JACC Vol. 19, No. 5
April 1992:918–25

Impaired Endothelium-Mediated Vasodilation in the Peripheral Vasculature of Patients With Congestive Heart Failure

STUART D. KATZ, MD,* LUIGI BIASUCCI, MD, CARLO SABBA, MD, JOEL A. STROM, MD, FACC, GUILLAUME JONDEAU, MD, MARIE GALVAO, RN, STEVEN SOLOMON, BS, SRDJAN D. NIKOLIC, PhD, ROBERT FORMAN, MD, FACC, THIERRY H. LEJEMTEL, MD

Bronx, New York

Impaired endothelial-dependent vasodilation has been demonstrated in two animal models of congestive heart failure and in the coronary circulation of patients with idiopathic dilated cardiomyopathy. To determine whether this impairment contributes to the abnormal peripheral vasomotor tone in patients with congestive heart failure, the local vascular response to intraarterial infusions of graded concentrations (10^{-8} *M* to 10^{-5} *M*) of acetylcholine (an endothelial-dependent vasodilator) and nitroglycerin (a direct-acting vasodilator) was studied in the superficial femoral artery of 19 patients with congestive heart failure (New York Heart Association classes I to IV) and 6 age-matched normal control subjects.

The local vascular response was determined from the arterial blood flow velocity pattern obtained by transcutaneous Doppler ultrasonography. Acetylcholine, 10^{-5} *M*, induced a pattern characteristic of vasodilation in all six normal subjects; mean blood flow velocity for the group significantly increased from 11.9 ± 2.7 to 44.8 ± 20.9 cm/s ($p < 0.05$). In contrast, the same dose of acetylcholine induced a blood flow velocity pattern characteristic of vasodilation in only 4 of the 19 patients with congestive heart failure. Group mean blood flow velocity did not change significantly. Nitroglycerin, 10^{-7} *M*, induced vasodilation in all 6 normal subjects but in only 1 of 19 patients. Nitroglycerin, 10^{-5} *M*, was administered to 10 patients; all 10 demonstrated a pattern characteristic of vasodilation.

Thus, acetylcholine-mediated endothelial-dependent vasodilation appears to be impaired in the peripheral vasculature of patients with congestive heart failure. Both endothelial dysfunction and abnormal vascular smooth muscle responsiveness may contribute to abnormal peripheral vasomotor tone.

(J Am Coll Cardiol 1992;19:918–25)

The skeletal muscle vasculature does not adequately dilate in response to exercise in patients with severe congestive heart failure (1). The absence of an adequate vasodilator response during exercise may be partially responsible for depressed peak aerobic capacity of these patients (2). Abnormal peripheral vasomotor tone at rest and during exercise has been attributed to increased sodium and water content in the arterial wall, neurohumoral activation and intrinsic abnormalities of the vascular smooth muscle (3).

Abnormalities of the vascular endothelium may also contribute to increased vasomotor tone in congestive heart failure. The vascular endothelium was recently shown to release vasoactive substances that play an important role in the normal regulation of vasomotor tone (4–6). Endothelium-derived relaxing factor modulates the vascular smooth muscle response to vasoactive hormones in both conduit and resistance vessels (7). It is chemically indistinguishable from nitric oxide and, like organic nitrate preparations used clinically, mediates vasorelaxation through activation of soluble guanylate cyclase in vascular smooth muscle (8). Release of endothelium-derived relaxing factor that is induced by increased shear stress at the arterial wall may be an important mediator of vasodilation during physiologic states of increased peripheral blood flow (9), such as those observed during maximal exercise. Impaired endothelial-dependent vasodilation has been reported in conduit vessels in two animal models of experimental heart failure (10,11) and in the coronary circulation of patients with idiopathic dilated cardiomyopathy (12,13). Whether this impaired vasodilation also contributes to abnormal vasomotor tone in the lower limb skeletal muscle vasculature of patients with congestive heart failure has not yet been described.

Accordingly, in this study we compared the local vascular responses to intraarterial infusions of an endothelial-dependent vasodilator, acetylcholine, and a direct-acting vasodilator, nitroglycerin, in the superficial femoral artery of normal subjects and patients with congestive heart failure.

From the Department of Medicine, Division of Cardiology, The Albert Einstein College of Medicine, Bronx, New York.

Manuscript received July 30, 1991; revised manuscript received September 18, 1991, accepted September 20, 1991.

*Present address: Montefiore Medical Center, 111 East 210 Street, Bronx, New York 10467.

Address for reprints: Thierry H. LeJemtel, MD, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, New York 10461.

0735-1097/92/$5.00

Table 1. Clinical Characteristics of 19 Patients

Pt No.	Age (yr)/ Gender	EF (%)	NYHA Class	Etiology	HTN History	DM	Serum Cholesterol (mg/dl)
1	65/M	15	III	CAD	–	–	240
2	66/M	20	III	CAD	–	+	216
3	73/M	23	II	CAD		+	251
4	60/M	15	IV	CAD	–	–	220
5	59/F	31	I	CAD	–	–	252
6	65/F	35	III	CAD	+	+	167
7	49/M	29	III	CAD	+	–	174
8	48/M	26	II	IDC	–	–	346
9	57/M	20	III	IDC	–	–	233
10	60/M	22	IV	IDC			148
11	52/F	21	II	IDC	–	+	350
12	47/M	12	III	IDC	–	–	174
13	59/M	14	II	IDC	–	+	178
14	73/M	20	III	IDC	–	–	264
15	40/F	20	II	IDC	–	+	179
16	35/M	18	III	IDC	–	–	131
17	62/F	15	III	IDC	–	–	234
18	71/M	27	II	IDC	–	–	170
19	61/M	20	III	IDC	–	–	125
Mean	58	22					
SD	12	6					

CAD = coronary artery disease; DM = diabetes mellitus; EF = ejection fraction (obtained by radionuclide ventriculography in all 19 subjects); F = female; HTN = hypertension; IDC = idiopathic dilated cardiomyopathy; M = male; NYHA Class = New York Heart Association functional class; Pt = patient.

Methods

Study patients. (Table 1). Nineteen ambulatory patients with chronic congestive heart failure of at least 3 months' duration were studied. Their clinical characteristics are summarized in Table 1. In seven patients left ventricular dysfunction was primarily due to coronary artery disease, as determined by coronary angiography or previous documented myocardial infarction. Three of the seven were insulin-dependent diabetic patients and two had a remote history of hypertension. The etiology of left ventricular dysfunction was idiopathic dilated cardiomyopathy in 12 patients. Three of these 12 also were insulin-dependent diabetic patients, and 1 had a history of labile hypertension that was not considered the primary cause of left ventricular dysfunction. No patient was hypertensive at the time of the study.

All patients were receiving medical therapy for congestive heart failure. The regimen included diuretic drugs in all patients, digoxin and angiotensin-converting enzyme inhibitors (either captopril [n = 14] or enalapril [n = 4]) in 18 patients, long-acting nitrate preparations in 6 patients and investigational phosphodiesterase-inhibiting agents in 11 patients. Heart failure was well compensated in all patients. None had peripheral edema.

Four men and two women served as normal control subjects. In view of the age dependence of endothelium-induced vasodilation (14), normal subjects were matched for age with the patients with congestive heart failure (mean age 50 ± 11 in the normal group vs. 58 ± 12 years in the patients [p = NS]). The normal subjects had no cardiac disease or chronic medical condition. The study protocol was approved by the Committee on Clinical Investigations of the Albert Einstein College of Medicine and written, informed consent was obtained from all patients and normal subjects.

Study protocol. Patients were studied in a fasting state, in a quiet temperature- and humidity-controlled room. All cardiovascular medications were withheld for ≥24 h before the study. After administration of 1% lidocaine for local anesthesia, a 3F, 10-cm polyethylene catheter was inserted into the common femoral artery for pressure monitoring and local intraarterial drug infusions. Proper positioning of the catheter tip was verified by two-dimensional ultrasound and an adequate pressure waveform. The subjects were then allowed to rest for ≥30 min.

All drugs were mixed in 5% dextrose in water on the day of the study. All concentrations are reported as final regional blood concentrations, assuming that an average common femoral blood flow was 400 ml/min (15). Drugs were infused at a rate of 1 ml/min for 2 min through the common femoral artery catheter by volumetric pump or by hand-held injection in the following sequence: mannitol, 10^{-3} *M* (vehicle control); acetylcholine, 10^{-8} *M*, 10^{-7} *M*, 10^{-6} *M* and 10^{-5} *M*; nitroglycerin, 10^{-7} *M* (20 ng/ml) and 10^{-5} *M* (2,000 ng/ml).

Acetylcholine, an endothelial-dependent vasodilating agent, was obtained from a commercially available pre-

paration (Miochol) containing acetylcholine, 2 mg/ml, in mannitol solution. Nitroglycerin (Tridil), a direct-acting endothelial-independent vasodilator, was obtained as a 5-mg/ml solution in 30% ethanol and 30% propylene glycol. Nitroglycerin was administered intraarterially at a dose of 10^{-7} *M* (20 ng/ml) to all 19 patients, which results in serum concentrations achieved during intravenous infusion of nitroglycerin at 50 to 100 μg/min (16). Because the response to nitroglycerin, 10^{-7} *M*, was minimal in the first 9 patients, the drug was also administered at a dose of 10^{-5} *M* in the last 10 patients.

Before and at completion of each 2-min infusion, mean arterial pressure and heart rate were recorded on photographic paper (Electronics for Medicine Model VR6). After each infusion, all catheters were cleared of active drug and flushed with heparinized solution. Subsequent infusions were administered at 5-min intervals when all variables had returned to prior baseline values. Superficial femoral artery blood flow velocity was determined before and throughout each drug infusion with use of the ultrasound techniques described here.

Doppler ultrasonography. Ultrasound examination was performed with use of a 7.5-MHz duplex mechanical transducer connected to a Hewlett-Packard Sonos 100 Ultrasound System. With the patient supine, the transducer was positioned on the medial portion of the patient's thigh at the inferior border of the femoral triangle. The superficial femoral artery was identified and carefully scanned to determine its origin and course and the presence and extent of atheroma, when relevant. Exclusion criteria included extensive arterial wall atheromatous changes, arterial narrowing or a Doppler signal consistent with significant proximal arterial stenosis, or both. The origin of the vessel was avoided because of changes in velocity profile at the branching point. Once the optimal portion of the artery was visualized, the position of the transducer was marked on the skin. Before any Doppler measurements were attempted, great care was taken to visualize the vessel at its largest diameter with vessel walls parallel in the two-dimensional sector image. When the walls of the vessel are parallel, one can assume that the ultrasound beam is directed parallel to the longitudinal axis of the vessel (angle theta close to 0°) (15). Transducer position was then adjusted to minimize the incident angle of the ultrasound beam. An angle of $<60°$ was obtained in all instances and was kept constant within any subject. Automatic internal correction for the Doppler angle was utilized with the aid of an on-screen cursor.

Once optimal visualization of the vessel had been achieved, a 2- × 3-mm sample volume was placed in the center of the vessel and fine adjustments of its position were made until a narrow Doppler spectrum with a whistling audio signal was obtained. Under these conditions, the maximal velocity, corresponding to the central position in a parabolic velocity profile, was recorded. This variable tended to overestimate the actual velocity across the whole cross section of the vessel (17); however, the overestimation was systematic in all patients studied. This approach was chosen to maximize reproducibility of the Doppler signal. The coefficient of variance for baseline determination of mean blood flow velocity was 6.3%. Doppler studies were recorded on 0.5-in. (1.27-cm) tape with a commercially available videocassette recorder for later analysis. Analysis of the Doppler mean velocity was performed by integrating the darkest portion of the spectral display throughout systole and diastole and dividing by the RR interval. The results of five consecutive cycles in the last 30 s of the infusion were averaged. In case of arrhythmias, extrasystolic and post-extrasystolic beats were excluded from analysis.

Transcutaneous Doppler flow velocity measurements in the superficial femoral artery were compared with determinations of blood flow obtained by venous occlusion plethysmography in the lower limbs of four normal subjects and five patients with congestive heart failure with use of a mercury-in-Silastic strain gauge, as previously described (18). Doppler blood flow velocity and limb blood flow by plethysmography were determined at 15-s intervals for 3 min after 5 min of circulatory arrest induced by a pressure cuff inflated above the knee to 240 mm Hg. In all subjects, the two methods were highly and significantly correlated ($r > 0.89$, $p < 0.01$). Similarly, we have reported (19) an excellent correlation between Doppler blood flow velocity in the superficial femoral artery and limb blood flow measured by plethysmography after intraarterial administration of amrinone and digoxin and after simultaneous administration of amrinone and digoxin.

Data analysis. All values are presented as mean values ± SD. Mean blood flow velocity, heart rate and mean arterial pressure were analyzed for normal subjects and patients with congestive heart failure with use of repeated measures analysis of variance followed by Scheffé post-hoc testing for statistical significance. Linear regression analysis was performed for selected clinical variables. Statistical significance was accepted at the 95% confidence level ($p < 0.05$).

Results

Response to Acetylcholine (Fig. 1 and 2)

Normal subjects (Table 2). A representative example of the change in Doppler-derived blood flow velocity profile in the superficial femoral artery induced by intraarterial administration of acetylcholine, 10^{-5} *M*, in a normal subject is depicted in Figure 1. At baseline the blood flow velocity profile at rest is characterized by an initial systolic forward flow velocity (positive) followed by an early diastolic flow reversal (negative velocity) and later in diastole by a second phase of forward flow. After administration of acetylcholine, changes in the blood flow velocity pattern characteristic of vasodilation are observed (20). Peak systolic and mean blood flow velocities increase, whereas early diastolic reversal of blood flow completely disappears and forward flow continues until the end of the cardiac cycle. Mean blood flow

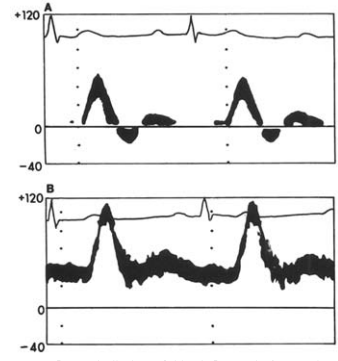

Figure 1. Spectral display of blood flow velocity obtained by transcutaneous Doppler ultrasonography in the superficial femoral artery of a normal subject. **A**, Baseline tracing. Mean blood flow velocity is 13 cm/s. **B**, After intraarterial infusion of acetylcholine, 10^{-5} *M*, mean blood flow velocity is increased to 59.9 cm/s.

velocity increases from a baseline value of 13 to 59.9 cm/s after acetylcholine infusion.

In normal subjects (Table 2) group mean blood flow velocity increased significantly from 11.9 ± 2.7 to 44.8 ± 20.9 cm/s ($p < 0.05$) after intraarterial administration of acetylcholine, 10^{-5} *M*, which produced a blood flow velocity pattern characteristic of vasodilation in every normal subject. Group mean blood flow velocity increased from 11.7 ± 4 to 33.8 ± 21.2 cm/s ($p < 0.05$) after administration of acetylcholine, 10^{-6} *M*, which produced a pattern characteristic of vasodilation in all but one normal subject. Acetylcholine, 10^{-7} *M* and 10^{-8} *M*, and mannitol vehicle control did not alter mean blood flow velocity or mean blood flow velocity pattern.

Figure 2. Group mean blood flow velocity at baseline and after increasing doses of acetylcholine in 6 normal subjects (**solid bars**), 7 patients with ischemic cardiomyopathy (**open bars**) and 12 patients with idiopathic dilated cardiomyopathy (**striped bars**). Values are mean values ± SEM. *Significant increase in mean velocity compared with baseline values ($p < 0.05$). [Ach] = acetylcholine concentration.

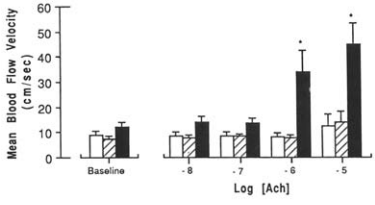

Table 2. Vascular Response to Acetylcholine, 10^{-5} *M*, in Six Normal Subjects

Subject No.	Age (yr)/ Gender	HR (min^{-1})		MAP (mm Hg)		MBFV (cm/s)	
		B	Ach	B	Ach	B	Ach
1	57/M	56	57	98	94	7.3	13.6
2	48/M	50	51	91	95	14.4	74.9
3	35/M	61	66	96	96	13	59.9
4	42/F	72	80	88	86	13	42.1
5	63/F	80	81	100	95	13.2	38.9
6	55/M	80	84	93	90	10.1	39.3
Mean	50	67	70	94	93	11.9	44.8
SD	11	13	17	5	4	2.7	20.9*

*$p < 0.05$ vs. baseline. Ach = acetylcholine; B = baseline; HR = heart rate; MAP = mean arterial pressure; MBFV = mean blood flow velocity.

Dilated cardiomyopathy (Table 3). In 12 patients with idiopathic dilated cardiomyopathy, group mean blood flow velocity did not change significantly (8.2 ± 4.1 cm/s before vs. 13.9 ± 15.4 cm/s after) in response to intraarterial

Table 3. Vascular Response to Acetylcholine, 10^{-5} *M*, in 19 Patients With Congestive Heart Failure

Pt No.	HR (min^{-1})		MAP (mm Hg)		MBFV (cm/s)	
	B	Ach	B	Ach	B	Ach
Ischemic cardiomyopathy (n = 7)						
1	104	105	91	85	13.1	12.9
2	67	71	44	51	3.5	4.3
3	77	60	103	103	4.4	4.7
4	97	79	91	97	2.5	3.4
5*	74	78	96	88	6.6	19.2
6*	85	87	94	93	14.9	36.5
7	87	87	97	102	6.2	6.1
Mean	84	81	88	88	7.3	12.4
SD	13	14	20	18	4.8	12.1
Idiopathic Dilated Cardiomyopathy (n = 12)						
8*	50	66	94	98	4.9	27.4
9	80	73	69	71	7.7	11
10	118	119	76	74	10.8	9.2
11*	88	95	105	105	12.9	57.6
12	112	116	88	91	9.5	12.6
13	82	87	90	104	17.2	16.8
14	72	75	66	70	3.8	2.4
15	90	98	94	98	8.3	8.5
16	96	99	85	97	7.5	5.1
17	63	80	102	97	4.2	3.7
18	60	68	89	94	8.3	8.7
19	103	101	97	100	2.8	4.3
Mean	85	90	88	92	8.2	13.9
SD	21	18	12	13	4.1	15.4

*Preserved vasodilator response to acetylcholine. All abbreviations as in Tables 1 and 2.

Table 4. Vascular Response to Nitroglycerin

	Normal Subjects (n = 6)	Patients With CHF (n = 19)	
	NTG, 10^{-7} *M* (n = 6)	NTG, 10^{-7} *M* (n = 19)	NTG, 10^{-5} *M* (n = 10)
Baseline*	10.7 ± 2.4	7.4 ± 4.7	5.7 ± 4.1
NTG*	21.7 ± 3.6†	10.8 ± 4.6†	28.7 ± 12†

*All values are mean blood flow velocity (cm/s). †$p < 0.05$ versus baseline. CHF = congestive heart failure; NTG = nitroglycerin.

administration of acetylcholine, 10^{-5} *M*, which produced a blood flow velocity pattern characteristic of vasodilation in only two patients (Cases 8 and 11). In the remaining 10 patients with idiopathic dilated cardiomyopathy, acetylcholine, 10^{-5} *M*, did not alter mean blood flow velocity. Acetylcholine, 10^{-6} *M*, 10^{-7} *M*, 10^{-8} *M*, and mannitol vehicle control did not affect mean blood flow velocity or blood flow velocity pattern.

Ischemic cardiomyopathy (Table 3). In seven patients with ischemic cardiomyopathy, group mean blood flow velocity did not change significantly, that is, 7.3 ± 4.8 versus 12.4 ± 12.1 cm/s after intraarterial administration of acetylcholine, 10^{-5} *M*, which produced a blood flow velocity pattern characteristic of vasodilation in only two patients (Cases 5 and 6). In the remaining five patients, acetylcholine, 10^{-5} *M*, did not change mean blood flow velocity. Acetylcholine, 10^{-6} *M*, 10^{-7} *M* and 10^{-8} *M*, and mannitol vehicle control did not alter mean blood flow velocity or blood flow velocity pattern.

Heart rate and mean superficial femoral artery pressure did not significantly change after intraarterial administration of acetylcholine at concentrations ranging from 10^{-8} *M* to 10^{-5} *M* in normal subjects and patients with idiopathic dilated or ischemic cardiomyopathies.

Response to Nitroglycerin (Table 4)

Normal subjects. Mean group blood flow velocity increased from 10.7 ± 2.4 to 21.7 ± 3.6 cm/s ($p < 0.05$) after intraarterial administration of nitroglycerin, 10^{-7} *M*, which produced a blood flow velocity pattern characteristic of vasodilation in every normal subject.

Patients with cardiomyopathy. In the 19 patients with idiopathic dilated and ischemic cardiomyopathy, intraarterial administration of nitroglycerin, 10^{-7} *M*, increased group mean blood flow velocity slightly from 7.4 ± 4.7 to 10.8 ± 4.6 cm/s ($p < 0.05$) and produced a blood flow velocity pattern characteristic of vasodilation in only 1 patient. To better assess the functional capacity of the cyclic guanosine monophosphate-dependent vasodilator mechanism in our patients, nitroglycerin at a dose of 10^{-5} *M* was administered to the last 10 patients. Group mean blood flow velocity increased from 5.7 ± 4.1 to 28.7 ± 12 cm/s ($p < 0.05$). All 10 patients demonstrated a blood flow velocity pattern characteristic of vasodilation.

Heart rate and mean superficial femoral artery pressure did not change significantly after intraarterial administration of nitroglycerin.

Discussion

The present data indicate that acetylcholine-mediated vasodilation is substantially reduced in the peripheral circulation of patients with congestive heart failure. This finding suggests that impaired endothelial-dependent vasodilation may contribute to the abnormal vasomotor tone present in congestive heart failure.

Previous studies. Our data in the lower limb peripheral vasculature are in agreement with preliminary reports in the forearm circulation in patients with congestive heart failure (21,22) and extend previous work by Treasure et al. (13) and Forstermann et al. (12) that demonstrated impaired acetylcholine-mediated vasodilation in the coronary circulation of patients with idiopathic dilated cardiomyopathy. Similar to Treasure et al. (13), who reported an acetylcholine-induced vasodilator response in only one of eight patients, we observed acetylcholine-mediated vasodilation in only one of our seven patients with primary idiopathic cardiomyopathy with normal cholesterol and without diabetes mellitus or a history of hypertension. Whereas Treasure et al. (13) only studied men with idiopathic dilated cardiomyopathy, our patient group included five women as well as seven patients with cardiomyopathy due to coronary artery disease. Three of the five women studied demonstrated a characteristic pattern of vasodilation in response to acetylcholine. Thus, three of our four patients who had a preserved vasodilator response to acetylcholine were women. The significance of this observation is unclear. Gender-related differences in endothelial-dependent relaxation have not been previously suggested and were not readily apparent in our normal control population.

Clinical predictors of response to acetylcholine. Left ventricular ejection fraction was significantly greater (27.8% vs. 20.3%, $p < 0.05$) and New York Heart Association functional class was lower (2 vs. 2.9, $p < 0.05$) in the 4 patients with preserved vasodilator response to acetylcholine than in the other 15 patients. However, neither left ventricular ejection fraction nor functional class were significantly correlated with mean blood flow velocity response to intraarterial administration of acetylcholine, 10^{-5} *M*. The clinical significance of these observations is uncertain in view of the small number of patients with a preserved response to acetylcholine.

Possible mechanisms of impaired endothelial-dependent vasodilation after nitroglycerin. The mechanisms that are responsible for impaired acetylcholine-mediated vasodilation in congestive heart failure are still poorly understood. Nitroglycerin was administered to assess the functional integrity of cyclic GMP-dependent vasorelaxation in the

vascular smooth muscle. In agreement with previous reports (7,23,24), the peripheral vasodilator response to nitroglycerin was reduced in patients with congestive heart failure when compared with values in normal subjects. A vasodilator response to nitroglycerin was elicited by increasing the dose of nitroglycerin to 10^{-5} *M*. Seven patients with idiopathic dilated cardiomyopathy who did not respond to 10^{-5} *M* of acetylcholine experienced a vasodilator response to 10^{-5} *M* of nitroglycerin. Thus, despite a reduced potency of nitroglycerin, the intracellular mechanisms mediating cyclic GMP-dependent vasodilation can be activated in patients with congestive heart failure. Nonetheless, our data suggest that an abnormal cyclic GMP-mediated vascular smooth muscle relaxation in patients with congestive heart failure may be partially responsible for the impaired response to acetylcholine (25,26).

A nonspecific attenuation of vasodilator capacity could have contributed to the blunted response to acetylcholine and nitroglycerin. However, patients with congestive heart failure have been reported to have preserved vascular responses to nonspecific vasodilating stimuli, such as postischemic reactive hyperemia (27) and adenosine (13). Activation of the sympathetic nervous system and other neurohumoral systems may also contribute to abnormal vasomotor tone in congestive heart failure. Heart rate and blood pressure were stable throughout our study, suggesting that the local intraarterial infusions used were not associated with acute activation of the sympathetic or other neurohumoral systems. The effects of chronic neurohumoral activation on endothelial-dependent vasodilation in congestive heart failure remain to be determined in future investigations.

Possible mechanisms of impaired vascular response to acetylcholine. The impaired vascular response to acetylcholine may be secondary to muscarinic receptor dysfunction or to abnormal endothelial synthesis or release of endothelial-derived relaxing factor, or both. In vitro experiments using thoracic aortic rings from rats with congestive heart failure after surgically induced myocardial infarction (11) and isolated vascular rings from epicardial coronary arteries from patients with idiopathic dilated cardiomyopathy (12) have demonstrated impaired acetylcholine-mediated vasorelaxation although the response to other nonmuscarinic endothelial-dependent vasorelaxing agents was preserved. Thus, a specific defect in peripheral vascular muscarinic receptor function could have contributed to the impaired vascular response to acetylcholine in our patients. Reduced density of myocardial muscarinic receptors has been previously reported in a canine model of chronic ventricular pressure overload (28) but not in the failing human heart (29).

The cause of reduced endothelial-dependent vasodilation in congestive heart failure is not apparent from our results. Preliminary work suggests that both prostaglandins and lymphokines are potential mediators of endothelial dysfunction. Cyclooxygenase inhibition with indomethacin has partially restored acetylcholine-mediated, endothelial-dependent vasodilation in a canine model of congestive heart failure (10). Tumor necrosis factor, which has been reported to be elevated in the serum of patients with congestive heart failure (30), and associated lymphokines, interleukin 2 and interleukin 6, have been demonstrated to inhibit endothelial-dependent vasodilation in vitro (31). Further clinical investigations will help to determine whether these mediators of inflammation are involved in the reduced endothelial-dependent vasodilation in patients with congestive heart failure.

Potential limitations. Although discontinued for ≥24 h before study, the medications administered to our patients may have influenced our results. Whereas digitalis glycosides have been demonstrated to inhibit both endothelial release of endothelial-derived relaxing factor and its action on vascular smooth muscle, acetylcholine did induce vasodilation in four patients treated with digoxin (32). Moreover, Treasure et al. (13) reported similar responses to intracoronary acetylcholine in patients with dilated cardiomyopathy whether or not they were treated with digoxin. The impaired response to acetylcholine and nitroglycerin is unlikely to have been secondary to nitrate tolerance. Indeed, only six patients were treated with long-acting nitrate preparations and a nitrate-free interval of 24 h appears adequate to avoid tolerance (33). Short-term administration of an angiotensin-converting enzyme inhibitor has been reported (34) to increase endothelial-dependent vasodilation in patients with essential hypertension. Nevertheless, 14 of 18 patients receiving maintenance therapy with an angiotensin-converting enzyme inhibitor did not demonstrate vasodilation in response to acetylcholine in the current study. Eleven patients were receiving various investigational cardiotonic agents that act primarily through specific phosphodiesterase inhibition. Although these agents mediate vasodilation primarily through cyclic adenosine monophosphate (AMP)-dependent protein phosphorylation, an effect on cyclic GMP-mediated vasodilation cannot be excluded (35).

As frequently observed clinically, several diseases may have contributed to the etiology of congestive heart failure in our patients. Atherosclerosis, present in the coronary vasculature of seven of our patients, has been demonstrated to impair acetylcholine-mediated vasodilation in an animal model (36) and in the coronary circulation in humans (37,38). Two of seven patients with documented coronary artery disease demonstrated a preserved peripheral vasodilator response to acetylcholine. Although none of our patients were hypertensive at the time of the study, a history of labile hypertension was elicited in three patients. Hypertension has been demonstrated to impair endothelial-dependent vasodilation both in animal models (39,40) and in the human forearm vasculature (41,42). One of the three patients with a history of hypertension demonstrated a preserved vasodilator response to acetylcholine, whereas the remaining two did not. Diabetes mellitus has been reported to impair acetylcholine-mediated vasodilation in animal models (43,44). Two of six patients with insulin-dependent diabetes mellitus had a preserved vasodilator response to acetylcholine. Hypercholesterolemia has also been demonstrated to attenuate

endothelial-dependent vasodilation, even in the absence of atherosclerosis (45,46). Of the seven patients with serum cholesterol levels ≥220 mg/dl, three had a preserved vasodilator response to acetylcholine.

Thus, although atherosclerosis, hypertension, diabetes mellitus and hypercholesterolemia may have contributed to the vascular responses observed in this study, these diseases cannot entirely account for our findings. Indeed, the vascular response to acetylcholine was impaired in six of the seven patients with idiopathic dilated cardiomyopathy who had no evidence of atherosclerosis, hypertension, diabetes mellitus or hypercholesterolemia. Abnormal endothelial-dependent vasodilation appears to be related solely to the presence of congestive heart failure in these seven patients.

The ultrasound techniques used in the present study did not allow accurate detection of small changes in blood vessel diameter. Thus, calculation of volumetric flow was not attempted. Although the increases in mean blood flow velocity observed could have resulted from a decrease in superficial femoral artery diameter, this appears unlikely for several reasons.

1. The blood flow velocity waveform after acetylcholine infusion, observed in the current study, is characteristic of peripheral vasodilation, as previously demonstrated in the canine femoral artery (20) and the human superficial femoral artery (47) with the use of invasive electromagnetic flow meters. The loss of diastolic flow reversal can be attributed to arteriolar vasodilation, with consequent decreased wave reflections from the distal vasculature (20,48). Thus, our findings demonstrate that acetylcholine mediates vasodilation in peripheral resistance vessels.

2. A 30% to 50% reduction in superficial femoral artery diameter would have been required to account for the two- to threefold increases in mean blood flow velocity observed. If present, such large changes in blood vessel diameter would have been detected by our ultrasound techniques.

3. The mean blood flow velocity obtained by Doppler ultrasound closely correlated with blood flow measurements obtained by venous occlusion plethysmography (19).

Conclusions. Acetylcholine-mediated vasodilation appears to be substantially reduced in the peripheral arterial circulation of patients with congestive heart failure, whereas a vasodilator response can still be elicited by a high dose of nitroglycerin. These findings suggest that vascular endothelial function is impaired in patients with congestive heart failure.

References

1. Zelis R, Mason DT, Braunwald E. A comparison of the effects of vasodilator stimuli on peripheral resistance vessels in normal subjects and in patients with congestive heart failure. J Clin Invest 1968;47:960–70.
2. Lelemtel TH, Maskin CS, Lucido D, Chadwick BJ. Failure to augment maximal limb blood flow in response to one-leg versus two-leg exercise in patients with severe heart failure. Circulation 1986;74:245–51.
3. Zelis R, Flaim SF. Alterations in vasomotor tone in congestive heart failure. Prog Cardiovasc Dis 1982;24:437–59.
4. Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 1980;288: 373–6.
5. Furchgott RF. Role of endothelium in responses to vascular smooth muscle. Circ Res 1983;53:557–73.
6. Griffith TM, Lewis MJ, Newby AC, Henderson AH. Endothelium-derived relaxing factor. J Am Coll Cardiol 1988;12:797–806.
7. Griffith TM, Edwards DH, Davies RLI, Harrison TJ, Evans KT. EDRF coordinates the behaviour of vascular resistance vessels. Nature 1987; 329:442–5.
8. Palmer RMJ, Ferrige AG, Moncada S. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. Nature 1987;327:524–6.
9. Rubanyi GM, Romero JC, Vanhoutte PM. Flow-induced release of endothelium-derived relaxing factor. Am J Physiol 1986;250:H1145–9.
10. Kaiser L, Spickard RC, Olivier NB. Heart failure depresses endothelium-dependent responses in canine femoral artery. Am J Physiol 1989;256: 1962–7.
11. Ontkean MT, Gay RG, Lewis HS, Greenberg BH. Diminished endothelium-derived relaxing factor (EDRF) activity in heart failure (abstr). Circulation 1989;80(suppl II):II-436.
12. Forstermann U, Mugge A, Alheid U, Haverich A, Frolich JC. Selective attenuation of endothelial mediated vasodilation in atherosclerotic human coronary arteries. Circ Res 1988;62:185–90.
13. Treasure CB, Vita JA, Cox DA, et al. Endothelial-dependent dilation of the coronary microvasculature is impaired in dilated cardiomyopathy. Circulation 1990;81:772–9.
14. Zeiher AM, Drexler H, Wollschlager H, Endothelian-dependent dilation of the coronary resistance vasculature declines with increasing age in normal humans (abstr). Circulation 1990;82(suppl III):III-443.
15. Lewis P, Psaila JV, Davies WT, McCarty K, Woodcock JP. Measurement of volume flow in the human common femoral artery using a duplex ultrasound system. Ultrasound Med Biol 1986;12:777–84.
16. Armstong PW, Armstrong JA, Marks GS. Pharmacokinetic-hemodynamic studies of intravenous nitroglycerin in congestive heart failure. Circulation 1980;62:160–6.
17. Hatle L, Angelsen B. Calculation of volumetric flow rate from the velocity profile. In: Hatle L, Angelsen B, eds. Doppler Ultrasound in Cardiology: Physical Principles and Clinical Applications. 2nd ed. Philadelphia: Lea & Febiger, 1985:321–4.
18. Greenfield ADM, Whitney RJ, Mowbray JF. Methods for the investigation of peripheral blood flow. Br Med Bull 1963;19:101–9.
19. Jondeau G, Klapholz M, Katz SD, et al. Control of arteriolar resistance in heart failure: partial attenuation of specific phosphodiesterase inhibitor-mediated vasodilation by digitalis glycosides. Circulation 1992;85:54–60.
20. O'Rourke MF, Taylor MG. Vascular impedance of the femoral bed. Circ Res 1966;28:126–39.
21. Kubo SH, Rector TS, Williams RE, Bank AJ, Heifetz SM. Vasodilation mediated by endothelial-derived relaxing factor is reduced in patients with heart failure (abstr). Circulation 1990;82(suppl III):III-592.
22. Hirooka Y, Imaizumi T, Takeshita A, Ando S, Harada S. Endothelial-dependent forearm vasodilation to intra-arterial acetylcholine is impaired in patients with heart failure (abstr). Circulation 1990;82(suppl III):III-591.
23. Imaizumi T, Takeshita A, Ashihara T, Nakamura M. The effects of sublingually administered nitroglycerin on forearm vascular resistance in patients with heart failure and in normal subjects. Circulation 1985;72: 747–52.
24. Flaim SF, Welzel RL, Zelis R. Mechanism of action of nitroglycerin during exercise in a rat model of heart failure. Circ Res 1981;49:458–68.
25. Rappaport RM, Murad F. Agonist-induced endothelium dependent relaxation in rat thoracic aorta may be mediated through cGMP. Circ Res 1983;52:352–7.
26. Holzmann S. Endothelium induced relaxation by acetylcholine associated with larger rises in cyclic GMP in coronary arterial strips. J Cyclic Nucleotide Res 1982;8:409–19.
27. Wilson JR, Wiener DH, Fink LI, Ferraro N. Vasodilatory behavior of skeletal muscle arterioles in patients with nonedematous chronic heart failure. Circulation 1986;74:775–9.
28. Vatner DE, Lee DL, Schwarz KR, et al. Impaired cardiac muscarinic

receptor function in dogs with heart failure. J Clin Invest 1988;81:1836–42.

29. Hershberger RE, Bristow MR. Receptor alterations in the failing human heart. Heart Failure 1988;3:230–8.
30. Levine B, Kalman J, Mayer L, Fillit HM, Packer M. Elevated circulating levels of tumor necrosis factor in severe chronic heart failure. N Engl J Med 1990;323:236–41.
31. Lefer AM, Tsao P, Aoki N, Palladino MA Jr. Mediation of cardioprotection by transforming growth factor-β. Science 1990;249:61–4.
32. Vanhoutte PM. Endothelial-derived relaxing and contracting factors. Heart Failure 1989;5:5–12.
33. Packer M, Lee WH, Kessler PD, Gottlieb SS, Medina N, Yushak M. Prevention and reversal of nitrate tolerance in patients with congestive heart failure. N Engl J Med 1987;317:799–804.
34. Hirooka Y, Imaizumi T, Takeshita A, Ando S, Haroda S. Impaired endothelial-dependent forearm vasodilation to acetylcholine in patients with essential hypertension and effects of captopril (abstr). Circulation 1990;82(suppl III):III-346.
35. Silver PJ, Lepore RE, O'Connor B, et al. Inhibition of the low Km cyclic AMP phosphodiesterase and activation of the cyclic AMP system in vascular smooth muscle by milrinone. J Pharmacol Exp Ther 1988;247: 34–42.
36. Freiman PC, Mitchell GC, Heistad DD, Armstrong ML, Harrison DG. Atherosclerosis impairs endothelium-dependent vascular relaxation to acetylcholine and thrombin in primates. Circ Res 1986;58:783–89.
37. Ludmer PL, Selwyn AP, Shook TL, et al. Paradoxical vasoconstriction induced by acetylcholine in atherosclerotic coronary arteries. N Engl J Med 1986;315:1046–51.
38. Werns SW, Walton JA, Hsia HH, Nabel EG, Sanz ML, Pitt B. Evidence of endothelial dysfunction in angiographically normal coronary arteries of patients with coronary artery disease. Circulation 1989;79:287–91.
39. Van de Voorde J, Leusen I. Endothelium-dependent and independent relaxation of aortic rings from hypertensive rats. Am J Physiol 1986;250: H711–7.
40. Miller MJS, Pinto A, Mullane KM. Impaired endothelium-dependent relaxation in rabbits subjected to aortic coarctation hypertension. Hypertension 1987;10:164–70.
41. Panza JA, Quyyumi AA, Brush JE Jr, Epstein SE. Abnormal endothelium-dependent vascular relaxation in patients with essential hypertension. N Engl J Med 1990;323:22–7.
42. Linder L, Kiowski W, Bubler FR, Luscher TF. Indirect evidence for release of endothelium-derived relaxing factor in human forearm circulation in vivo: blunted response in hypertension. Circulation 1990;81: 1762–7.
43. Oyama Y, Kawasaki H, Hattori Y, Hanno M. Attenuation of endothelium-dependent relaxation in aorta from diabetic rats. Eur J Pharmacol 1986;132:75–8.
44. Mayhan WG. Impairment of endothelium-dependent dilation of arterioles during diabetes mellitus. Am J Physiol 1989;256:H621–5.
45. Verbeuren TJ, Jordaens FH, Zonnekeyn LL, Herman AG. Effects of hypercholesterolemia on vascular reactivity in the rabbit. Circ Res 1986;58:552–64.
46. Creager MA, Cooke JP, Mendelsohn ME, et al. Impaired vasodilation of forearm resistance vessels in hypercholesterolemic humans. J Clin Invest 1990;86:228–34.
47. Furrer PJ, Maljovlak GS Jr, Johnson G Jr. Large vessel impedance in peripheral atherosclerosis. Circulation 1977;56(suppl II):II-171–8.
48. McDonald DA. Wave reflections. In: Blood Flow in Arteries. Baltimore: Williams & Wilkins, 1960:199–233.