Modulation of vascular endothelial gene expression by physical training in patients with chronic heart failure

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Background. **Abnormalities of the skeletal muscle vasculature, such as endothelial dysfunction and reduced microvascular density, can be reversed by physical training in patients with chronic heart failure. The molecular mechanisms that mediate the beneficial effects of physical training on the vascular endothelium are unknown.**

Methods. **Endothelial nitric oxide synthase (eNOS) and vascular endothelial growth factor (VEGF) gene expression in the skeletal muscle, peak oxygen consumption (VO_2) and calf peak reactive hyperemia were measured before and after 12 weeks of supervised physical training in 10 patients with chronic heart failure. Five patients with heart failure of similar severity who did not participate in the training program served as controls.**

Results. **The effects of physical training on eNOS and VEGF gene expression were heterogeneous. eNOS gene expression increased 3-4 fold in 4 patients while it remained constant in 6 patients. VEGF gene expression increased significantly in all patients who were not treated with beta-adrenergic blockade and remained constant in all patients who were treated with beta-adrenergic blockade. In contrast, physical training increased peak VO_2 and calf peak reactive hyperemia in all patients. Mean peak VO_2 increased from 13.13 ± 2.21 to 16.19 ± 2.69 ml/kg/min ($p < 0.001$) and calf peak reactive hyperemia increased from 19.7 ± 2.3 to 29.6 ± 4.0 ml*min^{-1}*100 ml^{-1} ($p < 0.001$).**

Conclusions. **A supervised program of physical training that consistently enhanced peak VO_2 and vascular reactivity in patients with chronic heart failure increased or left eNOS and VEGF gene expression unchanged in skeletal muscle. Changes in vascular endothelial gene expression may contribute to the benefits of training on vascular endothelial function but are not solely responsible for these benefits.**

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Introduction

Impaired endothelial function and reduced microvascular density have been documented in the skeletal muscle vasculature of patients with chronic heart failure[1,2]. Physical training, when resulting in increased peak aerobic capacity, is associated with improved vascular endothelial function in patients with chronic heart failure, and improved endothelial function and increased microvascular density in normal subjects[3-5]. Thus, functional and possibly anatomic alterations of the skeletal muscle vasculature are likely to contribute to the beneficial effects of physical training in chronic heart failure patients. However, the molecular mechanisms that mediate training-induced improvement in endothelial function in the skeletal muscle vasculature are poorly understood. We hypothesized that, as previously observed in experimental studies[6,7], physical training may consistently enhance the expression of vascular endothelial growth factor (VEGF) and endothelial nitric oxide synthase (eNOS) in the trained muscles of patients with chronic heart failure. Accordingly, eNOS and VEGF gene expression was measured in the skeletal muscle of patients with chronic heart failure who underwent 12 weeks of physical training.

Methods

Patient population. We studied 15 chronic heart failure patients who were in NYHA

functional class III despite compliance, for at least 6 months, with a medical regimen that included angiotensin-converting enzyme (ACE) inhibitors (enalapril or captopril at a daily dose of 40 and 75 mg, respectively), loop diuretics (furosemide at a daily dose ranging from 60 to 200 mg), and digoxin at a daily dose of 0.25 mg. Ten patients underwent physical training. Among these 5 were also treated with carvedilol at a dose of 25 mg bid. The other 5 patients remained sedentary and served as controls. None of the sedentary patients was treated with carvedilol. The baseline characteristics of the study population are detailed in table I. Their medical regimen did not change during the course of the study. The study was approved by the Committee on Clinical Investigations at the Albert Einstein College of Medicine. All patients signed informed consent.

Table I. Baseline characteristics of patients.

Characteristics	Physical training group		Sedentary TT
	TT alone	TT + βAB	
Age (years)	69 ± 6.4	66.4 ± 6.2	66 ± 6.3
Sex (M/F)	4/1	3/2	3/2
Cause (n=)			
Ischemic	2	2	3
Nonischemic	3	3	2
LVEF (%)	27.6 ± 2.9	28.6 ± 2.7	27.2 ± 4.6
Resting HR (b/min)	78 ± 12	59 ± 8	80 ± 11
Peak VO_2 (ml/kg/min)	12.5 ± 2.1	13.7 ± 2.4	13.1 ± 2.0

βAB = beta-adrenergic blockade with carvedilol; LVEF = left ventricular ejection fraction; HR = heart rate; TT = triple therapy (ACE inhibitors, diuretics, digoxin); VO_2 = oxygen consumption.

Measurement of peak oxygen consumption and physical training protocol. Peak oxygen consumption (peak VO_2, ml/kg/min) was measured on an upright bicycle ergometer using a 10 W/min ramp (Medical Graphics CPX System, St. Paul, MN, USA). Measurements were obtained twice before enrollment in the study, at the time of enrollment, and 6 and 12 weeks after enrollment. The highest value before enrollment was used for analysis. Patients exercised on a semi-recumbent bicycle (Tunturi 803) 4 times a week for 12 weeks at a workload corresponding to 50% of baseline peak VO_2. Initial sessions lasted 15 min, but patients were able to train for 60 min by the fourth week. Peak VO_2 was evaluated after 6 weeks of training and the workload was adjusted to maintain a training level corresponding to 50% of peak VO_2. Calf peak reactive hyperemia induced by a 5 min arterial occlusion was measured by venous occlusion plethysmography before and immediately after completion of the 12 week program of physical training.

RNA extraction and competitive polymerase chain reaction. Skeletal muscle biopsies were performed in the morning. The post-training biopsy was performed within 48 hours of the last training session. Percutaneous vastus lateralis biopsies were obtained following local anesthesia and skin incision at a site located 15 cm above the tip of the patella and immediately frozen in liquid N_2. Total RNA was isolated using TRI Reagent (Molecular Research Center, Cincinnati, OH, USA) and cDNA was synthesized using random hexamers and Avian Myeloblastosis virus reverse transcriptase (Promega, Madison, WI, USA). VEGF, eNOS and glyceraldehyde-phosphate-dehydrogenase (GAPDH) were amplified using AmpliTaq DNA polymerase (Perkin-Elmer/Cetus, Norwalk, CT, USA) and gene specific primers (VEGF: 5'-TCCAGGAGTACCCTGATGAG-3' and 5'-ATTCACATTTGTTGTGCTGT-3'; eNOS: 5'-GTGATGGCGAAGCGAGTGAAG-3' and 5'-CCGAGCCCGAACACACAGAAC-3'; GAPDH: 5'-CTGCACCACCAACTGC-3' and 5'-CCACCACTGACACGTT-3'). Primer sets were amplified across intron/exon boundaries to distinguish products amplified from cDNA and any contaminating genomic DNA (VEGF: 204 vs > 1100 bp; eNOS: 422 vs 946 bp; GAPDH: 278 vs 487 bp). Specific DNA competitors were constructed by introducing a small internal deletion into the amplified cDNAs using polymerase chain reaction followed by cloning and sequencing the resulting competitors. A fixed volume of muscle cDNA was amplified in the presence of a serial dilution of the competitor. The resulting polymerase chain reaction products were separated on 5% polyacrylamide gels, stained with SYBR-green (Molecular Probes, Eugene, OR, USA) and band intensities measured using a STORM Imager and the ImageQuant software (Molecular Dynamics, Sunnyvale, CA, USA). Intensities were plotted to determine the competitor concentration at which the target and competitor amplified equally.

Statistical analysis. Data are expressed as the mean ± SD. Differences between groups were assessed using ANOVA. Differences were considered significant when $p < 0.05$.

Results

Peak aerobic capacity and calf peak reactive hyperemia. After 12 weeks of physical training, peak VO_2 increased in all patients (Fig. 1). Mean peak VO_2 increased from 13.13 ± 2.21 to 16.19 ± 2.69 ml/kg/min ($p < 0.001$). Peak VO_2 was unchanged in patients who remained sedentary (13.12 ± 2.04 at baseline vs 12.82 ± 1.90 ml/kg/min after 12 weeks, p = NS). Calf peak reactive hyperemia increased in all patients who underwent physical training (Fig. 1). Mean calf peak reactive hyperemia increased from 19.7 ± 2.3 to 29.6 ± 4.0 ml*min^{-1}*100 ml^{-1} ($p < 0.001$). The increases in peak VO_2 and calf peak reactive hyperemia were similar in patients whether or not they were treated with carvedilol in addition to ACE inhibitors, diuretics and digoxin.

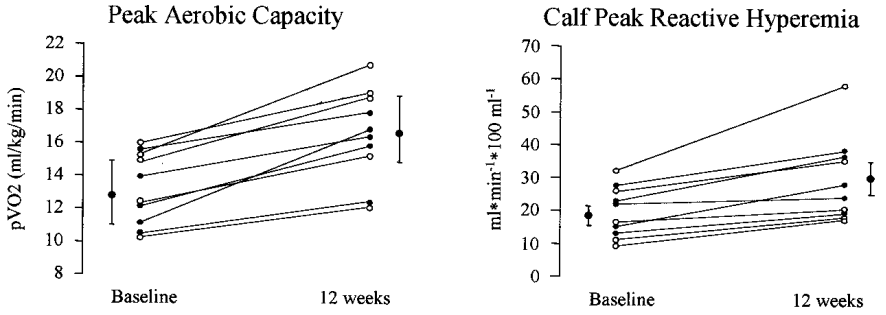

Figure 1. Peak VO_2 (pVO_2) and calf peak reactive hyperemia were measured in patients at baseline and after 12 weeks of physical training. Patients treated with triple therapy alone are indicated by solid circles and patients also treated with carvedilol are indicated by open circles. The mean for all patients is also indicated.

Vascular endothelial growth factor and endothelial nitric oxide synthase gene expression. We examined the mRNA levels of VEGF and eNOS in skeletal muscle biopsies obtained prior to and after 3 months of supervised physical training using a polymerase chain reaction-based analysis. GAPDH mRNA levels were also determined for the same preparations of cDNA and used for correction of any variations in cDNA quantity. When samples from all 10 patients who completed training were examined, the ratio of VEGF/GADPH mRNA levels in the trained muscle did not increase (Fig. 2[8], 1.5 fold increase, p = NS). However, VEGF mRNA expression in the trained skeletal muscle was affected by therapy. The ratio of VEGF/GADPH mRNA levels increased approximately 2 fold by physical training in all patients who were treated with ACE inhibitors, diuretics and digoxin alone while it remained unchanged in all patients who were also treated with carvedilol (Fig. 2). VEGF expression did not change in the skeletal muscles of the 5 sedentary patients. The ratio of eNOS/GADPH mRNA levels tended to increase after 12 weeks of physical training, but this increase did not reach any statistical significance (Fig. 2). The eNOS mRNA levels increased approximately 4 fold in 4 patients but remained constant in 6 patients. In contrast to VEGF, eNOS gene expression in the trained skeletal muscle was not affected by therapy with carvedilol. eNOS gene expression did not change in the vastus lateralis muscles of the 5 sedentary patients.

Discussion

Peak VO_2 and calf peak reactive hyperemia were markedly reduced in our patients when compared to values reported in age-matched normal subjects (13.13 ± 2.21 vs 29.8 ± 7.7 ml/kg/min, and 19.7 ± 2.3 vs 32.5 ± 3.5 $ml*min^{-1}*100\ ml^{-1}$, respectively)[9,10]. As expected with pretrained subjects who had markedly reduced peak oxygen uptake, patients with chronic heart failure showed a greater training-induced improvement in peak oxygen uptake than normal subjects: 23% in our patients vs 10-15% in the group of normal subjects older than 60 years studied by Blumenthal et al.[11]. Similarly, training-induced improvement in calf peak reactive hyperemia was greater in our patients than in normal subjects (50 vs 25%)[12].

In patients with chronic heart failure, physical training improves endothelial function in the skeletal muscle vasculature that is involved in training[3-5].

In healthy subjects, physical training increases capillary density in skeletal muscles, which in turn facilitates oxygen extraction by active skeletal muscles. Increased capillary density is likely to be mediated in part by increased VEGF expression[13,14].

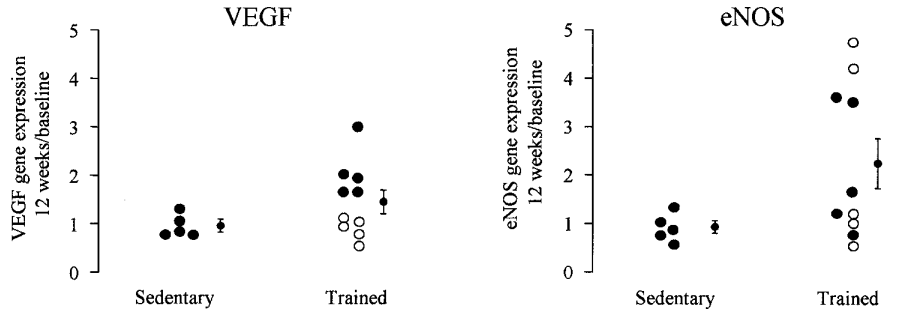

Figure 2. A competitive, quantitative reverse transcriptase-polymerase chain reaction strategy[8] was used to evaluate vascular endothelial growth factor (VEGF) and endothelial nitric oxide synthase (eNOS) gene expression in vastus lateralis biopsies. VEGF and eNOS gene expression was determined as the ratio between the concentrations of VEGF or eNOS and glyceraldehyde-phosphate-dehydrogenase cDNA in the same preparation of reversed transcribed RNA. Patients treated with triple therapy alone are indicated by solid circles and patients also treated with carvedilol are indicated by open circles. The means for the sedentary and trained patients (n = 10) are also indicated.

VEGF gene expression is low in the resting skeletal muscles of normal subjects at rest, with a ratio of VEGF mRNA to 18S of 0.38 ± 0.04[15]. Only the immediate effects of exercise on VEGF gene expression have been reported in normal subjects. Immediately after exercise, VEGF gene expression increases 1.78 to 4.8 fold in the skeletal muscle[16,17]. Although physical training consistently increases capillary density in skeletal muscles, concomitant changes in VEGF gene expression have not been documented in normal subjects.

We observed that, in the absence of treatment with carvedilol, VEGF gene expression increased by 2 fold after physical training for 12 weeks in patients with chronic heart failure receiving triple therapy with ACE inhibitors, digitalis and diuretics. In contrast, VEGF gene expression remained unaltered in patients who, in addition to triple therapy, were receiving carvedilol. To ascertain the reason for the disparate effect of physical training on VEGF gene expression, in the presence or absence of carvedilol, is beyond the scope of the present investigation. Carvedilol may hinder VEGF gene expression in skeletal muscle vasculature by modulating the cyclic AMP/protein kinase A-dependent pathway[18,19] and/or by reducing the inflammatory response via its antioxidant action and the glycoprotein 130/STAT pathway[20].

The increase in VEGF gene expression noted in patients who were not treated with carvedilol suggests that angiogenesis resulting in enhanced oxygen extraction by the active skeletal muscle may contribute to the training-induced increase in peak VO_2 in these patients. However, peak VO_2 was increased after 12 weeks of physical training in patients treated with carvedilol without a corresponding increase in skeletal muscle VEGF gene expression. Thus, mechanisms in addition to VEGF-mediated changes in skeletal muscle microvascular density are likely to be involved in training-induced improvement in peak VO_2.

Endothelial function is reduced in the limb vasculature of patients with chronic heart failure[21] but the mechanisms that are responsible for this reduction are still poorly understood. Depressed nitric oxide production and decreased nitric oxide levels have been reported in experimental models of heart failure suggesting that diminished capacity to generate nitric oxide may contribute to vascular endothelial dysfunction in heart failure[22,23]. Furthermore, a specific decrease in the synthetic activity of the L-arginine-nitric oxide pathway has been reported in patients with chronic heart failure[24]. Accordingly, the benefits of physical training on the vascular endothelial dysfunction have been attributed to increased nitric oxide production in experimental models of heart failure[6,7]. However, eNOS gene expression and vascular O_2^- production by an NADH-dependent oxidase have recently been found to have increased significantly in an ischemic model of heart failure, indicating that degradation of nitric oxide may play an important role in baseline vascular endothelial dysfunction in chronic heart failure[25]. Our data concerning the molecular mechanisms of training-induced improvement in vascular endothelial function corroborate these data. The lack of a consistent increase in eNOS gene expression in the trained skeletal muscles contrasts with an increase in nitric oxide production being the preponderant mechanism responsible for training-induced enhancement in vascular endothelial function in patients with chronic heart failure. Decreased degradation of nitric oxide or, alternatively, the increased production of substances other than nitric oxide such as the endothelium-derived hyperpolarizing factor or prostanoids[26] may be responsible for training-induced improvement in vascular endothelial function. In conclusion, increased eNOS and VEGF gene expression may contribute to the beneficial effects of physical training on vascular function. However, they do not appear to be solely responsible for the benefits of training on vascular function.

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