, Miami, FL, USA) was placed over a 0.036 cm guidewire containing a 12 MHz, pulsed-Doppler ultrasound velocimeter (FlowMAP, Cardiometrics, Endosonics, Rancho Cordova, CA, USA) to measure intracoronary blood flow velocity. The corresponding diameter of the coronary vessel was measured by quantitative angiography, and coronary blood flow was calculated by multiplying the coronary sectional area and the mean peak blood velocity. Endothelial function was assessed by administering the following through an intracoronary infusion catheter: saline (0.9%), acetylcholine (10 mg/mL; Dispersa, Germering, Germany), adenosine (3 mg/mL; Schwarz Pharma, Monheim Germany), acetylcholine in increasing doses (0.072, 0.72, and 7.2 μg/min), and again adenosine (2.4 mg/min). After return to baseline values, an intracoronary bolus of nitroglycerin (200 μg; 1 mg/mL; Schwarz Pharma) was injected. On coronary angiograms, the mean diameter of a 10 mm segment of interest was measured by an automated edge-detection algorithm (Medis, Leiden, The Netherlands), and its response to acetylcholine was compared with the initial diameter after saline infusion. Maximal flow-dependent coronary vasodilatation was calculated by measuring changes in the target-vessel diameter proximal to the tip of the infusion catheter after the administration of adenosine. The results were expressed in absolute values and in percentage of changes.

Intravascular ultrasound

An intravascular ultrasound (IVUS) catheter was advanced over the coronary guidewire (Ultracross™ 2.9, Boston Scientific, Natick, MA, USA). ECG-gated cross-sections of the vessel were obtained during motorized catheter pullback, digitally recorded and later analysed using the 3D IVUS Quantification Package software (Tomtec Imaging Systems, Unterschleissheim, Germany). Total plaque volume in the target vessel section was calculated by the software using Simpson's rule and divided by total vessel volume (also calculated by the software) to obtain the coronary plaque burden (%).[6]

mRNA expression of diabetes and oxidative stress candidate genes in skeletal muscle

Selection of diabetes candidate genes was based on our previous results[4,7] which demonstrated an effect of exercise on skeletal muscle gene expression. mRNA levels of adiponectin receptors R1 and R2 (AdipoR1 and AdipoR2), glucose transporter-4 (Glut4), IL-6, endothelial cell nitric oxide synthases (eNOS), peroxisome proliferator-activated receptor-α (PPARα) and -γ (PPARγ), PPARγ-coactivator-1α (PGC-1α), subunits of NADPH oxidase (gp91 and p22), and haem oxygenase 1 (HO-1) were measured. We homogenized muscle samples and isolated total RNA, which was reverse transcripted into cDNA by using random hexamer primer transcriptase (Superscript II; Invitrogen, Karlsruhe, Germany). Quantitative real-time PCR was performed using a Light Cycler System (Roche Diagnostic Inc., Mannheim, Germany) to analyse p22, gp 91, HO-1, PPARγ and -α. The results for mRNA expressions were normalized to the expression of 18S rRNA, which was amplified as house keeping gene (18s-rRNA-1: 5′-AAA CGG CTA CCA CAT CCA AG-3′, 18srRNA 2: 5′-CGC TCC CAA GAT CCA ACT AC-3′). AdipoR1 and AdipoR2, Glut4, IGF-1, eNOS, and PGC-1 were analysed by TAQMAN (Applied Biosystems; Darmstadt, Germany). All primers and the annealing temperatures will be made available upon request.

Assays

Plasma concentrations of insulin were measured as previously described[8] and plasma adiponectin was assessed using a radioimmunoassay kit (Linco Research, St Charles, MO, USA). Serum high-sensitive C-reactive protein was measured by immunonephelometry (Dade-Behring, Milan, Italy). Serum levels of sICAM-1 and sVCAM-1 were determined by the use of monoclonal antibody-based ELISA assays (R&D Systems, Oxford, UK) as previously described.[8] All samples were tested at least in duplicate. Both intraassay and interassay coefficients of variation were less than 5%.

Statistical analyses

Data are expressed as mean + SD. Absolute values and percent changes in relation to baseline measurements were analysed. Comparisons within groups were made using paired *t*-tests or the non-parametric Wilcoxon signed-rank test, where appropriate. Between group comparisons were performed by unpaired *t*-tests or the non-parametric Mann–Whitney *U* test, respectively. All statistical analyses were performed with SPSS 13.0 (SPSS Inc., Chicago, IL, USA). *P*-values less than 0.05 were considered statistically significant.

Results

Patients' characteristics at study begin

At the beginning of the study, patients' characteristics (*Table 1*) and patients' medication were well comparable between groups: intervention group: statins ($n = 10/11$), ACE-inhibitors ($n = 9/11$), aspirin/clopidogrel ($n = 10/11$), oral anti-diabetic drugs (OAD) ($n = 6/11$), or insulin ($n = 5/11$); control group: statins ($n = 10/12$), beta-blockers ($n = 11/12$), ACE-inhibitors ($n = 10/12$), aspirin/clopidogrel ($n = 10/12$), OAD ($n = 6/12$), or insulin ($n = 6/12$). Medication and dosage remained unchanged throughout the study period, with the exception of insulin, which was adjusted according to glucose levels.

Body weight, exercise capacity, glucose metabolism, and dyslipidaemia

In the intervention group, body weight was most strikingly reduced after 4 weeks in hospital ($P < 0.005$), but worsened during the following 5 months at home (*Table 1*). Exercise capacity and duration improved significantly. These parameters remained unchanged in the control group.

Only patients on oral anti-diabetic therapy were included in the analyses of fasting plasma insulin concentrations, which showed a significant improvement in the intervention group after 4 weeks and 6 months (*Table 1*). A significant correlation between fasting insulin plasma concentrations and HOMA-IR and improvement of endothelial dysfunction with gain of coronary blood flow after infusion of acetylcholine was found (both $r = -0.733$, $P = 0.025$). Fasting plasma glucose, HbA_1c, total and LDL-cholesterol were significantly reduced after 4 weeks of intervention, but at 6 months returned to values comparable to those at study begin. During the entire study, there were no significant changes in exercise capacity and metabolic parameters in the control group.

Coronary endothelial function

Comparing the vasoreactivity at study begin, we found that both groups showed paradoxical vasoconstriction to acetylcholine (*Table 2*; individual results: *Figures 1* and *2*).

In the intervention group (*Figures 1A* and *2A*), there were no changes observed after 4 weeks, but after 6 months there was

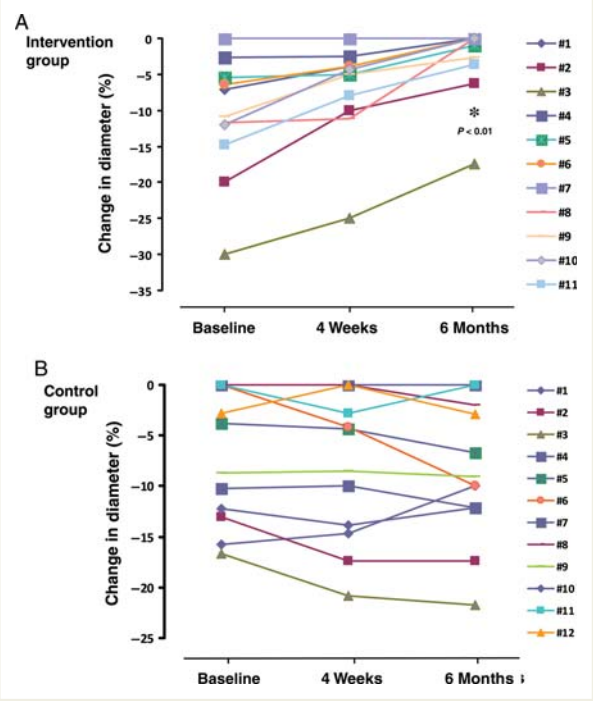

Figure 1 (*A* and *B*) Acetylcholine-induced changes in coronary artery diameter. Changes in coronary artery diameter in response to acetylcholine in a dose of 7.2 μg/min (*A*; $n = 11$) and control group (*B*; $n = 12$). *$P < 0.01$ study begin vs. 6 months within the study group.

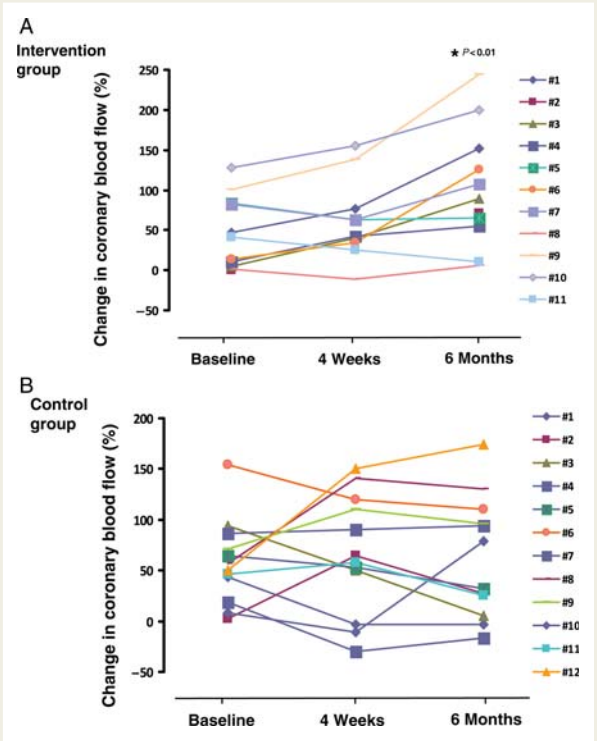

Figure 2 (*A* and *B*) Acetylcholine-induced changes in coronary artery blood flow. Changes in coronary flow in response to acetylcholine in a dose of 7.2 μg/min at study begin, after 4 weeks and 6 months in the intervention group (*A*; $n = 11$) and control group (*B*; $n = 12$). *$P < 0.01$ study begin vs. 6 months within the study group.

a significant attenuation of acetylcholine-induced vasoconstriction expressed in relative change of diameter (*Figure 1A*) and a relative increase in the change of coronary blood flow when compared with measurements obtained at the beginning of the study (*Figure 2A*), whereas results remained statistically unchanged in the control group (*Figures 1B* and *2B*). Between both groups no significant changes were seen.

These results were mirrored by adenosine infusion, where in the intervention group a significant increase in the relative change of coronary blood flow when compared with measurements obtained at the beginning of the study was not observed at 4 weeks but after 6 months. Furthermore, after 6 months, the percent change of coronary blood flow, i.e. the difference in blood flow after administration of acetylcholine or adenosine vs. saline infusion (=baseline), was significantly higher in the intervention group when compared with the control group ($P < 0.05$). The endothelium-independent vasodilatation induced by nitroglycerin was not statistically different within or between groups at any time (*Table 2*). Also, there were no significant differences between groups in any absolute variable measurement.

Intravascular ultrasound

Plaque burden did not vary between the two groups at any time point (all $P > 0.1$). During the entire study period, there were no significant changes in both the intervention group (study begin: 51.7 ± 18.9%; 4 weeks: 56.7 ± 23.3%, $P = 0.4$; 6 months: 57.8 ± 24.5%, $P = 0.08$) and the control group (study begin: 51.4 ± 11.8%; 4 weeks: 50.4 ± 11.3%, $P = 0.3$; 6 months: 54.2 ± 11.4%, $P = 1.0$).

Pro- and anti-inflammatory serum markers

In the intervention group, there was a strong trend for a decrease in sICAM-1 at 4 weeks ($P = 0.051$) as well as a significant reduction in high-sensitive C-reactive protein and adiponectin, which at 6 months all returned to levels comparable to those measured at study begin. In the control group, values remained essentially unchanged.

mRNA expression in skeletal muscle

mRNA expression of AdipoR1, AdipoR2, Glut4, IL-6, eNOS, PGC-1α showed significant increase after 4 weeks and 6 months (*Figure 3A*; $P < 0.05$). A different time course in mRNA expression was observed for oxidative stress related genes p22, gp91, and HO-1 as well as for the diabetes candidate genes PPARα and PPARγ (*Figure 3B*). Their maximum was after 4 weeks of intervention and showed a decline in expression after 6 months. In the control group, no significant changes were found.

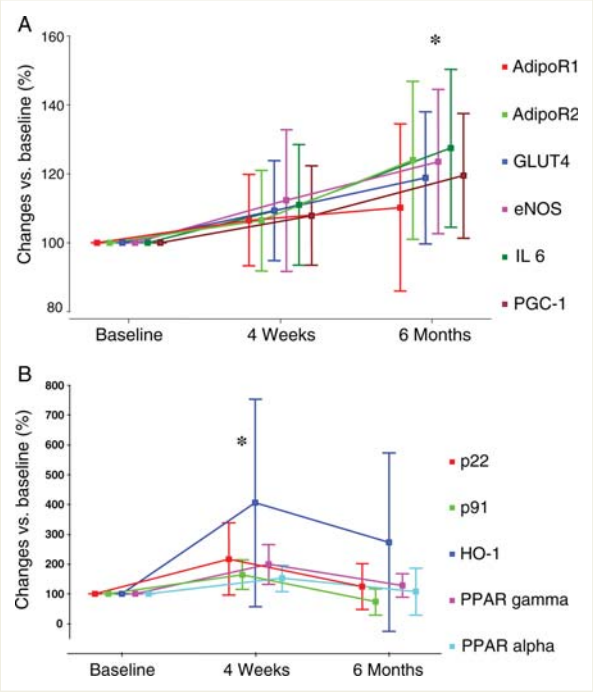

Figure 3 (*A* and *B*) mRNA expression of diabetes candidate genes in skeletal muscle. (*A*) mRNA expression of AdipoR1, AdipoR2, Glut4, IL-6, eNOS, PGC-1α showed a linear increase during the entire study period and reached a peak after 6 months. (*B*) Oxidative stress-related genes p22, gp91, haem oxygenase, PPARα, and PPARγ had a maximum mRNA expression after 4 weeks of intervention and showed a decline in expression after 6 months. Data in the control group remained unchanged (data not shown). $*P < 0.05$ study begin vs. 6 months within the study group.

Discussion

The major findings of our study are as follows.

(1) Multifactorial intervention with focus on exercise training improves coronary endothelial function in patients with T2DM.
(2) Intramural coronary lesions of less than 25% documented by quantitative coronary angiography and plaque burden analysed by IVUS remain unaffected.
(3) Serum markers of inflammation, and oxidative stress-related genes show most pronounced improvement after 4 weeks of intensive mulitfactorial intervention when compared with the subsequent 5 months of home-based exercise training during which patients still exercised far more than recommended by current guidelines.
(4) mRNA expression of diabetes candidate genes peak after 6 months of intervention, and thus delayed when compared with serum markers.

Endothelial dysfunction

Exercise training has been shown to improve coronary endothelial function in normoglycaemic patients,[1] but not yet in T2DM. However, in T2DM, there are several reports on improved exercise induced endothelial dysfunction in peripheral arteries.[9,10] Data from our group have identified exercise training to be more potent than the thiazolidinedione rosiglitazone.[11] Furthermore, there are several reports on beneficial diet-[12,13] and statin-induced changes[14] on the endothelium of the brachial artery.

In this study, we found that 6 months of multifactorial intervention which focused on vigorous exercise training significantly improved coronary endothelial function. Both coronary vasoconstriction in response to acetylcholine- and adenosine-induced flow-dependent vasodilatation were significantly attenuated. In comparison, in normoglycaemic patients endothelial dysfunction already improved after 4 weeks.[1] This suggests that altered glucose homeostasis and insulin resistance in T2DM contribute to a delayed restoration of endothelial function, possibly as a result of the more generalized atherosclerosis of the coronary tree with fewer healthy endothelium available to produce NO in response to exercise induced shear stress. Our findings are in line with a report by Giannuzzi *et al.*[15] in patients with ischaemic heart failure who could demonstrate a favourable effect on ventricular function and its anti-remodelling process after long-term rehabilitation.

Furthermore, multifactorial interventions which focus on exercise training have been shown to slow the progression and in some patients induce regression of coronary artery disease.[16,17] According to our data, it cannot be expected that a 6 months multifactorial intervention that focuses on exercise training induces detectable changes on the intramural plaque burden of stenoses $\leq$25%.

Glucose and lipid metabolism

Hyperglycaemia can affect endothelial function by adversely altering leucocyte adhesion, intercellular permeability, growth, and production of vasoactive substances.[18,19] Endothelial dysfunction can also be induced by combined adverse effects of hyperglycaemia and increased free fatty acids or reduced insulin action.[20] In our study, we did not find significant correlations between improved markers of acute or chronic hyperglycaemia and improved coronary endothelial dysfunction, suggesting that chronic hyperglycaemia is not the primary factor linking T2DM to endothelial dysfunction.

Insulin resistance may contribute to endothelial dysfunction via impaired insulin-mediated vasodilatation and diminished endothelial NO production.[21] In our study, fasting insulin plasma concentrations and HOMA-IR correlated with attenuated coronary vasoconstriction in response to acetylcholine. This suggests that beneficial effects may be primarily mediated by improved insulin sensitivity. In accordance with this, we found a continuously increased GLUT4 mRNA expression in skeletal muscle in response to training.

For fasting plasma glucose, high-sensitive C-reactive protein and HbA1c a significant improvement after 4 weeks but a return to levels comparable to those measured at study begin was observed after 6 months. There was also a slight re-gain in weight during the 5 months at home which correlated with increased levels of C-reactive protein and ICAM. This documents that the stricter surveillance during hospital stay resulted in superior effects when compared with the patient-chosen diet and a home-based exercise

training of lesser intensity, although this program still exceeded recommendations in current guidelines.[22] Data from our and other groups clearly indicate the need for long-term and preferably supervised out-patient rehabilitation programs in order to achieve life-long life style changes in these patients.

Patients with low high-sensitive C-reactive protein levels have better clinical cardiovascular outcomes independent of their LDL-cholesterol.[23] In this study, 4 weeks of training resulted in a significant decrease in high-sensitive C-reactive protein, whereas after 6 months high-sensitive C-reactive protein returned to levels comparable to those at study begin. Our findings suggest that improvement in inflammatory parameters precede the attenuation of endothelial dysfunction.

Plasma concentrations of adhesion molecules are elevated in patients with impaired glucose tolerance or T2DM[8] and exercise training leads to a near normalization of ICAM-1 and VCAM-1.[24] However, we did not find a relationship between improved coronary endothelial dysfunction and changes in adhesion molecule plasma concentrations.

Adiponectin is an adipocyte-derived peptide with anti-inflammatory and insulin-sensitizing properties, and high concentrations are independently associated with a reduced risk of T2DM.[25] There is some controversy about whether physical exercise increases adiponectin concentrations[7] or not.[26] In our study, we found an initial decrease of circulating adiponectin serum concentrations, but after 6 months, measurements were indistinguishable from those at study begin.

We further sought to identify changes in skeletal muscle gene expression in response to exercise training and were able to identify increased mRNA expression after 4 weeks with a near return after 6 months to levels comparable to those at study begin for oxidative stress-related genes p22, gp91, and haem oxygenase as well as for PPARα and PPARγ. NAD(P)H oxidase is a major source of reactive oxygen species[27,28] and it consists of several subunits of which p22 and gp91 are membrane associated. Increased expression of these NAD(P)H subunits may contribute to oxidative stress in skeletal muscle of patients with T2DM.[29] Our data confirm this concept by the finding that p22, gp91, and haem oxygenase are significantly increased after 4 weeks of exercise. The decrease after 6 months, although not reaching significance, could be considered an adaptation to chronic exercises training. This suggests that increased oxidative stress, which is present in T2DM, can be improved by an intervention that focuses on exercise training.

Peroxisome proliferator-activated receptors are transcriptional factors belonging to the family of ligand-inducible nuclear receptors and they are known to be involved in the control of chronic diseases-like diabetes, obesity, and atherosclerosis.[30] After 4 weeks of intervention, we observed increased expression of both PPARα and γ, whereas after 6 months expression returned to levels similar to those seen at study begin. It is still open to debate, whether an increase or decrease of PPARγ after physical exercise is beneficial.

mRNA expression of AdipoR1, AdipoR2, IL-6, eNOS, and PGC-1α increased after 4 weeks of intervention and were even higher after 6 months. For AdipoR1 and AdipoR2 expressions, an association with body composition, insulin sensitivity, and metabolic parameters has been reported.[31] Our results confirm previous observations that an exercise intervention of 4 weeks up-regulates expression of both adiponectin receptors in skeletal muscle,[32] which mediates improvement of insulin resistance and metabolic syndrome.

Interleukin-6 is affected by exercise intensity and duration, as well as energy availability.[33,34] In accordance with the previous reports,[35] we found a continuous increase in IL-6 mRNA expression over the entire study duration in skeletal muscle.

PGC-1α belongs to the family of transcriptional co-activators and is differentially regulated by training.[36] Increased PGC1α mRNA has been reported to have a beneficial influence on insulin sensitivity and cell proliferation and may therefore mediate the positive effects of training on endothelial dysfunction. Training generally increases PGC-1α, which is in accordance with our results.

Nitric oxide synthase (NOS) synthesizes NO, the major endogenous vasodilator in the vascular system,[37] which is stimulated by a variety of receptor agonists and shear stress produced by the flowing blood.[38,39] In this study, we found an induction of eNOS mRNA in skeletal muscle at 4 weeks and even higher levels at 6 months, confirming the role of NOS in chronic exercise training.

In conclusion, in patients with T2DM and coronary artery disease, a multifactorial intervention with focus on exercise training results in improved endothelial function after 6 months. Results obtained by IVUS show that an exercise program that exceeds recommendations of current guidelines, does not lead to a regression of haemodynamically not yet relevant intramural plaques.

Our intervention not only led to beneficial changes of markers of hyperglycaemia, insulin sensitivity, and inflammation, but also induced several previously unrecognized changes in mRNA expression in skeletal muscles, which could likely contribute to the beneficial effects of exercise on endothelial function.

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