Available online at www.sciencedirect.com
ScienceDirect
Metabolism Clinical and Experimental 55 (2006) 1508–1515
Metabolism
Clinical and Experimental
www.elsevier.com/locate/metabol

Endothelial function and biochemical vascular markers in first-degree relatives of type 2 diabetic patients: the effect of exercise training

Torben Østergård[a,b,*], Birgit Nyholm[a], Troels K. Hansen[a], Lars M. Rasmussen[c], Jørgen Ingerslev[d], Keld E. Sørensen[e], Hans E. Bøtker[e], Bengt Saltin[f], Ole Schmitz[a,b]

[a]Department of Endocrinology and Diabetes M, Aarhus University Hospital, Aarhus Sygehus, DK-8000 Aarhus C, Denmark
[b]Department of Clinical Pharmacology, University of Aarhus, DK-8000 Aarhus, Denmark
[c]Department of Clinical Biochemistry, Aarhus University Hospital, Aarhus Sygehus, DK-8000 Aarhus, Denmark
[d]Department of Clinical Immunology and Coagulation Laboratory, Aarhus University Hospital, Skejby Sygehus, DK-8200 Aarhus, Denmark
[e]Department of Cardiology, Aarhus University Hospital, Skejby Sygehus, DK-8200 Aarhus, Denmark
[f]Copenhagen Muscle Research Center, Rigshospitalet, DK-2400 Copenhagen, Denmark

Received 18 January 2006; accepted 29 June 2006

Abstract

Endothelial dysfunction (ED) is associated with the presence of atherosclerosis. However, ED is also considered a sign of the early vascular changes preceding atherosclerosis. By measuring flow-mediated vasodilation (FMD) and circulating markers of endothelial function we sought to explore whether impaired endothelial function is already present in healthy subjects at increased risk of developing type 2 diabetes mellitus. Furthermore, we aimed to assess the impact of short-term lifestyle intervention (10 weeks endurance exercise) on the potentially primary defects of endothelial function. Twenty-nine healthy but insulin-resistant first-degree relatives of patients diagnosed with type 2 diabetes mellitus (33 ± 5 years; body mass index, 26.3 ± 1.6 kg/m^2) were compared with 19 control subjects without a family history of diabetes mellitus (31 ± 5 years; body mass index, 25.8 ± 3.0 kg/m^2). At baseline the von Willebrand factor was significantly increased in the relatives ($P < .05$). Furthermore, mannose-binding lectin ($P = .06$), soluble intercellular adhesion molecule 1 ($P = .08$), and osteoprotegerin ($P = .08$) tended to be increased in relatives. The following markers of endothelial function were comparable at baseline: FMD, C-reactive protein, plasminogen activator inhibitor 1, and soluble vascular cell adhesion molecule 1. Exercise training resulted in a decrease in mannose-binding lectin ($P = .02$) and osteoprotegerin ($P < .01$) in relatives only, whereas other biochemical markers were unaffected in both groups. Moreover, the relatively high-intensity exercise training tended weakly to reduce FMD in the relatives ($P = .15$). In conclusion, healthy subjects predisposed for type 2 diabetes mellitus show only minor signs of endothelial dysfunction. Under these almost normal vascular conditions, exercise training has little effect on endothelial function.

1. Introduction

Type 2 diabetes mellitus develops gradually from asymptomatic insulin resistance, and beta-cell dysfunction through impaired glucose tolerance and ultimately turning into overt type 2 diabetes mellitus [1]. During the phases of deteriorating insulin sensitivity and glucose tolerance the pathophysiologic processes giving rise to the diabetic complications may also be in progress [2]. The main mechanisms behind the complications of diabetes mellitus are functional and structural abnormalities in the micro- and macrovascular system. Thus, in type 2 diabetic subjects, atherosclerosis is more extensive and diffuse compared with a healthy population, and diabetic patients have a considerably higher risk of developing cardiovascular disease (CVD) [3]. Endothelial dysfunction, most often defined as the reduced capacity of endothelial nitric oxide (NO) production, is known to precede atherosclerosis and is considered a risk marker for future development of CVD [4]. In type 2 diabetic subjects, endothelial dysfunction is a very consistent finding [5]. The processes leading to atherosclerosis are thought to be related to a subclinical systemic inflammatory condition. In line with this hypothesis, patients with CVD and type 2 diabetes

* Corresponding author. Department of Medicine M (Endocrinology and Diabetes), Aarhus University Hospital, Aarhus Sygehus, DK-8000 Aarhus C, Denmark. Tel.: +45 8949 2015; fax: +45 8949 2010.
E-mail address: oest@dadlnet.dk (T. Østergård).

0026-0495/$ – see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2006.06.024

mellitus have increased levels of inflammatory markers [6]. It is controversial whether endothelial dysfunction in diabetic subjects is a primary abnormality in the vasculature, or is induced by an inflammatory condition or the deranged metabolic milieu. First-degree relatives of patients with type 2 diabetes mellitus (offspring) have a high risk of developing type 2 diabetes mellitus later on in life [7]. Thus, the study of healthy and normal glucose–tolerant offspring may give indications of the primary events in the development of type 2 diabetes mellitus and its complications.

Efforts to stop or slow the progression of the atherosclerotic processes should have high priority in potentially prediabetic subjects to prevent future disease. Exercise reduces the risk of type 2 diabetes mellitus in subjects with impaired glucose tolerance [8,9] and also improves endothelial function in people with overt type 2 diabetes mellitus [10]. However, whether the improvement of endothelial function by exercise in those studies is a direct effect on the vasculature or may be mediated by the concomitant metabolic improvement is not clear.

Consequently, the present study was undertaken to investigate whether endothelial dysfunction is already present in potentially prediabetic subjects not yet influenced by significant metabolic derangement (diabetes or impaired glucose tolerance). Moreover, we wanted to explore whether offspring respond to exercise training with respect to markers of vascular function similar to healthy subjects without a family history of diabetes. To this end we assessed the capability of flow-mediated vasodilation (FMD) and measured markers of endothelial dysfunction, inflammation, endothelial activation, and coagulation/fibrinolysis in first-degree relatives of type 2 diabetic subjects and a matched control group before and after endurance exercise training. C-reactive protein (CRP) and mannose-binding lectin (MBL) are 2 circulating substances synthesized by hepatocytes and involved in the acute-phase response [11,12]. The von Willebrand factor (vWF) is an endothelial-derived glycoprotein that is involved in primary hemostasis. Elevated plasma concentrations of vWF are considered a measure of endothelial dysfunction [13]. Plasminogen activator inhibitor 1 regulates fibrinolysis, and high levels have been shown to predict development of CVD and type 2 diabetes mellitus [14,15]. Soluble vascular cell adhesion molecule 1 (s-VCAM-1) and soluble intercellular adhesion molecule 1 (s-ICAM-1) are expressed on endothelial cells after stimulation by inflammatory cytokines. They mediate leukocyte adhesion, and increased expression is related to increased risk of CVD and insulin resistance [16,17]. In addition, we measured a novel putative marker of endothelial matrix changes, osteoprotegerin (OPG).

2. Methods

2.1. Subjects

Twenty-nine first-degree relatives of patients diagnosed with type 2 diabetes mellitus were compared with 19 control subjects without any family history of diabetes and matched groupwise by sex, age, and body mass index (BMI). Four offspring and 3 controls did not volunteer for the assessment of FMD. They did not differ anthropometrically or metabolically from the remainder. The offspring were recruited either through their parents diagnosed with type 2 diabetes mellitus from the outpatient clinic at Medical Department M, Aarhus University Hospital, Denmark, or like control subjects, through advertisements in local newspapers. All subjects were required to be of white inheritance; aged 20 to 50 years; with a BMI of less than 30 kg/m^2; and to exhibit a normal glucose tolerance, a sedentary lifestyle (not engaged in regular physical activity), and maximum oxygen consumption (V_{O_2}max) of less than 50 mL kg^{-1} min^{-1}. In the offspring group, 3 subjects had one known family member with type 2 diabetes mellitus (a parent) and 26 subjects had 2 or more family members (at least one parent) known to be diagnosed with type 2 diabetes mellitus. Eight had a maternal history and 21 had a paternal family history of type 2 diabetes mellitus. None (offspring and controls) were related. All subjects gave written consent to participate in the study, which was approved by the local ethical committee of the county of Aarhus, Denmark. The study complies with the guidelines proposed in the Declaration of Helsinki.

2.2. Experimental design

All subjects were examined before and after 10 weeks of exercise training. The investigational program consisted of an initial evaluation with respect to general health status and the fulfillment of inclusion and exclusion criteria. A standard oral glucose tolerance test was performed to ascertain normal glucose tolerance, and an exercise test was performed to assess aerobic physical capacity. Fasting levels of plasma lipids and the levels of circulating endothelial-related substances were also assessed. One week later, eligible subjects were examined by the hyperinsulinemic euglycemic clamp to determine peripheral insulin-stimulated glucose uptake (insulin sensitivity = M value). Finally, on a separate day, subjects were evaluated with respect to FMD by a noninvasive ultrasonographic method. All investigational procedures were repeated after 10 weeks of exercise training timed 7 days from the last exercise bout to observe the long-term effects of exercise training.

2.3. Oral glucose tolerance test

Subjects were admitted to the research unit in the morning after an overnight fast (10-12 hours). At time 0 minutes, 75 g glucose was ingested and blood samples for plasma glucose were obtained at t = 0 and t = 120 minutes.

2.4. Hyperinsulinemic euglycemic clamp

Subjects were admitted to the research unit in the morning (08:00 AM) after an overnight fast. They were placed in a bed and intravenous catheters were inserted in an antecubital vein for infusions and in a heated dorsal hand vein on

the opposite arm for blood samples. Subjects rested for 150 minutes before start of the clamp. From $t = 150$ minutes, insulin was infused at a rate of 1.0 mU kg^{-1} min^{-1} and plasma glucose was kept at 5 mmol/L by a variable infusion of glucose (200 mg/L). The interval 270 to 300 minutes was considered a "steady-state" situation, and the average glucose infusion rate in this period was taken as an expression of insulin-stimulated glucose uptake (*M* value).

2.5. Exercise tests

The exercise tests were carried out as maximal exhaustive incremental exercise tests on a cycle ergometer. However, 5 offspring and 5 control subjects were examined by a submaximal test. Each individual was assessed by the same method before and after the exercise program.

2.6. Maximal incremental test

Subjects were placed on a bicycle ergometer and were connected to a spiroergometer system by which heart rate, oxygen consumption, and carbon dioxide production were monitored continuously throughout the test (Oxycon Delta, Erich Jaeger, Würtzburg, Germany). After a short warm-up, the exercise intensity was increased by 20 or 30 W/min so that the total time of the test would not be more than 10 to 12 minutes. The test was considered sufficient if (1) subjects felt exhaustive, (2) if the respiratory exchange ratio was more than 1.1, and/or (3) heart rate was close to expected maximal heart rate (220 − age). Peak oxygen consumption (mL/min) was divided by body weight and $V_{O_2}max$ accordingly given as milliliters per kilogram per minute.

2.7. Submaximal test

Subjects were placed on a cycle ergometer and heart rate was monitored during the test. They were instructed to pedal constantly with 50 rpm, and by increasing the exercise intensity in steps of 25 W (women) or 50 W (men), heart rate was aimed to reach 125 beats per minute or more and kept constant during the fourth and sixth minute of the test. By using the exercise intensity and the actual heart rate at 4 to 6 minutes, $V_{O_2}max$ was estimated by extrapolation [18].

2.8. Exercise training regimen

All study subjects were instructed to perform aerobic exercise training on a bicycle ergometer for 10 weeks. The duration of the exercise program was chosen to be able to observe the potential long-term effects by exercise training. The exercise sessions were performed 3 times weekly, 45 minutes each session at 70% of $V_{O_2}max$. Subjects either exercised at local fitness centers or were provided an ergometer bicycle to use at home. The exercise intensity was prescribed, adjusted and monitored by heart rate. By the initial exercise test the maximal pulse rate of each individual was assessed simultaneously with the maximal aerobic capacity ($V_{O_2}max$). From these parameters the pulse rate corresponding to approximately 70% of $V_{O_2}max$ was estimated and passed on to each subject to aim for in their training sessions. Each subject was supplied a diary in which they were instructed to enter date, actual duration of each exercise session, and pulse rate at 15, 30, and 45 minutes after commencing each training session.

2.9. Measurements of endothelial function

2.9.1. Flow-mediated vasodilation

Changes in right brachial artery diameter in response to reactive hyperemia and nitroglycerin were measured with high-resolution ultrasound (Acuson 128XP/10 with a 7.0-MHz linear-array transducer, Mountain View, CA). After being placed in the supine position, blood pressure was measured before commencing the scanning sequence. The artery was scanned in longitudinal sections immediately below the elbow. Depth and gain settings were optimized to identify the lumen-to-vessel wall interface and were kept constant during each study. After a baseline scan, a blood pressure cuff placed around upper arm was inflated to more than 250 mm Hg. After 4 1/2 minutes, the cuff was released and the artery scanned continuously from 30 seconds before to 90 seconds after cuff deflation. A second baseline scan was recorded 10 minutes later. Finally, glyceryl trinitrate (GTN) (400-μg spray) was administered sublingually and the artery was scanned 3 minutes later. All scans were recorded on super-VHS tapes, and arterial diameters were measured directly from the tape by 2 independent observers blinded to the scan sequence and the identity of the subject. Vessel internal diameters were measured from the anterior to the posterior interface between the media and the adventitia. An average diameter was calculated from 4 cardiac cycles incident with the R wave on the electrocardiogram. The technique has been shown to be reliable and reproducible [19].

2.9.2. Markers of inflammation

C-reactive protein was measured by a commercially available high-sensitive CRP enzyme-linked immunosorbent (ELISA) kit (DakoCytomation, Glostrup, Denmark). Serum MBL concentrations were measured using an in-house time-resolved immunofluorometric assay [20].

2.9.3. Markers of coagulation/fibrinolysis

The vWF was measured by a sandwich ELISA technique as previously described [21]. Plasminogen activator inhibitor 1 was measured by a commercially available ELISA kit (Technoclone, Surrey, UK).

2.9.4. Markers of endothelial activation

Serum s-ICAM-1 and s-VCAM-1 were measured by commercially available ELISA kits, as described by the manufacturer (R&D Systems, Minneapolis, MN, catalog no. BBE1B, BBE3, and DY809, respectively).

2.9.5. Osteoprotegerin

Osteoprotegerin is a novel putative marker of vascular matrix changes. Plasma concentration of OPG was measured

Table 1
Baseline characteristics of study groups

	Offspring (n = 29)	Controls (n = 19)
Sex (M/F)	19/10	14/5
Age (y)	33 ± 5	31 ± 5
BMI (kg/m^2)	26.3 ± 1.6	25.8 ± 3.0
Fasting glucose (mmol/L)	5.3 ± 0.5	5.1 ± 0.3
HbA_{1c} (%)	5.2 ± 0.4	5.1 ± 0.3
M (mg kg^{-1} min^{-1})	5.5 ± 1.9*	7.2 ± 2.6
Systolic blood pressure (mm Hg)	130 ± 8	129 ± 7
Diastolic blood pressure (mm Hg)	78 ± 4	77 ± 3
V_{O_2}max (mL kg^{-1} min^{-1})	38 ± 6.5	40.8 ± 6.1

HbA_{1c} indicates hemoglobin A_{1c}.
* $P < .05$ (Student *t* test).

in duplicate by a sandwich ELISA method as previously described [22] (R&D Systems, catalog no. DY805).

2.9.6. Other laboratory procedures

Plasma glucose was measured in duplicate immediately after sampling (Beckman Instruments, Palo Alto, CA). Hemoglobin A_{1c} was determined by high-pressure liquid chromatography (reference range, 4.8%-6.4%). Plasma lipids (cholesterol, low-density lipoprotein [LDL] cholesterol, high-density lipoprotein cholesterol, and triglycerides) were analyzed at the Department of Clinical Biochemistry, Aarhus University Hospital, by an enzymatic colorimetric method (Cobas Integra 700, Hoffmann-La Roche, Basel, Switzerland).

2.10. Statistical analyses

Data are given as mean ± SD for parametrically distributed data. Nonparametrically distributed data are given as geometric mean or median and interquartile range. Student *t* test for independent data or Mann-Whitney *U* test was used to test differences between groups. Pre-post values were analyzed by Student *t* test for paired data or the Wilcoxon signed rank test. Bivariate correlations were done by the Pearson product moment correlation test or the Spearman correlation test. All statistical analyses were performed using SPSS for Windows version 11.0 (SPSS, Chicago, IL).

3. Results

3.1. Baseline characteristics

The offspring group was insulin-resistant as shown by the reduced *M* value, but was otherwise comparable to control subjects. Systemic blood pressure was comparable in the 2 groups, and controlling for blood pressure in the analyses did not affect the results. In response to the training program, both offspring and controls exhibited significant improvements in V_{O_2}max (14.1% ± 11.3% [$P < .05$] vs 16.1% ± 14.2% [$P < .05$]; P = not significant [NS]) and insulin sensitivity (9.3% ± 22.0% [$P < .001$] vs 11.4% ± 33.2% [$P < .001$]; P = NS). Adherence to the training protocol was similar in both groups (97% ± 6% vs 97% ± 5%; P = NS). Weight changes were comparable in the 2 groups (−0.7 ± 1.6 vs 0.9 ± 2.6 kg; P = NS) (Table 1).

3.2. Lipids and markers of endothelial function

3.2.1. Endothelium-dependent vasodilation

There were no differences between the 2 groups in the diameter of the brachial artery before and after exercise training (Table 2). At baseline, neither FMD nor GTN-provoked vasodilation differed between offspring and control subjects. There were no significant changes in FMD after exercise training in either group, although a weak tendency of reduced FMD was present in first-degree relatives (ΔFMD [%], −1.34%; P = .15) (Fig. 1).

Table 2
Markers of endothelial function and circulating lipids before and after 10 weeks of exercise training in healthy relatives of type 2 diabetic subjects (offspring) and a matched control group of subjects without diabetic predisposition

	Preexercise training		*P*	Postexercise training		*P*	*P* (ex-effects)[a]
	Offspring	Controls		Offspring	Controls		
FMD (%)	10.0 ± 3.6	8.3 ± 5.8	NS	8.6 ± 3.5	8.3 ± 3.9	NS	.15/.98
GTN (%)	23.0 ± 7.5	22.0 ± 10.8	NS	21.7 ± 6.0	23.8 ± 7.1	NS	NS
Artery diameter (mm)	2.87 ± 0.52	2.82 ± 0.53	NS	2.88 ± 0.49	2.85 ± 0.52	NS	NS
Hyperemia (%)	618 ± 331	732 ± 422	NS	715 ± 397	754 ± 371	NS	NS
Cholesterol (mmol/L)	5.1 ± 0.8	4.6 ± 0.9	<.05	5.1 ± 0.9	4.5 ± 1.1	.07	NS
LDL (mmol/L)	3.2 ± 0.6	2.7 ± 1.0	<.05	3.2 ± 0.7	2.7 ± 0.9	<.05	NS
HDL (mmol/L)	1.2 ± 0.3	1.3 ± 0.3	NS	1.2 ± 0.3	1.4 ± 0.4	NS	NS
Triglycerides (mmol/L)	1.2 (0.8-1.5)	1.1 (0.8-1.5)	NS	1.3 (0.9-1.8)	1.0 (0.7-1.3)	.08	NS
MBL (ng/mL)	1510 (310-2225)	353 (100-1280)	.06	1203 (259-2445)	288 (82-1453)	<.05	.02/.10
CRP (mg/L)	0.16 (0.10-0.28)	0.13 (0.06-0.19)	NS	0.14 (0.08-0.23)	0.14 (0.08-0.20)	NS	NS
vWF (U/mL)	0.89 (0.62-1.17)	0.67 (0.55-0.82)	<.05	0.92 (0.72-1.16)	0.60 (0.52-0.79)	<.001	NS
PAI-1 (U/mL)	10.6 (5.7-15.3)	13.0 (7.0-24.8)	NS	12.6 (9.2-15.8)	7.2 (3.8-20.0)	NS	NS
s-ICAM (μg/L)	229 (161-331)	168 (130-235)	.08	219 (152-293)	178 (139-239)	NS	NS
s-VCAM (μg/L)	594 ± 124	595 ± 114	NS	605 ± 121	625 ± 117	NS	NS
OPG (μg/mL)	1.2 (1.1-3.5)	1.1 (0.9-1.4)	.08	1.1 (0.9-3.2)	1.0 (0.9-1.4)	NS	.005/.59

Data are expressed as mean ± SD or geometric mean/median (IQR). HDL indicates high-density lipoprotein; PAI-1, plasminogen activator inhibitor 1.
[a] Offspring/controls.

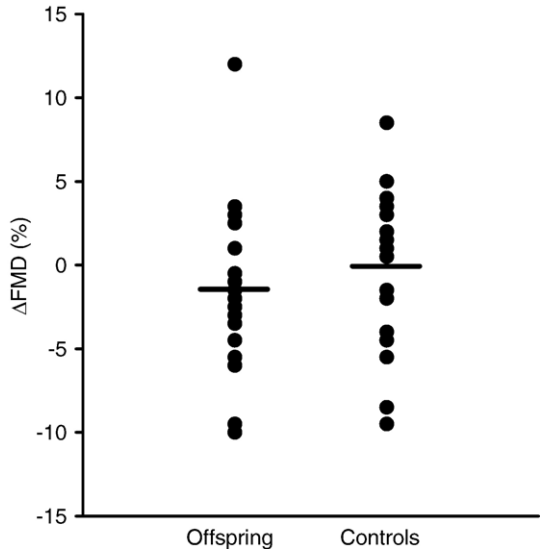

Fig. 1. Changes in FMD [ΔFMD (%)] after 10 weeks of endurance exercise in healthy offspring of type 2 diabetic patients and control subjects. No significant changes were observed, albeit a weak tendency of reduced FMD in offspring ($P = .15$).

Flow-mediated vasodilation was not correlated with insulin sensitivity (M value) or Vo_2max in any of the groups at baseline. Furthermore, changes in FMD were not associated to changes in M or Vo_2max in any of the groups.

3.2.2. Plasma lipids and inflammatory markers

Total cholesterol and LDL cholesterol levels were higher in offspring before and after the training program. We did not observe any significant changes in lipid parameters after exercise. Mannose-binding lectin was higher in offspring both before ($P = .06$) and after exercise training ($P < .05$). Furthermore, MBL was significantly reduced by exercise in offspring (ΔMBL, −13.1%; $P < .05$) and a trend toward a reduction was found in controls (ΔMBL, −14.8%; $P = .10$). Mannose-binding lectin concentrations did not correlate with insulin sensitivity or Vo_2max (baseline values or changes). C-reactive protein concentrations were similar between groups and did not change after exercise training.

3.2.3. Markers of coagulation and fibrinolysis

The vWF was increased in offspring both at baseline ($P < .05$) and after exercise training ($P < .001$). The vWF was not influenced by exercise in any of the groups. Plasminogen activator inhibitor 1 levels were similar in offspring and controls at baseline and did not change significantly in any of the groups after exercise.

3.2.4. Markers of endothelial activation

At baseline, s-ICAM-1 tended to be higher in offspring ($P = .08$). Exercise did not change s-ICAM-1 levels significantly in any group. Soluble vascular cell adhesion molecule 1 concentrations were comparable between groups at baseline and did not change after exercise training.

3.2.5. Osteoprotegerin

Initially, no statistical difference between offspring and controls could be detected in the distribution of OPG at baseline ($P = .17$). However, the statistical analysis was clearly skewed by one subject in the control group with an extremely high OPG concentration both pre- and postexercise training (25-30 μg/mL [subject] vs 1-2 μg/mL [the rest of the group]). The reason for this was not obvious. Excluding this subject, a trend toward higher OPG levels in offspring at baseline could be demonstrated (1.22 [1.07-3.46] vs 1.12 [0.94-1.42] μg/mL; $P = .08$). Moreover, OPG concentrations were significantly reduced in offspring after exercise, whereas in controls, no change in OPG levels could be detected (Table 2). At baseline, low insulin sensitivity (M value) was related to higher OPG concentrations in controls ($r = -0.47$; $P = .06$). Changes in insulin sensitivity after exercise however were not related to changes in OPG concentrations. We were not able to demonstrate associations of OPG to endothelial function (FMD), lipid parameters, or the inflammatory markers assessed in this study. Likewise, changes in these parameters after exercise were not related to changes in OPG concentrations.

4. Discussion

In the present study we demonstrate that offspring of type 2 diabetic patients only show few signs of endothelial dysfunction at a stage when they are still healthy, normotensive, and normal glucose–tolerant but insulin-resistant. Moreover, we show that exercise training affects vascular function minimally when no prior significant impairment of endothelial function is present.

Impaired endothelial function is typically seen in subjects with clinically overt metabolic dysregulation, for example, type 2 diabetes mellitus [23-25]. Nonetheless, endothelial dysfunction has previously been demonstrated in healthy and normal glucose–tolerant subjects who were predisposed for development of type 2 diabetes mellitus. In the study by Caballero et al [25], healthy relatives of type 2 diabetic patients were found to have reduced endothelium-dependent vasodilation compared with controls. However, their subjects were 20 years older than the population studied here. In a cohort more similar to the present, Balletshofer et al [26] failed to show a significant difference in FMD between offspring and controls, but were able to demonstrate an association between endothelial dysfunction and insulin resistance in offspring. It has previously been proposed that endothelial dysfunction is a condition progressing with the severity of insulin resistance [27], and this hypothesis seems to be in accordance with our findings of almost normal endothelial function in healthy offspring. Apparently, the metabolic impairment in our population was not as pronounced yet as to have caused endothelial dysfunction detectable by the methods applied here.

In the current study, endothelial function as assessed by FMD did not improve in either offspring or controls after

exercise training. Indeed, in contrast to what we anticipated, FMD tended weakly to decrease in offspring after the training period. Although such tendency obviously is of uncertain significance it seems to be in contrast with the generally favorable effects of exercise on cardiovascular morbidity and mortality that have been shown in previous studies (eg, reference [28]). Furthermore, a positive effect of exercise on the endothelium has been suggested as an important mechanism by which exercise diminishes the risk of CVD [29]. Endothelium-dependent vasodilation has previously been reported to increase after exercise training in healthy subjects [30,31] and in individuals with the polymetabolic syndrome [32]. However, Bergholm et al [33] reported lower FMD after 12 weeks of high-intensity running and presented evidence of a simultaneous reduction in circulating antioxidant concentrations. Differences in both the exercise regimen and exercise intensity may explain these discrepancies. Accordingly, Goto et al [34] demonstrated that only moderate-intensity exercise improved endothelial function in healthy subjects, whereas both low-intensity and high-intensity exercise did not. The previously sedentary subjects studied in the current study performed relatively high-intensity exercise 3 times weekly for 10 weeks. The present data could suggest that this regimen after 10 weeks did not affect endothelial function markedly, but may in some have resulted in a worsening of endothelial function as assessed by FMD. As proposed [33], one possible mechanism may well have been a reduction in circulating antioxidants. Interestingly, type 2 diabetes mellitus and insulin resistance have been associated with mitochondrial dysfunction [35-37], and we have been able to show indications of reduced oxidative capacity in the offspring participating in the present study [38]. Thus, as the tendency toward a reduction in FMD in our study only was apparent in offspring, one could hypothesize that the clearance of free radicals produced by intense exercise might be impaired in offspring characterized by a degree of mitochondrial dysfunction. Obviously, these hypotheses need further testing.

The changes in endothelial function induced by exercise may also be time-dependent. Animal studies suggest that short-term exercise probably affects endothelial function by enhanced NO production, whereas in later stages, structural changes in the vasculature may predominate and consequently endothelial-dependent vasodilation will normalize [39,40]. Clearly, our results cannot be extrapolated to other training regimens and to already physically active subjects. Still, our study poses the question how exercise should be prescribed to reduce CVD risk.

We demonstrate that MBL concentrations were higher in offspring, whereas no difference was detected in CRP levels between offspring and control subjects. Furthermore, only MBL concentrations decreased after exercise training. The mechanisms responsible for these results are not apparent from this study. Elevated CRP concentration is considered a marker of subclinical inflammation and is associated with an increased risk of developing CVD [41] and diabetes [15]. Mannose-binding lectin is an important component of the innate immune system and low levels of MBL are associated with a greater susceptibility for infections [42]. However, comparable to other acute-phase proteins, MBL levels increase during acute-phase responses [12], although the changes are much slower and less pronounced than changes in CRP concentrations. In our view the increased MBL levels in offspring at baseline may in part support previous reports of the presence of low-grade inflammation in subjects at risk for CVD or diabetes [6]. Furthermore, the reduction in MBL only occurred in subjects with increased levels, suggesting a greater impact of exercise in subjects with some degree of metabolic impairment (insulin resistance). C-reactive protein levels were comparable between groups and did not change after exercise training. This might be due to a higher within-subject variation in CRP levels opposed to MBL. Moreover, the importance of CRP as a marker of increased risk for vascular disease has recently been challenged [43]. As an indication of some degree of endothelial dysfunction in offspring we found that vWF was increased in potentially prediabetic subjects compared with control subjects. Increased vWF has been shown to indicate a higher risk of future thrombotic events [13]. However, other markers of coagulability and endothelial activation were not different between the 2 groups and the variables were not affected by exercise. Overall, these data seem to indicate only minor endothelial dysfunction in subjects predisposed for type 2 diabetes mellitus at a stage when they are still healthy and glucose-tolerant but insulin-resistant.

Finally, we evaluated a potential new vascular marker, OPG. Osteoprotegerin, which was first discovered in bone, is a member of the tumor necrosis factor receptor superfamily. In addition to bone tissue, OPG is present in the arterial wall, where it has been linked to the development of vascular calcifications in both experimental [44] and human studies [45,46]. Because an increased incidence of linear media calcifications is seen in patients with diabetes [47,48] it is interesting that augmented amounts of OPG are found in individuals with diabetes [49]. Osteoprotegerin circulates in plasma, and increased concentrations have been reported both in patients with diabetes [22,46,50] and in individuals with CVD [46,50,51]. Thus, it is possible that plasma OPG, at least partly, reflects the matrix changes, which develop in parallel with vascular calcification, either as part of atherosclerotic plaques or as part of linear media calcifications in the diabetic macroangiopathy. It should be noted however that the role of OPG is still unclear, and that the production of this molecule is induced by pro-inflammatory peptides, but inhibited by insulin [49] and peroxisome proliferator-activated receptor-gamma (PPAR-γ) ligands [52], and that data suggest that it operates as an endothelial "survival factor," that is, a decoy receptor for the receptor activator of nuclear factor–B ligand and tumor necrosis factor–related apoptosis-inducing ligand. Our results, showing a tendency toward increased

OPG concentrations in offspring, significantly reduced by exercise, are compatible with the idea that plasma OPG concentrations mark the presence of early diabetic arterial changes, which can be influenced by hormonal and metabolic factors.

One limitation to the interpretation of the results from this study is the relatively low number of subjects in each group. The study was carried out as a supplement to a study examining primarily insulin sensitivity and exercise capacity in which the size of the study population was appropriate [38]. Furthermore, as in other studies examining FMD, the variance in the measurements of FMD and GTN should be taken into consideration when evaluating the data. Thus, some of the results from the present study probably may only serve as indications of mechanisms taking place in first-degree relatives, which should be confirmed in larger studies.

In conclusion, our results indicate that significant endothelial dysfunction (indications of impaired NO production, inflammation and hypercoagulability) is not present in young and healthy, but insulin-resistant offspring of type 2 diabetic patients. We speculate whether the metabolic derangement in subjects with more severe insulin resistance or type 2 diabetes mellitus may aggravate endothelial dysfunction rather than the other way around. Furthermore, based on data from this study one could speculate that intensive exercise may influence endothelial function adversely in previously unfit individuals and poses the question how exercise should be prescribed to improve endothelial function optimally.

Acknowledgments

The study was supported by grants from the Danish Diabetes Association, the Institute of Experimental Clinical Research, University of Aarhus, and the Danish Medical Research Council.

The expert assistance by Ms Bente Mortensen is highly appreciated. Ms Annette Mengel and Ms Lene Ring are thanked for their excellent technical assistance.

References

[1] Reaven GM. Banting lecture 1988 Role of insulin resistance in human disease. Diabetes 1988;37:1595-607.
[2] Haffner SM, Stern MP, Hazuda HP, et al. Cardiovascular risk factors in confirmed prediabetic individuals. Does the clock for coronary heart disease start ticking before the onset of clinical diabetes? JAMA 1990;263:2893-8.
[3] Haffner SM, Lehto S, Ronnemaa T, et al. Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. N Engl J Med 1998;339:229-34.
[4] Heitzer T, Schlinzig T, Krohn K, et al. Endothelial dysfunction, oxidative stress, and risk of cardiovascular events in patients with coronary artery disease. Circulation 2001;104:2673-8.
[5] Yki-Jarvinen H. Insulin resistance and endothelial dysfunction. Best Pract Res Clin Endocrinol Metab 2003;17:411-30.
[6] Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. Circulation 2002;105:1135-43.
[7] Martin BC, Warram JH, Krolewski AS, et al. Role of glucose and insulin resistance in development of type 2 diabetes mellitus: results of a 25-year follow-up study. Lancet 1992;340:925-9.
[8] Tuomilehto J, Lindstrom J, Eriksson JG, et al. Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. N Engl J Med 2001;344:1343-50.
[9] Knowler WC, Barrett-Connor E, Fowler SE, et al. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. N Engl J Med 2002;346:393-403.
[10] Maiorana A, O'Driscoll G, Cheetham C, et al. The effect of combined aerobic and resistance exercise training on vascular function in type 2 diabetes. J Am Coll Cardiol 2001;38:860-6.
[11] Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. N Engl J Med 1999;340:448-54.
[12] Hansen TK, Thiel S, Wouters PJ, et al. Intensive insulin therapy exerts antiinflammatory effects in critically ill patients and counteracts the adverse effect of low mannose-binding lectin levels. J Clin Endocrinol Metab 2003;88:1082-8.
[13] Lip GY, Blann A. von Willebrand factor: a marker of endothelial dysfunction in vascular disorders? Cardiovasc Res 1997;34:255-65.
[14] Thogersen AM, Jansson JH, Boman K, et al. High plasminogen activator inhibitor and tissue plasminogen activator levels in plasma precede a first acute myocardial infarction in both men and women: evidence for the fibrinolytic system as an independent primary risk factor. Circulation 1998;98:2241-7.
[15] Festa A, D'Agostino Jr R, Tracy RP, et al. Elevated levels of acute-phase proteins and plasminogen activator inhibitor-1 predict the development of type 2 diabetes: the insulin resistance atherosclerosis study. Diabetes 2002;51:1131-7.
[16] Huo Y, Ley K. Adhesion molecules and atherogenesis. Acta Physiol Scand 2001;173:35-43.
[17] Meigs JB, Hu FB, Rifai N, et al. Biomarkers of endothelial dysfunction and risk of type 2 diabetes mellitus. JAMA 2004;291:1978-86.
[18] Aastrand I. Aerobic work performance in men and women with special references to age. Acta Physiol Scand 1960;49:1-92.
[19] Sorensen KE, Celermajer DS, Spiegelhalter DJ, et al. Non-invasive measurement of human endothelium dependent arterial responses: accuracy and reproducibility. Br Heart J 1995;74:247-53.
[20] Thiel S, Moller-Kristensen M, Jensen L, et al. Assays for the functional activity of the mannan-binding lectin pathway of complement activation. Immunobiology 2002;205:446-54.
[21] Ingerslev J. A sensitive ELISA for von Willebrand factor (vWf:Ag). Scand J Clin Lab Invest 1987;47:143-9.
[22] Knudsen ST, Foss CH, Poulsen PL, et al. Increased plasma concentrations of osteoprotegerin in type 2 diabetic patients with microvascular complications. Eur J Endocrinol 2003;149:39-42.
[23] McVeigh GE, Brennan GM, Johnston GD, et al. Impaired endothelium-dependent and independent vasodilation in patients with type 2 (non–insulin-dependent) diabetes mellitus. Diabetologia 1992;35:771-6.
[24] Johnstone MT, Creager SJ, Scales KM, et al. Impaired endothelium-dependent vasodilation in patients with insulin-dependent diabetes mellitus. Circulation 1993;88:2510-6.
[25] Caballero AE, Arora S, Saouaf R, et al. Microvascular and macrovascular reactivity is reduced in subjects at risk for type 2 diabetes. Diabetes 1999;48:1856-62.
[26] Balletshofer BM, Rittig K, Enderle MD, et al. Endothelial dysfunction is detectable in young normotensive first-degree relatives of subjects with type 2 diabetes in association with insulin resistance. Circulation 2000;101:1780-4.
[27] Caballero AE. Endothelial dysfunction in obesity and insulin resistance: a road to diabetes and heart disease. Obes Res 2003;11:1278-89.
[28] Myers J, Prakash M, Froelicher V, et al. Exercise capacity and mortality among men referred for exercise testing. N Engl J Med 2002;346:793-801.

[29] Higashi Y, Yoshizumi M. Exercise and endothelial function: role of endothelium-derived nitric oxide and oxidative stress in healthy subjects and hypertensive patients. Pharmacol Ther 2004;102:87-96.
[30] Clarkson P, Montgomery HE, Mullen MJ, et al. Exercise training enhances endothelial function in young men. J Am Coll Cardiol 1999;33:1379-85.
[31] DeSouza CA, Shapiro LF, Clevenger CM, et al. Regular aerobic exercise prevents and restores age-related declines in endothelium-dependent vasodilation in healthy men. Circulation 2000;102:1351-7.
[32] Lavrencic A, Salobir BG, Keber I. Physical training improves flow-mediated dilation in patients with the polymetabolic syndrome. Arterioscler Thromb Vasc Biol 2000;20:551-5.
[33] Bergholm R, Makimattila S, Valkonen M, et al. Intense physical training decreases circulating antioxidants and endothelium-dependent vasodilatation in vivo. Atherosclerosis 1999;145:341-9.
[34] Goto C, Higashi Y, Kimura M, et al. Effect of different intensities of exercise on endothelium-dependent vasodilation in humans: role of endothelium-dependent nitric oxide and oxidative stress. Circulation 2003;108:530-5.
[35] Petersen KF, Dufour S, Befroy D, et al. Impaired mitochondrial activity in the insulin-resistant offspring of patients with type 2 diabetes. N Engl J Med 2004;350:664-71.
[36] Kelley DE, He J, Menshikova EV, et al. Dysfunction of mitochondria in human skeletal muscle in type 2 diabetes. Diabetes 2002; 51:2944-50.
[37] Lowell BB, Shulman GI. Mitochondrial dysfunction and type 2 diabetes. Science 2005;307:384-7.
[38] Ostergard T, Andersen JL, Nyholm B, et al. Impact of exercise training on insulin sensitivity, physical fitness, and muscle oxidative capacity in first-degree relatives of type 2 diabetic patients. Am J Physiol Endocrinol Metab 2006;290:E998-E1005.
[39] Oltman CL, Parker JL, Adams HR, et al. Effects of exercise training on vasomotor reactivity of porcine coronary arteries. Am J Physiol 1992;263:H372-82.
[40] Maiorana A, O'Driscoll G, Taylor R, et al. Exercise and the nitric oxide vasodilator system. Sports Med 2003;33:1013-35.
[41] Jager A, van Hinsbergh VW, Kostense PJ, et al. von Willebrand factor, C-reactive protein, and 5-year mortality in diabetic and nondiabetic subjects: the Hoorn Study. Arterioscler Thromb Vasc Biol 1999;19: 3071-8.
[42] Koch A, Melbye M, Sorensen P, et al. Acute respiratory tract infections and mannose-binding lectin insufficiency during early childhood. JAMA 2001;285:1316-21.
[43] Levinson SS, Miller JJ, Elin RJ. Poor predictive value of high-sensitivity C-reactive protein indicates need for reassessment. Clin Chem 2004;50:1733-5.
[44] Bucay N, Sarosi I, Dunstan CR, et al. Osteoprotegerin-deficient mice develop early onset osteoporosis and arterial calcification. Genes Dev 1998;12:1260-8.
[45] Dhore CR, Cleutjens JP, Lutgens E, et al. Differential expression of bone matrix regulatory proteins in human atherosclerotic plaques. Arterioscler Thromb Vasc Biol 2001;21:1998-2003.
[46] Schoppet M, Al Fakhri N, Franke FE, et al. Localization of osteoprotegerin, tumor necrosis factor-related apoptosis-inducing ligand, and receptor activator of nuclear factor–kappaB ligand in Monckeberg's sclerosis and atherosclerosis. J Clin Endocrinol Metab 2004;89:4104-12.
[47] Neubauer B, Christensen NJ, Christensen T, et al. Diabetic macro-angiopathy. Medial calcifications, narrowing, rugosities, stiffness, norepinephrine depletion and reduced blood flow capacity in the leg arteries. Acta Med Scand Suppl 1984;687:37-45.
[48] Niskanen LK, Suhonen M, Siitonen O, et al. Aortic and lower limb artery calcification in type 2 (non–insulin-dependent) diabetic patients and non-diabetic control subjects. A five year follow-up study. Atherosclerosis 1990;84:61-71.
[49] Olesen P, Ledet T, Rasmussen LM. Arterial osteoprotegerin: increased amounts in diabetes and modifiable synthesis from vascular smooth muscle cells by insulin and TNF-alpha. Diabetologia 2005;48:561-8.
[50] Browner WS, Lui LY, Cummings SR. Associations of serum osteoprotegerin levels with diabetes, stroke, bone density, fractures, and mortality in elderly women. J Clin Endocrinol Metab 2001;86: 631-7.
[51] Kiechl S, Schett G, Wenning G, et al. Osteoprotegerin is a risk factor for progressive atherosclerosis and cardiovascular disease. Circulation 2004;109:2175-80.
[52] Fu M, Zhang J, Lin YY, et al. Activation of peroxisome proliferator-activated receptor gamma inhibits osteoprotegerin gene expression in human aortic smooth muscle cells. Biochem Biophys Res Commun 2002;294:597-601.