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Effects of L-Arginine on Impaired Acetylcholine-Induced and Ischemic Vasodilation of the Forearm in Patients With Heart Failure

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Background Endothelium-dependent vasodilation in response to acetylcholine (ACh) and ischemic vasodilation during reactive hyperemia are attenuated in the forearm of patients with heart failure (HF). It has been shown that L-arginine augments endothelium-dependent vasodilation in healthy subjects. Thus, the aim of the present study was to determine if L-arginine improves endothelium-dependent and ischemic vasodilation in the forearm in HF.

Methods and Results Forearm blood flow was measured by a strain-gauge plethysmograph in 20 patients with HF and in 24 age-matched control subjects (C). Resting forearm vascular resistance (FVR) was significantly higher in HF than in C (37 ± 4 versus 22 ± 2 U, $P < .01$). Intra-arterial infusions of ACh or sodium nitroprusside (SNP) at graded doses progressively decreased FVR in HF as well as in C. The magnitude of

ACh-induced vasodilation was attenuated in HF ($P < .01$), whereas SNP-induced vasodilation was similar between the two groups. The minimal FVR during reactive hyperemia after 10 minutes of arterial occlusion was significantly higher in HF ($n=12$) than in C ($n=12$) (3.2 ± 0.4 versus 2.1 ± 0.1 U, $P < .05$). L-Arginine significantly augmented maximal vasodilation evoked with ACh and decreased minimal FVR during reactive hyperemia in HF ($P < .01$) but not in C. L-Arginine did not affect SNP-induced vasodilation in HF or C.

Conclusions Our results suggest that defective endothelial function may contribute to impaired ischemic vasodilator capacity in HF. (*Circulation*. 1994;90:658-668.)

Key Words • acetylcholine • heart failure, congestive • endothelium • relaxing factors • nitric oxide

It has been shown that ischemia- and exercise-induced vasodilation of the extremities of patients with heart failure is markedly attenuated.^{1,2} It is likely that decreased exercise tolerance in patients with heart failure is due not only to impaired pump function of the heart but also to inadequate increases in muscle blood flow resulting from impaired vasodilation during exercise.^{1,2} The mechanism(s) of impaired ischemia- and exercise-induced vasodilation in heart failure are not known. It also has been shown in humans³⁻⁶ as well as in animals⁷⁻⁹ that endothelium-dependent vasodilation evoked with acetylcholine is impaired in heart failure. Thus, it is possible that endothelial dysfunction may contribute to impaired vasodilation during exercise or ischemia in patients with heart failure.

L-Arginine is a precursor of endothelium-derived nitric oxide.^{10,11} L-Arginine augments acetylcholine-induced vasodilation in hypercholesterolemic humans¹² and animals.¹³⁻¹⁵ It was reported in healthy humans that intravenously infused L-arginine decreased blood pressure¹⁶ and that intra-arterially infused L-arginine dilated forearm blood vessels and augmented acetylcholine-induced vasodilation.^{17,18} These results suggest that supplementation of L-arginine facilitates production of

nitric oxide and augments endothelium-dependent vasodilation.

The aim of the present study was to examine whether intra-arterially infused L-arginine augments endothelium-dependent vasodilation to acetylcholine and ischemic vasodilation during reactive hyperemia in the forearm of patients with heart failure. If L-arginine augments ischemic vasodilation as well as endothelium-dependent vasodilation, such results would suggest the possibility that defective endothelial function may contribute to impaired ischemic vasodilator capacity in patients with heart failure.

Methods

Subjects

Twenty patients with heart failure (14 men and 6 women) and 24 control subjects (20 men and 4 women) were studied. The ages of the patients with heart failure were 37 to 74 years with a mean of 57 ± 3 years, and ages of healthy subjects were 36 to 71 years with a mean of 52 ± 2 years ($P = NS$). The clinical profiles of patients with heart failure are summarized in Table 1. The underlying heart disease was dilated cardiomyopathy in 11 patients and valvular heart disease in 9 patients. All patients had several signs and symptoms suggestive of congestive heart failure on admission, such as dyspnea on exertion, orthopnea, fatigue, nocturia, rales, congestion in the lungs as assessed by chest roentgenography, pleural effusion, liver congestion, or pedal edema. New York Heart Association functional classification shown in Table 1 was that on admission. Some patients underwent cardiac catheterization when they were stable. Six patients with mitral regurgitation had somewhat better ejection fraction as assessed by echocardiography than did patients with dilated cardiomyopathy. However, all patients with mitral regurgitation had elevated pul-

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TABLE 1. Clinical Profiles of Patients With Heart Failure

Patient	Age, y	Sex	Diagnosis	EF, %		NYHA Class	Symptom	T-chol, mg/dL	BP, mm Hg	FBS, mg/dL
				Echo	LVG					
1	44	M	DCM	30	19	IV	Orthopnea	233	116/66	74
2	63	F	DCM	29	26	IV	Orthopnea	146	124/52	104*
3	38	M	DCM	24	18	IV	Orthopnea	177	110/50	99
4	37	F	DCM	49	...	III	Dyspnea	221	116/64	79
5	51	M	DCM	34	24	IV	Orthopnea	156	130/78	81
6	47	M	DCM	42	...	II	Fatigue	168	118/70	96
7	63	M	DCM	27	...	III	Orthopnea	205	136/68	90
8	53	M	DCM	42	...	III	Orthopnea	233	126/72	119
9	70	M	DCM	60	54	III	Dyspnea	220	140/60	143†
10	62	M	DCM	64	37	III	Dyspnea	159	128/60	102
11	64	M	AR	45	...	II	Dyspnea	254	140/80	80
12	74	F	TR	73	...	IV	Dyspnea	187	104/76	78
13	55	M	MR	55	34	IV	Orthopnea	180	122/66	102
14	59	M	MR	73	59	IV	Orthopnea	144	140/50	87
15	56	F	MR	80	75	IV	Orthopnea	213	122/64	96
16	66	F	MR	62	37	III	Dyspnea	181	154/78	91
17	57	M	AR	52	36	III	Dyspnea	235	166/32	104
18	68	F	DCM	34	...	IV	Orthopnea	140	108/58	91
19	42	M	MR	86	77	IV	Orthopnea	105	120/64	77
20	69	M	MR	67	58	III	Orthopnea	211	102/70	105

EF indicates ejection fraction; LVG, left ventriculography; NYHA, New York Heart Association; TR, tricuspid regurgitation; T-chol, total cholesterol; BP, blood pressure; FBS, fasting blood sugar; DCM, dilated cardiomyopathy; AR, aortic regurgitation; and MR, mitral regurgitation.

*Treated with 1 tablet of glibenclamide per day.

†Treated with 0.5 tablet of glibenclamide per day.

monary capillary wedge pressure (14 to 33 mm Hg; mean, 21 mm Hg). They were treated medically for several days to a few weeks before the study was undertaken. The study was done when they were stable and could lie supine. All patients were receiving digoxin and diuretics at the time of the study, and all other medications were discontinued at least 2 days before the study. No patient had hypercholesterolemia. One patient had isolated systolic hypertension, and 2 had a mild degree of diabetes mellitus. Both patients with diabetes were receiving glibenclamide (0.5 to 1 tablet per day). Ten control subjects were admitted to our hospital for the work-up of chest pain, and 4 subjects were admitted for the work-up of arrhythmias. Patients with chest pain had angiographically normal coronary arteries. These 14 patients had normal cardiac function as evaluated by echocardiography and/or cardiac catheterization. Exercise performance was also normal. Another 10 control subjects were volunteers who were healthy hospital employees. Control subjects did not have apparent hypertension, hyperlipidemia, or diabetes mellitus. The study protocol was fully explained, and written informed consent was obtained from each subject. The study was approved by the Human Investigation Committee of our institution.

General Procedures

The study was done with the participants in a supine position and in a postabsorptive state in an air-conditioned room with room temperature at 25° to 26°C. Under local anesthesia with 2% procaine, the left brachial artery was cannulated with a 20-gauge intravascular over-the-needle poly(tetrafluoroethylene) cannula (Quick-Cath, Travenol Lab-

oratories, Inc, Baxter Healthcare Corp) for drug infusion, and the cannula was connected by a three-way stopcock to a pressure transducer (Vigo-Spectramed) for direct measurement of arterial pressure. The arterial line was kept open by infusing heparinized saline (0.1 mL/min) while no drug was being infused. In some subjects, a vein in the antecubital region of the ipsilateral arm was cannulated with the same cannula to obtain blood samples. Heart rate was obtained by counting the pulse rate for a few minutes on arterial pressure recordings.

Measurements of Forearm Blood Flow

Forearm blood flow was measured by using a mercury-in-Silastic strain-gauge plethysmograph with a venous occlusion technique.^{19,20} The strain gauge was placed approximately 5 cm below the antecubital crease. Forearm blood flow (milliliters per minute per 100 mL of forearm tissue) was calculated from the rate of increase in forearm volume while venous return from the forearm was prevented by inflating the cuff on the upper arm. The pressure in the venous occlusion or congesting cuff was 40 mm Hg. Circulation to the hand was arrested by a cuff inflated around the wrist. The wrist cuff was inflated before the determination of forearm blood flow and continuously throughout the measurements. An average of four flow measurements made at a 15-second interval, calculated by two of the authors independently, was used for later analysis. Forearm vascular resistance was calculated by dividing the mean arterial pressure (diastolic pressure plus one third of the pulse pressure in mm Hg) by the forearm blood

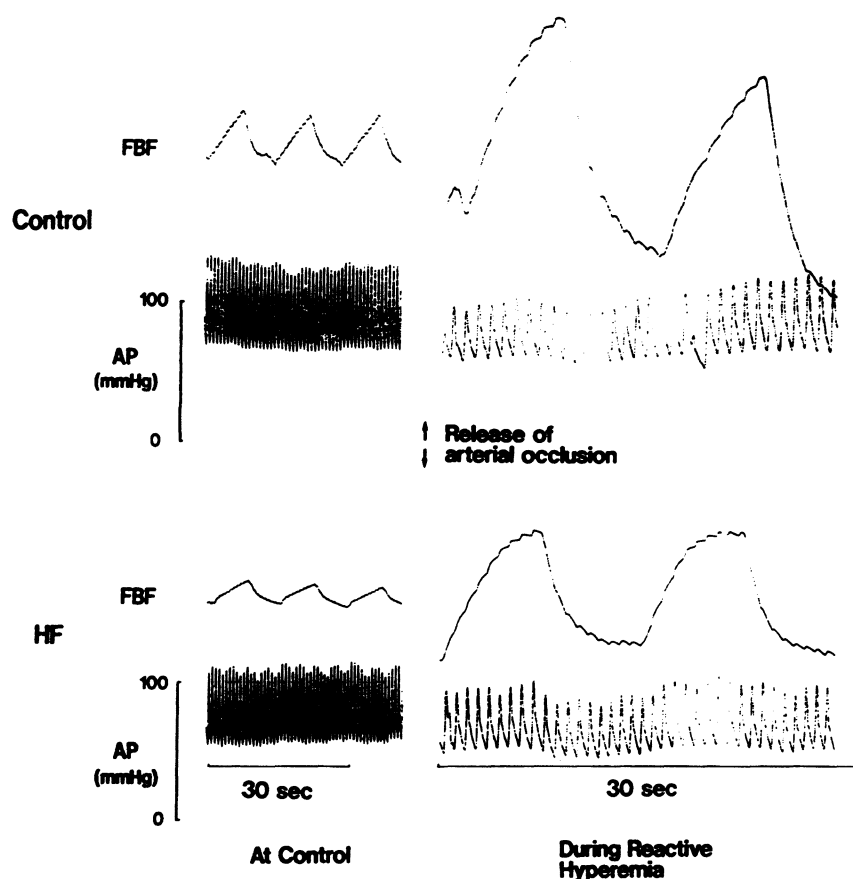


FIG 1. Representative recordings of reactive hyperemic flows after 10 minutes of arterial occlusion in a control subject and a patient with heart failure. Note attenuated flow responses in the patient with heart failure. AP indicates arterial pressure; FBF, forearm blood flow; and HF, heart failure.

flow. These values are expressed as units throughout this report.

Reactive Hyperemia

To produce reactive hyperemia, blood flow to the forearm was occluded by inflating a cuff on the upper arm to suprasystolic pressure for 10 minutes.^{21,22} After the release of arterial occlusion, forearm blood flow was measured at 5 seconds after release and every 15 seconds thereafter for 3 minutes. Reactive hyperemic flow was recorded at a paper speed of 30 cm/min, which was three times faster than that for control forearm blood flow measurements. The linear portions of plethysmographic recordings were used for calculation. Two authors calculated blood flow independently, and the average peak blood flow was used for analysis. It has been shown that maximal vasodilation of forearm resistance vessels is achieved during peak reactive hyperemia following 10 minutes of arterial occlusion and that minimal forearm vascular resistance at peak reactive hyperemia is reproducible.^{21,23} Typical recordings of forearm blood flow at rest and during reactive hyperemia in a control subject and in a patient with heart failure are shown in Fig 1.

Forearm Vascular Responses to Drugs

We examined forearm vasodilator responses to intra-arterial infusion of acetylcholine ($n=18$ for heart failure patients and $n=18$ for control subjects) or sodium nitroprusside ($n=15$ for heart failure patients and $n=18$ for control subjects) at graded doses. Acetylcholine (4, 8, 16, and 22 $\mu\text{g}/\text{min}$) or sodium nitroprusside (0.2, 0.4, 0.8, 1.2, and 1.6 $\mu\text{g}/\text{min}$) was infused intra-arterially for 2 minutes at each dose. The dose of drug infusion was altered by changing infusion volume. The maximal infusion volume for the maximal dose of acetylcholine or sodium nitroprusside was 0.8 mL/min. We have previously shown that infusion of saline at 0.6 mL/min did not affect

forearm blood flow.²⁴ The order of acetylcholine and sodium nitroprusside infusion was alternated. Infusion of the second drug was begun after forearm blood flow had returned to the baseline level. Forearm blood flow was measured continuously at a 15-second interval in the ipsilateral arm during drug infusion. Because forearm blood flow reached the steady state by 1 minute after starting infusion of each drug, we used the last 1-minute measurements during drug infusion of each dose for later analysis.

Protocols

After the placement of cannulas and a strain-gauge plethysmograph, at least 15 minutes were allowed for subjects to become accustomed to the study conditions before the experiments were begun. Forearm blood flow, arterial pressure, and heart rate were measured at rest and during graded doses of acetylcholine or sodium nitroprusside. After recovery, the forearm blood flow measurements during reactive hyperemia following 10 minutes of arterial occlusion were obtained in 12 control subjects and 12 patients with heart failure. After forearm blood flow had returned to the baseline level, L-arginine was infused into the brachial artery at 10 mg/min (0.1 mL/min) for 5 minutes, and then acetylcholine or sodium nitroprusside was infused in the same way as before L-arginine while L-arginine was infused simultaneously and continuously. During arterial occlusion by a cuff on the upper arm for reactive hyperemia, L-arginine was continuously infused at 10 mg/min. After the release of occlusion, reactive hyperemic flows were recorded in the same way as before infusion of L-arginine. L-Arginine at 10 mg/min was chosen because this dose does not alter baseline forearm blood flow but augments vasodilator responses to acetylcholine.¹⁷ In three patients with heart failure, reactive hyperemic responses before and during simultaneous infusion of D-arginine (10 mg/min) were examined. We reported previously that D-arginine did not augment

TABLE 2. Baseline Values for Control Subjects and Patients With Heart Failure

	Mean BP, mm Hg	HR, bpm	FBF, mL · min ⁻¹ · 100 mL ⁻¹	FVR, U
Control subjects (n=24)	87.0±1.9	63.4±2.1	4.4±0.3	22.4±1.9
Patients with heart failure (n=20)	82.0±3.0	73.5±2.4*	2.6±0.2†	37.0±3.6†

BP indicates blood pressure; HR, heart rate; bpm, beats per minute; FBF, forearm blood flow; and FVR, forearm vascular resistance. * $P < .05$ and † $P < .01$ vs control values.

acetylcholine-induced forearm vasodilation.¹⁷ In some patients, the reactive hyperemia study was done without studies with acetylcholine and sodium nitroprusside.

Measurements of Plasma Levels of Renin, Norepinephrine, and Atrial Natriuretic Peptide

Before experiments, we sampled venous blood from the contralateral arm for measurements of plasma renin activity, norepinephrine, and atrial natriuretic peptide concentrations. Venous blood was sampled into the tube containing EDTA-2Na (1 mg/mL) and promptly chilled in an ice bath. After plasma was removed, aliquots of the plasma sample were stored at -20°C until use. Plasma renin activity and atrial natriuretic peptide concentration were determined by radioimmunoassay (RIA). Plasma norepinephrine concentration was determined by high-performance liquid chromatography. All measurements were done at a commercially available laboratory (SRL).

Drugs

Because acetylcholine is unstable in solution, 100 mg of acetylcholine (Daiichi Pharmaceutical) was lyophilized and stored in a vial (0.4 mg acetylcholine per vial). It was dissolved in physiological saline immediately before use. Sodium nitroprusside (Wakou Junyaku Kogyo) was dissolved in physiological saline at a concentration of 2000 ng/mL. Special care was taken not to expose sodium nitroprusside to light. For the infusion of L-arginine, commercially available L-arginine solution (0.1 g of L-arginine per milliliter; Morishita Pharmaceutical) was used. The pH of L-arginine solution was 5.0 to 6.0. D-Arginine was obtained from Sigma Chemical Co and prepared for human use at the pharmaceutical division of our hospital.

Statistical Analysis

Values at rest were compared by unpaired *t* test between control subjects and patients with heart failure. The effects of L-arginine on the resting values were analyzed by paired *t* test. One-way or two-way ANOVA was used when appropriate. When they were significantly different by two-way ANOVA, values at each dose or at same time after arterial occlusion were compared by paired or unpaired *t* test. Bonferroni's correction was used when necessary. Because resting forearm vascular resistance was significantly different between control subjects and patients with heart failure, responses in forearm vascular resistance to graded doses of drugs were normalized by baseline forearm vascular resistance and compared by two-way ANOVA. All values are expressed as mean±SEM. $P < .05$ was considered to be statistically significant.

Results

Baseline Hemodynamics and Hormonal Values

Baseline hemodynamic values are shown in Table 2. Mean blood pressure at rest did not significantly differ between patients with heart failure and control subjects. Heart rate at rest was significantly higher ($P < .05$) in patients with heart failure than in control subjects. Forearm blood flow at rest was smaller ($P < .01$) and forearm vascular resistance at rest was greater ($P < .01$) in patients with heart failure than in control subjects.

Plasma renin activity (5.5 ± 1.1 versus 1.7 ± 0.6 ng/mL per hour), norepinephrine concentration (0.51 ± 0.11 versus 0.15 ± 0.02 ng/mL) and atrial natriuretic peptide concentration (142 ± 30 versus 38 ± 3 pg/mL) in patients with heart failure (n=9) were significantly higher than in control subjects (n=8) ($P < .01$ for each).

Forearm Responses to Drugs and After 10 Minutes of Arterial Occlusion

Intra-arterial infusions of acetylcholine or sodium nitroprusside did not alter mean blood pressure or heart rate (data not shown). The graded doses of acetylcholine and sodium nitroprusside caused progressive increases in forearm blood flow ($P < .01$) in control subjects and patients with heart failure (Fig 2). The magnitudes of increases in forearm blood flow in response to acetylcholine were less in patients with heart failure than in control subjects ($P < .01$). However, the magnitudes of increases in forearm blood flow in response to sodium nitroprusside did not differ between the two groups. Fig 3 shows percent decreases in forearm vascular resistance during infusions of acetylcholine and sodium nitroprusside at graded doses in the two groups. In patients with heart failure, the percent decreases in forearm vascular resistance during graded infusions of acetylcholine were attenuated ($P < .01$) compared with those in control subjects. However, the percent decreases in forearm vascular resistance during graded infusions of sodium nitroprusside did not differ between the two groups.

Time courses of reactive hyperemic flow and vascular resistance in control subjects and patients with heart failure are shown in Fig 4. Maximal forearm blood flow during peak reactive hyperemia following release of 10 minutes of arterial occlusion was significantly lower in patients with heart failure (n=12) than in control subjects (n=12) (30 ± 3 versus 43 ± 3 mL/min per 100 mL, $P < .01$). Minimal forearm vascular resistance during peak reactive hyperemia was significantly higher in patients with heart failure than in control subjects (3.2 ± 0.4 versus 2.1 ± 0.1 U, $P < .05$). To examine the correlation between the degree of endothelial dysfunction assessed by acetylcholine and the degree of reactive hyperemia impairment, we plotted the maximal flow responses to acetylcholine against those during reactive hyperemia in Fig 5. There was a weak but significant ($P < .05$) correlation between them.

Effects of L-Arginine

Infusion of L-arginine at the dose used in this study did not alter forearm blood flow, arterial pressure, or heart rate in control subjects or patients with heart failure (Table 3). Figs 6 and 7 show forearm vascular responses to infusion of acetylcholine and sodium nitroprusside in control subjects (Fig 6) and patients with heart failure (Fig 7) before and after L-arginine. In

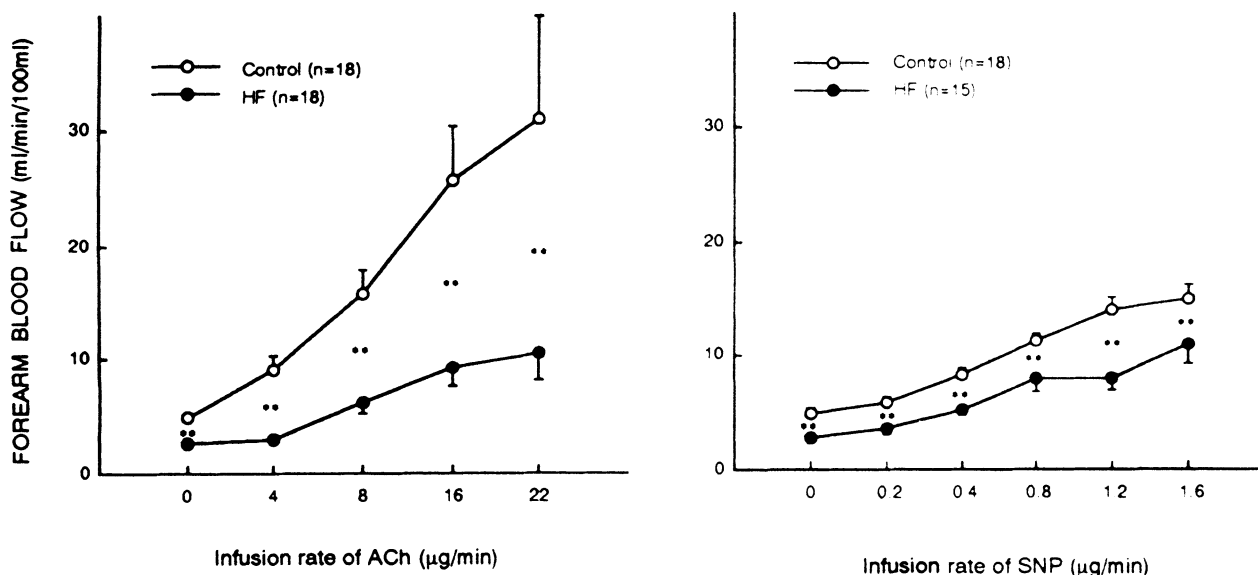


FIG 2. Plots showing responses of forearm blood flow (FBF) to acetylcholine (ACh) and sodium nitroprusside (SNP) in patients with heart failure (●) and in control subjects (○). The FBF at rest and during infusion of ACh and SNP was less in patients with heart failure than in control subjects. The magnitudes of the increases in FBF in response to ACh were less in patients with heart failure than in control subjects, but those in response to SNP were comparable. HF indicates heart failure. ** $P < .01$.

control subjects, pretreatment with L-arginine significantly augmented acetylcholine-induced vasodilation at the lower doses but not at the higher doses, whereas in patients with heart failure, pretreatment with L-arginine augmented acetylcholine-induced vasodilation not only at the lower doses but also at the higher doses. Thus, pretreatment with L-arginine augmented acetylcholine-induced maximal vasodilation only in patients with heart failure. Pretreatment with L-arginine did not alter responses of forearm vascular resistance to sodium nitroprusside in control subjects or in patients with heart failure.

Pretreatment with L-arginine did not affect the maximal reactive hyperemic flow or minimal forearm vascular resistance (Fig 8) in control subjects. Pretreatment with L-arginine augmented maximal reactive hyperemic flow (Figs 9 and 10) and decreased minimal forearm vascular resistance ($P < .01$) (Fig 10) in patients with

heart failure. Pretreatment with D-arginine did not change heart rate, arterial pressure, forearm blood flow, or forearm vascular resistance at rest and reactive hyperemic responses in patients with heart failure (data not shown).

Discussion

Major study findings are that endothelium-dependent forearm vasodilation to acetylcholine was impaired but endothelium-independent vasodilation to sodium nitroprusside was not altered in patients with heart failure and that pretreatment with L-arginine (a precursor of endothelium-derived nitric oxide) improved the magnitude of maximal vasodilation induced by acetylcholine and vasodilation during peak reactive hyperemia only in patients with heart failure. These findings suggest that decreased maximal vasodilation to ischemic stimuli in

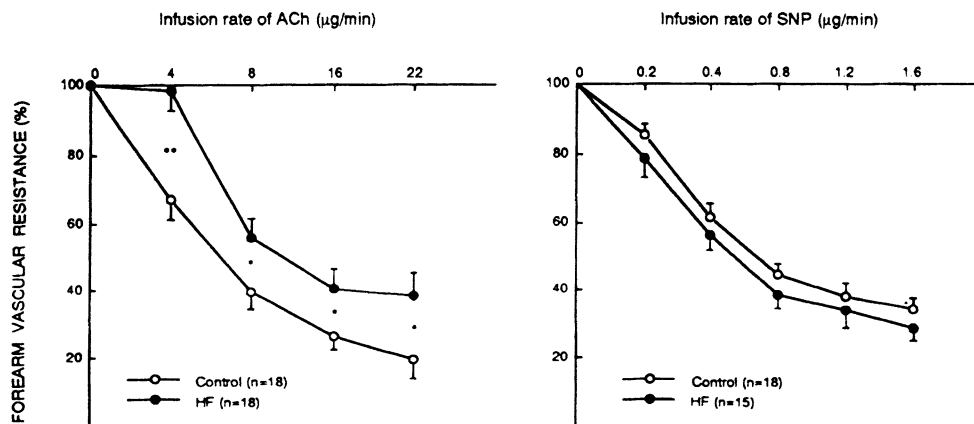


FIG 3. Plots showing responses of forearm vascular resistance (FVR) (percent changes from the baseline) in response to acetylcholine (ACh) and sodium nitroprusside (SNP) in patients with heart failure (●) and in control subjects (○). The percent decreases in FVR to ACh were attenuated in patients with heart failure versus control subjects. The percent decreases in FVR to SNP did not differ. The values before drug infusion are shown as 100%. HF indicates heart failure. * $P < .05$, ** $P < .01$.

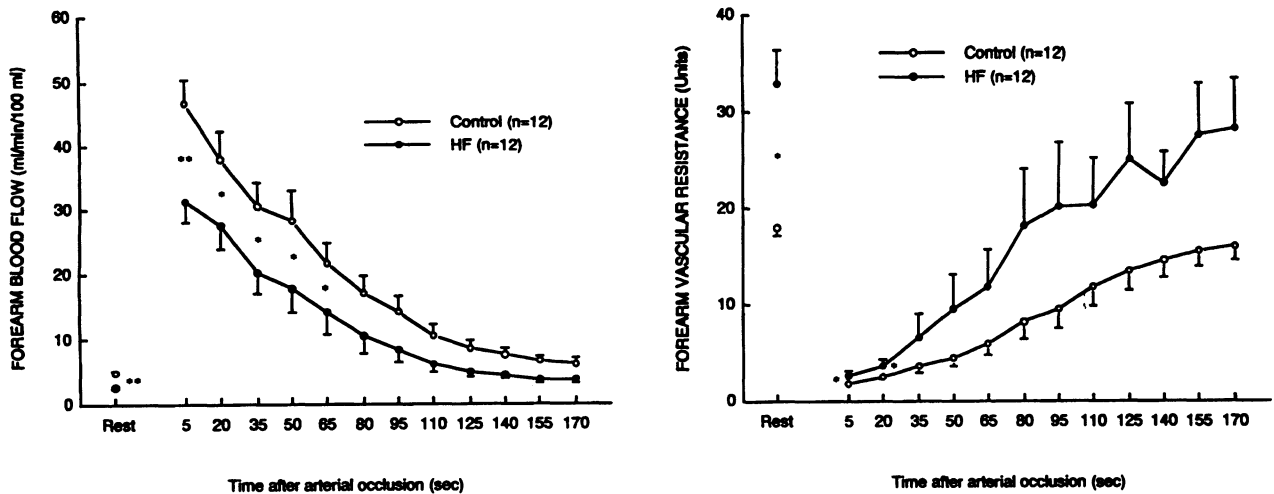


FIG 4. Plots of time courses of reactive hyperemic flows (left) and vascular resistance (right) in control subjects (○) (n=12) and patients with heart failure (●) (n=12). HF indicates heart failure. * $P < .05$, ** $P < .01$.

patients with heart failure may be in part due to a defect in the release of nitric oxide from the endothelium.

Decreased Vasodilator Responses to Acetylcholine in Patients With Heart Failure

Previous studies have demonstrated that acetylcholine-induced vasodilation is impaired in heart failure in animals⁷⁻⁹ as well as humans.³⁻⁶ In this study, we also demonstrated that the magnitude of forearm vasodilation evoked with acetylcholine was much less in patients with heart failure than in control subjects, whereas the magnitude of forearm vasodilation evoked with sodium nitroprusside (an endothelium-independent vasodilator) was comparable between the two groups. Although one patient had isolated systolic hypertension due to aortic regurgitation and two had mild degree of diabetes mellitus, forearm blood flow responses to acetylcholine were still abnormal when these three patients were excluded (data not shown). Thus, the present and previous studies suggest that endothelium-dependent vasodilation evoked with acetylcholine is impaired in patients with heart failure.

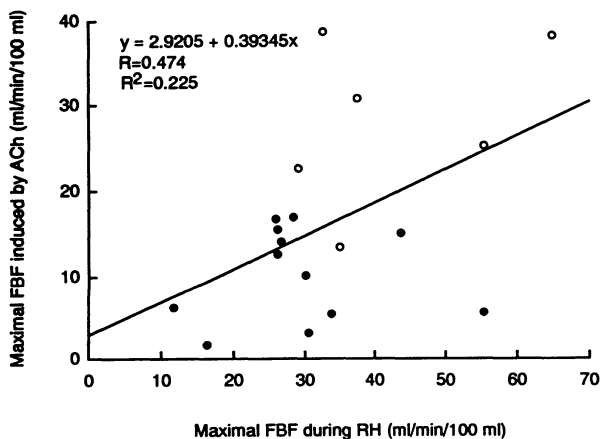


FIG 5. Scatterplot of correlation between the degree of endothelial dysfunction assessed by acetylcholine (ACh) and the degree of reactive hyperemia (RH) impairment. ○ Indicates control subjects; ●, patients with heart failure. FBF indicates forearm blood flow.

Several mechanisms have been considered to account for attenuated forearm vasodilation evoked with acetylcholine in patients with heart failure, which include defective release of nitric oxide from the endothelium, reduced responsiveness of vascular smooth muscle to nitric oxide, and facilitated breakdown of nitric oxide. As was discussed by previous investigators,³⁻⁶ reduced responsiveness of vascular smooth muscle to nitric oxide was not likely since the magnitude of vasodilation evoked with sodium nitroprusside was comparable between patients with heart failure and control subjects. Facilitated breakdown of nitric oxide in heart failure is also unlikely, since we have previously shown that endothelium-dependent vasodilation to substance P did not differ between patients with heart failure and control subjects.⁴ We examined the possibility of defective release of nitric oxide by studying the effects of L-arginine supplementation on acetylcholine-induced forearm vasodilation.

L-Arginine is a precursor of endothelium-derived nitric oxide.^{10,11} Recently, we¹⁷ and Panza et al¹⁸ showed that pretreatment with L-arginine augmented acetylcholine-induced vasodilation but did not alter sodium nitroprusside-induced vasodilation in the forearm of healthy humans. We also showed that higher doses of L-arginine caused vasodilation.¹⁷ D-Arginine did not have such effects.^{17,18} Thus, it appears that supplementation of L-arginine facilitates production of nitric oxide in the forearm of healthy humans. This study further demonstrated that supplementation of L-arginine augmented acetylcholine-induced vasodilation in patients with heart failure as well as in control subjects. Of note is that supplementation of L-arginine augmented vasodilation evoked with acetylcholine at the higher doses in patients with heart failure but not in control subjects. The reason for the difference in these results is not known, but we consider that in control subjects, maximal endothelium-dependent vasodilation that could be evoked with acetylcholine had been achieved in control subjects so that supplementation of L-arginine did not further augment vasodilation to acetylcholine at the higher doses in control subjects. Nevertheless, supplementation of L-arginine augmented vasodilation to ace-

TABLE 3. Baseline Values Before and After L-Arginine

	Mean BP, mm Hg	HR, bpm	FBF, mL · min ⁻¹ · 100 mL ⁻¹	FVR, U
Control subjects (n=12)				
Before L-arginine	86.6±2.4	61±2.2	4.4±0.4	21.3±2.2
After L-arginine	87.8±2.4	58±2.8	4.5±0.4	21.2±1.7
Patients with heart failure (n=15)				
Before L-arginine	80.0±3.8	75±3.0	2.8±0.3	34.4±4.0
After L-arginine	84.3±2.8	74±3.1	2.9±0.3	35.0±4.3

BP indicates blood pressure; HR, heart rate; bpm, beats per minute; FBF, forearm blood flow; and FVR, forearm vascular resistance. Values are not different before and after L-arginine in either control subjects or patients with heart failure.

tylcholine at the higher doses in patients with heart failure, and maximal vasodilation evoked with acetylcholine after L-arginine supplementation was comparable between patients with heart failure and control subjects. These results suggest that the decreased vasodilation to acetylcholine at the higher doses in patients with heart failure was most likely due to defective release of nitric oxide from the endothelium.

Because venous pressure is high and there may be organic changes in the arteriole such as the increase in

sodium and water content in vessel walls in patients with heart failure, these mechanical factors could have been responsible for attenuated forearm vasodilation to acetylcholine in patients with heart failure. However, the vasodilator responses to sodium nitroprusside were comparable between the two groups, and supplementation of L-arginine caused further acetylcholine-induced vasodilation in patients with heart failure. Moreover, the magnitudes of maximal vasodilation evoked with acetylcholine after L-arginine did not differ between the

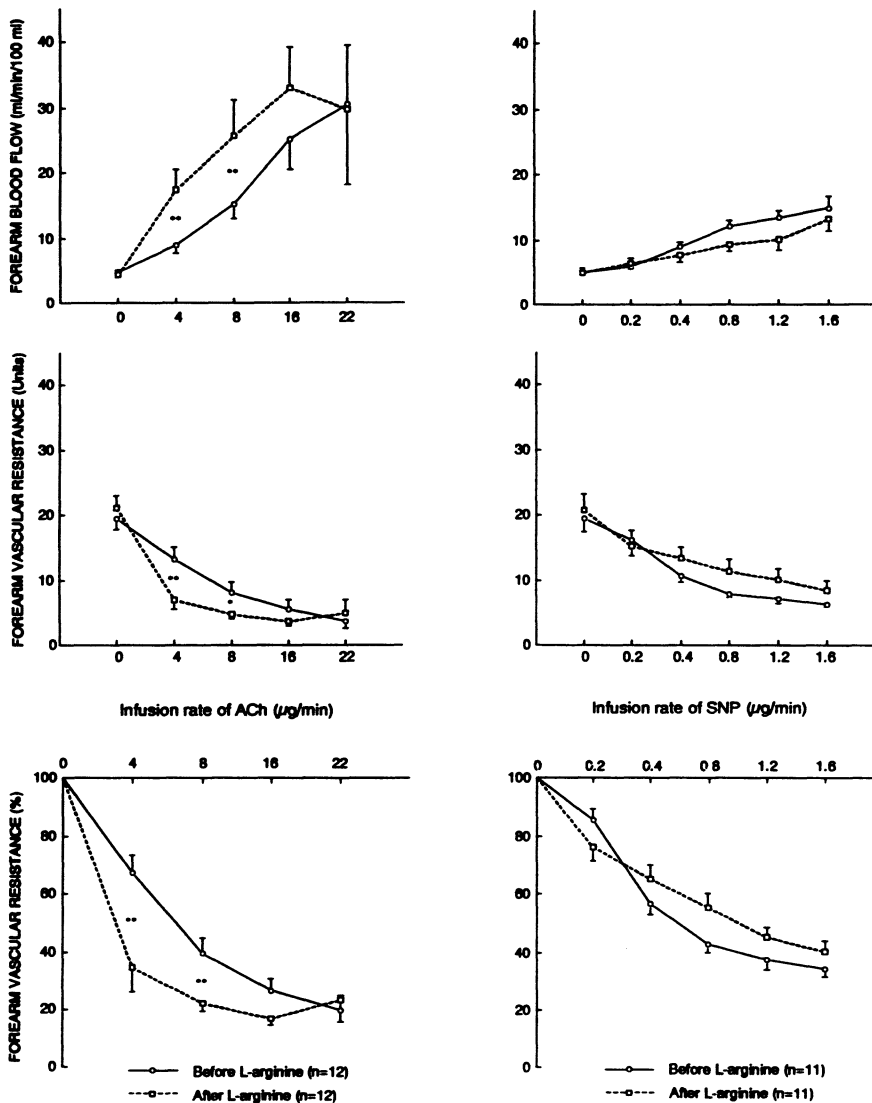


FIG 6. Plots showing responses of forearm blood flow and forearm vascular resistance (absolute values and percent changes from the baseline) in response to acetylcholine (ACh) and sodium nitroprusside (SNP) in control subjects before (○) and after L-arginine (□). L-Arginine augmented the vasodilator responses to ACh at the lower doses but not at the higher doses. L-Arginine did not alter vasodilator responses to SNP. The values of forearm vascular resistance before drug infusion are shown as 100%. * $P < .05$, ** $P < .01$.

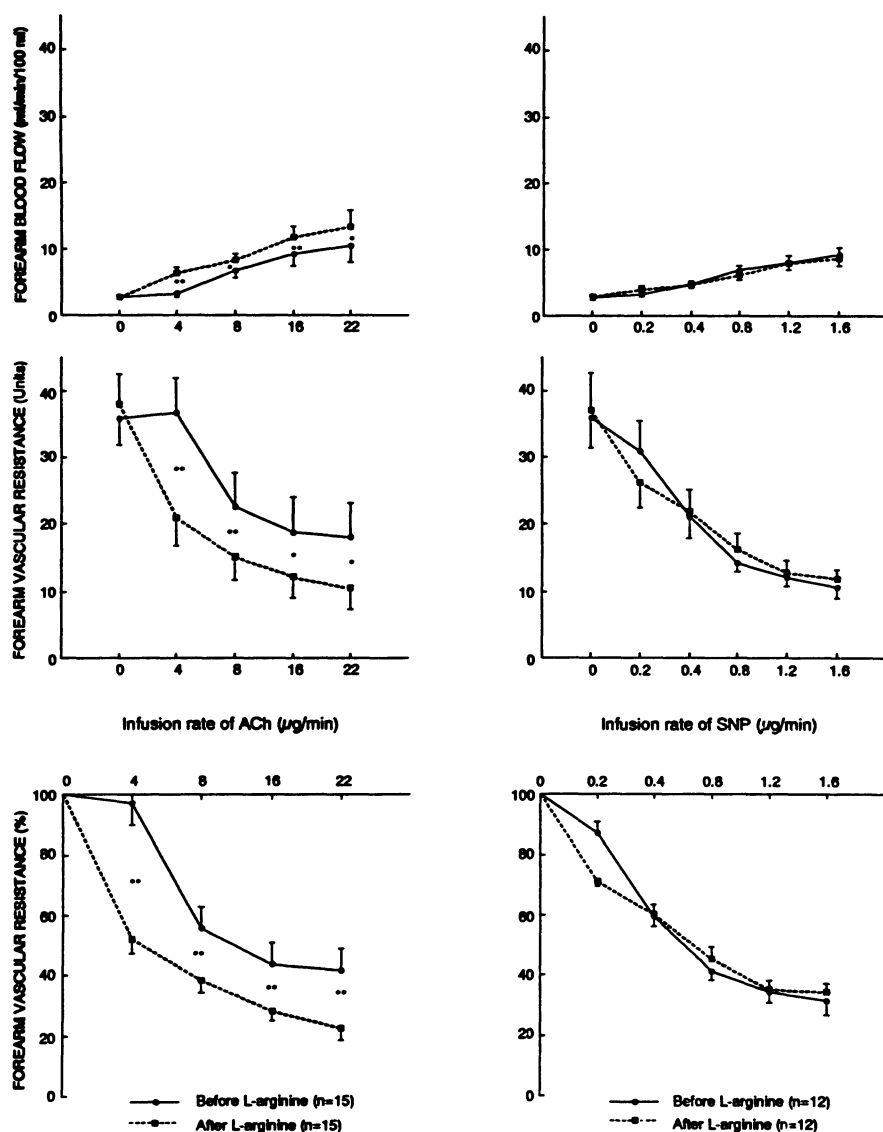


FIG 7. Plots showing responses of forearm blood flow and forearm vascular resistance (absolute values and percent changes from the baseline) in response to acetylcholine (ACh) and sodium nitroprusside (SNP) in patients with heart failure before (●) and after L-arginine (■). L-Arginine augmented the vasodilator responses to ACh at the lower doses and at the higher doses. L-Arginine did not alter vasodilator responses to SNP. The values of forearm vascular resistance before drug infusion are shown as 100%. * $P < .05$, ** $P < .01$.

two groups. These results strongly suggest that attenuated forearm vasodilation to acetylcholine before L-arginine did not result from mechanical factors, since L-arginine should not affect these factors within several minutes.

The effects of L-arginine were not nonspecific since D-arginine did not affect maximal forearm blood flow in some patients with heart failure. Moreover, we¹⁷ and Panza et al¹⁸ have previously shown that D-arginine did not affect acetylcholine-induced vasodilation.

Acetylcholine causes vasodilation by other mechanisms such as releases of prostacyclin²⁵ and endothelium-derived hyperpolarizing factor (EDHF)²⁶ or inhibition of norepinephrine release.²⁵ Although Linder et al²⁷ have shown in healthy controls and patients with hypertension that pretreatment with aspirin or phentolamine did not affect the magnitude of acetylcholine-induced forearm vasodilation, there are no data on the contribution to these factors in patients with heart failure. Contribution of an EDHF is also unknown. Further studies are needed to clarify these possibilities.

Decreased Maximal Vasodilation During Reactive Hyperemia in Patients With Heart Failure

Previous studies suggest that arterial occlusion for 10 minutes is the most potent vasodilator stimulus that can be applied to the study in human forearm.^{1,28,29} It was previously shown that adding repetitive handgrip exercise to 10 minutes of ischemia did not further lower minimal forearm vascular resistance compared with that following 10 minutes of arterial occlusion alone and that infusion of vasoconstrictor drugs or reflex sympathetic stimulation did not limit peak reactive hyperemia flow following 10 minutes of arterial occlusion.^{1,21,23,30} Thus, we applied 10 minutes of arterial occlusion to examine vasodilator capacity in our patients with heart failure and control subjects.

In this study, the peak forearm blood flow during reactive hyperemia following 10 minutes of arterial occlusion was significantly less and the minimal forearm vascular resistance was significantly higher in patients with heart failure than in control subjects. Our findings are consistent with those by Zelis et al,^{1,2} who showed that maximal vasodilation during reactive hyperemia

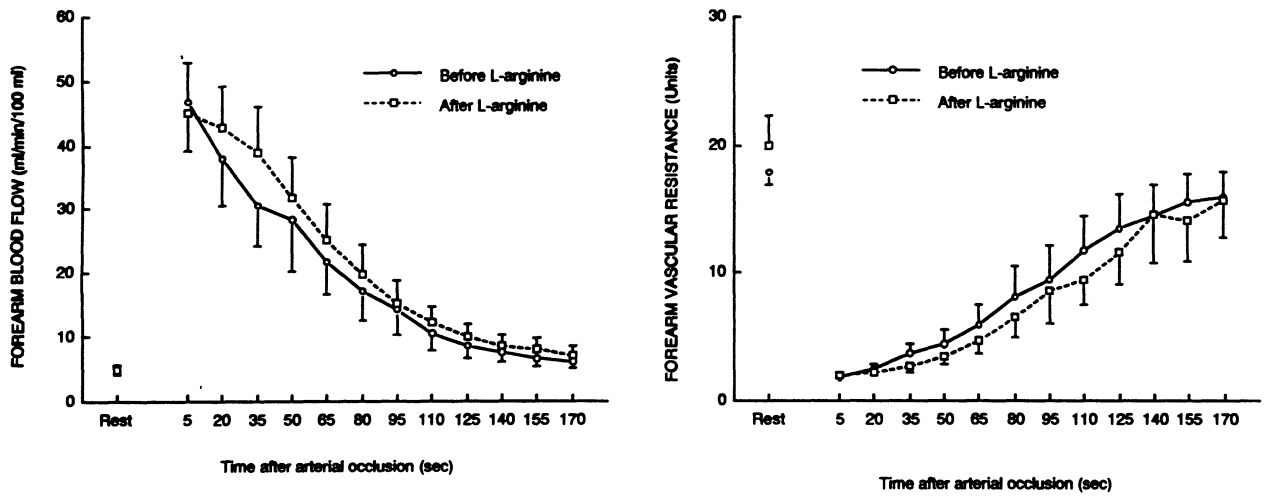


FIG 8. Plots showing pooled data of the time course of forearm blood flow and forearm vascular resistance during reactive hyperemia in control subjects before (○) and after L-arginine (□). L-Arginine did not affect maximal forearm blood flow or minimal forearm vascular resistance.

was reduced in patients with heart failure and suggest that patients with heart failure have impaired ischemic vasodilator mechanisms. The minimal forearm vascular resistance in control subjects was 2.1 ± 0.1 U, and that in

patients with heart failure was 3.2 ± 0.4 U. These values are comparable with those in previous reports.^{1,31} Several mechanisms are considered to account for the decreased ischemic vasodilator capacity in patients with

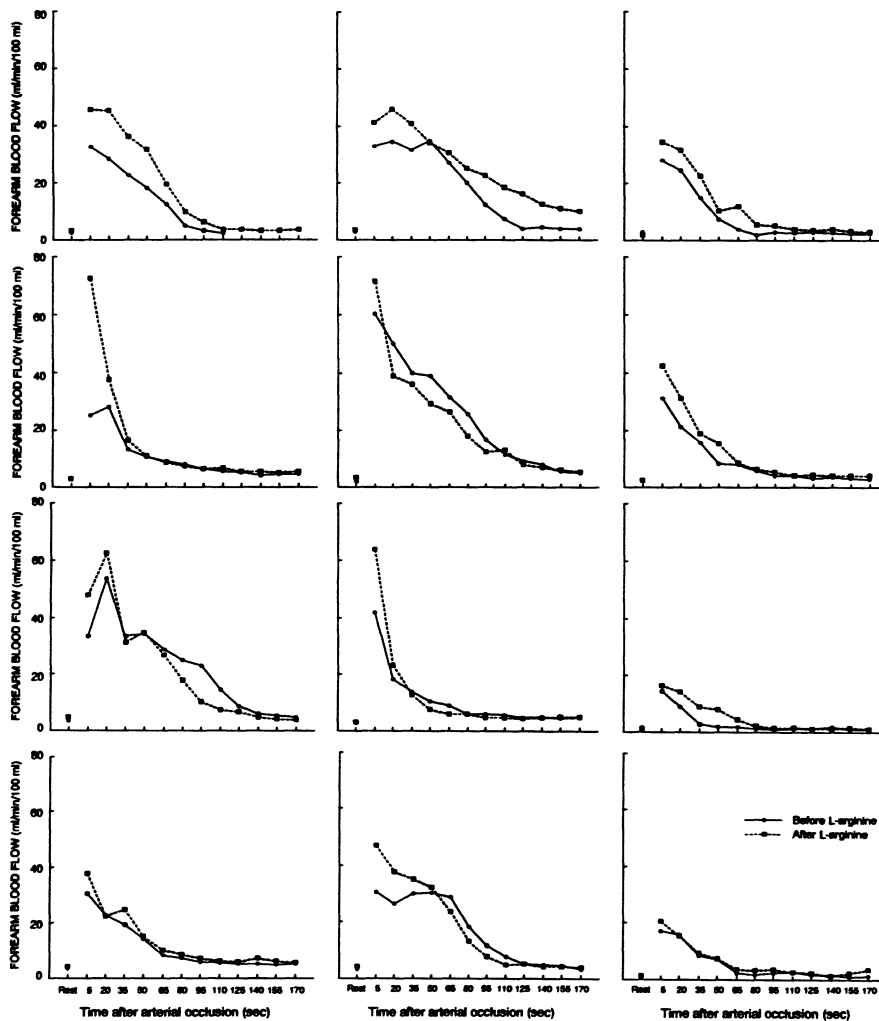


FIG 9. Plots showing the individual data of the time-course of forearm blood flow during reactive hyperemia in patients with heart failure before (●) and after L-arginine (■).

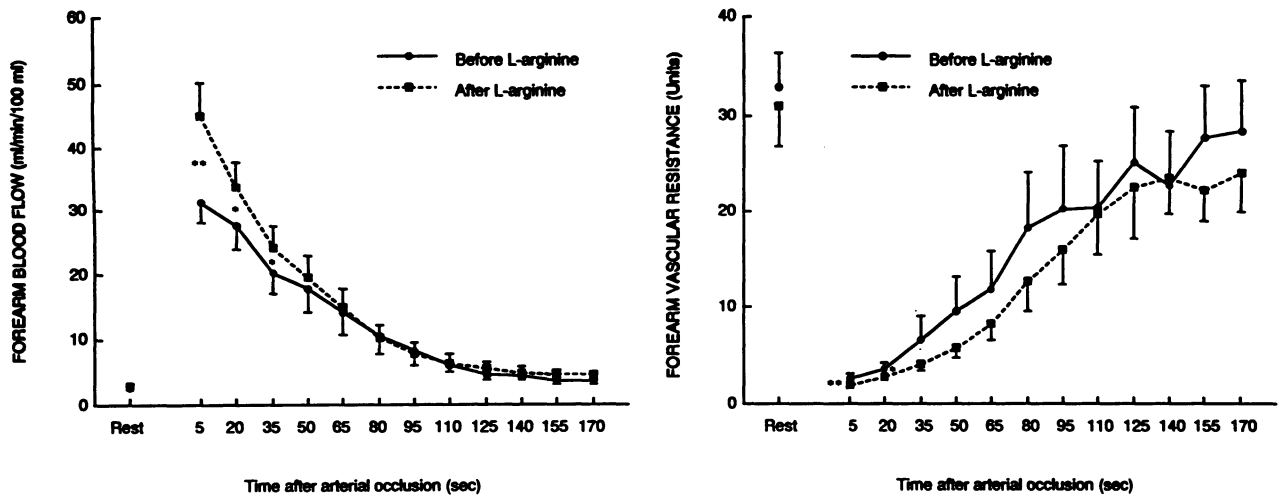


FIG 10. Plots showing pooled data of the time-course of forearm blood flow (left) and forearm vascular resistance (right) during reactive hyperemia in patients with heart failure before (●) and after L-arginine (■). L-Arginine increased maximal forearm blood flow and decreased minimal forearm vascular resistance. * $P < .05$, ** $P < .01$.

heart failure. Zelis et al^{1,31} considered that it was due to the increase in the sodium and water content in vessel walls, because diuretic therapy improved the impaired maximal vasodilator capacity in edematous patients with heart failure. However, a mechanism other than sodium and water retention in vessel walls is involved because peak reactive hyperemic flow after diuretic therapy was still 32% below normal.³¹ Activated neuro-humoral factors or increased venous pressure were unlikely to contribute to the decreased ischemic vasodilator capacity in patients with heart failure.^{1,2}

The important and unexpected finding of this study is that pretreatment with L-arginine nearly restored impaired ischemic vasodilation during reactive hyperemia in patients with heart failure, who did not have peripheral edema at the time of study. This finding may suggest that impaired ischemic vasodilation in our patients with heart failure resulted largely from a defect in release of nitric oxide from the endothelium. The significant correlation between the maximal blood flow responses to acetylcholine and peak reactive hyperemic flow (Fig 5) may support this notion.

Recently, it has been demonstrated in coronary³²⁻³⁴ and peripheral circulation³⁵⁻³⁷ of animals that a nitric oxide synthase inhibitor did not affect peak reactive hyperemic flow. These results may suggest in healthy animals that nitric oxide release may not contribute to peak reactive hyperemia. In this study, peak reactive hyperemic flow was not affected by pretreatment with L-arginine in control subjects. This finding in control subjects accords with those in animals. Thus, supplementation of L-arginine augmented maximal vasodilation evoked with acetylcholine and during reactive hyperemia only in patients with heart failure but not in control subjects.

It has been shown that endothelin is elevated in plasma in patients with heart failure,^{38,39} which may contribute to control of vasomotion in heart failure. Although it is possible that drugs for heart failure may affect endothelin-dependent vasomotion, it is not likely that drugs for heart failure affected endothelin-dependent vasomotion because no drugs, except digoxin and

diuretics, were being taken by the patients at the time of the study.

Clinical Implications

It has been suggested that fatigue in patients with heart failure is in part due to impaired vasodilation in skeletal muscle.² Our results suggest that defective endothelial function may contribute to impaired vasodilation in skeletal muscle. It is possible that improved endothelium function may lead to the increase in exercise tolerance. It has been shown that angiotensin-converting enzyme inhibitors improve impaired vasodilator capacity and increase exercise tolerance in patients with heart failure.^{40,41} Furthermore, it has been shown that angiotensin-converting enzyme inhibitors improve impaired endothelial function in hypertensive animals⁴² as well as humans.⁴³ Thus, it is assumed that the beneficial effects of an angiotensin-converting enzyme inhibitor on skeletal muscle vasodilator capacity and fatigue in patients with heart failure may be secondary to improved endothelial function. However, conclusion should be awaited until L-arginine is shown to improve impaired vasodilator capacity in skeletal muscle during exercise in patients with heart failure.

In summary, our results suggest that impaired forearm vasodilation to ischemic stimuli in patients with heart failure may be in part due to a defect in the release of nitric oxide from the endothelium.

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