Augmentation of endothelial function following exercise training is associated with increased L-arginine transport in human heart failure

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ABSTRACT

We have reported previously a decrease in the clearance of the NO (nitric oxide) precursor L-arginine in the forearm circulation of CHF (congestive heart failure) patients, suggesting a potential rate-limiting mechanism contributing to the common finding of endothelial dysfunction in CHF. Given data that show exercise training augments endothelial function in CHF, the aim of the present study was to investigate whether these improvements were due to an increase in L-arginine transport. Measures of L-arginine transport, endothelial function and exercise capacity were repeated before and after 8 weeks of ‘usual living’ or exercise training in 21 CHF patients [NYHA (New York Heart Association) class II/III]. Exercise capacity (6-min walk test) increased following exercise training (496 ± 21 to 561 ± 17 m; \( P = 0.005 \)), whereas the control group demonstrated no change [488 ± 18 to 484 ± 21 m; \( P = \text{ns} \) (not significant)]. Basal FBF (forearm blood flow) remained stable following exercise training (2.68 ± 0.55 to 2.46 ± 0.32 ml·min\(^{-1} \)·100 ml\(^{-1} \) of tissue) and ‘usual living’ (2.16 ± 0.37 to 2.91 ± 0.55 ml·min\(^{-1} \)·100 ml\(^{-1} \) of tissue). FBF responses to ACh (acetylcholine) increased following exercise by 49.6 ± 17.7% (area under curve; \( P = 0.01 \)) demonstrating augmented endothelial function. FBF responses to SNP (sodium nitroprusside) were also improved following exercise training (30.8 ± 8.2%; \( P = 0.02 \)). There was no change in vascular function in the ‘usual living’ group. The clearance of L-arginine was significantly increased following involvement in the exercise programme (69.4 ± 7.8 to 101.0 ± 9.5 ml/min; \( P = 0.04 \)), whereas there was no change in the ‘usual living’ group (78.4 ± 17.5 to 81.0 ± 14.9 ml/min; \( P = \text{ns} \)). In conclusion, the augmentation in endothelial function observed following exercise may be due, in part, to an increase in the transport of L-arginine in CHF patients.

INTRODUCTION

The systemic vasoconstriction observed in CHF (congestive heart failure) represents the combined effect of activation of neurohormonal systems, such as the sympathetic nervous system [1] and renin–angiotensin system [2], as well as endothelin levels [3]. Heightened peripheral vasoconstriction has an important role in influencing haemodynamics and exercise tolerance in this patient group. Although initial studies suggested that neurohormonal factors were the leading cause of systemic vasoconstriction, subsequent studies have highlighted the important role of endothelial dysfunction in contributing to abnormal vascular tone in CHF [4,5].

Endothelial dysfunction is well documented in both the coronary [6] and peripheral circulation [7] of CHF patients, most commonly by observing diminished blood flow responses to endothelium-dependent vasodilators.
such as ACh (acetylcholine). In addition, as a surrogate for NO (nitric oxide) synthesis, Katz and co-workers [8] demonstrated a reduced urinary excretion of [15N]-
nitrates in CHF patients when compared with healthy controls.

Many studies suggest that only discrete endothelial dysfunction is present, whereas the ability of vascular smooth muscle to respond to direct vasodilator stimuli [e.g. SNP (sodium nitroprusside)] remains intact in CHF patients [5]. However, others have demonstrated both endothelium-dependent and -independent impairment, observing abnormal FBF (forearm blood flow) responses to NO donors, such as GTN (glyceryl trinitrate) [9,10] and SNP [5,11]. These studies suggest that a decrease in smooth muscle function may also contribute to the dampened ACh response. Thus there is continuing debate on the role of vascular smooth muscle and endothelium dysfunction in enhanced vascular tone. It is appreciated that the abnormal vasoconstriction is multifactorial, including sympathetic and angiotensin systems, endothelin levels and endothelial function, as well as structural vascular changes limiting vasodilatory capacity [12].

It has become increasingly apparent that exercise training augments endothelial function, both in healthy individuals and CHF patients [4,13]. Furthermore, a range of interventions in CHF, including daily handgrip, cycling, aerobic and circuit training [4,13–15], have all been demonstrated to improve endothelial function. In this context, many studies have suggested a role for reduced eNOS (endothelial NO synthase) expression in the setting of CHF [16]. The observation that L-arginine supplementation can restore endothelial function in heart failure [17], however, highlights a possible role for reduced substrate availability for eNOS. Our group has shown that L-arginine transport is reduced in human heart failure [18], suggesting a possible role for impaired L-arginine transport in endothelial dysfunction in CHF. In that study [18], we found that the expression of the system y+ cationic amino acid transporter CAT-1 was reduced, providing a potential explanation for reduced L-arginine transport in CHF.

To further define the role of L-arginine transport in the setting of endothelial dysfunction in CHF patients, the aim of the present study was to determine the effects of 8 weeks of exercise training on exercise capacity, endothelial function and L-arginine transport in CHF patients.

METHODS

Subjects

Adult male CHF patients \( n = 21 \); NYHA (New York Heart Association) class II/III, LVEF (left ventricular ejection fraction) \(< 40\%\) aged between 18–70, without co-morbidities, such as hypertension and hyperlipidaemia, were recruited from the Alfred Heart Centre Heart Failure Clinic. Subjects gave written informed consent, and the study was performed in accordance with the Declaration of Helsinki (2000) of the World Medical Association and with the approval of The Alfred Health-Care Group Ethics Committee. Patients were randomly allocated to one of two groups: an 8-week exercise training programme or 8 weeks of ‘usual living’ (control group). The patients allocated to the ‘usual living’ group were instructed not to change their regular daily activities for the 8-week study duration.

The exercise programme was conducted over 8 weeks, where patients attended a specific heart failure rehabilitation unit three times/week. Patients were required to exercise at 50–60 % of their predetermined maximum heart rate, during an individualized programme of walking, light hand weights and stationary cycling. This programme was tailored according to individual’s capacities and progressively increased over the 8-week period according to individual responses. Patients were also encouraged to participate in a home-based programme, where they were instructed to progressively increase their exercise duration from three 30 min sessions/week to approx. 60 min/day on 5–7 days/week.

Study protocol

Subjects attended the clinical research laboratory on two occasions, at study enrolment and at the conclusion of the 8-week period. Patients began each study by completing a 6-min walk test [19]. Subjects then rested for 20 min before undertaking the next stage of the protocol. Patients were placed in a comfortable supine position, with their non-dominant arm positioned at an angle of approx. 80° to the body and 30° vertically above the level of the heart. Subjects remained in this position in a comfortable relaxed environment at a constant temperature of 22 °C for the duration of the study.

Endothelial function and L-arginine transport were measured as described previously [18]. Briefly, the brachial artery was cannulated under local anaesthesia for local drug infusions and blood pressure monitoring. A 5 French cannula was retrogradely inserted percutaneously into a deep antecubital forearm vein under local anaesthesia for venous blood sampling. After an initial bolus of 1 µCi of \([4,5-\text{H}]\)-arginine (New England Nuclear) in 2 ml of 0.9 % NaCl, a continuous intra-arterial infusion of 100 nCi/min of \([4,5-\text{H}]\)-arginine was commenced and continued throughout the study (maximum exposure was 1 µSv). Venous blood samples were drawn at 10-min intervals during the initial 40 min of \([\text{H}]\)-arginine infusion. Samples were immediately placed on ice, centrifuged at 4 °C, and plasma was stored at – 80 °C. Endothelial function was assessed by examining FBF responses to local infusions of endothelium-dependent and -independent agonists using venous occlusion plethysmography [20]. In brief, a sealed alloy-filled (gallium
Baseline concentrations of [3H]-SDMA (points 2 min into infusions of ACh and SNP at the indicated doses). 

Assessment of L-arginine clearance in human plasma

Upon completion of the study, blood samples were centrifuged at 4°C and plasma was stored at −80°C. Plasma concentrations of [3H]-L-arginine were determined using ion-exchange chromatography as described previously [18]. Radioactivity was determined by liquid scintillation spectroscopy.

L-Arginine clearance was calculated using the following formula [18]:

\[
\text{L-arginine clearance} = \frac{\text{infusion rate}}{\text{venous concentration}}
\]

Determination of L-arginine and dimethylarginines

Baseline and 8-week plasma concentrations of arginine, citrulline, ADMA (N\text{G},N\text{G}'-dimethylarginine) and SDMA (N\text{G},N\text{G}'-dimethylarginine) were measured by reversed-phase liquid chromatography with a time-controlled ortho-phthalaldehyde precolumn derivatization, as described previously [21].

Statistical analysis

Data are presented as means ± S.E.M. Between-group comparisons were performed using paired or unpaired Student’s t tests, where appropriate. Comparison of the intervention with the ‘usual living’ control group was performed by two-way ANOVA. Statistical analysis was performed using SPSS Version 10.0. The significance level employed was \( P < 0.05 \).

RESULTS

The two groups were similar at baseline with respect to age, BMI (body mass index), diagnosis, drug regime, NYHA class and LVEF (Table 1). In addition, there was no difference in blood pressure, plasma arginine metabolites and lipid profile (Table 2); however, triacylglycerols (triglycerides) were unexpectedly somewhat higher in the exercise group \( (P = 0.01) \). These parameters remained stable throughout the 8-week study period.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>'Usual living' group</th>
<th>Exercise group</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>( n )</td>
<td>10</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>54 ± 3</td>
<td>55 ± 3</td>
<td>0.764</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27 ± 1.3</td>
<td>28 ± 1.3</td>
<td>0.473</td>
</tr>
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<td>Diagnosis (isch/non-isch)</td>
<td>5/5</td>
<td>2/9</td>
<td>0.135</td>
</tr>
<tr>
<td>NYHA class (II/III)</td>
<td>10/0</td>
<td>10/1</td>
<td>0.353</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>20.8 ± 2.4</td>
<td>21.1 ± 2.5</td>
<td>0.926</td>
</tr>
<tr>
<td>ACEI (%)</td>
<td>100</td>
<td>100</td>
<td>1</td>
</tr>
<tr>
<td>Diuretic (%)</td>
<td>90</td>
<td>87.5</td>
<td>0.614</td>
</tr>
<tr>
<td>Aspirin (%)</td>
<td>50</td>
<td>37.5</td>
<td>0.308</td>
</tr>
<tr>
<td>Digoxin (%)</td>
<td>70</td>
<td>37.5</td>
<td>0.136</td>
</tr>
<tr>
<td>ß-Blocker (%)</td>
<td>80</td>
<td>100</td>
<td>0.500</td>
</tr>
<tr>
<td>Warfarin (%)</td>
<td>50</td>
<td>25</td>
<td>0.418</td>
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</table>

Exercise capacity

Following the exercise training programme, CHF patients significantly increased their 6-min walk distance \( (496 ± 21 \text{ to } 561 ± 17 \text{ m}; P = 0.005) \), indicating an increase in exercise capacity, whereas there was no change in the ‘usual living’ group \( (488 ± 18 \text{ to } 484 ± 21 \text{ m}; P = \text{ns} \) (not significant)).

Endothelial function

Basal FBF was not different between the two groups at baseline \( (2.16 ± 0.37 \text{ ml} \cdot \text{min}^{-1} \cdot 100 \text{ ml}^{-1} \text{ of tissue}), \text{NS} \) group compared with \( 2.68 ± 0.55 \text{ ml} \cdot \text{min}^{-1} \cdot 100 \text{ ml}^{-1} \text{ of tissue} \), \text{Ex} \text{group}; \( P = 0.45 \)). These values did not change following exercise training \( (2.46 ± 0.32 \text{ ml} \cdot \text{min}^{-1} \cdot 100 \text{ ml}^{-1} \text{ of tissue}), \text{Ex} \text{group} \text{vs} \text{Us} \text{group}; \( P = 0.006 \)).

At baseline, there was no difference in FBF response to ACh between the exercise and ‘usual living’ groups \( (P = 0.8) \). Following 8 weeks of exercise training, FBF response to ACh significantly increased \( (\text{Us}: 18 \text{ to } 484 \text{ ml} \cdot \text{min}^{-1} \cdot 100 \text{ ml}^{-1} \text{ of tissue}) \), \text{Ex} \text{group}: \( P = 0.03 \), \text{Us} \text{group}: \( P = 0.01 \)). As such, these results are consistent with an augmentation of endothelial function following 8 weeks of exercise training. In addition, although forearm vascular responses to SNP did not change during ‘usual living’ \( (\text{Us}: 18 \text{ to } 484 \text{ ml} \cdot \text{min}^{-1} \cdot 100 \text{ ml}^{-1} \text{ of tissue}) \), \text{Ex} \text{group}: \( P = 0.43 \). As such, these results are consistent with an augmentation of vascular wall function that may be endothelium independent.

L-Arginine transport

L-Arginine transport was measured at baseline and at follow-up in ten subjects in the exercise group and ten subjects in the ‘usual living’ group. At baseline, the two study groups were well matched with respect to forearm L-arginine clearance \( (69.41 ± 7.79 \text{ ml/min in the exercise group}) \).
Table 2  Lipid profile and haemodynamics
Values are means \( \pm \) S.E.M. * \( P = 0.01 \) compared with the 'usual living' group. DBP, diastolic blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MAP, mean arterial pressure; NOHA, \( \text{N}^\text{G} \)-hydroxy-L-arginine; NMMA, \( \text{N}^\text{G} \)-monomethyl-L-arginine; SBP, systolic blood pressure.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>8 weeks</th>
<th>Exercise group</th>
<th>Baseline</th>
<th>8 weeks</th>
</tr>
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<tbody>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>3.78 ( \pm ) 0.19</td>
<td>3.85 ( \pm ) 0.23</td>
<td>4.38 ( \pm ) 0.24</td>
<td>4.38 ( \pm ) 0.31</td>
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<tr>
<td>HDL-cholesterol (mmol/l)</td>
<td>1.06 ( \pm ) 0.13</td>
<td>1.10 ( \pm ) 0.11</td>
<td>0.93 ( \pm ) 0.10</td>
<td>0.91 ( \pm ) 0.09</td>
<td></td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/l)</td>
<td>1.95 ( \pm ) 0.24</td>
<td>2.03 ( \pm ) 0.28</td>
<td>2.15 ( \pm ) 0.29</td>
<td>2.17 ( \pm ) 0.28</td>
<td></td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>6.44 ( \pm ) 0.42</td>
<td>5.54 ( \pm ) 0.20</td>
<td>7.35 ( \pm ) 1.06</td>
<td>7.68 ( \pm ) 1.09</td>
<td></td>
</tr>
<tr>
<td>Triglycerols (mmol/l)</td>
<td>1.69 ( \pm ) 0.21</td>
<td>1.72 ( \pm ) 0.28</td>
<td>2.81 ( \pm ) 0.34*</td>
<td>2.80 ( \pm ) 0.35</td>
<td></td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>111 ( \pm ) 4</td>
<td>111 ( \pm ) 4</td>
<td>107 ( \pm ) 4</td>
<td>104 ( \pm ) 4</td>
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<tr>
<td>DBP (mmHg)</td>
<td>60 ( \pm ) 2</td>
<td>59 ( \pm ) 4</td>
<td>55 ( \pm ) 2</td>
<td>53 ( \pm ) 2</td>
<td></td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>77 ( \pm ) 2</td>
<td>77 ( \pm ) 3</td>
<td>73 ( \pm ) 3</td>
<td>70 ( \pm ) 3</td>
<td></td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>67 ( \pm ) 3</td>
<td>64 ( \pm ) 4</td>
<td>67 ( \pm ) 3</td>
<td>70 ( \pm ) 3</td>
<td></td>
</tr>
<tr>
<td>Arginine (( \mu \text{mol/l} ))</td>
<td>140.7 ( \pm ) 20.5</td>
<td>133.0 ( \pm ) 15.6</td>
<td>168.2 ( \pm ) 11.4</td>
<td>159.7 ( \pm ) 21.7</td>
<td></td>
</tr>
<tr>
<td>Citrulline (( \mu \text{mol/l} ))</td>
<td>70.9 ( \pm ) 7.5</td>
<td>86.9 ( \pm ) 7.4</td>
<td>97.6 ( \pm ) 15.4</td>
<td>80.3 ( \pm ) 11.4</td>
<td></td>
</tr>
<tr>
<td>NOHA (( \mu \text{mol/l} ))</td>
<td>15.1 ( \pm ) 2.0</td>
<td>18.9 ( \pm ) 2.5</td>
<td>16.48 ( \pm ) 1.7</td>
<td>17.82 ( \pm ) 3.09</td>
<td></td>
</tr>
<tr>
<td>NMMA (( \mu \text{mol/l} ))</td>
<td>0.22 ( \pm ) 0.04</td>
<td>0.25 ( \pm ) 0.04</td>
<td>0.26 ( \pm ) 0.08</td>
<td>0.40 ( \pm ) 0.11</td>
<td></td>
</tr>
<tr>
<td>ADMA (( \mu \text{mol/l} ))</td>
<td>1.24 ( \pm ) 0.40</td>
<td>1.39 ( \pm ) 0.45</td>
<td>1.25 ( \pm ) 0.34</td>
<td>0.93 ( \pm ) 0.15</td>
<td></td>
</tr>
<tr>
<td>SDMA (( \mu \text{mol/l} ))</td>
<td>3.88 ( \pm ) 1.29</td>
<td>3.48 ( \pm ) 1.4</td>
<td>6.33 ( \pm ) 2.5</td>
<td>5.09 ( \pm ) 2.58</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1  Basal FBF and FBF responses to two infusions of ACh in (a) the exercise training group (\( n = 11 \)) and (b) the 'usual living' group (\( n = 10 \)) at baseline and 8 weeks
ACh infusions were at 9.25 and 37 \( \mu \text{g/min} \). (■) Baseline; (○) 8 weeks. The area under the curve is significantly enhanced following 8 weeks in the exercise group (\( * P = 0.01 \)), whereas there was no change following 8 weeks of 'usual living', illustrating augmented endothelial function following exercise training.

Figure 2  Basal FBF and FBF responses to two infusions of SNP in (a) the exercise training group (\( n = 10 \)) and (b) the ‘usual living’ group (\( n = 10 \))
SNP infusions were at 2 and 8 \( \mu \text{g/min} \). (■) Baseline; (○) 8 weeks. There was a significantly increased FBF to infusion of SNP following the exercise training programme (\( * P = 0.006 \)), whereas no change was evident in the ‘usual living’ group.
Exercise training and heart failure

Figure 3 Plasma L-arginine clearance at baseline and 8 weeks in both the ‘usual living’ group (n = 10) and exercise group (n = 10)

Closed bars, ‘usual living’ group; open bars, exercise group. Exercise training significantly increased L-arginine clearance, whereas there was no change in the ‘usual living’ group. *P = 0.04 compared with baseline.

Figure 4 Correlation between the change in FBF response to ACh and change in L-arginine transport following exercise training (n = 10)

This relationship (r = 0.69, P = 0.02) suggests that the improvements in endothelial function following exercise training may be due to augmentation of L-arginine transport.

The existence of endothelial dysfunction in CHF is now well established. Depression of endothelial function in CHF has been found in many vascular beds, including the forearm [20], lower limb [5] and coronary [22] and isolated resistance [23] arteries. The endothelium plays a critical role in regulating vascular function and, in circumstances where endothelial function is reduced, heightened vascular tone is commonly observed. Accordingly, considerable attention has been directed at identifying interventions that improve endothelial function.

The present study demonstrated an improvement in exercise capacity following involvement in a combined aerobic and light resistance weight programme. This supports previous findings in our laboratory using this exercise protocol in CHF patients [24]. A period of 8 weeks of combined aerobic and light resistance training augmented FBF responses to ACh, consistent with enhanced endothelial function. These findings support previous work suggesting both local [13,15,25,26] and systemic [4] endothelial improvements with exercise training in CHF. In addition, the present study demonstrates for the first time that exercise training increases L-arginine transport in this patient group. Previous studies to date have suggested reduced L-arginine transport in CHF patients [18], highlighting the importance of exercise training in this regard.

During acute bouts of aerobic exercise training, blood flow to active skeletal muscle increases, leading to flow-mediated vasodilation. Animal studies have shown that trained skeletal muscle arterioles dilate 25–100% more than sedentary ones [27], and that arteries upstream of the active muscle contribute significantly to the total flow response [28]. These findings suggest that the repetitive increases in blood flow may act to increase NO production, potentially via shear-stress-induced mechanisms. The findings in the present study suggest that chronic exercise training, which repetitively increases shear stress, alters the endothelium which can be detected via infusions of ACh.

In the present study we also observed enhanced FBF responses to SNP following exercise training in CHF patients, potentially confounding the interpretation of the findings in regard to ACh responses. Of note, a study using a similar 8-week exercise programme has also demonstrated augmented FBF responses to both ACh and SNP [15]. Taken together, the data in regard to changes in SNP and ACh responses suggest that the apparent beneficial effects of exercise training on endothelial function in CHF are mediated by a number of complementary processes. In part, the effect may be mediated by an enhanced sensitivity of smooth muscle to NO in association with an increased capacity of the endothelium to generate NO. The present study supports the contention that increased NO production plays at least a part of this process, by virtue of the relatively greater increase in the response to ACh in comparison with SNP, and our finding of increased arginine transport provides a plausible mechanism for this observation.

In addition, several studies investigating the role of exercise training on vascular function have reported changes
in endothelium-independent function. Kingwell et al. [29] found an approx. 20% reduction in forearm vascular resistance in young athletes compared with sedentary controls. Rywik et al. [30] observed a trend for higher non-endothelium-dependent dilation in endurance-trained older men when compared with controls. Following 6 months of aerobic exercise, a 21–25% enhancement of vasodilatory capacity was observed in an older population (64 ± 3 years) [31]. Further studies demonstrated a slight, but non-significant, attenuation in SNP FBF responses in sedentary compared with exercise-trained individuals [32]. Indeed, exercise training appears to mediate both endothelial and vascular smooth muscle improvements.

Several mechanisms have been suggested to produce endothelial dysfunction in the setting of CHF. It is therefore possible that the increase in endothelial function following exercise training may be mediated via several pathways. Sympathetic activity has been demonstrated to decrease following exercise training in CHF [33], which has been suggested to mediate the related decrease in vascular tone. Sympathetic activity was not directly measured in the present study and, although this cannot be excluded, resting heart rate remained stable throughout the study period in both groups, suggesting no change in autonomic nervous system activity. eNOS expression has been demonstrated to increase following exercise training in rats [34] and, therefore, may have contributed to the enhanced endothelial function observed in the present study.

The observation that L-arginine supplementation augments endothelial function [26] suggests a preference for extracellular L-arginine as a substrate for eNOS. In addition, the decreased expression of the y+ cationic amino acid transporter CAT-1 demonstrated in CHF [18] highlights further the importance of substrate delivery to eNOS. Therefore the reduced L-arginine transport observed in CHF [18] may result in diminished substrate delivery to eNOS, thus reducing NO production and ultimately impaired endothelium-dependent vasodilation. Katz et al. [8] interpreted their finding of reduced [15N]nitrate following infusions of [15N]L-arginine as a reduced eNOS activity. Instead, it could be suggested that this may reflect reduced L-arginine transport. That the present study demonstrated a good correlation between the exercise-training-augmented endothelial function and L-arginine transport highlights further the importance of extracellular substrate supply mediating endothelial function.

The importance of endothelial cellular L-arginine uptake as the rate-limiting factor for the production of NO is becoming increasingly recognized. Much of the work in this area has been limited to in vitro investigations, with our laboratory recently devising a method of investigating L-arginine transport in the clinical setting. Previous studies have shown that exposure to 40 min of shear stress evokes an increase in L-arginine uptake in porcine aortic endothelial cells [35]. Further studies have supported these findings and have recognized an dependence of L-arginine uptake on the production of NO [36,37]. The present study is the first to demonstrate augmented L-arginine transport following an exercise training programme in vivo, suggesting that L-arginine transport may be a potential mediator for the observed augmented endothelial function following exercise training in CHF. However, we, cannot exclude the possibility that the observation of a positive correlation between a change in response to ACh and the change in L-arginine clearance with exercise is associative rather than reflecting a causative process.

It is thought that circulating inhibitors of L-arginine transport, such as ADMA, may be elevated in CHF. Further speculation suggests that ADMA concentrations may decrease following exercise, potentially mediating the increase in L-arginine transport observed in the present study. However, it is unlikely that ADMA would exert a significant effect on either L-arginine transport or endothelial function in the range of plasma concentrations reported in the present study. In addition, there was no change in ADMA following exercise training, which is consistent with a study investigating exercise in CHF patients [38].

Study limitations
There was no difference between the two CHF patient groups at enrolment, excluding triacylglycerols, which were unexpectedly elevated in the exercise group at baseline. Throughout the 8-week period, triacylglycerol levels did not change within both groups. Many of the patients enrolled in the present study had a past history of cardiovascular risk factors and were taking additional therapy to control these risk factors. However, this is unlikely to have influenced our findings, as previous studies in CHF have not withdrawn vasoactive treatment and still observed endothelial dysfunction [22]. Most importantly, all drug therapy remained stable for at least 2 weeks prior to commencement and throughout the 8-week study period, thus could not have confounded our findings.

Conclusions
The present study demonstrates that a clinically relevant, combined aerobic and light resistance 8-week exercise training programme increased exercise capacity in CHF patients. Our study shows that this mode of exercise training improves vascular function, with improvements in both endothelium-dependent and -independent vasodilator capacity. It is likely that these responses are explained by an increase in smooth muscle NO responsiveness and NO generation. The present study demonstrates that the exercise-induced improvement in
endothelial function may, in part, be mediated by an increase in $l$-arginine transport.

**ACKNOWLEDGMENTS**

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