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# Allopurinol Improves Endothelial Dysfunction in Chronic Heart Failure

Colin A.J. Farquharson, MBChB, MRCP(UK); Robert Butler, MD, MRCP(UK); Alexander Hill, PhD; Jill J.F. Belch, MD, FRCP; Allan D. Struthers, MD, FRCP

**Background**—Increased oxidative stress in chronic heart failure is thought to contribute to endothelial dysfunction. Xanthine oxidase produces oxidative stress and therefore we examined whether allopurinol improved endothelial dysfunction in chronic heart failure.

**Methods and Results**—We performed a randomized, placebo-controlled, double-blind crossover study on 11 patients with New York Heart Association class II-III chronic heart failure, comparing 300 mg allopurinol daily (1 month) versus placebo. Endothelial function was assessed by standard forearm venous occlusion plethysmography with acetylcholine, nitroprusside, and verapamil. Plasma malondialdehyde levels were also compared to assess significant changes in oxidative stress. Allopurinol significantly increased the forearm blood flow response to acetylcholine (percentage change in forearm blood flow [mean±SEM]: 181±19% versus 120±22% allopurinol versus placebo;  $P=0.003$ ). There were no significant differences in the forearm blood flow changes between the placebo and allopurinol treatment arms with regard to sodium nitroprusside or verapamil. Plasma malondialdehyde was significantly reduced with allopurinol treatment (346±128 nmol/L versus 461±101 nmol/L, allopurinol versus placebo;  $P=0.03$ ), consistent with reduced oxidative stress with allopurinol therapy.

**Conclusions**—We have shown that allopurinol improves endothelial dysfunction in chronic heart failure. This raises the distinct possibility that allopurinol might reduce cardiovascular events and even improve exercise capacity in chronic heart failure. (*Circulation*. 2002;106:221-226.)

**Key Words:** antioxidants ■ heart failure ■ exercise ■ endothelium

Chronic heart failure (CHF) is a common and disabling condition that causes substantial morbidity and mortality despite current therapeutic strategies and is a major consumer of health service resources.<sup>1</sup> This complex clinical syndrome is associated with a wide spectrum of abnormalities including endothelial dysfunction and increased oxidative stress.<sup>2-6</sup>

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It is possible that the increased oxidative stress of CHF actually contributes to the endothelial dysfunction seen in this syndrome, since superoxide anions have been shown to inactivate nitric oxide<sup>7</sup> and inhibit endothelium-dependent vasorelaxation.<sup>8</sup> To reduce these harmful effects of oxidative stress, most work has concentrated on using antioxidant vitamins to negate the effect of the superoxide anions. However, it may also be possible to use the alternative strategy of preventing the formation of superoxide anions. In fact, superoxide anions are generated from a number of different sources. In the vascular endothelium, the xanthine oxidase (XO) system is one of the main producers of superoxide anions.<sup>9</sup> Therefore, XO inhibition with allopurinol

prevents the formation of superoxide free radicals, which could lead to better endothelial function.<sup>10</sup> Also supporting a role for allopurinol in heart failure is the reported association between death and uric acid concentration.<sup>11-13</sup>

In this study, we therefore investigated whether allopurinol as an XO inhibitor could improve endothelial dysfunction in CHF. We did this by using a standard forearm venous occlusion plethysmography protocol, with brachial artery infusion of acetylcholine as a stimulator of endothelium-derived vasodilation,<sup>14</sup> sodium nitroprusside as an endothelium-independent vasodilator, and verapamil as a control vasodilator (a nonspecific endothelium-independent vasodilator). Plasma malondialdehyde (MDA) levels were also compared with indirectly assess any change in oxidative stress between the allopurinol and placebo groups.

## Methods

### Study Population

Eleven patients with compensated mild to moderate CHF were studied. All subjects gave written informed consent to participate in the study, which had prior approval by the Tayside Committee on

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From the Department of Clinical Pharmacology and Therapeutics (C.A.J.F., R.B., A.D.S.) and the Department of Medicine (A.H., J.J.F.B.), Ninewells Hospital and Medical School, Dundee, UK.

Correspondence to Prof A.D. Struthers, Department of Clinical Pharmacology and Therapeutics, Ninewells Hospital and Medical School, Dundee DD1 9SY UK. E-mail a.d.struthers@dundee.ac.uk

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**TABLE 1. Baseline Demographic, Hemodynamic, Humoral, and Treatment Characteristics of Patients**

Parameter	Mean (SD)
Age, y	67.5 (5.5)
Sex, male/female, n	10/1
NYHA class II/III, n	6/5
Time from first diagnosis, mo	47 (26)
Smoking history: previous/never-smokers	8/3
Average smoking duration, pack-years	35.5 (6.4)
Systolic blood pressure, mm Hg	129 (6)
Diastolic blood pressure, mm Hg	74 (3)
Mean arterial pressure, mm Hg	93 (3)
Left ventricular ejection fraction, %	29.7 (4.6)
Left ventricular fractional shortening, %	20.4 (5.5)
Serum urea, mmol/L	7.3 (1.1)
Serum creatinine, $\mu$ mol/L	116 (22)
Serum total cholesterol, mmol/L	4.6 (0.8)
Serum urate, mmol/L	0.42 (0.8)
Serum ACE activity, IU/L	7 (2)
Plasma MDA, nmol/L	425 (142)
Plasma glucose (random), mmol/L	5.4 (0.8)
Furosemide dose, mg/d	40 (15)
Daily aspirin dose: 75 mg/150 mg	5/6
Concomitant medication	
Nitrates	4
Digoxin	2
Calcium-channel blocking drugs	4
$\beta$ -Blockers	5

Medical Research Ethics. The recruits underwent screening by clinical history and physical examination, routine hematology and biochemical analysis, urinalysis, and ECG. Subjects were excluded if there was a history of diabetes mellitus, hypercholesterolemia (total cholesterol  $>5.5$  mmol/L), uncontrolled hypertension (blood pressure  $>160/90$  mm Hg), or previous adverse reaction to allopurinol.

The baseline clinical characteristics of the patients are summarized in Table 1. All recruits had been clinically stable for at least 3 months before the study commencement, with no modifications to drug therapy. The study was performed before the widespread use of  $\beta$ -blockers. None of the patients were current smokers. Patients had not taken any cholesterol-lowering agents in the previous 3 months or any antioxidant multivitamin supplementation within the preceding 6 months, and all were receiving loop diuretic and ACE inhibitor therapy (lisinopril,  $15 \pm 6$  mg/d,  $n=6$ ; enalapril,  $12 \pm 2$  mg/d). The one female subject recruited to this study was not taking estrogen replacement therapy. All patients were in sinus rhythm at the time of study. All had evidence of left ventricular systolic dysfunction as manifested by either a left ventricular ejection fraction of  $<35\%$  to 40% or echocardiographic fractional shortening of  $<25\%$ , or both. The underlying cause of CHF was ischemic heart disease in all recruits.

### Study Protocol

After initial screening, subjects were given either 300 mg oral allopurinol daily or placebo in a randomized, double-blind, crossover fashion. After 1 month of treatment, each subject attended a 4-hour study morning to evaluate the effects of allopurinol and placebo on endothelial function. Mornings were selected to avoid the recognized diurnal fluctuations in endothelial cell function.<sup>15,16</sup> The patients

fasted overnight during which alcohol- and caffeine-containing beverages were excluded, but they took all their usual morning medication in the same way on each study day, so that the only difference between the two study days was allopurinol versus placebo. They attended a temperature-controlled laboratory ( $24^\circ$  to  $26^\circ\text{C}$ ) in our research unit. After 20 minutes of supine rest, basal blood pressure measurements were recorded and venepuncture for blood sampling of plasma MDA levels (vide infra) was carried out. Subjects then underwent cannulation of the nondominant brachial artery with a 27-gauge steel needle mounted onto a 16-gauge epidural catheter under local anesthesia. After a period of 30 minutes, during which physiological saline was infused at a rate of 1 mL/min to allow resting blood flow to stabilize, baseline forearm blood flow (FBF) was measured by means of forearm venous occlusion plethysmography.<sup>17</sup> Mercury-in-silastic strain gauges (Medasonics) were placed around both forearms and connected to a plethysmograph (Medasonics Vasculab SPG-16). An occlusion cuff was placed at the wrist and a congestion cuff was positioned at the elbow. The wrist cuffs were inflated to above systolic blood pressure (200 mm Hg) with a rapid cuff inflator (Hokanson E-2) to exclude the hand circulation. The congestion cuffs were inflated rapidly to 30 mm Hg during each measurement to record FBF for  $\approx 10$  seconds in every 15-second cycle. The first 60 seconds of each recording period was disregarded, as this is often associated with reflex vasoconstriction.<sup>17</sup> The mean of the final 5 measurements of each recording period was used for analysis.

When resting FBFs had been established, drug solutions according to the study infusion protocol were infused into the study arm at 1 mL/min, with the use of a constant rate infuser (Braun Perfusor). FBF was measured at each baseline and then during the last 2 minutes of each drug infusion. Acetylcholine (CIBA Vision) was infused at 25, 50, and 100 nmol/min each for 7 minutes to produce a cumulative dose-response curve. After an appropriate recovery period to allow FBF in the infused arm to normalize to baseline, sodium nitroprusside (David Bull Laboratories) was infused at rates of 4.2, 12.6, and 37.8 nmol/min for 7 minutes each. Thereafter, verapamil (Knoll Ltd) at 10, 20, and 40 nmol/min each for 7 minutes was administered. Between different drugs, the drug infusion set was flushed with saline, and sufficient time was allowed for the FBF to return to baseline values ( $\approx 20$  to 30 minutes).

All FBF values were expressed as mL/min per 100 mL forearm volume. These blood flows were then converted to the ratio between the increase in blood flow in the infused arm and the blood flow in the control arm, expressed as the percentage change in FBF from the baseline immediately preceding each drug administration (mean  $\pm$  SEM), calculated according to the method of Whitney.<sup>18</sup> Although using the noninfused arm data in this way minimizes the confounding effects of blood flow changes caused by minor external factors,<sup>19,20</sup> it can introduce a different problem, which is that measurement error is increased because this way of calculating drug effects now includes measurements from two arms rather than just one. Blood pressure was measured with a semiautomated, noninvasive oscillometric sphygmomanometer (Dinamap) in the noninfused (control) arm before each infusion period and at the conclusion of the study. The person conducting the detailed vascular studies was blinded to the treatment being taken on each occasion.

Plasma MDA levels were assayed in the department using a method developed by Tatum et al,<sup>21</sup> with modification. In brief, free radical attack on plasma lipoprotein polyunsaturated fatty acids results in increased formation of lipid peroxides. Acid hydrolysis of these peroxides then releases MDA, which reacts with thiobarbituric acid to form a fluorescent adduct which, on further processing and separation by high-performance liquid chromatography, correlates indirectly with free radical activity. Effectively in this assay, MDA acts as an indirect measure of oxidized LDL because it is only the oxidized form of LDL that is converted to MDA by the acid conditions of the assay.

### Statistical Analysis

Clinical characteristics between the placebo and allopurinol study visits were compared by means of independent Student's *t* tests.

**TABLE 2. Absolute FBF (Infused Arm) in Response to Infusion of Acetylcholine, Sodium Nitroprusside, and Verapamil in Placebo and Allopurinol Treatment Arms (Mean±SEM)**

	FBF, mL/min per 100 mL	
	Placebo	Allopurinol
Baseline	3.01±0.19	3.36±0.14
Acetylcholine		
25 nmol/min	4.35±0.61	5.05±0.52
50 nmol/min	5.10±0.80	6.81±0.61
100 nmol/min	5.88±0.90	7.24±0.64
Baseline	2.66±0.37	2.95±0.25
Sodium nitroprusside		
4.2 nmol/min	4.87±0.54	5.52±0.20
12.6 nmol/min	7.63±0.87	8.25±0.23
37.8 nmol/min	9.42±1.32	9.37±0.65
Baseline	2.54±0.20	2.62±0.20
Verapamil		
10 nmol/min	3.71±0.41	4.42±0.30
20 nmol/min	4.64±0.45	5.49±0.35
40 nmol/min	5.78±0.62	6.91±0.45

Statistical analysis of FBF measurements for individual subjects were compared between treatments by means of repeated-measures ANOVA and the Bonferroni method for calculating 95% confidence intervals, correcting for multiple comparisons for within group effects, and order of treatment periods. A probability value of <0.05 was considered significant and a value of <0.01 as highly significant. All results of clinical parameters were expressed as mean±SD, apart from the plethysmographic data, which was expressed as mean±SEM.

## Results

Allopurinol had the expected effect on plasma urate (baseline, 0.42±0.08 mmol/L; placebo, 0.44±0.11 mmol/L; allopurinol, 0.17±0.08 mmol/L).

### Heart Rate, Blood Pressure, and Basal Blood Flow

Baseline FBF between the placebo and treatment study visits was not significantly different (3.01±0.19 mL per min per 100 mL versus 3.36±0.14 mL/min per 100 mL placebo versus allopurinol;  $P=0.26$ ). In addition, during both sets of study visits, there was no significant change in absolute FBF of the noninfused (control) arm during the infusion periods. The absolute values of FBF in the infused arm are shown in Table 2.

There was also no significant difference between baseline blood pressure and heart rate between the two treatment groups. Throughout the study visits, during the infusion of the different vasoactive substances, no significant change from baseline in blood pressure was observed (Table 3).

### Vascular Response to Acetylcholine, Sodium Nitroprusside, and Verapamil

A significant improvement in endothelium-dependent vasodilation in the allopurinol treatment group in response to acetylcholine was seen (Figure 1, maximal absolute value, 7.24±0.64 versus 5.88±0.90 mL/min per 100 mL, allopuri-

**TABLE 3. Comparisons in Blood Pressure Readings (Mean±SD) From Control (Noninfused) Arm During Forearm Venous Occlusion Plethysmography Between Placebo and Allopurinol Treatment Arms**

	Placebo	Allopurinol	<i>P</i>
Baseline, mm Hg			
SBP	128±17	127±20	0.89
DBP	74±11	70±8	0.12
MAP	92±12	89±10	0.24
After Ach, mm Hg			
SBP	129±12	126±17	0.33
DBP	74±10	73±9	0.60
MAP	93±9	91±10	0.26
After SNP, mm Hg			
SBP	130±13	127±18	0.50
DBP	74±9	71±9	0.28
MAP	93±8	90±10	0.12
After verapamil, mm Hg			
SBP	128±14	127±17	0.76
DBP	74±8	71±6	0.21
MAP	92±9	90±8	0.27

Ach indicates acetylcholine; SNP, sodium nitroprusside; SBP, systolic blood pressure; DBP, diastolic blood pressure; and MAP, mean arterial pressure.

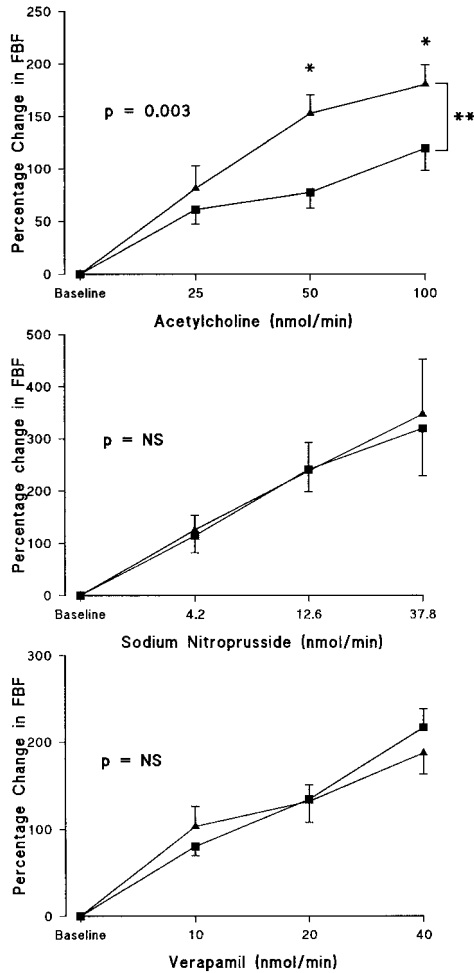
nol versus placebo percentage change in blood flow, 181±19% versus 120±22%, allopurinol versus placebo;  $P=0.003$ ). Even if the data from the noninfused arm are ignored, allopurinol produced a significant ( $P=0.03$  ANOVA) improvement in acetylcholine responses over placebo (Figure 2). There was no significant difference with the FBF responses between allopurinol and placebo with regards the endothelium-independent vasodilators sodium nitroprusside (maximal absolute value, 9.37±0.65 versus 9.42±1.32 mL/min per 100 mL allopurinol versus placebo, percentage change in blood flow from baseline, 347±106% versus 319±91% allopurinol versus placebo;  $P=0.84$ ) or verapamil (maximal absolute value, 6.01±0.45 versus 5.78±0.62 mL/min per 100 mL, allopurinol versus placebo, percentage change in blood flow from baseline, 187±24% versus 217±22%, allopurinol versus placebo;  $P=0.89$ ) (Figures 1, 3, and 4).

### Plasma MDA Levels

With regard to plasma MDA, a significant reduction with allopurinol therapy was observed (346±128 nmol/L versus 461±101 nmol/L allopurinol versus placebo;  $P=0.03$ ) (Figure 5).

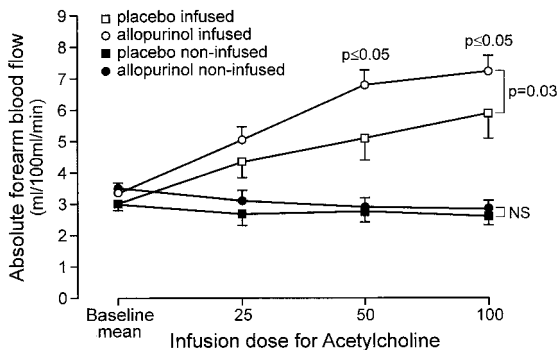
## Discussion

These above results show for the first time that treatment with allopurinol can improve endothelium-dependent vasodilation in CHF as assessed by the response to acetylcholine and nitroprusside, which are the traditional ways of assessing endothelial dysfunction. Our findings are consistent with similar work done by our group that showed an improvement in endothelium-dependent vasodilation with allopurinol (300

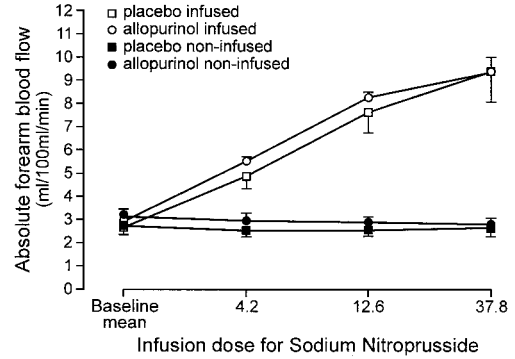


**Figure 1.** Percentage changes in FBF (corrected for noninfused arm data) with increasing doses of acetylcholine (25, 50, and 100 nmol/min), sodium nitroprusside (4.2, 12.6, and 37.8 nmol/min), and verapamil (10, 20, and 40 nmol/min) in placebo and allopurinol treatment arms (mean±SEM) ■ indicates placebo; ▲, allopurinol. \* $P < 0.05$ , \*\* $P < 0.01$ .

mg/d) in patients with type II diabetes.<sup>22</sup> It also agrees with data showing that oxyprinol improves endothelial function in hypercholesterolemia.<sup>23</sup> However, the data presented here on CHF are particularly noteworthy because allopurinol is often prescribed anyway in patients with heart failure, although for other reasons, such as diuretic-induced gout,



**Figure 2.** Absolute FBF data for allopurinol vs placebo-infused and noninfused arm data.



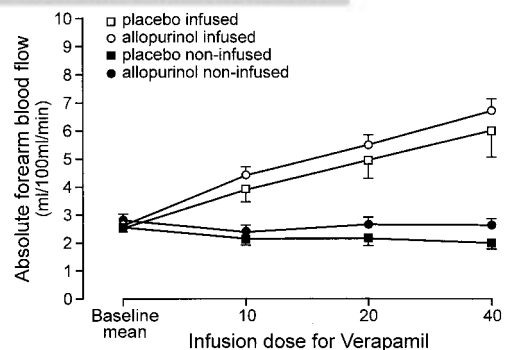
P-values (between placebo and allopurinol)

	Non-infused arm	Infused arm
SNP 4.2	0.26	0.29
SNP 12.6	0.32	0.48
SNP 37.8	0.61	0.97

**Figure 3.** Absolute FBF response to nitroprusside for allopurinol vs placebo.

whereas it is not part of normal therapy in diabetes mellitus or hypercholesterolemia.

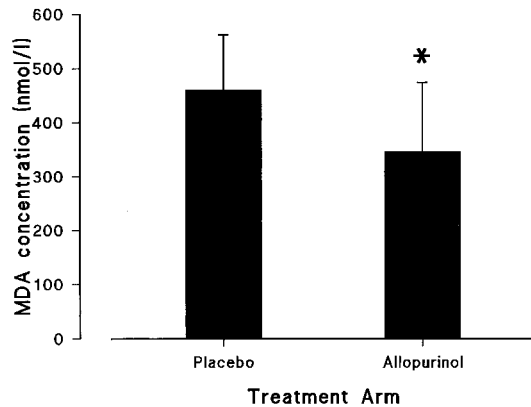
Blood pressure and baseline FBF parameters in our study group were unaffected by chronic treatment with allopurinol, which shows that changes in hemodynamic factors per se were not responsible for our findings. In addition, the vascular responses to sodium nitroprusside and verapamil were similar in both the placebo and allopurinol treatment arms, implying that the beneficial effect of allopurinol was not related to correcting a generally abnormal responsiveness to vasodilator stimuli. It is unlikely, although not impossible, that the improvement in acetylcholine-mediated vasodilation seen here is mediated by altered prostaglandin synthesis by vascular cyclooxygenase, since all of the subjects were taking soluble aspirin chronically (mean duration of treatment, 6.5 years) at a dose of at least 75 mg daily, which is above the 35- to 50-mg daily dose known to cause vascular cyclooxygenase inhibition in humans.<sup>24,25</sup> Furthermore, all subjects were studied in the morning, when the aspirin was exerting its maximum effect.



P-values (between placebo and allopurinol)

	Non-infused arm	Infused arm
VMP 10	0.29	0.39
VMP 20	0.14	0.38
VMP 40	0.10	0.40

**Figure 4.** Absolute FBF response to verapamil for allopurinol vs placebo.



**Figure 5.** Differences in MDA concentrations between placebo and allopurinol treatment groups (mean $\pm$ SD).

Therefore, on the basis of the above findings, the most likely mechanism for the observed increase in endothelium-dependent vasodilation is that allopurinol indirectly increases the in vivo bioavailability of endothelium-derived relaxing factor such as nitric oxide, presumably by blocking the production of reactive oxygen species mediated by XO. This is further substantiated by our finding of a significant reduction in plasma MDA with allopurinol. This is because oxidative stress caused by oxidized LDL is a major determinant of endothelial dysfunction and MDA is released from oxidized LDL itself.<sup>26</sup> In fact, the assay that we used measures MDA released from oxidized LDL.<sup>21</sup> Therefore, our finding of a significant decrease in plasma MDA with allopurinol suggests, albeit indirectly, that allopurinol has indeed reduced oxidative stress.

Recent data suggest another possible benefit with allopurinol, that is, it has been shown that raised serum uric acid levels correlate independently with cardiovascular death in hypertensive patients. Recent work has also suggested that in CHF, serum uric acid correlates strongly not only with mortality rates (risk ratio, 4:23) but also with circulating markers of chronic inflammation in CHF,<sup>12,27</sup> which implies a relation between XO activation and endothelial injury secondary to increased oxidative stress. However, an intriguing point about our study is that the vast majority of our subjects had baseline serum urate levels in the normal range, suggesting that blocking XO even in the absence of hyperuricemia may still confer benefit with regard to improving endothelial dysfunction. Clearly, we cannot tell from this study whether allopurinol produced its benefit by way of decreasing superoxide anions or decreasing uric acid or both.

Previous data with other therapies would suggest that the beneficial effects that we have seen on endothelial function in the brachial artery may be mirrored by similar changes in the coronary circulation and may be associated with reduced cardiovascular events. Several articles in *Circulation* now show a link between endothelial dysfunction and future cardiac events, including two studies using forearm endothelial dysfunction.<sup>28–30</sup> There are also several treatments in which their effect on endothelial dysfunction is paralleled by their effect on future cardiovascular events, for example, statins, ACE inhibitors, and spironolactone improve both,<sup>31,32</sup>

whereas the effect of vitamin E on both is usually neutral.<sup>33,34</sup> The only treatment so far in which there is dissociation between its effect on endothelial function and its effect on cardiac events is hormone replacement therapy. However, taken as a whole, our observation that allopurinol improves endothelial dysfunction in CHF raises the possibility that the beneficial effects that we have seen in this study with allopurinol may be translated into beneficial effects in coronary arteries and perhaps even in real cardiovascular events, as suggested by some preliminary data.<sup>12</sup> A further possibility highlighted by Drexler<sup>35</sup> is that treatments that improve endothelial function in CHF are likely also to improve exercise capacity, which makes it possible that allopurinol will improve exercise capacity in patients with heart failure, even if it turns out that it has no effect on mortality rates. The limitations of our study are the relatively small number of subjects (n=11) and the fact that correcting for the noninfused arm data appears to increase the level of statistical significance with acetylcholine (Figure 1 versus Figure 2), which means that the noninfused arm data partly contributes by chance to our positive result in Figure 1, although conventional statistical significance is achieved whichever way the acetylcholine data are analyzed.

In summary, these data show for the first time that allopurinol improves the impaired endothelial vasodilator function in patients with CHF. Allopurinol may therefore be a relatively safe and inexpensive way to reduce oxidative stress and improve endothelial dysfunction in CHF. Further definitive studies are now required to examine whether this inexpensive treatment also improves exercise tolerance and reduces cardiovascular events in patients with CHF.

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