

# Correction of Endothelial Dysfunction in Chronic Heart Failure: Additional Effects of Exercise Training and Oral L-Arginine Supplementation

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- OBJECTIVES** The aim of this study was to analyze whether L-arginine (L-arg.) has comparable or additive effects to physical exercise regarding endothelium-dependent vasodilation in patients with chronic heart failure (CHF).
- BACKGROUND** Endothelial dysfunction in patients with CHF can be corrected by both dietary supplementation with L-arg. and regular physical exercise.
- METHODS** Forty patients with severe CHF (left ventricular ejection fraction  $19 \pm 9\%$ ) were randomized to an L-arg. group (8 g/day), a training group (T) with daily handgrip training, L-arg. and T (L-arg. + T) or an inactive control group (C). The mean internal radial artery diameter was determined at the beginning and after four weeks in response to brachial arterial administration of acetylcholine (ACh) (7.5, 15, 30  $\mu\text{g}/\text{min}$ ) and nitroglycerin (0.2 mg/min) with a transcutaneous high-resolution 10 MHz A-mode echo tracking system coupled with a Doppler device. The power of the study to detect clinically significant differences in endothelium-dependent vasodilation was 96.6%.
- RESULTS** At the beginning, the mean endothelium-dependent vasodilation in response to ACh, 30  $\mu\text{g}/\text{min}$  was  $2.54 \pm 0.09\%$  ( $p = \text{NS}$  between groups). After four weeks, internal radial artery diameter increased by  $8.8 \pm 0.9\%$  after ACh 30  $\mu\text{g}/\text{min}$  in L-arg. ( $p < 0.001$  vs. C), by  $8.6 \pm 0.9\%$  in T ( $p < 0.001$  vs. C) and by  $12.0 \pm 0.3\%$  in L-arg. + T ( $p < 0.005$  vs. C, L-arg. and T). Endothelium-independent vasodilation as assessed by infusion of nitroglycerin was similar in all groups at the beginning and at the end of the study.
- CONCLUSIONS** Dietary supplementation of L-arg. as well as regular physical exercise improved agonist-mediated, endothelium-dependent vasodilation to a similar extent. Both interventions together seem to produce additive effects with respect to endothelium-dependent vasodilation. (J Am Coll Cardiol 2000;35:706–13) © 2000 by the American College of Cardiology

Advances in heart failure research have introduced a new pathophysiological concept of chronic heart failure (CHF) as a systemic rather than a cardiac disorder involving hemodynamic, neurohormonal and peripheral derangements. Alterations in skeletal muscle metabolism and impaired vasodilation during exercise have been identified as factors contributing to key symptoms of CHF like exercise intolerance (1–4).

Endothelial dysfunction with attenuated vasodilation in response to acetylcholine (ACh) and reduced ischemic vasodilation during reactive hyperemia have been demon-

strated in patients with CHF (4,5). The effects of ACh are mediated by nitric oxide (NO), which is synthesized by endothelial nitric oxide synthase (eNOS) from the terminal guanidino nitrogen of its amino acid precursor L-arginine (L-arg.). Although L-arg. is stored in significant amounts in intracellular depots, oral supplementation of L-arg. has been shown to increase exercise-induced blood-flow in patients with CHF (6).

Endothelial shear stress serves as the primary stimulus for endothelium-mediated vasodilation during exercise and initiates the release of NO via receptor-independent activation of endothelial potassium channels (7,8) and increase of calcium influx (9). The expression of mRNA for endothelial nitric oxide synthase (eNOS) is upregulated in cultured endothelial cells exposed to laminar shear stress (10). These findings are consistent with recent observations in human

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#### Abbreviations and Acronyms

ACh	=	acetylcholine
c	=	control
CHF	=	chronic heart failure
DCM	=	dilated cardiomyopathy
eNOS	=	endothelial nitric oxide synthase
FDD	=	flow-dependent dilation
L-arg.	=	L-arginine
NO	=	nitric oxide
NOS	=	nitric oxide synthase
RH	=	reactive hyperemia
T	=	training

studies that exercise training enhances NO-mediated vasodilation in patients with CHF (11–13).

As L-arg. supplementation increased endothelial substrate availability and exercise training augmented eNOS expression and activity, we hypothesized that a combination of physical exercise and L-arg. might be additive with regard to correcting endothelial dysfunction in CHF.

## METHODS

**Subjects.** In this study male patients  $\leq 70$  years with CHF were studied. All patients had clinical, radiological and echocardiographical signs of CHF and a reduced left ventricular ejection fraction  $\leq 40\%$  as assessed by angiography. Exclusion criteria were exercise-induced myocardial ischemia, significant valvular heart disease, diabetes mellitus, smoking ( $>10$  cigarettes/day), hypertension ( $>165$  mm Hg systolic blood pressure), overt atherosclerotic peripheral vascular disease and hypercholesterolemia ( $\geq 240$  mg/dL;  $\geq 6.2$  mmol/L).

**Study protocol.** The study was approved by the ethics committee of the University of Leipzig and written informed consent was obtained from all patients. All patients were in a clinically stable condition for at least three months prior to enrollment. With the exception of the control group, all patients stayed in the hospital for the duration of the study (four weeks). At baseline patients were studied in a fasting state in a quiet temperature and humidity controlled room. All cardiovascular medications were withheld for  $>24$  h before assessment of endothelium-dependent vasodilation.

A 20 g arterial catheter was placed in the brachial artery of the nondominant arm, and the patient rested for 20 min in supine position. After adjustment of both ultrasound and Doppler transducer over the radial artery, measurements of arterial diameter and flow velocity were performed.

**Intraarterial infusions.** Baseline measurements of arterial diameter were performed during infusion of 5% glucose at a constant rate of 1 mL/min for 3 min. Endothelium-dependent vasodilation was assessed by infusion of increasing doses of ACh: 7.5  $\mu\text{g}/\text{min}$ , 15  $\mu\text{g}/\text{min}$  and 30  $\mu\text{g}/\text{min}$

were administered at a constant flow rate of 1 mL/min for 5 min at each concentration level.

For determination of flow-dependent vasodilation (FDD), a blood pressure cuff was inflated to 50 mm Hg above the systolic blood pressure for 5 min. After deflation of the cuff, maximal reactive vasodilation was measured noninvasively. It is known that blood flow in reactive hyperemia peaks shortly after release of cuff pressure and shows an exponential decline falling to 50% of maximal flow as early as after 25 s (14). The maximal flow-dependent vasodilation, however, occurs after approximately 40 to 60 s (15). We, therefore, measured radial artery internal diameter in 5 s intervals for 2 min after cuff deflation. The maximal internal diameter was recorded as a measure of FDD (11).

Finally, nitroglycerin was given at a rate of 0.2 mg/min, 1 mL/min for 5 min to assess endothelium-independent vasodilation.

Between the different measurements described above, an interval of 2 min was allowed to assure the return of endothelial function to baseline levels.

**Randomization.** After baseline measurements, patients were randomized to one of the following four groups: 1) control, 2) L-arg. supplementation alone, 3) forearm exercise training alone, and 4) L-arg. supplementation plus forearm exercise training.

- 1) Control patients received measurements of endothelial function as described above at the beginning of the study and after four weeks and continued their sedentary lifestyle.
- 2) *L-arg. supplementation.* Patients received 8 g L-arg. per day split into three single doses of 3 g, 2 g and 3 g. L-arg. was administered as capsules containing 0.5 g L-arg. each, and intake of medication was closely supervised.
- 3) *Forearm exercise training.* Handgrip training (T) was performed at 70% (i.e., 60 N) of the maximal exercise capacity six times per day using a handgrip ergometer. Each training session lasted for the time previously determined in the maximal exercise test.
- 4) L-arg. plus training combined L-arg. supplementation and forearm exercise training into a comprehensive endothelial treatment scheme.

All measurements were repeated after four weeks. Cardiac medication remained unchanged during the study period.

**Pharmacokinetic study.** In a pharmacokinetic study involving 12 patients with CHF (all 10 patients of the L-arg. group and the first two patients of the L-arg. + T group), the pharmacokinetic properties of oral L-arg. were assessed at the first and the last day of therapy. Patients received oral L-arg. in gelatin capsules each containing 0.5 g of L-arg. at a dosage of 3 g at 8:00 AM, 2 g at 2:00 PM and 3 g at 8:00 PM. Between 8:00 AM and 12:00 PM, blood samples were drawn at 1 h intervals to determine plasma L-arg. levels.

**Table 1.** Baseline Characteristics

	Control	L-Arg.	Training	L-Arg. + Training
n	10	10	10	10
Age (yr)	56 ± 3	55 ± 2	55 ± 4	55 ± 2
LVEF (%)	19 ± 3	18 ± 2	18 ± 3	19 ± 3
LVEDD (mm)	67 ± 2	69 ± 1	68 ± 3	69 ± 3
DCM/ischemic CMP	7/3	6/4	6/4	5/5
NYHA II	1	2	1	2
III	9	8	9	8
HR (beats/min)	86 ± 2	86 ± 4	87 ± 5	84 ± 4
RR systolic (mm Hg)	118 ± 4	118 ± 3	116 ± 6	113 ± 3
RR diastolic (mm Hg)	74 ± 2	73 ± 3	76 ± 3	73 ± 2

Data are mean ± SEM.

CMP = cardiomyopathy; DCM = dilated cardiomyopathy; HR = heart rate; L-arg. = L-arginine; LVEDD = left ventricular end-diastolic diameter; LVEF = left ventricular ejection fraction; NYHA = New York Heart Association; RR = arterial blood pressure.

**Ultrasound measurement of radial artery diameter.** A high-resolution echo-tracking angiometer (NIUS 02, Asulab Research Laboratory, Neuchâtel, Switzerland) was used for noninvasive measurement of mean radial artery diameter. In brief, short ultrasonic pulses of 10 MHz center frequency are generated at a pulse repetition frequency of 500 Hz. The radio frequency echo line permits accurate determination of the internal artery diameter over time by automatic tracking of the anterior and posterior wall (spatial resolution: 2  $\mu$ m) (16). During measurement the patient was in supine position with the arm resting on a special support to avoid unintentional motions. It has previously been demonstrated that ultrasound measurement of artery diameter correlate with plethysmographic data (17).

**Doppler measurement of intravascular blood flow velocity.** Intravascular blood flow velocity was continuously determined using a fixed Doppler probe (Doptek 2003, Deltex France SA, Montpellier, France) with a Doppler frequency of 8 MHz. Blood flow was calculated as average peak velocity multiplied by cross sectional area, yielding flow in mL/min.

**Exercise testing.** For testing of maximal forearm exercise capacity, patients performed dynamic handgrip exercise with the nondominant forearm using a hand ergometer set at a force of 80 N and a travel of 0.03 m. Contractions were repeated at a frequency of 20 per min, and the time until the patient stopped due to fatigue was determined.

**Statistics.** All variables were calculated as mean ± standard error. Both absolute values and percentage changes from baseline were used for statistical analysis and yielded similar p values. Both intragroup and intergroup comparisons were made using the two-way repeated measures analysis of variance followed by the Tukey post-hoc test (SigmaStat 2.0.3 for Windows, SPSS Inc., Chicago, Illinois). Data were tested for normal distribution using the Kolmogorov-Smirnov test and for homogeneity of variances with Lev-

ene's test. A p value <0.05 was considered statistically significant.

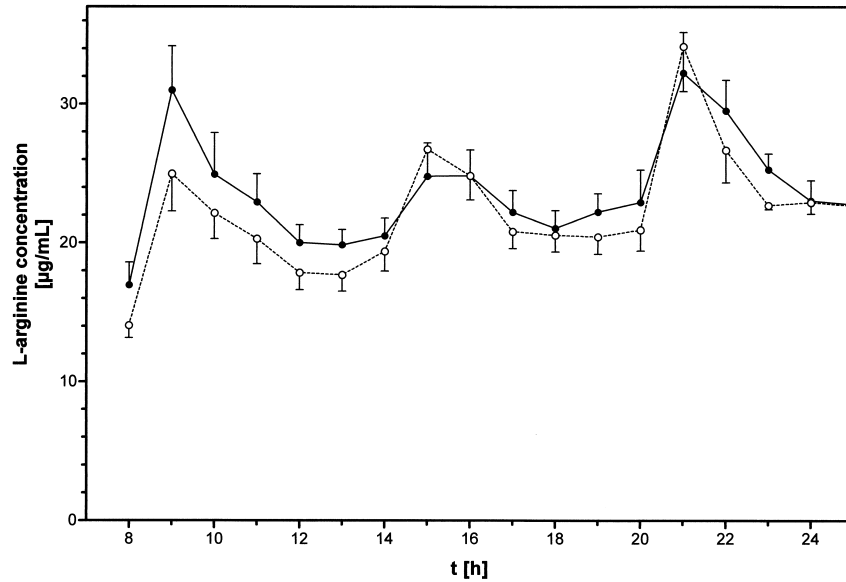
In a previous validation of the NIUS-02 high-resolution echo-tracking angiometer, we determined a standard deviation of 82  $\mu$ m for measurements of radial artery diameter changes at 30  $\mu$ g/min ACh after a local training intervention in patients with CHF (n = 7, age 55 ± 4 years, left ventricular ejection fraction 15 ± 3%). We therefore estimated a standard deviation of 100  $\mu$ m for the assessment of endothelium-dependent vasodilation in this study. A difference in radial artery diameter exceeding two standard deviations (i.e., 200  $\mu$ m) was considered to be clinically relevant. Based on a sample size of n = 10 for each of the four treatment groups, the power of the study to detect clinically significant differences in endothelium-dependent vasodilation was calculated to be 96.6%.

## RESULTS

**Patient characteristics.** In this study 40 male patients with CHF were studied. The etiology of heart failure was related to ischemic heart disease in 16 patients. In the absence of identifiable causes of ventricular dysfunction, 24 patients were diagnosed as having dilated cardiomyopathy (DCM). Baseline characteristics were similar for all variables in the four groups (Table 1). Medication was not changed during the study period in any patient. All patients were taking angiotensin conversion enzyme inhibitors; 39 patients were on diuretics and 30 on digitalis.

One patient in the control group died from sudden arrhythmogenic complications of CHF during the study period, unrelated to participation in the study. Another control group patient declined to participate in the follow-up examination, so that complete follow-up was available for 38 patients only.

**Pharmacokinetic study.** Oral L-arg. was effective in doubling the normal serum levels of L-arg. Peak plasma levels were reached about an hour after administration, and the plasma



**Figure 1.** Average plasma concentrations in 12 chronic heart failure patients taking oral L-arginine at a dose of 8 g/day (3 g at 8:00 AM, 2 g at 2:00 PM, 3 g at 8:00 PM). The **straight line** denotes plasma levels on the first day of therapy; the **dotted line** after four weeks of continuous treatment.

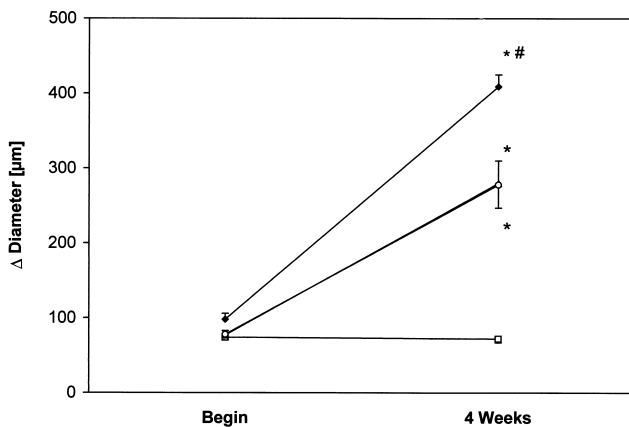
half-life period ranged between 2 and 3 h (Fig. 1). No adverse effects with regard to hypotension were observed in the pilot study. Apart from mild intermittent diarrhea in one patient, no further side effects were observed in any other patient. No significant change in bioavailability was observed between the beginning and the end of the treatment period (Fig. 1).

**Vasodilative response to acetylcholine.** *Baseline measurements.* The baseline vasodilative response was not significantly different between the groups after administration of

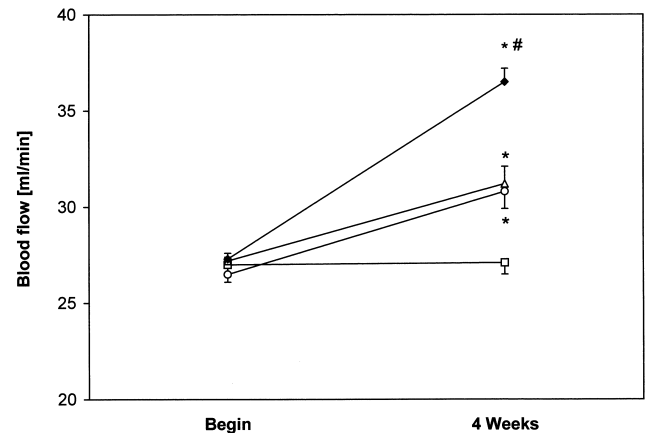
ACh 30 µg/min as determined by radial artery diameter (Fig. 2) and blood flow (Fig. 3). Baseline vascular diameter did not change between the beginning and the end of the study in any patient group.

*Control.* The response of radial artery diameter to ACh remained unchanged after four weeks.

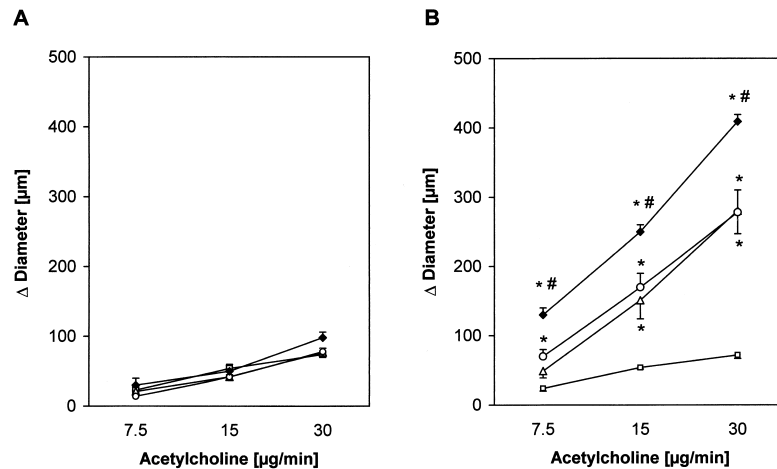
*L-arg.* After four weeks of L-arg. supplementation at a dose of 8 g/day, the vasodilative response to ACh 30 µg/min as compared with baseline (glucose 5%) increased significantly from 77 ± 5 µm to 280 ± 33 µm (from 2.2 ±



**Figure 2.** Vasodilative effect of 30 µg/min acetylcholine at the beginning and after four weeks of L-arg. (**triangle**), handgrip training alone (**circle**) and handgrip training plus L-arg. (**black rhombus**). The absolute changes in radial artery internal diameter as compared with infusion of 5% glucose are presented as mean ± standard error. \*Denotes p < 0.001 vs. control (**square**) and begin; #denotes p < 0.001 vs. L-arg. alone and training alone. L-arg. = L-arginine.



**Figure 3.** Effects of 30 µg/min acetylcholine on blood flow at the beginning and after four weeks of L-arg. (**triangle**), handgrip training alone (**circle**) and handgrip training plus L-arg. (**black rhombus**). The absolute changes in blood flow as compared with infusion of 5% glucose are presented as mean ± SE. \*Denotes p < 0.001 vs. control (**square**) and begin; #denotes p < 0.001 vs. L-arg. alone and training alone. L-arg. = L-arginine.



**Figure 4.** Dose-response curves for acetylcholine-induced endothelium-dependent vasodilation before (A) and after four weeks (B) of L-arg. (triangle), handgrip training alone (circle) and handgrip training plus L-arg. (black rhombus) vs. baseline. \*Denotes  $p < 0.01$  vs. control (square); #denotes  $p < 0.005$  vs. L-arg. alone and training alone. L-arg. = L-arginine.

0.2 to  $8.8 \pm 0.9\%$ ,  $p < 0.001$  vs. begin). Similar changes were observed with respect to blood flow (Fig. 3).

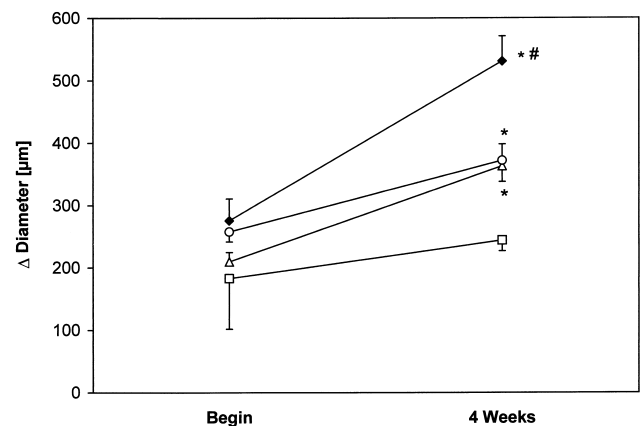
**Local exercise training.** After four weeks of handgrip exercise training, ACh 30  $\mu\text{g}/\text{min}$  caused an increase of internal diameter change from  $78 \pm 5 \mu\text{m}$  to  $278 \pm 32 \mu\text{m}$  (from  $2.1 \pm 0.1\%$  to  $8.6 \pm 0.9\%$ ,  $p < 0.001$  vs. begin) (Fig. 2 and 4). Blood flow changes are given in Fig. 3.

**Combination of L-arg. supplementation and training.** In comparison with both the L-arg. and the training group, the vasodilative response to ACh 30  $\mu\text{g}/\text{min}$  was significantly enhanced after four weeks of L-arg. plus handgrip exercise training: from  $98 \pm 8 \mu\text{m}$  to  $409 \pm 16 \mu\text{m}$  (from  $2.9 \pm 0.2\%$  to  $12.0 \pm 0.3\%$ ,  $p < 0.001$  vs. begin and vs. control,  $p < 0.001$  vs. L-arg. and training) (Fig. 2 and 4). Acetylcholine induced blood flow augmentation increased from  $27.3 \pm 0.1 \text{ mL}/\text{min}$  to  $36.5 \pm 0.7 \text{ mL}/\text{min}$  ( $p < 0.001$  vs. begin, control, L-arg. and training) (Fig. 3).

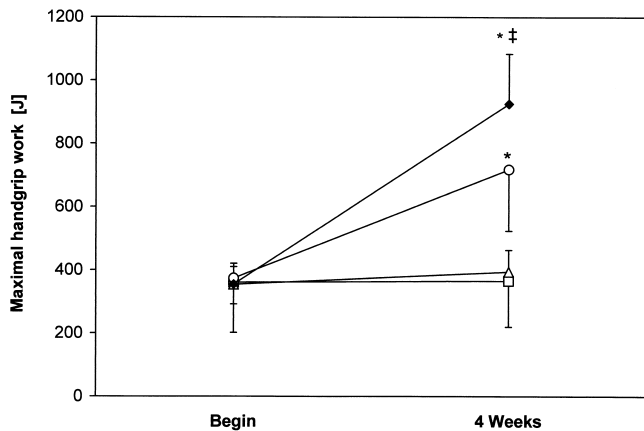
**Vasodilative response during reactive hyperemia.** After 5 min of complete limb ischemia, maximal flow-dependent vasodilation during reactive hyperemia (RH) was measured. In all intervention groups (L-arg., training and L-arg. plus training), RH-induced vasodilation was significantly enhanced after four weeks of treatment (Fig. 5). Vasodilation was more pronounced in the L-arg. + training group than in L-arg. or training alone ( $15.5 \pm 1.0\%$  vs.  $11.3 \pm 1.2\%$  in L-arg. [ $p = 0.044$ ] and  $11.4 \pm 0.9\%$  in training [ $p = 0.037$ ]).

**Vasodilative response to nitroglycerin.** After administration of nitroglycerin 0.2 mg/min, no significant differences between baseline and follow-up measurements were determined (control:  $402 \pm 23 \mu\text{m}$  vs.  $394 \pm 23 \mu\text{m}$ ; L-arg.:  $469 \pm 19 \mu\text{m}$  vs.  $492 \pm 29 \mu\text{m}$ ; training:  $474 \pm 22 \mu\text{m}$  vs.  $474 \pm 21 \mu\text{m}$ ; L-arg. plus training:  $481 \pm 25 \mu\text{m}$  vs.  $481 \pm 24 \mu\text{m}$ ).

**Exercise capacity.** Maximal handgrip work did not change in the control group (Fig. 6). In the nontraining L-arg. group, a slight nonsignificant increase of maximal work was noted (+11% from  $354 \pm 67 \text{ J}$  to  $394 \pm 69 \text{ J}$ ,  $p = \text{NS}$ ). More pronounced were the changes in the training groups: training alone doubled maximal handgrip work from  $374 \pm 82 \text{ J}$  to  $718 \pm 194 \text{ J}$  ( $p < 0.001$  vs. begin). The addition of L-arg. supplementation to exercise training further enhanced exercise capacity from  $355 \pm 159 \text{ J}$  to  $926 \pm 158 \text{ J}$  ( $p < 0.001$  vs. begin and  $p = 0.017$  vs. control and L-arg.). However, the difference between the effects of training + L-arg. versus training alone with respect to local exercise capacity did not reach statistical significance.



**Figure 5.** Vasodilative effect of reactive hyperemia. The absolute changes in radial artery internal diameter as compared with infusion of 5% glucose are presented as mean  $\pm$  SE. \*denotes  $p < 0.02$  vs. begin and control (square); #denotes  $p < 0.05$  vs. L-arginine (triangle) and training (circle); combination of L-arginine and training (black rhombus).



**Figure 6.** Maximal handgrip exercise capacity before and after intervention. \*Denotes  $p < 0.001$  vs. begin; ‡denotes  $p = 0.017$  vs. control and L-arg. Control (square); L-arg. (triangle); training (circle); L-arg. + training (black rhombus). L-arg. = L-arginine.

## DISCUSSION

Endothelial dysfunction has been corrected in patients with CHF by oral L-arg. supplementation (6) and physical exercise training (11-13,18). Since both interventions influence different sections of the L-arg.—NO—pathway, the question arises whether the combination of oral L-arg. and exercise training might be superior to separate interventions alone.

Two major messages emerge from this first prospective randomized clinical trial comparing the effects on endothelial dysfunction in CHF of L-arg. alone, training alone and the combination of L-arg. and training: 1) supplemental oral L-arg. improves endothelium-dependent vasodilation, most likely by increased endothelial release of NO. Local exercise training had similar beneficial effects as compared with exercise training with respect to ACh-mediated vasodilation. 2) The effects of L-arg. supplementation and exercise training on endothelium-dependent vasodilation seem to be additive. This result suggests that improved endothelial function may be a sum effect of increased substrate availability and shear stress induced upregulation of eNOS.

It has long been established that CHF is characterized by peripheral vasoconstriction and abnormal vascular compliance, both of which may be related, in part, to endothelial dysfunction of peripheral resistance and conduit vessels (4,19). Indeed, numerous studies have documented a reduced endothelium-dependent vasodilation in CHF patients (4,16). In addition, it has been found that endothelium-independent relaxation is not different between CHF patients and healthy controls (20).

In this study a bifactorial treatment approach to correct endothelial function in CHF was evaluated: supplementation of L-arg. and exercise training.

**Effects of L-arg. supplementation.** Oral supplementation of L-arg. at a dose of 8 g per day leads to a fourfold increase in endothelium dependent vasodilation in patients with CHF after four weeks of treatment. This study confirmed the beneficial effect of L-arg. supplementation on ACh-mediated vasodilation in patients with CHF (6,20).

In contrast with this result, Chin-Dusting et al. (21) reported that oral supplementation of L-arg. at a dose of 20 g per day failed to improve endothelial dysfunction in patients with CHF. Several differences in this study are to be noted:

- 1) L-arg. was administered in a water-based syrup solution, which was stored at the patients' homes for the study period of 28 days.
- 2) No attempt was made to determine the bioavailability of oral L-arg. in this water-soluble form.
- 3) The patients studied by Chin-Dusting et al. were in more advanced stages of heart failure than our patients with 7/20 in New York Heart Association class IV.
- 4) The methodological approach for measuring ACh-induced vasodilation (forearm venous occlusion plethysmography) differed from our study.

*Mechanisms involved in the effects of L-arg. supplementation.* In pathological states like reperfusion injury, hypercholesterolemia, diabetes mellitus and CHF, reduced NO activity has been described (5,22-25). L-arg. supplementation may partially restore NO levels in these situations by a variety of actions:

- 1) In reperfusion injury, NO is rapidly degraded by oxygen radicals. L-arg. supplementation corrected endothelium-dependent vasodilation by competing with molecular oxygen as a substrate for electron transfer and reduced superoxide generation.
- 2) Elevated levels of asymmetrical dimethylarginine have been observed in hypercholesterolemic rabbits (26). Asymmetrical dimethylarginine has been proposed as an endogenous regulator of nitric oxide synthase (NOS) activity and may act via a competitive mechanism, which is reversed by L-arg.
- 3) In CHF, elevated levels of free oxygen radicals have been described (27). L-arg. may act as a radical scavenger and reduce oxygen radicals, thereby increasing NO half-life (28).

**Effects of exercise training.** Local training by submaximal handgrip exercise six times per day was effective in significantly improving endothelial function within four weeks; ACh-induced vasodilation increased fourfold from  $2.1 \pm 0.1\%$  to  $8.6 \pm 0.9\%$  ( $p < 0.001$ ). Cell culture experiments have demonstrated that eNOS activity and NO release are modulated by alterations of blood flow and shear stress (8-10,29). Shear stress responsive elements have been identified within the promoter region of the NOS gene (30).

Animal studies have confirmed that short-term (31) and

long-term exercise (31-33) increased the mRNA expression of eNOS. The effectiveness of exercise training to reverse endothelial dysfunction has also been documented in humans (11,13,18).

**Effects of a combination of L-arg. supplementation and exercise training.** This study is the first prospective randomized clinical trial to demonstrate that the combination of oral L-arg. supplementation and regular submaximal exercise training has additive effects as compared with L-arg. or exercise training alone. Combination treatment leads to a difference in agonist-mediated endothelium-dependent vasodilation of  $12 \pm 0.3\%$  as compared with  $8.8 \pm 0.9\%$  in L-arg. and  $8.6 \pm 0.9\%$  in training, respectively. This finding is consistent with the possible mechanisms of actions of both interventions outlined above because they interfere at different points of the NO-pathway.

**Reactive hyperemia and flow-dependent vasodilation.** Arterial occlusion is the most potent stimulus for vasodilation in the human forearm (19). Similar to the endothelium-dependent vasodilation in response to acetylcholine, ischemic vascular relaxation is also significantly attenuated in patients with CHF (19,20).

Different mechanisms are involved in reactive hyperemia: peak flow seems to be mediated primarily by vasodilating prostaglandins and myogenic factors (34,35). The initial rise in blood flow exceeds the perfusion required to repay the metabolic debt and leads to a wash-out of any locally acting factors. It has, therefore, been argued that prostaglandins and myogenic factors are of minor importance for the vasodilation seen about 40 to 60 s after release of the arterial occlusion (36). As inhibition of eNOS by  $N^G$ -monomethyl-L-arginine has no additional effect on peak hyperemia in the presence of cyclooxygenase inhibitors but further reduces total excess blood flow (36), NO has been suggested to mediate the flow-dependent vasodilation in late reactive hyperemia (35).

As endothelial factors contribute substantially to reactive hyperemia, we expected significant changes after L-arg. supplementation or training. Maximal FDD was significantly enhanced after both interventions (after L-arg. +74%,  $p = 0.001$ , after training +47%,  $p = 0.009$ ). The combination of L-arg. and training further augmented FDD: +90%,  $p = 0.037$  vs. training and  $p = 0.044$  vs. L-arg.).

**Clinical implications.** One of the key symptoms of CHF is exercise intolerance, accompanied by early muscular fatigue. It has been shown that exercise intolerance correlates poorly with central hemodynamics (37). Therefore, therapeutic strategies in CHF focussing on improving cardiac function may fail to enhance exercise capacity.

Intact endothelial function seems to play an important role for adequate peripheral perfusion during exercise. In this study we confirmed that a combination of supplement-

ation of oral L-arg. and local exercise training seems to have superior effects on endothelium-dependent vasodilation in patients with CHF as compared with separate interventions.

Although the feasibility of long-term oral L-arg. supplementation may be debated for practical reasons, our study underlines the potential benefits of augmenting endothelial NO production or release in patients with CHF.

In this study endothelial function was assessed in the trained extremity, suggesting that local exercise leads only to local effects. In a consecutive trial we are currently challenging this traditional concept by investigating whether a similar effect on forearm endothelial function can be achieved with bicycle ergometer training. Thereby it should be possible to differentiate between local and systemic effects of exercise training on the skeletal muscle vasculature in CHF.

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