Exercise Training Improves Vascular Endothelial Function in Patients with Type 1 Diabetes

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OBJECTIVE — Impaired endothelial function of resistance and conduit arteries can be detected in patients with type 1 diabetes. We studied whether a persistent improvement of endothelial function can be achieved by regular physical training.

RESEARCH DESIGN AND METHODS — The study included 26 patients with type 1 diabetes of 20 ± 10 years' duration and no overt angiopathy; 18 patients (42 ± 10 years old) participated in a bicycle exercise training program, and 8 patients with type 1 diabetes (33 ± 11 years old) served as control subjects. Vascular function of conduit arteries was assessed by flow-mediated and endothelium-independent dilation of the brachial artery and of resistance vessels by the response of ocular fundus pulsation amplitudes to intravenous N^G-monomethyl-L-arginine (L-NMMA) at baseline, after 2 and 4 months of training, and 8 months after cessation of regular exercise.

RESULTS — Training increased peak oxygen uptake ($V_{O_{2max}}$) by 13% after 2 months and by 27% after 4 months ($P = 0.04$). Flow-mediated dilation (FMD) of the brachial artery increased from 6.5 ± 1.1 to 9.8 ± 1.1% ($P = 0.04$) by training. L-NMMA administration decreased fundus pulsation amplitude (FPA) by 9.1 ± 0.9% before training and by 13.4 ± 1.5% after 4 months of training ($P = 0.02$). $V_{O_{2max}}$, FMD, and FPA were unchanged in the control group. Vascular effects from training were abrogated 8 months after cessation of exercise.

CONCLUSIONS — Our study demonstrates that aerobic exercise training can improve endothelial function in different vascular beds in patients with long-standing type 1 diabetes, who are at considerable risk for diabetic angiopathy. However, the beneficial effect on vascular function is not maintained in the absence of exercise.

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Cardiovascular morbidity and mortality in patients with type 1 diabetes are caused by micro- and macrovascular complications, with clinical manifestation beginning 15–20 years after the onset of diabetes (1,2). It is generally accepted that the endothelium plays a pivotal role in the maintenance of vascular function. Impaired endothelial function is detectable in patients with diseases associated with vascular complications, such as hypercholesterolemia (3), hypertension (4), or diabetes (5), in the absence of macroscopic changes of the vasculature. An important functional consequence of endothelial dysfunction is the inability to release nitric oxide (NO), the vasodilator of the underlying vascular smooth muscle cells.

Studies in patients with hypertension and hypercholesterolemia (6) or coronary artery disease (7) have suggested that improvement of endothelial function could be a surrogate therapeutic target for interventions to reduce the development of regular symptoms or clinical events. Lifestyle changes such as regular physical exercise may influence endothelial function and may in turn reduce the cardiovascular risk profile. It has been demonstrated that regular physical exercise can correct endothelial dysfunction in patients with chronic heart failure (8), hypercholesterolemia (9), and the polymetabolic syndrome (10). This would also represent an easy and generally applicable intervention to preserve or restore vascular function in diabetes.

The aim of the present study was to investigate whether increased physical activity could also influence vascular endothelial function in patients with long-standing type 1 diabetes, who are at considerable risk for vascular complications. Subjects participated in a standardized exercise training program over 4 months. The functional properties of conduit vessels were assessed by measurement of endothelium-dependent and -independent vasodilation of the brachial artery (11); the functional properties of the resistance vessels were assessed by the responses of systemic hemodynamics and ocular blood flow to systemic administration of N^G-monomethyl-L-arginine (L-NMMA) (12), an inhibitor of constitutive NO formation. To investigate if beneficial effects by training persist, vascular function tests were repeated 8 months after cessation of regular exercise.

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Abbreviations: FMD, flow-mediated dilation; FPA, fundus pulsation amplitude; GTN, nitroglycerin; L-NMMA, N^G-monomethyl-L-arginine; MAHC, modified Airlie House classification; MMT, manual muscle test; $V_{O_{2max}}$, peak oxygen uptake.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

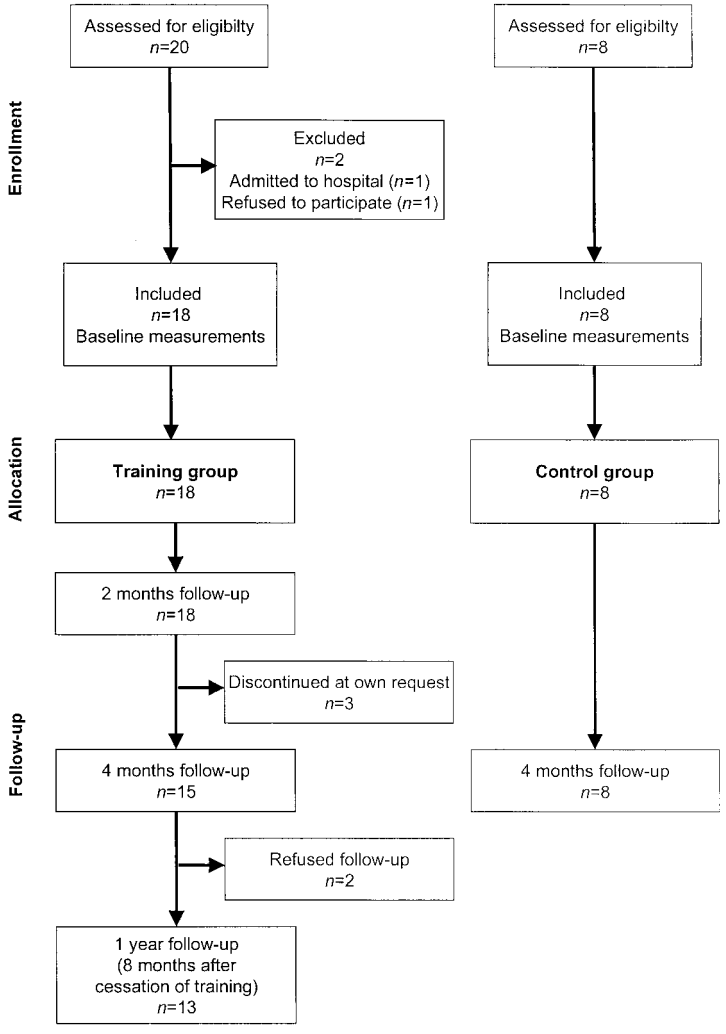

Figure 1—*Study design and participants.*

RESEARCH DESIGN AND METHODS

— The study was approved by the Ethics Committee of the University of Vienna. The investigation complies with the principles of the Declaration of Helsinki including current revisions and the Good Clinical Practice guidelines of the European Union. All subjects gave written informed consent.

Subjects

The open parallel-group study included 26 patients aged 40 ± 10 years with type 1 diabetes of 20 ± 10 years' duration (Fig. 1). Eighteen patients with type 1 diabetes (11 women and 7 men aged 42 ± 10 years) with a sedentary lifestyle before enrollment (physical exercise once a week) were included in the intervention group. Subsequently, eight patients with type 1 diabetes (three women and five men aged 33 ± 11 years), who claimed to exercise more than once weekly and to be in a good physical condition, were recruited and served as control subjects (Fig. 1). There were 13 smokers in the training group and 5 among control subjects. Physical and metabolic patient characteristics are summarized in Tables 1 and 2.

All patients were screened for diabetic microvascular complications. Two patients (one in the treatment group and one control subject) had increased albumin excretion of 36 and 32 μg/min, respectively. Microalbuminuria was <20 μg/min in all other patients. Diabetic retinopathy was classified according to the modified Airlie House classification (MAHC) (13). In the training group, 11 patients had no signs of diabetic retinopathy, 1 patient had hemorrhages and/or microaneurysms (MAHC level 2), and 6 other patients had hemorrhages and/or microaneurysms (one of them also soft exudates) (MAHC level 3). Among control subjects, seven patients were without diabetic retinopathy. One patient had hemorrhages and/or microaneurysms (MAHC level 3).

None of the subjects had uncontrolled moderate or severe systemic hypertension. Four patients had a medical history of hypertension; accordingly, two patients in the training group and two control subjects received concomitant therapy with calcium channel blockers. Hypercholesterolemia was present in four patients in the training group and two control subjects, and two patients in the training group received statin therapy. There was no evidence of autonomic cardiac neuropathy in any of the patients. Three women in the training group were postmenopausal but did not receive hormone replacement therapy. Cardiopulmonary dysfunction was excluded by transthoracic echocardiography and a lung function test (forced expiratory volume in 1 s, vital capacity) before the study.

Assessment of outcome parameters

Outcome parameters in the intervention group were measured before training, after 2 and 4 months of the aerobic training program, and 8 months after the end of the training program. Patients in the control group were followed over 4 months to confirm the reproducibility of the measurements. Exercise and muscle strength tests were performed on different days than were vascular function tests. Plasma glucose levels in the training group before vascular function tests were 141 ± 16 mg/dl before training, 127 ± 16 and 192 ± 18 mg/dl after 2 and 4 months, respectively, and 113 ± 11 mg/dl at the 8-month follow-up. Plasma glucose levels in the control group were 132 ± 38 mg/dl at baseline and 124 ± 20 mg/dl after 4 months.

Peak oxygen uptake and muscle strength

Exercise studies were performed using a symptom-limited, incremental cycle ergometer protocol. The work rate was increased by 25 W every 2 min. Ventilatory parameters were measured breath by breath using a computer-based device (Sensor Medics 2900 System; Sensormedics, Yorba Linda, CA). Peak oxygen uptake ($V_{O_{2max}}$) was defined as the maximum oxygen consumption obtained during the exercise test.

Table 1—*Physical and laboratory parameters at baseline and after 2 and 4 months of training and 8 months after regular exercise training in patients with diabetes*

	Baseline	Training period		8 months after training
		2 months	4 months	
n	18	18	15	13
Body weight (kg)	70 ± 3	69 ± 3	68 ± 3	70 ± 3
BMI (kg/m^2)	24.7 ± 0.9	24.7 ± 0.9	23.8 ± 0.8	24.4 ± 1.0
Mean arterial blood pressure (mmHg)	79 ± 2	76 ± 2	75 ± 2	79 ± 2
Resting heart rate (beats/min)	78 ± 3	76 ± 3	71 ± 3	80 ± 3
$V_{O_{2max}}$ (ml · kg^{-1} · min^{-1})	28.1 ± 1.2	31.8 ± 2.0	35.7 ± 2.8*	28.4 ± 1.8
Maximum exercise capacity (W)	151 ± 12	165 ± 14	183 ± 19	174 ± 17
Total cholesterol (mmol/l)	5.4 ± 0.3	5.1 ± 0.4	4.9 ± 0.3	4.9 ± 0.2
LDL cholesterol (mmol/l)	3.1 ± 0.3	3.0 ± 0.3	2.8 ± 0.3	2.7 ± 0.2
HDL cholesterol (mmol/l)	1.9 ± 0.1	1.7 ± 0.1	1.7 ± 0.1	1.7 ± 0.1
Triglycerides (mmol/l)	1.0 ± 0.1	1.0 ± 0.2	0.8 ± 0.1	0.8 ± 0.1
HbA_{1c} (%)	7.3 ± 0.2	7.7 ± 0.3	7.5 ± 0.3	7.0 ± 0.2
Insulin dose (units · kg^{-1} · day^{-1})	0.62 ± 0.07	—	0.51 ± 0.05*	0.60 ± 0.04

Results are means ± SE. *$P < 0.05$ vs. baseline.

Mean manual muscle test (MMT) scores for the hip flexors and knee extensors were measured in all subjects (CYBEX 6000 Isokinetic dynamometer; Lumex, Ronkonkoma, NY) (14). Handgrip muscle strength of the dominant and nondominant hands was assessed using a portable device (Digital Hand dynamometer; Jamar, Clifton, NJ). The best of three attempts was recorded.

Ultrasound measurements

A high-resolution ultrasound system with a 10-MHz transducer (Vingmed System Five; GE Medical Systems, Waukesha, WI) was used to measure brachial artery diameter (11). Each subject was in supine position with the left arm supported on a foam block and a cuff placed on the upper arm. The probe was fixed in an adjustable swivel arm to maintain an identical position on the forearm during the experiments. The brachial artery was scanned in a longitudinal section proximal to its bifurcation, which was used as an anatomical marker, and the diameter was measured at end-diastole. All measurements were performed by the same experienced operator.

Baseline vessel wall diameter was assessed as the mean of three consecutive readings. The cuff on the upper arm was inflated to suprasystolic pressure (250 mmHg) for 4.5 min and then released. Vessel diameter was measured every 30 s for the following 2 min. After a resting period of 15 min, baseline measurements were repeated and a single sublingual dose of 0.8 mg nitroglycerin (GTN) was administered. Measurements of vessel diameter were performed 5 min after GTN application.

Flow-mediated dilation of the brachial artery was expressed as percentage change of diameter following reactive hyperemia (mean of four measurements) from baseline. Endothelial-independent dilation to GTN was expressed as percentage change of diameter following drug administration (mean of four measurements) from baseline.

Ocular fundus pulsation amplitude

Pulse synchronous pulsations of the eye fundus as a measure of choroidal blood flow were assessed by laser interferometry (15). Briefly, the right eye was illuminated by the beam of a single-mode laser along the optical axis. The light was reflected at both the front side of the cornea and the retina. The two re-emitted waves produced interference fringes from which the distance changes between cornea and retina during a cardiac cycle were calculated. The method has been shown to detect changes in the pulsatile choroidal blood flow with high sensitivity and high topographical resolution (16). Fundus pulsation amplitude was calculated as the mean of three to five cardiac cycles.

Measurements of fundus pulsation

Table 2—*Outcome variables in the diabetic control group*

	Baseline	4 months
Body weight (kg)	84 ± 2	86 ± 2
BMI (kg/m^2)	26.7 ± 1.4	27.4 ± 1.4
Mean arterial blood pressure (mmHg)	80 ± 3	85 ± 2
Resting heart rate (beats/min)	76 ± 3	78 ± 4
$V_{O_{2max}}$ (ml · kg^{-1} · min^{-1})	29.6 ± 2.3	29.7 ± 2.4
Maximum exercise capacity (W)	207 ± 16	204 ± 16
Total cholesterol (mmol/l)	5.1 ± 0.4	5.0 ± 0.4
LDL cholesterol (mmol/l)	3.1 ± 0.4	2.9 ± 0.3
HDL cholesterol (mmol/l)	1.8 ± 0.3	1.6 ± 0.1
Triglycerides (mmol/l)	0.8 ± 0.1	1.0 ± 0.2
HbA_{1c} (%)	7.4 ± 0.4	7.4 ± 0.2
Insulin dose (units · kg^{-1} · day^{-1})	0.66 ± 0.10	0.64 ± 0.10
FMD (%)	9.8 ± 1.1	9.1 ± 1.4
GTN-induced dilation (%)	10.1 ± 1.2	8.9 ± 1.1
FPA reduction by L-NMMA (%)	−12.9 ± 2.3	−12.3 ± 2.1

Results are means ± SE; $n = 8$.

Table 3—*Isometric muscle strength of legs (newton meter) and hands (kilopond) before and after training*

	Before training	Training period	
		2 months	4 months
n	18	18	15
Left leg			
Extensors	165.3 ± 16.4	166.9 ± 14.1	175.4 ± 13.0
Flexors	95.7 ± 9.5	90.6 ± 7.3	99.2 ± 8.9
Right leg			
Extensors	178.9 ± 18.2	182.4 ± 16.2	193.1 ± 16.5
Flexors	102.7 ± 8.3	96.6 ± 8.0	102.5 ± 8.6
Dominant hand	60.4 ± 5.9	59.0 ± 6.3	64.6 ± 5.6
Nondominant hand	56.3 ± 5.3	55.2 ± 5.8	60.6 ± 5.9

Results are presented as means ± SE. No differences were noted after 2 and 4 months of training.

amplitude were performed with subjects in sitting position after a 15-min equilibration period. A bolus of 5 mg/kg L-NMMA (Clinalfa, Läufelfingen, Switzerland) was then administered intravenously, and measurements were repeated 15 and 30 min after start of bolus administration. The maximum effect on fundus pulsation amplitude (FPA) was used for statistical analysis.

Training program

Patients were assigned to groups of four to five patients, who participated in a 4-month training program of stationary cycling. Each exercise session lasted for ~1 h. At each training session, cycling workload was slowly increased on an individual basis. Following a 3- to 5-min warm-up, resistance was increased until a heart rate of 60–70% of the previously established difference from resting to maximum heart rate was achieved. This workload was maintained over 40 min, followed by a 5-min cool-down. During the first 2 weeks, training was carried out twice a week, and during the remaining study period, three times a week. The training was guided and supervised by a physician. The patients were allowed to carry out an additional training program at home, but compliance to training sessions had to be >60% for eligibility.

Statistical analysis

All data sets were tested for normal distribution using the Kolmogorov-Smirnov test. Baseline measurements were compared between groups using unpaired Student's *t* test or the Mann-Whitney *U* test for normally and nonnormally distributed variables, respectively. Within groups, variables were tested by the paired Student's *t* test or the Wilcoxon signed-rank test, respectively. All calculations were performed using the Statistica software package (release 4.5; StatSoft, Tulsa, OK). $P < 0.05$ was considered significant. Values are expressed as means ± SE unless indicated otherwise.

RESULTS — Figure 1 summarizes patients in the study. Eighteen patients with type 1 diabetes completed training over 2 months, and 15 patients were eligible for analysis after 4 months. Three patients discontinued the study after 2 months of training because of time constraints. Exercise session attendance (compliance) was 81 ± 7%. All eight control patients with type 1 diabetes completed the 4-month study period. Thirteen patients of the training group repeated assessment of outcome parameters 8 months after the end of the training program. None of these patients continued regular training. Two patients discontinued the study for personal reasons.

Effects of training on physical and biochemical parameters

The hemodynamic and metabolic parameters from baseline, after 2 and 4 months of aerobic exercise, and 8 months after training are presented in Table 1. $V_{O_{2max}}$ increased by 13.4 ± 8.0% after 2 months and by 27.3 ± 12.9% after 4 months of training ($P = 0.04$). Exercise capacity was increased and resting heart rate was decreased with training, but these changes did not reach the level of significance. Isometric muscle strength of both legs tended to increase with training (Table 3). Cholesterol tended to decrease, and a significant reduction of insulin requirement by 18% was observed in the training group after 4 months ($P < 0.05$). The significant effect of training on $V_{O_{2max}}$, exercise capacity and resting heart rate, and insulin requirement were abolished 8 months after the end of the training program, when the values were comparable to those before lifestyle intervention.

Maximum exercise capacity of control subjects was higher than that of the training group before exercise sessions, reflecting their good physical condition. Physical and metabolic conditions were unchanged in control subjects after 4 months (Table 2).

Conduit artery function

Brachial artery diameter was 4.0 ± 0.7 mm before training, 4.0 ± 0.6 mm after 2 months, and 4.0 ± 0.6 mm after 4 months of training (NS). The arterial diameters of the control group were 4.1 ± 0.6 mm and 3.9 ± 0.5 mm at baseline and after 4 months, respectively (NS). The endothelium-dependent vasodilatory response to reactive hyperemia (flow-mediated dilation [FMD]) was only slightly improved after 2 months, but it significantly increased after 4 months of training ($P = 0.04$) (Fig. 2). Endothelium-independent dilation to GTN also tended to increase with training (Fig. 2). FMD and endothelium-independent dilation returned to baseline values 8 months after training (Table 4).

FMD tended to be higher in the control group ($P = 0.08$ vs. training group). No changes in FMD or GTN-induced vasodilation were detectable in the control group during the 4-month observation period.

Resistance vessel responsiveness

Baseline FPA was 4.3 ± 0.3 μm before training and did not change after 2 months (4.2 ± 0.3 μm) or 4 months (4.4 ± 0.3 μm) of exercise. Before training, L-NMMA reduced FPA to a maximum of 4.0 ± 0.3 μm ($P < 0.0001$) (Fig. 3). This responsiveness was significantly enhanced by regular exercise, and a reduction of FPA to a maximum of 3.7 ± 0.3 μm and 3.8 ± 0.3 μm with L-NMMA was seen after 2 and 4 months, respectively ($P < 0.05$ vs. pretraining; NS between 2 and 4 months) (Fig. 3). FPA responsiveness correlated with $V_{O_{2max}}$ after 4 months of training ($r = 0.54$; $P < 0.05$). Again, the effect of training on FPA was no longer apparent 8 months after training, and baseline responsiveness had been reestablished (Table 4).

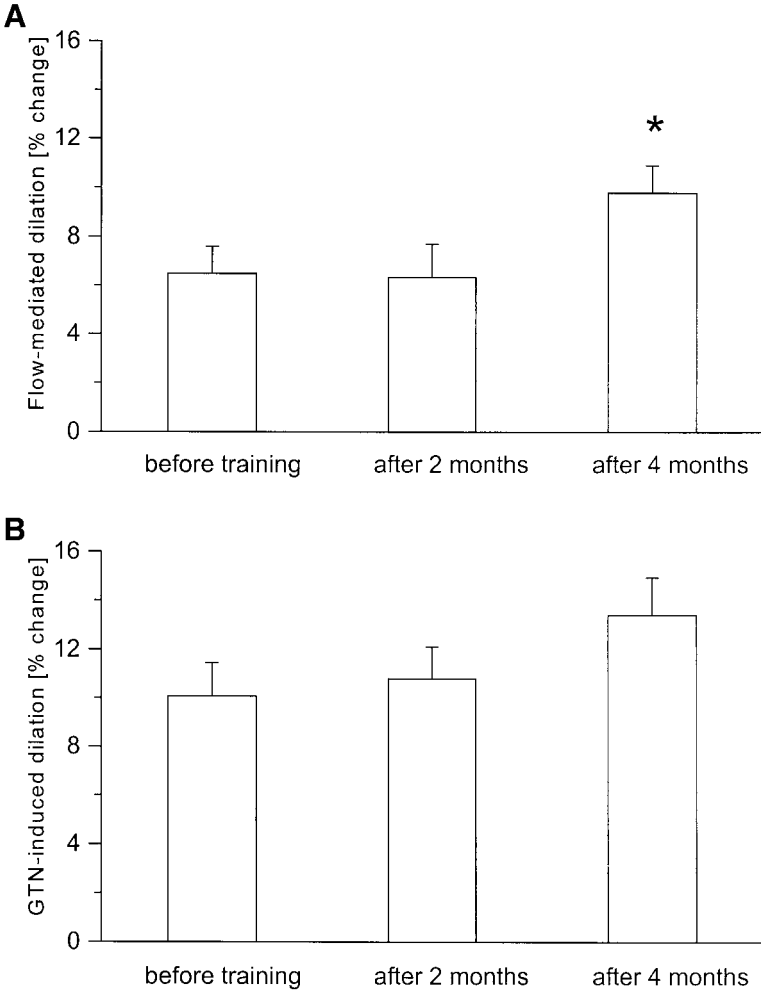

Figure 2—*Flow-mediated* (A) *and GTN-induced* (B) *dilation of the brachial artery before training* (n = *18) and after 2* (n = *18) and 4* (n = *15) months of training in patients with diabetes. Vasodilation is expressed as percent change from baseline diameter; results are means ± SE. Exercise significantly increased flow-mediated vasodilation,* *P < *0.05 (ANOVA) vs. before training.*

FPA in the control group was 4.4 ± 0.5 μm before and 4.5 ± 0.4 μm after 4 months. The reduction of FPA by L-NMMA to 3.9 ± 0.5 μm was greater than in the training group, but the difference did not reach the level of significance ($P = 0.1$). The responsiveness of FPA to L-NMMA was unchanged in control subjects after 4 months (Table 2).

CONCLUSIONS — The present study demonstrates that endothelial function of conduit and resistance vessels can be improved by regular aerobic training in patients with type 1 diabetes, and the study has important implications for these patients. First, this effect was shown in different vascular beds including the ocular vasculature, which is a target of diabetic vascular damage. Second, there is also evidence, for the first time, that the beneficial effects of training on different vascular beds are not maintained 8 months after discontinuation of a regular training program.

Endothelial dysfunction is associated with arteriosclerosis and is regarded as a risk factor for cardiovascular events (17). Improvement of endothelial function is therefore an important goal in patients with type 1 diabetes, since these patients have a two- to fourfold risk of developing cardiovascular disease (18). FMD has been used to investigate the presence of endothelial dysfunction in type 1 diabetes, which was also seen in this study cohort (19). After 4 months of training, significant changes in FMD of the brachial artery were seen in our experiments and paralleled by a small increase in endothelium-independent vasodilation. Our results and the magnitude of effect on conduit artery function are in good agreement with other training studies in different patient cohorts (10,20). It has further been demonstrated that the dilating capacity to nitrates is also enhanced in trained men (21). Thus, it is possible that high-intensity training could also increase NO sensitivity.

There is no direct evidence of which mechanisms contribute to the functional improvement of the vasculature in our study. Most authors have discussed the role of increased shear stress, which affects the vascular NO system in many ways (22). Endothelial L-arginine uptake, the substrate of NO production, is increased (23); further, NO synthase gene expression in endothelial cells is augmented (24) and NO release of endothelial cells is increased (25). In animals, enhanced NO synthase gene expression, higher NO production, and increased endothelium-dependent dilation of coronary arteries were associated with training (26). Inactivation of NO by oxygen-derived free radicals is an important mechanism of endothelial dysfunction in diabetes (27). Hyperglycemia can promote superoxide production as a consequence of glucose auto-oxidation, formation of advanced glycation end products, and abnormal arachidonic acid metabolism by activating protein kinase C, depleting tetrahydrobiopterin, and increasing the activity of nitric oxide syn-

Table 4—*Vascular function parameters of 13 patients with type 1 diabetes before training, after 4 months of training, and after 8 months without training*

	Before training	4 months of training	8 months after training
FMD (%)	7.7 ± 1.4	9.9 ± 1.0*	7.3 ± 0.4
GTN-induced dilation (%)	10.4 ± 1.5	14.1 ± 1.5	10.1 ± 0.7
FPA reduction by L-NMMA (%)	−9.5 ± 1.0	−13.4 ± 1.4*	−10.9 ± 1.2

Results are means ± SE; $n = 13$. *$P < 0.05$ vs. before training.

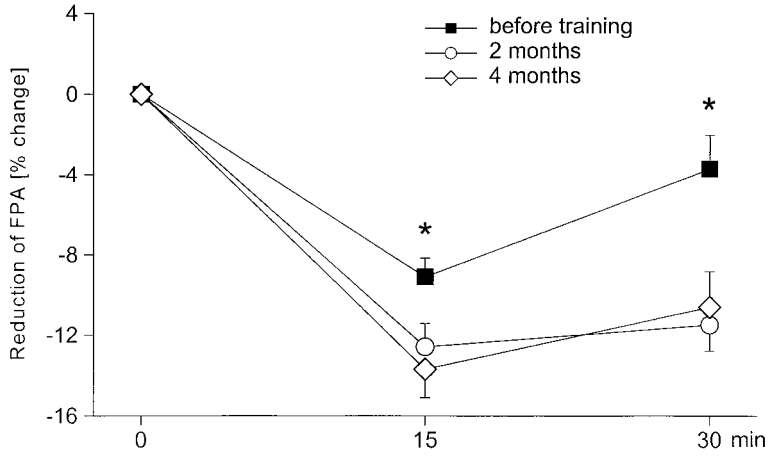

Figure 3— *Effect of L-NMMA on ocular fundus pulsation amplitude before training* (n = *18) and after 2* (n = *18) and 4* (n = *15) months of training in patients with diabetes. Results are means ± SE. Exercise significantly increased the vasoconstrictor response of choroidal resistance arteries,* *P < *0.05 (ANOVA) vs. 2 and 4 months.*

thase, which could also be a source of superoxide (28). Training could have increased the antioxidative capacity by increased expression of the potent antioxidative extracellular enzyme superoxide dismutase, as shown in animal studies (29), or indirectly by enhanced NO formation (30).

A role of increased NO synthesis, release of NO, or altered NO sensitivity by training is also supported by our results obtained in the ocular vasculature. NO is an important regulator of ocular blood flow, and the choroidal vasculature is particularly sensitive to changes in NO production (31). We have previously demonstrated that the ocular responsiveness to L-NMMA is reduced in patients with long-standing type 1 diabetes (12), which is compatible with the results of the present study. Maximum effects of training on L-NMMA responsiveness in the ocular vasculature were already seen after 2 months. It is unclear if the effect of exercise on resistance arteries is present before changes in conduit arteries are detectable or if this is due to the different methodology or a higher sensitivity of pharmacological testing. Nevertheless, our results in the ocular vasculature are important, since diabetic ocular angiopathy is associated with altered ocular blood flow (32).

The training group had a small reduction in total and LDL cholesterol levels; reduction of LDL cholesterol is also associated with improved endothelial function (33). It is possible that reduction of cholesterol could have contributed to improvement of vascular function in this group. Daily insulin requirement was also reduced. This finding is in good agreement with former exercise studies in patients with type 1 diabetes (34). Experimental studies demonstrated exercise-increased expression and function of several proteins involved in insulin-signal transduction (35). Insulin resistance has been linked to impaired endothelial function (36). Therefore, improvement of glucose metabolism and insulin utilization could have influenced vascular function in our patients. However, no clamp investigations were performed, and previous studies found no connection between reduced insulin requirements and insulin resistance (34).

Regardless of the underlying mechanism, the beneficial effect of training is independent of blood glucose level and apparently not limited to the muscle group under training, since bicycle training improved endothelial function in the brachial artery and in the eye. The observation that vascular function can be improved by up to 50% in type 1 diabetic patients implies that regular exercise is beneficial even at a later time point of diabetes, because potent effects may still be achieved. Importantly, the impact of training was independent of patient age or presence of microvascular complications. However, the beneficial effects of training are limited to the period of regular exercise, and the functional improvement does not persist.

One limitation of our study is that we did not follow a randomized design. It was not possible to recruit an appropriate number of sedentary patients with long-standing type 1 diabetes who were willing to comply with the training protocol for randomization, and control subjects were therefore slightly more trained. However, the FBF and FPA measurements are robust against a potential observer bias.

In conclusion, our data show that physical training significantly improves vascular function in patients with long-standing type 1 diabetes, who are at considerable risk for developing micro- and macroangiopathy. Whether improved endothelial function can reduce cardiovascular morbidity and mortality in these patients remains to be established. Further studies with continued training programs and hard clinical end points are required to answer this question.

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