L-Arginine supplementation improves exercise capacity after a heart transplant[1–3]

Stéphane Doutreleau, Olivier Rouyer, Paola Di Marco, Evelyne Lonsdorfer, Ruddy Richard, François Piquard, and Bernard Geny

ABSTRACT

Background: Endothelial dysfunction is associated with the decreased exercise capacity observed in heart-transplant (HTx) recipients. L-Arginine supplementation (LAS) stimulates the nitric oxide (NO) pathway and restores endothelial function.

Objective: We compared exercise capacity in healthy subjects and HTx patients and investigated whether chronic LAS might improve exercise capacity and NO/endothelin balance after an HTx.

Design: Clinical, echocardiographic, and exercise characteristics were measured in 11 control subjects and 22 HTx recipients. In a prospective, double-blind study, the 22 HTx recipients performed a 6-min exercise [6-min-walk test (6MWT)] and a maximal bicycle exercise test before and after a 6-wk period of placebo intake or LAS. Endothelial function was measured by analyzing blood NO metabolites, endothelin, and the resulting NO/endothelin balance.

Results: Exercise capacity decreased after transplantation. Unlike with the placebo intake, 6 wk of LAS improved quality of life in HTx recipients (mean ± SEM Minnesota Score: from 15.3 ± 1.3 to 10.6 ± 1.1; $P < 0.001$) and their submaximal exercise capacity. The distance walked during the 6MWT increased (from 525 ± 20 to 580 ± 20 m; $P = 0.002$), and the ventilatory threshold during the incremental test was delayed by 1.2 min ($P = 0.01$). Central factors such as resting stroke volume, systolic pulmonary arterial pressure, cardiac systolodiastolic functions, and heart-rate reserve were not modified, but LAS significantly increased the NO:endothelin ratio (from 2.49 ± 0.38 to 3.31 ± 0.39; $P = 0.03$).

Conclusion: Oral LAS may be a useful adjuvant therapeutic to improve quality of life and exercise tolerance in HTx recipients. *Am J Clin Nutr* 2010;91:1261–7.

INTRODUCTION

Exercise capacity is generally reduced after a heart transplant (HTx). Although functional status and exercise performance steadily increase during the first 3 mo after surgery, HTx patients still have a lower exercise capacity than matched sedentary persons (1, 2). This is an important clinical problem because lower exercise capacity indicates a lower quality of life.

Central and peripheral factors limit exercise capacity (3). Cardiac diastolic dysfunction and chronotropic insufficiency as well as muscular (4) and endothelial dysfunction (5, 6) appear to be the main limiting factors of exercise capacity after HTx. An increase in training-induced exercise capacity is related to peripheral factors including an improvement in muscular or endothelial function (3, 7–9). This is not surprising because the endothelium modulates vascular tone, structure, and function (10) and plays a pivotal role in redistributing blood flow toward active muscles during exercise through the secretion of vasomotor factors. The vasodilator nitric oxide (NO) and the vasoconstrictor endothelin-1 are mainly secreted by endothelial cells and modulate blood flow and oxygen supply to working muscles (11). As NO and endothelin synthesis are interdependent, the NO/endothelin balance may be a key factor in peripheral limitation of exercise capacity. The NO/endothelin balance was shown to have increased and to be related to the degree of improvement in the aerobic exercise capacity of individuals in response to training (12).

After an HTx, the disruption of endothelial homeostasis is common (13, 14). In addition to preexisting factors and cytokine elevation, immunosuppressive therapy is largely implicated in such abnormalities. In particular, cyclosporine induces endothelial dysfunction through an elevation in endothelin-1 release by capillaries (15, 16). Such endothelial dysfunction is characterized by abnormal resting and exercise vasomotor functions that are associated with decreased NO bioavailability and an increased endothelin synthesis in muscular and coronary arteries (13, 17–20).

NO bioavailability can be restored by L-arginine supplementation (LAS) because L-arginine, a semiessential amino acid, is the precursor of NO production. Although LAS is controversial, and its effectiveness probably depends on the degree of NO-pathway impairment (11), LAS was shown to increase NO concentrations and to decrease endothelin-1 plasma concentrations. LAS also improved endothelial function and exercise capacity in healthy subjects, patients with heart failure, and patients with diabetes (21–26).

Because the limitation of exercise capacity is related to endothelial dysfunction (27, 28) and impaired NO production in HTx patients (29), and LAS might attenuate coronary (30) or peripheral endothelial dysfunction (6–31) after an HTx, we hypothesized that chronic LAS might increase NO bioavailability

[1] From the Medicine Faculty Physiology Institute, Strasbourg, France (SD, OR, PDM, EL, RR, FP, and BG), and the Physiology Department, Nouvel Hôpital Civil, Strasbourg, France (SD, OR, PDM, EL, RR, and BG).

[2] L-arginine was supplied by Novartis, France.

[3] Address correspondence to S Doutreleau, Nouvel Hôpital Civil, 1 Place de l'hôpital, 67000 Strasbourg, France. E-mail: stephane.doutreleau@chru-strasbourg.fr.

Received April 3, 2009. Accepted for publication February 2, 2010.

First published online March 3, 2010; doi: 10.3945/ajcn.2009.27881.

and thus improve the NO/endothelin balance and exercise capacity in HTx patients.

Therefore, we examined the biological and clinical effects of a 6-wk intake of oral LAS compared with a placebo and investigated the exercise capacity of HTx recipients in a 6-min-walk test (6MWT) and a maximal bicycle exercise test.

SUBJECTS AND METHODS

Population

Twenty-two male sedentary patients (>18 y old), who had transplants ≥6 mo before the study and were in sinus rhythm and clinically stable (reject free for ≥3 mo), participated in the study. Exclusion criteria included an unstable cardiac pathology, obesity [body mass index (in kg/m^2) > 40], participation in another study in the past month, chronic renal failure (creatinine clearance < 20 mL/min), renal transplantation, diabetes, impossibility to practice an exercise test, or nitrate therapy.

HTx patients were randomly assigned to the L-arginine group (6 g L-arginine twice/d; Dynamisan; Novartis, Rueil Malmaison, France) or placebo group.

To measure normal clinical and exercise values, 11 healthy sedentary age- and sex-matched subjects served as control subjects.

Study design

Clinical data and all tests (6MWT, maximal bicycle test, echocardiography, and blood sampling) were performed at baseline (before any supplementation) and after 6 wk of placebo intake or LAS.

In this prospective, double-blind study, after a standardized meal, patients performed the 6MWT and, after a 2-h resting period, a maximal upright bicycle cardiopulmonary exercise test in a quiet air-conditioned room (21°C). To avoid circadian variation, all exercise tests were performed between 1400 and 1600.

All patients gave written informed consent, and the hospital and the university review board for human studies approved the study.

Variables measured

Echocardiograms were recorded in all subjects with the ATL HDI 5000 echocardiography system (Bothell, WA). Subjects were examined in the left lateral position by using parasternal (long and short axes) and apical views. The same trained cardiac sonographer, who was blinded to the patient's group, took all images and measurements following the American Society of Echocardiography recommendations (32). Systolic function was assessed by the left ejection fraction (%), which was determined in the 4 and 2 apical views with the Simpson's method. Cardiac output [CO (L/min)] was calculated by using the following equation:

$$CO = VTI \times HR \times LVOT\ area \qquad (1)$$

where VTI is the aortic velocity time integral, HR is the heart rate, and LVOT is the left ventricular outflow track with LVOT area = $\pi \times r^2$ (r = radius), which is derived from the measure of the valve annulus diameter. Stroke volume [SV (mL)] was also calculated by dividing the cardiac output by HR.

To assess diastolic function, mitral valve pulsed Doppler waves were recorded from the apical 4-chamber view with the sample volume placed at the mitral leaflet tips. Peak early transmitral filling velocity (E) and peak late transmitral atrial filling velocity (A) were measured, and E:A ratios were calculated. Tissue Doppler imaging (TDI) of the lateral mitral annulus at end expiration from the apical 4-chamber view was done by using a 5-mm sample volume. The peak early diastolic mitral annulus velocity (Ea) and diastolic velocity of the posterior wall were measured with TDI. The E:Ea ratio was calculated and used to estimate the left ventricular filling pressure. When possible, tricuspid velocity regurgitation was measured [TVR (m/s)] to estimate the systolic pulmonary artery pressure (sPAP):

$$sPAP\ (mm\ Hg) = 4 \times TVR^2 + RAP \qquad (2)$$

where RAP is the estimated right atrial pressure (mm Hg).

For each variable, 3 consecutive beats were measured, and the average value was recorded.

Biological variables

Blood samples were taken at rest and immediately centrifuged, separated, and stored at −80°C for subsequent analysis.

The amino acid plasma concentration was measured by high-performance liquid chromatography by using liquid chromatography separation and fluorimetric detection with orthophtaldialdehyde (Liquimat IV Kontron; Zurich, Switzerland) with a mean CV of 6.5%.

Endothelin-1 was measured by radioimmunoassay (Commercial kits; Amersham Pharmacia Biotech, Buckinghamshire, United Kingdom) after extraction by ethanol-acetic acid solution on Sep-Pak C18 cartridges (Waters Associated, Inc, Milford, MA). The intraassay CV for duplicate samples was 4.5% for the observed values.

Plasma nitrates and nitrites (NOx) were measured by the colorimetric method on the basis of the Griess reaction.

Exercise tests

6MWT

The 6MWT was coached to patients in a calibrated corridor by the same physicians according to American Thoracic Society recommendations (33). Results were expressed as the actual distance walked in meters. Before the test, patients rested in a chair near the starting position for 10 min. HR and pulse oxygen saturation (%) were continuously measured with a finger pulse oxymeter (Oxypleth; Novametrix, Wallingford, CT) during the test and first 3 min of recovery.

Maximal cardiopulmonary bicycle exercise test

All HTX patients performed a maximal bicycle exercise test between 1400 and 1600 with an electronically braked bicycle ergometer (Medifit 1000 S; Medifit, Maarn, Netherlands) before and after a 6-wk period of placebo intake or LAS.

We used the same protocol for all patients, and after a warm-up on the bicycle for 3 min set at 30 W, the exercise workload

was increased by 15 W/min. Patients were asked to exercise until exhausted. Exhaustion was defined as the inability to maintain the pedal frequency >50 rpm because of leg fatigue or dyspnea.

While sitting on the bicycle, patients were connected to a respiratory gas analysis system both before and during exercise. Breath-by-breath oxygen uptake [$\dot{V}O_2$ ($\cdot$ $mL^{-1} \cdot min^{-1} \cdot kg^{-1}$)], carbon dioxide output [$\dot{V}CO_2$ ($\cdot$ $mL^{-1} \cdot min^{-1} \cdot kg^{-1}$)], and minute ventilation [$\dot{V}_E$ (L/min)] were measured with a ergospirometer (Vmax 229; Sensormedics, Yorba Linda, CA), averaged, and printed every 30 s. We calculated the oxygen pulse [O_2/HR (mL O_2/b)] as an index of peripheral oxygen availability. Peak oxygen uptake was defined as the highest value of oxygen consumption during the last minute of exercise. Two blinded examiners used the V-slope method to manually measure the ventilatory threshold. The mean ± SEM interobserver variability for the ventilatory threshold measurement was 4.8 ± 1.64%.

Electrocardiographic activity was monitored continuously (Cardiovit CS-200; Schiller, Zurich, Switzerland), and systemic arterial blood pressure was registered every 2 min with a sphygmomanometer.

Patient quality of life

Patient quality of life was measured with the Minnesota Living with Heart Failure questionnaire (34).

Statistical analyses

All values are expressed as means ± SEMs. Comparisons of the 3 groups (control, placebo, and L-arginine) were tested by a one-factor analysis of variance. Changes in cardio-respiratory and biological variables were assessed by a 2-factor analysis of variance with repeated measures, considering the effect of the group (placebo or L-arginine) and the effect of time (before and after). $P < 0.05$ was considered statistically significant, and we used a post hoc multiple-comparison procedure (corrected Bonferroni method) to investigate these differences. We also indicated the ranger of the power of the performed tests with $\alpha = 0.05$. All statistical analysis was done with SigmaStat (version 3.5 for Windows; Systat Software, Point Richmond, CA).

RESULTS

Three patients did not complete the protocol, one patient in the L-arginine group did not complete the protocol because of exacerbated hypertension, and 2 patients in the placebo group did not complete the protocol because of personal convenience. These 3 patients were excluded from the study. With LAS, one HTx patient experienced vertigo at the beginning of the study without any objective clinical confirmation. This sensation disappeared, and the patient completed the protocol. No other potential adverse effects were noted. The power of the performed tests ranged, when not significant, between 0.05 and 0.57.

Clinical and biological characteristics of the subjects

As shown in **Table 1**, the control subjects and HTx patients were similar concerning age, body mass index, and mean arterial systemic pressure. The average HR of HTx patients was higher than the average HR of control subjects, most likely because of surgically induced cardiac denervation (5, 35). Immunosuppressive therapy, cyclosporine plasma concentrations, and time since transplantation were similar in both HTx patient groups. Except for one patient, all patients received transplants because of a dilated cardiomyopathy. All patients had systemic hypertension treated with an angiotensin-converting enzyme inhibitor ($n = 15$) or calcium antagonist ($n = 9$) but none of them took β-blockers. There were no current smokers in the HTx or control groups (before having HTxs, there was one former smoker in the placebo group and one former smoker in the L-arginine group). All HTx patients were systematically treated with pravastatin (20 mg/d).

Echocardiographic characteristics of the subjects: systolic and diastolic function

As shown in **Table 2**, all HTx patients in the 2 groups had normal left ventricular systolic function (ejection fraction) and SV. Although echocardiographic assessment of diastolic function was not previously validated in cardiac-transplant recipients, we measured the E and A. The resultant E:A ratio and the normal E:Ea ratio confirmed normal diastolic function and normal left ventricular filling. No significant difference was shown with the control group for systolic or diastolic function.

TABLE 1
Baseline clinical, biological, and hemodynamic characteristics of the control, placebo, and L-arginine groups[1]

	Control ($n = 11$)	Placebo ($n = 10$)	L-Arginine ($n = 9$)	P
Age (y)	57.9 ± 1.2	54.5 ± 2.5	57.1 ± 2.9	NS
BMI (kg/m^2)	25.7 ± 0.59	26.1 ± 0.95	25.4 ± 1.83	NS
Time since transplant (mo)	—	57.8 ± 15.2	58.6 ± 13.9	NS
Cyclosporine (mg/d)	—	174.5 ± 17.5	207.8 ± 19.6	NS
Prednisolone (mg/d)	—	7.0 ± 1.0	7.5 ± 1.4	NS
Mycophenolate mofetil (mg/d)	—	1583 ± 327	1143 ± 142	NS
Cyclosporine plasma concentrations (ng/ml)	—	100.6 ± 10.9	101.7 ± 8.4	NS
HR (beats/min)	74.6 ± 3.5[2]	97.7 ± 2.4	102.1 ± 2.6	<0.0001
MAP (mm Hg)	95.6 ± 3.1	97.8 ± 2.6	97.2 ± 2.3	NS

[1] All values are means ± SEMs. HR, heart rate; MAP, mean arterial pressure. Significance was determined by using one-factor ANOVA.

[2] $P < 0.0001$ between the control group and heart-transplant patients (placebo and L-arginine groups).

TABLE 2
Echocardiography and Doppler variables: systolic and diastolic left heart function and pulmonary arterial pressure before and after 6 wk in the placebo and L-arginine groups[1]

	Placebo (n = 10)		L-Arginine (n = 9)		
	Before	After	Before	After	P
EF (%)	65.7 ± 1.4	65.7 ± 1.4	65.3 ± 3.3	67.7 ± 2.6	NS
SV (mL)	81.5 ± 6.4	82.4 ± 5.4	78.7 ± 5.5	82.0 ± 5.7	NS
E/A	1.73 ± 0.10	1.70 ± 0.12	1.64 ± 0.06	1.74 ± 0.04	NS
Ea (cm/s)	16.1 ± 0.87	17.0 ± 0.77	16.7 ± 0.9	16.7 ± 0.8	NS
E/Ea	4.95 ± 0.52	4.55 ± 0.99	4.27 ± 0.21	4.13 ± 0.29	NS
TDI_{pw} (cm/s)	18.9 ± 0.8	20.9 ± 1.2	17.29 ± 1.2	18.28 ± 1.6	NS
TVR (m/s)	2.4 ± 0.14	2.5 ± 0.16	2.3 ± 0.13	2.2 ± 0.13	NS
sPAP (mm Hg)	29.2 ± 3.6	31.8 ± 3.9	27.2 ± 2.5	24.4 ± 2.2	NS

[1] All values are means ± SEMs. EF, ejection fraction; SV, stroke volume; E, peak early transmitral filling velocity; A, peak late transmitral filling velocity; Ea, peak early diastolic mitral annulus velocity; TDI_{pw}, diastolic velocity of the posterior wall in tissue Doppler imaging; TVR, tricuspid velocity regurgitation peak velocity; sPAP, estimated systolic pulmonary arterial pressure. P values were adjusted by using Bonferroni correction. There were no significant interactions and no significant differences between groups (2-factor ANOVA).

No change was observed in either the placebo or L-arginine echocardiographic values after 6 wk in the HTx groups.

Quality of life

The quality of life in both groups was similar to that previously reported after an HTx (1). The quality of life remained unchanged after 6 wk in the placebo group. However, after 6 wk of oral LAS, the quality of life increased in the L-arginine group, as inferred by the significant decrease in the Minnesota Score (from 15.3 ± 1.3 to 10.6 ± 1.1; $P < 0.001$) (**Figure 1**).

Exercise capacity

6MWT

At baseline, the distances walked were similar in both patient groups (520 ± 21 and 525 ± 20 m in the placebo and L-arginine groups, respectively), which were significantly lower than the distance walked in the control group (608 ± 12 m; $P < 0.01$).

After 6 wk, the walking distance significantly increased only after LAS (to 580 ± 20 m; +9.4 ± 1%, $P = 0.002$) and remained unchanged after placebo intake (510 ± 15 m; NS) (**Figure 2**A).

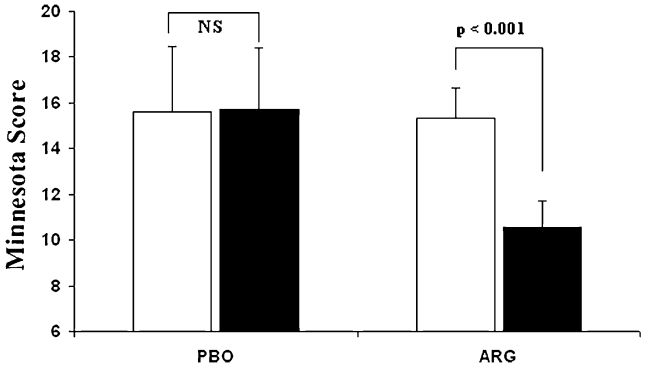

FIGURE 1. Mean (±SEM) quality-of-life (Minnesota Score) values in the placebo (PBO; n = 10; left panel) and L-arginine (ARG; n = 9; right panel) groups before (white bars) and after (black bars) 6 wk. There were no significant differences at baseline between the 2 groups. The time-by-treatment interaction was significant at $P < 0.001$ (2-factor ANOVA).

Bicycle exercise test

At baseline, the maximal exercise performance of our HTx recipients was significantly lower than that the sedentary healthy control group. The maximal oxygen uptakes were 23.7 ± 1.5, 23.6 ± 1.8, and 30.0 ± 1.1 · mL^{-1} · min^{-1} · kg^{-1} in the placebo, L-arginine, and control groups, respectively ($P < 0.001$), and the maximal power was 136.5 ± 14.4, 136.5 ± 13.1, and 178.3 ± 15.2 W in the same 3 groups, respectively ($P < 0.001$).

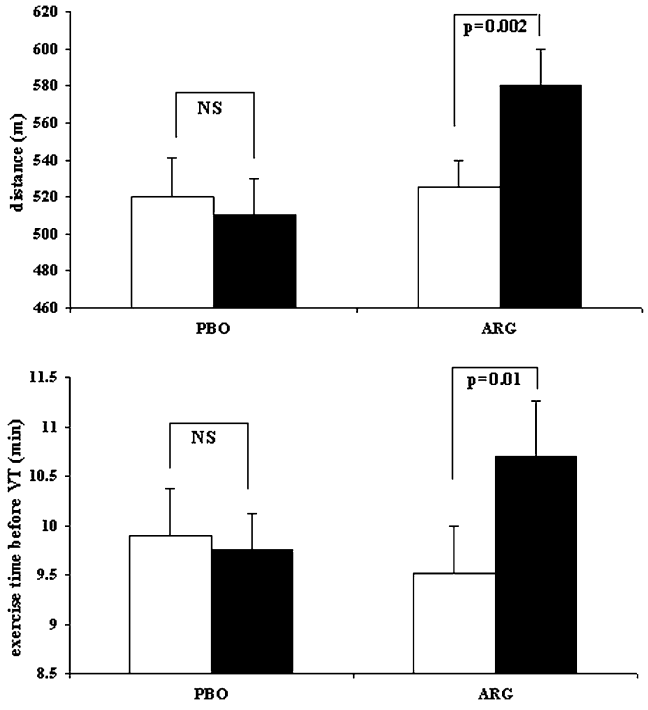

FIGURE 2. Mean (±SEM) exercise capacity in both groups: distance (m) walked during the 6-min-walk test (A) and exercise time (min) before the ventilatory threshold (VT) (B) before (white bars) and after (black bars) 6 wk in the placebo (PBO; n = 10; left panel) and L-arginine (ARG; n = 9; right panel) groups. There were no significant differences at baseline between the 2 groups. The time-by-treatment interaction was significant at $P < 0.001$ (2-factor ANOVA).

The patients stopped exercising because of leg fatigue ($n = 18$) or dyspnea ($n = 3$). The HR and blood pressure responses were similar in both HTx groups: HR increased from 97.7 ± 2.4 to 146.8 ± 5.6 and 102.1 ± 2.6 to 148.6 ± 5.3 beats/min in the placebo and L-arginine groups, respectively; and mean arterial blood pressure increased from rest to the end of exercise from 97.8 ± 2.6 to 116.3 ± 2.8 and 94.8 ± 2.4 to 110.7 ± 3.1 mm Hg in the placebo and L-arginine groups respectively. In the control group, the HR increased from 74.6 ± 3.5 to 172.4 ± 0.9 beats/min.

After 6 wk, resting and maximal exercise HRs were lower in the L-arginine group (**Table 3**), without any variation in the HR reserve (HRR, calculated as peak HR − resting HR; Table 3) or mean arterial pressure compared with baseline values. However, in the L-arginine group, we observed a significant delay in the ventilatory threshold (+1.2 min; $P = 0.01$; Figure 2B) and an increase in simultaneous increased oxygen uptake (16.5 ± 0.8 to 18.5 ± 1.1 · mL^{-1} · min^{-1} · kg^{-1}; $P = 0.037$).

In contrast, maximal exercise $\dot{V}O_2$ was not modified after placebo intake or LAS (23.7 ± 1.5 to 22.2 ± 1.3 and 23.6 ± 1.8 to 24.5 ± 1.7 · mL^{-1} · min^{-1} · kg^{-1} in the placebo and L-arginine groups, respectively).

The maximal oxygen pulse, product of the SV and arteriovenous difference in oxygen (A–V), an indirect index of oxygen extraction, only increased significantly in the L-arginine group from 11.6 ± 0.8–12.6 ± 0.8 mL O_2/b ($P = 0.002$).

L-Arginine, endothelin-1, and NOx

The L-arginine plasma concentration increased significantly only after supplementation (from 113 ± 11.7 to 243.6 ± 19.5 μmol/L; +125%; $P < 0.001$) in the L-arginine group compared with placebo (from 106.7 ± 6.3 to 112.7 ± 7.1 μmol/L).

Before supplementation, plasma endothelin-1 and NOx were similar in both groups (18.2 ± 0.8 and 17.5 ± 0.9 pmol/L and 36.3 ± 5.1 and 41 ± 5.6 μmol/L for endothelin-1 and NOx for the placebo and L-arginine groups, respectively). After supplementation, the NOx:endothelin-1 ratio significantly increased in the L-arginine group (from 2.49 ± 0.38 to 3.31 ± 0.39, $P = 0.03$, and from 2.02 ± 0.28 to 2.33 ± 0.34, NS, for the L-arginine and placebo groups, respectively) (**Figure 3**).

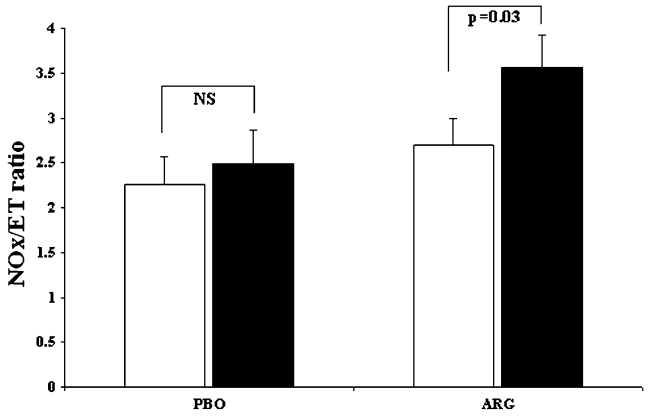

FIGURE 3. Mean (±SEM) nitric oxide/endothelin (NOx/ET) ratio before (white bars) and after (black bars) 6 wk in both placebo (PBO; $n = 10$; left panel) and L-arginine groups (ARG; $n = 9$; right panel). There were no significant differences at baseline between the 2 groups. The time-by-treatment interaction was significant at $P = 0.002$ (2-factor ANOVA).

DISCUSSION

The major finding of this study is that chronic LAS significantly improved the quality of life and submaximal exercise capacity in HTx recipients. LAS increased the distance walked during the 6MWT and delayed the onset of the ventilatory threshold during the maximal bicycle exercise test.

Rather than depending on central factors (no change in resting HR or cardiac function), this improvement in exercise capacity could be dependent on peripheral factors including the NO/endothelin balance.

Exercise capacity after an HTx

Despite having an HTx, the exercise capacity of HTx recipients remains lower than expected and their exercise tolerance is reduced (1). Accordingly, the HTx patients is the current study has significantly reduced values of exercise capacity in both exercise tests compared with the values for control subjects.

The 6MWT is relatively easy to perform and is a valuable prognostic tool for cardiovascular disease. This test is commonly used in patients with heart failure (36), patients with pulmonary hypertension (37), and patients with chronic obstructive pulmonary disease or older healthy people (38). This test corresponds to real-life situations. In the current study, the 6MWT and maximal exercise test showed a reduced submaximal exercise capacity and that the ventilatory threshold occurred earlier in the maximal exercise test. We also observed a reduction in values of maximal exercise capacity in HTx patients compared with normal values (4, 7).

TABLE 3
Heart rate (HR) at rest and at end of exercise (EE) and heart-rate reserve (HRR) before and after 6 wk in the placebo and L-arginine groups[1]

	Placebo ($n = 10$)		L-Arginine ($n = 9$)	
	Before	After	Before	After
Rest HR (beats/min)	97.7 ± 2.4	98.1 ± 2.9	102.1 ± 2.6	94.1 ± 3.8[2]
EE HR (beats/min)	146.8 ± 5.6	150.7 ± 5.7	148.6 ± 5.3	143.2 ± 5.1[3]
HRR (beats/min)	49 ± 7.4	52.6 ± 6.1	46.4 ± 4.7	49.1 ± 5.5

[1] All values are means ± SEMs. There were significant interactions for rest HR ($P = 0.039$) and EE HR ($P = 0.018$) and no significant differences at baseline between the placebo and L-arginine groups (2-factor ANOVA).

[2,3] Change from baseline in the L-arginine group: [2]$P = 0.009$, [3]$P = 0.01$.

Several mechanisms have been proposed that explain these limitations. Before surgery, the primary disease and long-term inactivity play a role. After surgery, central factors (cardiac diastolic dysfunction and cardiac denervation resulting in chronotropic incompetence) as well as peripheral factors such as endothelial and muscular dysfunctions most likely contribute to a reduced exercise capacity (1–9).

In our study group, which included HTx patients with no complications, it is unlikely that central factors played an important limiting role. Indeed, cardiac systolic and diastolic functions were similar to that of healthy control subjects. Although a reduced HRR was shown in our HTx patients, which was caused by increased resting HR after surgical cardiac denervation, no relation was observed between HRR and exercise capacity. Such a relation has been observed mainly in highly trained HTx patients, suggesting that greater exercise intensity may be necessary to reveal the central limitation of exercise capacity after an HTx (4).

The role of vascular and muscular alterations should be considered peripheral factors. Muscular alterations may be due to a direct deleterious effect of deconditioning or the immunosuppressive therapy. Indeed, both glucocorticoids (39) and cyclosporine (40) were shown to damage the muscles of HTx recipients by interfering with mitochondrial function. Skeletal muscle dysfunction might also be the outcome of relative hypoxia from inadequate perfusion, and as a result, vascular dysfunction is related to exercise-capacity reduction after an HTx (28). In this study, we investigated the endothelial function of our patients by measuring their NO/endothelin balance before and after the 6-wk LAS or placebo intake.

Effect of chronic LAS

We confirmed our hypothesis that chronic LAS significantly improved the submaximal exercise capacity of patients after an HTx. Indeed, both the distance walked during the 6MWT and the time elapsed before the ventilatory threshold during the bicycle test, known to be independent of the patient's motivation, increased after LAS, whereas these factors were unchanged after the placebo intake. Such results are of particular clinical importance because these variables correlate better with daily life symptoms than peak $\dot{V}O_2$ (41). Accordingly, the patients described an improved quality of life after L-arginine supplementation.

The mechanisms of LAS effects on HR should be investigated. LAS significantly decreased the resting HRs of our patients, and this may theoretically improve their exercise capacity by reversing the chronotropic insufficiency of an HTx. An L-arginine-related HR decrease was described in healthy and in heart-failure patients (42). The mechanisms involved remain unclear, but a direct or indirect increase in parasympathetic activity might play a key role (42, 43). Such a hypothesis might well apply in cardiac-denervated HTx patients because the cardiac hormone atrial natriuretic peptide may have a cardiac-sympathoinhibitory effect in patients after an HTx (44).

However, the exercise-induced HR increase was also blunted after LAS in the current study. The HRR was not enhanced, making it unlikely that the beneficial effect of LAS on exercise capacity was obtained through this central pathway.

Because potential central limiting factors were not improved, LAS might have worked through peripheral mechanisms. Indeed, HTx endothelial dysfunction was related to the exercise capacity of patients (5, 29), and LAS reduced impairments in the vascular endothelial NO pathway (6). Furthermore, LAS increased NO and decreased endothelin-1 plasma concentrations, thereby improving endothelial function and exercise capacity in heart-failure or HTx patients (21, 45). Therefore, we chose to measure the NOx/endothelin balance as an endothelial biological function in our patients before and after supplementation.

The NOx/endothelin ratio significantly increased after chronic LAS, suggesting that the beneficial effects of L-arginine on the exercise capacity of patients after an HTx might, at least partly, be related to an improvement in skeletal muscle vasodilatation and oxygen delivery and extraction during exercise as suggested by the significant increase in the oxygen pulse after LAS.

In conclusion, oral LAS increased submaximal exercise performance and the NOx:endothelin ratio in HTx recipients. These results suggest that LAS might improve muscular oxygen supply through an improvement in endothelial function. Therefore, oral LAS might be useful as a therapeutic adjuvant to improve the quality of life and exercise tolerance of patients after an HTx.

Study limitations

This pilot study provides support that LAS might improve the exercise capacity of patients after an HTx. A larger-scale study should help to determine whether LAS may be useful for all HTx recipients. It should also better define the mechanisms involved and particularly analyze changes at the capillary level in muscle perfusion and/or muscular endothelial function.

We thank the HTx recipients for their enthusiastic participation in the study and their daily courage in life. We also thank I Bentz and F Goupilleau for their expert technical assistance.

The authors' responsibilities were as follows—SD: design of the experiment, collection and analysis of data, and writing of the manuscript; OR, PDM, EL, and RR: collection and analysis of data; FP: statistic analysis; and BG: design of experiment and writing of manuscript. None of the authors reported a conflict of interest.

REFERENCES

1. Marconi C, Marzorati M. Exercise after heart transplantation. Eur J Appl Physiol 2003;90:250–9.
2. Kobashigawa JA, Leaf DA, Lee N, et al. A controlled trial of exercise rehabilitation after heart transplantation. N Engl J Med 1999;340:272–7.
3. Kao AC, Van Trigt P, Shaeffer-McCall GS, et al. Central and peripheral limitations to upright exercise in untrained cardiac transplant recipients. Circulation 1994;89:2605–15.
4. Richard R, Verdier JC, Doutreleau S, Piquard F, Geny B, Rieu M. Exercise limitation in trained heart and kidney transplant recipients: central and peripheral limitations. J Heart Lung Transplant 2005;24:1774–80.
5. Richard R, Zoll J, Mettauer B, Piquard F, Geny B. Counterpoint: Cardiac denervation does not play a major role in exercise limitation after heart transplantation. J Appl Physiol 2008;104:560–2, discussion 562–4.
6. Lim DS, Mooradian SJ, Goldberg CS. Effect of oral L-arginine on oxidant stress, endothelial dysfunction, and systemic arterial pressure in young cardiac transplant recipients. Am J Cardiol 2004;94:828–31.
7. Geny B, Charloux A, Lampert E, Lonsdorfer J, Haberey P, Piquard F. Enhanced brain natriuretic peptide response to peak exercise in heart transplant recipients. J Appl Physiol 1998;85:2270–6.
8. Geny B, Saini J, Mettauer B, et al. Effect of short-term endurance training on exercise capacity, haemodynamics and atrial natriuretic peptide secretion in heart transplant recipients. Eur J Appl Physiol Occup Physiol 1996;73:259–66.
9. Zoll J, N'Guessan B, Ribera F, et al. Preserved response of mitochondrial function to short-term endurance training in skeletal muscle of heart transplant recipients. J Am Coll Cardiol 2003;42:126–32.

10. Hurairah H, Ferro A. The role of the endothelium in the control of vascular function. Int J Clin Pract 2004;58:173–83.
11. Böger RH. L-Arginine therapy in cardiovascular pathologies: beneficial or dangerous? Curr Opin Clin Nutr Metab Care 2008;11:55–61.
12. Maeda S, Tanabe T, Miyauchi T, et al. Aerobic exercise training reduces plasma endothelin-1 concentration in older women. J Appl Physiol 2003;95:336–41.
13. Braith RW, Schofield RS, Hill JA, Casey DP, Pierce GL. Exercise training attenuates progressive decline in brachial artery reactivity in heart transplant recipients. J Heart Lung Transplant 2008;27:52–9.
14. Kubo SH, Rector TS, Bank AJ, et al. Effects of cardiac transplantation on endothelium-dependent dilation of the peripheral vasculature in congestive heart failure. Am J Cardiol 1993;71:88–93.
15. Nickel T, Schlichting CL, Weis M. Drugs modulating endothelial function after transplantation. Transplantation 2006;82:S41–6.
16. Wilasrusmee C, Da Silva M, Siddiqui J, et al. Role of endothelin-1 in microvascular dysfunction caused by cyclosporin A. J Am Coll Surg 2003;196:584–91.
17. Berkenboom G, Crasset V, Giot C, Unger P, Vachiery JL, LeClerc JL. Endothelial function of internal mammary artery in patients with coronary artery disease and in cardiac transplant recipients. Am Heart J 1998;135:488–94.
18. Geny B, Piquard F, Lonsdorfer J, Haberey P. Endothelin and heart transplantation. Cardiovasc Res 1998;39:556–62.
19. Petrakopoulou P, Anthopoulou L, Muscholl M, et al. Coronary endothelial vasomotor function and vascular remodeling in heart transplant recipients randomized for tacrolimus or cyclosporine immunosuppression. J Am Coll Cardiol 2006;47:1622–9.
20. Schmidt A, Pleiner J, Bayerle-Eder M, et al. Regular physical exercise improves endothelial function in heart transplant recipients. Clin Transplant 2002;16:137–43.
21. Rector TS, Bank AJ, Mullen KA, et al. Randomized, double-blind, placebo-controlled study of supplemental oral L-arginine in patients with heart failure. Circulation 1996;93:2135–41.
22. Martina V, Masha A, Gigliardi VR, et al. Long term N-acetylcysteine and L-arginine administration reduces endothelial activation and systolic blood pressure in hypertensive patients with type 2 diabetes mellitus. Diabetes Care 2008;31:940–4.
23. Poelzl G, Frick M, Lackner B, et al. Short-term improvement in submaximal exercise capacity by optimized therapy with ACE inhibitors and beta blockers in heart failure patients is associated with restoration of peripheral endothelial function. Int J Cardiol 2006;108: 48–54.
24. Schaefer A, Piquard F, Geny B, et al. L-Arginine reduces exercise-induced increase in plasma lactate and ammonia. Int J Sports Med 2002; 23:403–7.
25. Doutreleau S, Mettauer B, Piquard F, et al. Chronic but not acute oral L-arginine supplementation delays the ventilatory threshold during exercise in heart failure patients. Can J Appl Physiol 2005;30:419–32.
26. Brown AA, Hu F. Dietary modulation of endothelial function: implications for cardiovascular disease. Am J Clin Nutr 2001;73:673–86.
27. Børsheim E, Bui QU, Tissier S, Kobayashi H, Ferrando AA, Wolfe RR. Effect of amino acid supplementation on muscle mass, strength and physical function in elderly. Clin Nutr 2008;27:189–95.
28. Patel AR, Kuvin JT, DeNofrio D, et al. Peripheral vascular endothelial function correlates with exercise capacity in cardiac transplant recipients. Am J Cardiol 2003;91:897–9.
29. Schaefer A, Piquard F, Doutreleau S, et al. Reduced exercise capacity is associated with reduced nitric oxide production after heart transplantation. J Thorac Cardiovasc Surg 2001;122:821–2.
30. Drexler H, Fischell TA, Pinto FJ, et al. Effect of L-arginine on coronary endothelial function in cardiac transplant recipients. Relation to vessel wall morphology. Circulation 1994;89:1615–23.
31. Bai Y, Sun L, Yang T, Sun K, Chen J, Hui R. Increase in fasting endothelial function after short-term oral L-arginine is effective when baseline flow-mediated dilatation is low: a meta-analysis of randomized controlled trials. Am J Clin Nutr 2009;89:77–84.
32. Gottdiener JS, Bednarz J, Devereux R, et al; American Society of Echocardiography. American Society of Echocardiography recommendations for use of echocardiography in clinical trials. J Am Soc Echocardiogr 2004;17:1086–119.
33. ATS Committee on Proficiency Standards for Clinical Pulmonary Function Laboratories. ATS statement: guidelines for the six-minute walk test. Am J Respir Crit Care Med 2002;166:111–7.
34. Rector TS, Cohn JN. Assessment of patient outcome with the Minnesota Living with Heart Failure questionnaire: reliability and validity during a randomized, double-blind placebo-controlled trial of pimobendan. Am Heart J 1992;124:1017–25.
35. Piquard F, Charloux A, Mettauer B, et al. Exercise-induced increase in circulating adrenomedullin is related to mean blood pressure in heart transplant recipients. J Clin Endocrinol Metab 2000;85:2828–31.
36. Lewis ME, Newall C, Townend JN, Hill SL, Bonser RS. Incremental shuttle walk test in the assessment of patients for heart transplantation. Heart 2001;86:183–7.
37. Oudiz RJ, Barst RJ, Hansen JE, et al. Cardiopulmonary exercise testing and six-minute walk correlations in pulmonary arterial hypertension. Am J Cardiol 2006;97:123–6.
38. Simonsick EM, Fan E, Fleg JL. Estimating cardiorespiratory fitness in well-functioning older adults: treadmill validation of the long distance corridor walk. J Am Geriatr Soc 2006;54:127–32.
39. Dirks-Naylor AJ, Griffiths CL. Glucocorticoid-induced apoptosis and cellular mechanisms of myopathy. J Steroid Biochem Mol Biol 2009;117:1–7.
40. Mercier JG, Hokanson JF, Brooks GA. Effects of cyclosporine A on skeletal muscle mitochondrial respiration and endurance time in rats. Am J Respir Crit Care Med 1995;151:1532–6.
41. Jones NL, Ehrsam RE. The anaerobic threshold. Exerc Sport Sci Rev 1982;10:49–83.
42. Doutreleau S, Mettauer B, Piquard F, et al. Chronic L-arginine supplementation enhances endurance exercise tolerance in heart failure patients. Int J Sports Med 2006;27:567–72.
43. Chowdhary S, Marsh AM, Coote JH, Townend JN. Nitric oxide and cardiac muscarinic control in humans. Hypertension 2004;43:1023–8.
44. Geny B, Charloux A, Brandenberger G, Piquard F. Despite cardiac denervation, atrial natriuretic peptide possess a cardiac sympatho-inhibitory effect after heart transplantation. J Thorac Cardiovasc Surg 2006;131:1417–8.
45. Doutreleau S, Piquard F, Lonsdorfer E, et al. Improving exercise capacity, 6 weeks training tends to reduce circulating endothelin after heart transplantation. Clin Transplant 2004;18:672–5.