Relation between serum uric acid and lower limb blood flow in patients with chronic heart failure

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Abstract
Objective—To determine whether lower limb blood flow is related to serum uric acid concentrations in patients with chronic heart failure, taking into account the hyperuricaemic effects of diuretic treatment and insulin resistance.
Design—Lower limb blood flow was measured at rest and after maximum exercise followed by a five minute period of ischaemia (maximum blood flow) using strain gauge venous occlusion plethysmography. All patients underwent a metabolic assessment, which included an intravenous glucose tolerance test (IVGTT)—to obtain an index of insulin sensitivity—and measurement of serum uric acid.
Setting—University and hospital departments specialising in cardiology and metabolic medicine.
Subjects—22 patients with chronic heart failure.
Results—Mean (SEM) resting and maximum blood flow values were 2·87 (0·23) and 24·00 (1·83) ml/100 ml/min, respectively. Patients in the upper tertile of serum uric acid had lower maximum blood flow than those in the lowest tertile (15·6 (2·2) v 31·0 (2·1) ml/100 ml/min, P = 0·003). Serum uric acid correlated with maximum blood flow ($r = -0·86$, P < 0·001), but not with resting blood flow. In stepwise regression analysis, uric acid emerged as the only predictor of maximum blood flow (standardised coefficient = $-0·83$ (P < 0·001), R^2 = 0·68 (P < 0·001)), independently of diuretic dose, age, body mass index, plasma creatinine, fasting and IVGTT glucose and insulin, insulin sensitivity, maximum oxygen uptake and exercise time during the treadmill exercise test, and alcohol intake.
Conclusions—There is a strong inverse relation between serum uric acid concentrations and maximum leg blood flow in patients with chronic heart failure. Further studies are needed to determine whether serum uric acid can be used as an index of vascular function in cardiovascular diseases.

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Keywords: uric acid; xanthine oxidase; blood flow; chronic heart failure

In healthy individuals, lower limb blood flow increases by as much as 20-fold during exercise.[1] This response is severely compromised in patients with chronic heart failure[2-4] and partly accounts for the fatigue and impaired exercise tolerance experienced by patients with this condition.[5]

No unifying pathophysiological mechanism has been proposed for the common association of hyperuricaemia with cardiovascular disease and its risk factors. The observation that interruption of arterial flow to the limbs results in an increase in serum uric acid[6] suggests that disturbances in blood flow may be related to hyperuricaemia. Lower limb ischaemia, as assessed by the presence of intermittent claudication or by a reactive hyperaemia test, is related to serum uric acid concentrations.[7] In the heart, increases in serum uric acid have been observed in the coronary sinus following balloon inflations during coronary angioplasty[8 9] and during coronary artery bypass operations.[10] These findings suggest a link between serum uric acid and impairment of regional blood flow.

On this basis, we hypothesised that in chronic heart failure, serum uric acid concentrations might be inversely related to lower limb blood flow. In testing this hypothesis, it is necessary to consider that chronic heart failure is an insulin resistant, hyperinsulinaemic state,[11] and that diuretic treatment,[12] adiposity,[13] plasma insulin, and insulin resistance[14 15] are also correlated with serum uric acid concentrations.

Methods
Twenty two patients with chronic heart failure due to coronary heart disease (n = 12) or idiopathic dilated cardiomyopathy (n = 10) were included in the study. In the chronic heart failure group, 18 patients were taking angiotensin converting enzyme inhibitors, 17 were taking loop diuretics, nine were taking thiazide diuretics, and nine were taking potassium sparing diuretics, either alone or in combination. No patients were taking hypouricaemic drugs. All patients had been in chronic heart failure for more than three months. Patients with chronic lung disease, haemodynamically important valve disease, neuromuscular disorders, myocardial infarction in the preceding three months, severe renal failure, symptomatic peripheral vascular disease, or excessive alcohol intake were

excluded from the study. All patients gave written informed consent, and the protocol was approved by the local ethics committee.

METABOLIC STUDY
Metabolic studies were carried out in our metabolic day ward as described previously.[11] Briefly, blood samples were taken for measurement of fasting plasma glucose and insulin concentrations and serum uric acid. Participants then underwent an intravenous glucose tolerance test (IVGTT) (0·5 g/kg body weight dextrose given as a 50% solution) with sampling for plasma glucose and insulin at 3, 5, 7, 10, 15, 20, 30, 45, 60, 90, 120, 150, and 180 minutes after injection of the glucose solution. All metabolic studies were carried out in the morning and all physical measurements in the afternoon of the day of assessment.

FUNCTIONAL CAPACITY
All patients were encouraged to exercise to exhaustion while undergoing a symptom limited treadmill exercise test. A standard Bruce protocol was employed, with the addition of a stage "0" (three minutes of exercise at one mile per hour with a gradient of 5%) for patients with chronic heart failure. A respiratory mass spectrometer (Amis 2000, Odense, Denmark) connected to by a one way valve was used for measurement of maximum oxygen consumption using a standard inert gas dilution technique. Left ventricular ejection fraction was estimated by radionuclide ventriculography using a stannous fluoride red cell labelling agent, a bolus injection of radiolabelled ^{99}Tc, and gamma camera imaging.

LEG BLOOD FLOW
Leg blood flow in the right leg was determined using a mercury-in-Silastic strain gauge venous occlusion plethysmography.[16] Patients were rested in the supine position for at least 10 minutes, with the leg slightly elevated. A cuff placed around the right thigh was connected to a rapid inflation pump (Hokanson, Bellevue, USA). The strain gauge was placed at the largest part of the calf and connected to a plethysmograph (EC4, Hokanson, Bellevue, USA). Measurement of resting blood flow

were taken after inflation of the cuff to 40 mm Hg. A further measurement of leg blood flow was obtained immediately after a maximal exercise test (described above) followed by a five minute period of ischaemia (to achieve maximum vasodilator capacity). This was achieved by inflating the cuff to suprasystolic pressure (30 mm Hg above the systolic blood pressure measured at the peak of exercise) for five minutes. The time period between finishing the exercise test and this blood flow measurement did not exceed six minutes (one minute to the start of cuff inflation and five minutes for ischaemia). Blood flow was measured at five seconds, 15 seconds, and then every 10 seconds until the flow decreased. The highest flow results were considered to be the maximum leg blood flow. All results for leg blood flow are expressed as millilitres of blood flow per 100 ml tissue per minute (ml/100 ml/min).

LABORATORY DETERMINATIONS
Plasma glucose was determined on the same day by glucose oxidase procedure using aminophenazone.[17] Plasma insulin concentrations were measured on samples stored at $-20°C$ using the radioimmunoassay procedure.[18] Serum uric acid was determined by the uricase-peroxidase method[19] using a Cobas Mira discrete analyser (Roche, Switzerland). Within and between batch precision was monitored throughout the study using frozen plasma and serum pools and commercially available freeze dried sera, and by participation in national quality assurance schemes.

DATA ANALYSES
Fasting plasma concentrations of glucose and insulin were taken as the mean of the two pretest samples. Incremental areas under the IVGTT concentration profiles were calculated using the trapezium rule. In the derivation of mean values, insulin measures were logarithmically transformed and insulin sensitivity square root transformed. The latter—inversely related to insulin resistance—was assessed using the minimal model approach.[20] Univariate Pearson correlation coefficients were derived. Stepwise multiple linear regression analyses adopted a tolerance of 0·01 for entry into the models. Group differences were assessed using the Mann-Whitney U test or analysis of variance, as appropriate. Statistical analyses were carried out using the SYSTAT (Evanston, Illinois, USA) statistical package.
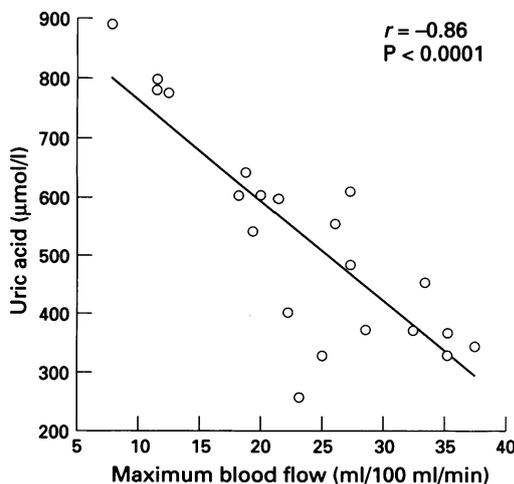
Results
The study group consisted of 22 patients with chronic heart failure aged 62·3 (SEM 2·7) years, with a body mass index of 23·0 (0·6) kg/m² (table 1). There were no significant differences in blood flow between the group with chronic heart failure due to coronary heart disease and those with idiopathic dilated cardiomyopathy (resting blood flow: coronary heart disease = 3·04 (0·33), dilated cardiomyopathy = 2·77 (0·32); maximum blood flow: coronary heart disease = 26·72 (2·45), dilated

Table 1 Characteristics of study group

Age (years)	62·3 (2·7)
Body mass index (kg/m²)	23·0 (0·6)
Systolic blood pressure (mm Hg)	109·2 (4·0)
Diastolic blood pressure (mm Hg)	67·1 (2·5)
Creatinine (μmol/l)	129·6 (9·4)
Uric acid (μmol/l)	527·2 (37·8)
Fasting glucose (μmol/l)	6·1 (0·6)
IVGTT glucose (mmol/l/min)	595·1 (50·8)
Fasting insulin (pmol/l)	38·8 (−6·01, 7·10)
IVGTT insulin (pmol/l/1·10⁻⁴)	1·67 (−0·22, 0·26)
Insulin sensitivity (10⁵/min/[pmol/l])	1·71 (−0·33, 0·36)
Diuretic dose (mg)	88·2 (12·8)
Resting blood flow (ml/100 ml/min)	2·87 (0·23)
Maximum blood flow (ml/100 ml/min)	24·00 (1·84)
Left ventricular ejection fraction (%)	25·8 (3·4)
NYHA class	
I	1
II	8
III	11
IV	2

Results are expressed as mean (SEM), except for insulin concentrations and insulin sensitivity, which are expressed as mean (−SEM, +SEM).
IVGTT, intravenous glucose tolerance test; NYHA, New York Heart Association.

Scatterplot of serum uric acid concentrations against maximum blood flow in patients with chronic heart failure.

$r = -0.86$
$P < 0.0001$

Table 3 *Results of stepwise multiple regression analysis using maximum blood flow as the dependent variable*

	Standardised coefficient	P value
Independent variables		
Uric acid	−0·83	< 0·001
Variables that failed to enter into regression model		
Diuretic dose*		
Fasting glucose		
Incremental glucose area		
Fasting insulin		
Incremental insulin area		
Creatinine		
Insulin sensitivity index		
Body mass index		
Age		
Alcohol intake		
Maximum oxygen uptake		
Exercise time		
R²	0·68	< 0·001

*Entered as frusemide equivalent dose.

cardiomyopathy = 20·49 (2·51)) . There were no differences in any of the other variables between the group with chronic heart failure due to coronary heart disease and those with idiopathic dilated cardiomyopathy (data not shown).

In univariate correlation analyses, serum uric acid was negatively correlated with maximum blood flow ($r = -0.86$, P ≪ 0·001) (figure), but no significant correlation emerged between serum uric acid and resting blood flow. Left ventricular ejection fraction did not correlate with maximum blood flow ($r = 0.27$). As shown in table 2, patients in the upper tertile of serum uric acid concentrations had lower maximum blood flow than those in the lowest tertile (P = 0·003). In contrast, no differences were detected with respect to resting blood flow with increasing tertiles of uric acid.

In stepwise regression analyses, serum uric acid emerged as the only significant predictor of maximum blood flow (table 3), independently of diuretic dose, age, body mass index, plasma creatinine, fasting glucose and insulin, IVGTT glucose and insulin, insulin sensitivity, exercise time, maximum oxygen uptake, and alcohol intake. In further analyses, aetiology of chronic heart failure failed to enter into stepwise regression models (data not shown). Similar results were obtained when age

adjusted values were employed. On stepwise analysis, only IVGTT insulin area emerged as a significant predictor of resting blood flow (standardised coefficient = −0·43, P = 0·027; R² = 0·64, P = 0·0012 for analysis) (data not shown).

Discussion

To our knowledge, this is the first report of an association between serum uric acid concentrations and arterial blood flow in patients with chronic heart failure. The hyperuricaemic effects of concurrent diuretic treatment[12] might reasonably be suspected as the cause of this relation. However, neither diuretic dose nor any other metabolic factor which is known to affect serum uric acid metabolism achieved statistical significance in multivariate regression analyses.

Interestingly, serum uric acid was strongly associated with maximum blood flow, but not with resting blood flow. This suggests that serum uric acid is somehow related to blood flow responses after exercise and ischaemia. In this respect, we have recently shown that in patients with chronic heart failure serum uric acid is inversely related to exercise tolerance and to maximum oxygen uptake during a treadmill exercise test.[21] This is consistent with reports of increased serum uric acid in various

Table 2 *Clinical and metabolic variables of patients with chronic heart failure, grouped according to tertiles of serum uric acid concentrations*

	Tertiles of serum uric acid			P value	
	1	2	3	2 v 1	3 v 1
Uric acid (μmol/l)	339·6 (15·2)	500·0 (26·6)	711·8 (40·5)		
Age (years)	55·1 (5·0)	64·3 (3·2)	67·1 (4·7)	NS	NS
Body mass index (kg/m²)	22·1 (2·2)	24·4 (1·5)	22·45 (0·9)	NS	NS
Systolic blood pressure (mm Hg)	108·6 (4·0)	118·6 (7·8)	102·5 (8·2)	NS	NS
Diastolic blood pressure (mm Hg)	68·9 (3·6)	69·7 (2·20)	62·3 (5·7)	NS	NS
Alcohol intake (7units/week)	19·6 (3·0)	3·0 (1·1)	5·0 (2·4)	NS	NS
Creatinine (μmol/l)	100·4 (3·9)	121·4 (10·6)	160·5 (19·6)	NS	0·021
Fasting glucose (mmol/l)	5·4 (0·3)	5·4 (0·2)	7·7 (1·6)	NS	NS
IVGTT glucose (mmol/l/min)	497·9 (56·7)	564·1 (46·2)	743·6 (130·9)	NS	NS
Fasting insulin (pmol/l)	20·7 (−11·6, 26·1)	62·4 (−17·7, 24·7)	47·5 (−26·2, 58·4)	0·009	NS
IVGTT insulin (pmol/l·10⁻⁴)	1·3 (−0·5, 0·8)	2·7 (−0·1, 0·2)	1·3 (−0·7, 0·1)	0·025	NS
Insulin sensitivity (10⁵/min/[pmol/l])	2·01 (−1·55, 2·64)	1·99 (−1·04, 1·37)	1·39 (−1·21, 2·32)	NS	NS
Diuretic dose*	51·4 (14·4)	82·9 (9·2)	135·0 (25·0)	NS	0·01
Resting blood flow (ml/100 ml/min)	2·5 (0·21)	3·1 (0·4)	3·1 (0·5)	NS	NS
Maximum blood flow (ml/100 ml/min)	31·0 (2·1)	26·1 (2·1)	15·6 (2·2)	NS	0·003
Left ventricular ejection fraction (%)	27·4 (5·1)	19·9 (7·5)	15·9 (2·9)	NS	0·049

Results are expressed as mean (SEM), except for insulin concentrations and insulin sensitivity, which are expressed as mean (−SEM, +SEM).
IVGTT, intravenous glucose tolerance test.
*Entered as frusemide equivalent dose (1 mg bumetanide = 40 mg frusemide).

hypoxic states.[22-26] Xanthine oxidase activity has been shown to increase during ischaemia and to intensify during reperfusion in coronary endothelial cells.[27] It is also noteworthy that interruption of regional blood leads to an increase in uric acid production.[28] Thus failure to meet the tissue demand for oxygen during exercise might explain our observation of an inverse relation between serum uric acid and maximum blood flow in patients with chronic heart failure.

We found no significant association between maximum blood flow and left ventricular ejection fraction. This is consistent with numerous studies highlighting the lack of an association between exercise tolerance and haemodynamic indices of cardiac function in patients with chronic heart failure.[29 30] While left ventricular ejection fraction did not correlate with serum uric acid on univariate analysis, it was significantly lower in patients in highest tertile of serum uric acid than in those in the lowest tertile. An association between serum uric acid and cardiac function might be expected in view of the relation between the degree of xanthine oxidase inhibition by allopurinol and improvement of postischaemic left ventricular function in isolated hearts subjected to global ischaemia.[31] Studies in animals have clearly shown that allopurinol has a potent effect on myocardial infarct limitation when given before coronary occlusion.[32] In addition, allopurinol has also been shown to enhance the recovery of stunned myocardium.[33] In humans, allopurinol has been shown to exert a dose related protective effect on the myocardium of patients undergoing heart operations.[34]

There is increasing evidence that free radicals play a major role in the impairment of vasomotor control in cardiovascular disorders. The reaction catalysed by xanthine oxidase is an important source of superoxide free radicals.[35] Inhibition of xanthine oxidase by allopurinol has been shown to improve endothelium dependent vasomotor responses in animals.[36-38] To our knowledge, no studies have examined the effects of allopurinol on vasomotor control in humans. Our finding of a relation between blood flow at the peak of exercise and serum uric acid concentrations could be related to the deleterious effects of xanthine oxidase derived free radicals on vascular function.

Our group has previously shown that impairment of lower limb blood flow in chronic heart failure is linked to an impairment of both endothelium independent and endothelium dependent mechanisms.[39] Accumulating evidence suggests that such disturbances are related to the release of nitric oxide. Interestingly, both impaired endothelial release of nitric oxide[40] and increased activity of xanthine oxidase/dehydrogenase[36-38] occur in disorders of vasomotion. Moreover, nitric oxide is known to exert a potent dose dependent inhibition of xanthine oxidase/dehydrogenase.[41-43] On the basis that xanthine oxidase is a major source of toxic free radicals, it has been proposed that inactivation of this enzyme by

nitric oxide could represent a protective role of nitric oxide in the endothelium.[42] On the other hand, nitric oxide reacts with vasodilator superoxide ions to yield (non-vasodilator) peroxynitrite.[44] Thus release of superoxide ions during uric acid production could attenuate the vasodilator effects of nitric oxide[45] by increasing its conversion to peroxynitrite. This is consistent with the finding that inhibition of xanthine oxidase by allopurinol potentiates nitric oxide mediated vasorelaxation.

In conclusion, we have shown a strong association between serum uric acid concentrations and maximum leg blood flow in patients with chronic heart failure. Whether this association is related to an involvement of xanthine oxidase in the impairment of vascular tone that characterises chronic heart failure remains unresolved. Further studies are needed to determine whether serum uric acid can be used as an index of vascular function in cardiovascular diseases. The possible effects of xanthine oxidase inhibitors on vasomotor responses in patients with chronic heart failure warrant further investigation.

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