Increased metaboreflex activity is related to exercise intolerance in heart transplant patients

Anne Houssière,2 Marko Gujic,2 Gael Deboeck,1 Agnieszka Ciarka,2 Robert Naeije,1 and Philippe van de Borne2

Departments of 1Physiology and 2Cardiology, Erasme Hospital, Brussels, Belgium

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HEART TRANSPLANTATION RESTORES close to normal cardiac function in patients with severe end-stage heart failure (20). However, it does not restore normal exercise capacity, as these patients continue to show a similar ventilatory response to exercise as patients with moderate degrees of heart failure (4, 20, 24). Moreover, heart transplant recipients (HTRs) have increased sympathetic activity at rest (4, 5).

The peripheral chemoreceptors are located in the aortic and carotid bodies and respond primarily to a reduction in oxygen and to acidosis by increasing minute ventilation (Ve) and muscle sympathetic nerve activity (MSNA) (8, 10, 23, 43). Our laboratory recently reported that the increased ventilatory response to exercise observed in HTRs is related to enhance resting peripheral chemoreflex sensitivity (4). We have also observed that an increased resting peripheral chemoreflex drive contributed to the increased resting MSNA in HTRs (4).

Several other reflex mechanisms may, however, also contribute to increased MSNA and exercise intolerance in HTRs. One of these mechanisms is the muscle metaboreflex, which increases MSNA during exercise, and which is believed to act by increasing blood pressure (BP) and preventing a mismatch between blood flow and metabolism in the contracting muscle (8–10, 19). This reflex is mediated by small afferents in the skeletal muscle. The endings of the afferents are associated with collagen structures and vessels in the muscles that are activated by the metabolic products of exercise (14, 32). There is evidence that overactivation of the metaboreflex contributes to exercise hyperventilation and reduces exercise tolerance in patients with left ventricular dysfunction (27). This overactivation could be because of abnormal muscle oxidative metabolism, which persists after cardiac transplantation (2, 15).

We decided to test the hypothesis that metaboreflex regulation remains abnormal after cardiac transplantation. We are not aware of a previous study on metaboreflex control in patients after heart transplantation. We examined the effects of isometric (ischemic) and rhythmic handgrip exercise at 30% of maximal voluntary contraction (MVC) on MSNA in HTRs and in closely matched normal subjects. Changes in sympathetic activity during a local circulatory arrest after handgrip were assessed to isolate the metaboreflex contribution to the exercise response (8–10).

We further tested the hypothesis that increased metaboreflex control is related to exercise intolerance in HTRs and asked all participants to perform a maximal, symptom-limited cardiovascular exercise test. We also exposed the subjects to an acute isocapnic hypoxic challenge to assess their peripheral chemoreflex sensitivity and to determine the respective contributions of increased metaboreflex and peripheral chemoreflex activity to exercise intolerance after heart transplantation.

METHODS

Subjects

The study included 11 heart transplant patients [51 ± 8 yr, all men, body mass index (BMI) 24 ± 3 kg/m²]. Mean time after heart transplantation was 8 yr (range, 4–15 yr). HTRs had a normal left ventricular ejection fraction of 69 ± 10% (range, 53–85%), as estimated by routine echocardiography, performed according to the European Society of Cardiology Guidelines. HTRs were being treated with various combinations of immunosuppressive drugs and were

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receiving cyclosporine (n = 9), methylprednisolone (n = 11), tacrolimus (n = 2), and mofetil mycophenolate (n = 3). Hypertension was being treated with diuretics (n = 3), B-blockers (n = 2), calcium channel blockers (n = 7), angiotensin-converting enzyme inhibitors (n = 9), vasodilators (n = 2), and an imidazoline agonist (n = 1). The origin of the heart failure was ischemic heart disease (n = 4), idiopathic dilated cardiomyopathy (n = 3), viral cardiomyopathy (n = 2), and valvular heart disease (n = 2). Patients had either no or modest left ventricular hypertrophy (both interventricular and posterior end-diastolic thicknesses were 11 ± 1 mm), as measured by routine echocardiography. The HTRs had normal renal function and were not taking any hypoglycemic medication. No patients suffered from acute allograft rejection at the time of the study. For ethical reasons, medication was not altered in the HTRs during the study period.

The control group consisted of 10 healthy control subjects (52 ± 5 yr, all men, BMI 25 ± 2 kg/m²). No control subjects were receiving any medication or had any chronic diseases.

The Ethical Committee of Erasme University Hospital approved the study protocol, and informed, written consent was obtained from each subject.

**Measurements**

We obtained continuous recordings of the electrocardiogram (Siemens Medical, ECG Monitoring, Erlangen, Germany), Ve (pneumotachometer, Medical Electronic Equipment, Brussels, Belgium), oxygen saturation (Nellcor N-100 C Pulse Oxyimeter, Pleasanton, CA) and end-tidal CO₂ (Normocap 200 Capnometer, Datex-Ohmeda, Hatfield, UK). BP (Physiocontrol Colin BP-880 sphygmomanometer, Colin Medical, ECG Monitoring, Erlangen, Germany), V˙E (pneumotacorn, ECG Monitoring, Erlangen, Germany), and time (baseline and the 3 min of interventions) as factors. Another ANOVA for repeated measurements with groups (HTRs vs. control) was performed to determine whether the interventions modified the recorded parameters.

**Protocol and Interventions**

*Metaboreflex activation.* Isometric and rhythmic handgrip exercises of the dominant arm were performed at 30% of the MVCs for 3 min in all subjects (8–10). The exercise rhythm of the dynamic handgrips was provided by a metronome and consisted of a 1-s contraction followed by a 1-s rest (30 contractions/min). These two interventions were performed in random order after a 5-min baseline period of stable ventilation, with subjects breathing room air.

Both interventions were followed by 2 min of local circulatory arrest to the upper arm, without handgrip, while subjects breathed room air. Local circulatory arrest was produced by inflating a standard BP cuff to 240 mmHg on the upper arm, 5 s before the end of each intervention. The subjects were instructed to relax their grip after the cuff was inflated. This procedure is used to trap metabolites released by the muscle contraction and dissociate the mechanics of the muscle contraction (mechanoreceptor reflex) and volitional effects (central command) from stimulation of metabolically sensitive muscle afferents (metaboreflex). This allows determination of the contribution of metaboreflex activation to the hemodynamic and sympathetic changes during exercise, in the absence of other reflex mechanisms that can mask, attenuate, or inhibit metaboreflex activation (13, 29, 45).

*Chemoreflex activation.* The protocol used to test the chemoreceptor responses to isocapnic hypoxia was the same as in previous studies (8–10, 39). After a 5-min baseline period of stable ventilation, peripheral chemoreceptors were activated for 3 min by exposure to hypoxia (10% O₂ in 90% of N₂). Activation of central chemoreceptors was prevented during these 3 min by adding CO₂ to the inspired gas mixture. The subjects breathed through a two-way mouthpiece to avoid rebreathing, with the use of a nose clip to ensure exclusive mouth breathing. End-tidal CO₂ was measured at the expiratory part of the mouthpiece. Small amounts of 100% CO₂ were added to the inspired gas and titrated to maintain end-tidal CO₂ at baseline value during the hypoxic challenge.

*Cardiopulmonary exercise testing.* Ten HTRs and 10 control subjects underwent a maximum, symptom-limited cardiopulmonary exercise test on a cycle ergometer. The patients started with 1-min unloaded pedaling, and the load was subsequently increased by 10 W every minute. Expired gas was collected in the mixing chamber and sampled using an O₂ and CO₂ analyzer, while Ve was also recorded (SensorMedics, Yorba Linda, CA). Ventilation and gas concentrations were averaged over 30 s, and, from these values, Ve, the rate of oxygen uptake (V˙O₂), the rate of CO₂ production (V˙CO₂), and the respiratory exchange ratio were derived. Heart rate (HR) was recorded by a continuously monitored ECG, while systolic and diastolic BP were determined at the end of each workload by an automatic sphygmomanometer. Peak V˙O₂ was defined as the V˙O₂ during the last 30 s of peak exercise.

The adequacy of ventilation-perfusion matching was assessed by determining the physiological dead space-to-tidal volume ratio (V˙DS/V˙T) at the beginning of the exercise (while subjects were pedaling at 20 W for 30 s) and at peak exercise.

The physiological V˙DS/V˙T, an index of lung gas exchange efficiency, is a useful indicator of the matching of ventilation to perfusion, both at rest and during exercise. An increased V˙DS/V˙T at rest, or the failure of V˙DS/V˙T to decrease appropriately with exercise, serves to identify uneven ventilation-perfusion relationships (47). The V˙DS/V˙T can be derived from the modified Bohr’s equation under steady-state conditions:

\[
\frac{V_{DS}}{V_{T}} = \frac{P_{ACO_2} - P_{ECO_2}}{V_{DM}}
\]

where \(P_{ACO_2}\) is arterial PCO₂, \(P_{ECO_2}\) is expired PCO₂, VDM is mechanical dead space volume (in liters), and VT is tidal volume (in liters). End-tidal CO₂ was used as an estimate of arterial \(P_{ACO_2}\) (12).

**Data Analysis**

Sympathetic recordings and cardiovascular changes during the exercises were analyzed during the 5-min baseline periods, during the 3 min of dynamic and isometric exercises, and during the 2 min of post-handgrip ischemia. Changes in sympathetic activity and cardiorespiratory variables during peripheral chemoreceptor testing were analyzed during the 3 min of isocapnic hypoxia.

Sympathetic bursts were identified by careful inspection of the mean voltage neurogram by a single, trained observer blinded to the interventions (8–10). The amplitude of each burst was determined, and sympathetic activity was calculated as bursts per minute, multiplied by mean burst amplitude (arbitrary units), and expressed as percent increase from baseline values. Burst amplitude depends on neural signal amplification, which varies from one subject to another. Thus we used percent increase in amplitude from baseline values to compare changes in sympathetic nerve activity between the patients and the control subjects.

**Statistical Analysis**

Results are expressed as means ± SD. Statistical analysis was performed with the Statview 5.0 program (SAS). Subjects’ characteristics and baseline variables were compared by unpaired two-tailed Student’s t-tests.

We first performed an ANOVA for repeated measurements to determine whether the interventions modified the recorded parameters. Next, to determine whether the cardiovascular and sympathetic responses differed between the HTRs and the control subjects during isometric exercise, dynamic exercise, and hypoxia, we performed an ANOVA for repeated measurements with groups (HTRs vs. control) and time (baseline and the 3 min of interventions) as factors.
ANOVA for repeated measurements was performed with groups and time (baseline and the 2 min of the local ischemia post-handgrips) as factors to determine the contribution of abnormal metaboreflex regulation on the differences in response observed during exercise.

Peripheral chemoreflex sensitivity was expressed as the increase in ventilation and MSNA per percent reduction in arterial saturation during hypoxia. This was related by simple regression analysis to measurements of exercise parameters during cardiopulmonary exercise testing, to evaluate the contribution of chemoreflex sensitivity to the responses to exercise. We also related changes in ventilation and sympathetic activity during local ischemia after isometric exercise to the exercise parameters, to further examine whether metaboreflex dysregulation contributed to abnormal exercise tolerance after cardiac transplantation. Finally, a stepwise regression analysis was performed to assess whether deranged metaboreflex or chemoreflex activity were independent predictors of exercise intolerance in HTRs. A $P < 0.05$ was considered significant.

**RESULTS**

**Subjects’ Characteristics**

HTRs and control subjects were matched for sex, age, height, weight, and BMI (all $P > 0.05$) (Table 1). The MVC of the dominant forearm was also identical in both groups ($P = 0.3$).

**Baseline-Room Air Breathing**

HTRs had higher resting MSNA (47 ± 19 vs. 36 ± 16 bursts/min, $P < 0.01$) and faster HRs (86 ± 8 vs. 67 ± 7 beats/min, $P < 0.01$) than the control subjects (Table 1). At rest, MSNA, expressed as bursts per 100 heartbeats, did not differ among HTRs and control subjects (60 ± 19 bursts/100 heartbeats in the HTRs and 59 ± 20 bursts/100 heart beats in control subjects, $P = 0.8$).

Mean arterial BP (106 ± 9 mmHg) was slightly higher in the HTRs than in the control subjects (103 ± 6 mmHg), but the difference was not significant ($P > 0.05$). Both groups had identical arterial blood oxygen saturations (96 ± 3 vs. 97 ± 2% in the HTRs and the controls, respectively, $P > 0.05$). $V_e$ was also similar between groups (7.1 ± 1.1 vs. 7 ± 0.8 l/min; $P > 0.05$).

**Isometric Exercise**

See Figs. 1–3. Three minutes of isometric handgrip increased MSNA, $V_e$, mean BP, and HR (ANOVA, all $P < 0.001$) in HTRs and control subjects. The percent increase in MSNA above baseline levels was more marked in the HTRs than in the control group (22 ± 77% of baseline values in the

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**Table 1. Subjects characteristics**

<table>
<thead>
<tr>
<th></th>
<th>Heart Transplant</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>51 ± 8</td>
<td>52 ± 5</td>
</tr>
<tr>
<td>Height, cm</td>
<td>174 ± 11</td>
<td>176 ± 6</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>75 ± 17</td>
<td>77 ± 7</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>24 ± 3</td>
<td>25 ± 2</td>
</tr>
<tr>
<td>MSNA, bursts/min</td>
<td>47 ± 19</td>
<td>36 ± 16*</td>
</tr>
<tr>
<td>MBP, mmHg</td>
<td>106 ± 9</td>
<td>103 ± 6</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>86 ± 8</td>
<td>67 ± 7*</td>
</tr>
<tr>
<td>$V_e$, l/min</td>
<td>7.1 ± 1.1</td>
<td>7 ± 0.8</td>
</tr>
<tr>
<td>Arterial saturation, %</td>
<td>96 ± 3</td>
<td>97 ± 2</td>
</tr>
<tr>
<td>MVC, kg</td>
<td>35 ± 16</td>
<td>37 ± 16</td>
</tr>
</tbody>
</table>

Values are recorded at rest and expressed as means ± SD. Comparisons are shown between heart transplant patients and control subjects for age, height, weight, body mass index (BMI), muscle sympathetic nerve activity (MSNA), mean blood pressure (MBP), heart rate (HR), minute ventilation ($V_e$), arterial saturation, and maximal voluntary contraction (MVC). *Significant differences between groups ($P < 0.05$).
During isometric exercise, the increase in MSNA, expressed as bursts per 100 heartbeats, was also more marked in the HTRs than in the control group (81 ± 17 bursts/100 heartbeats in the HTRs and 64 ± 17 bursts/100 heartbeats in the controls, \( P = 0.038 \)). However, the increase in MBP during exercise was comparable in the two groups (to 126 ± 8 mmHg in the HTRs and to 125 ± 12 mmHg in the controls, ANOVA, \( P = 0.89 \)). V˙E during isometric exercise tended to be larger in the HTRs (10.2 ± 1.8 l/min in the HTRs and 9.2 ± 1.1 l/min in the control subjects, ANOVA, \( P = 0.15 \)). The increase in HR did not differ between groups (to 95 ± 13 beats/min in the HTRs and 76 ± 9 beats/min in the controls, ANOVA, \( P = 0.92 \)).

During the local circulatory arrest after isometric handgrip, which isolates the metaboreflex contribution, MSNA and V˙E remained elevated above baseline values (ANOVA, both \( P < 0.001 \)) in both groups. This persistent increase in MSNA (179 ± 73% of baseline values in the HTR and 134 ± 26% in the control subjects; ANOVA, \( P < 0.05 \)) and V˙E (9.6 ± 2.5 l/min in the HTRs and 8 ± 1.1 l/min in the control subjects; ANOVA, \( P < 0.02 \)) remained larger in HTRs than in the control subjects.

BP also remained elevated above baseline values (\( P < 0.0001 \), with no differences between groups (ANOVA, \( P > 0.9 \)). Similarly, HR returned to baseline levels during the local circulatory arrest after handgrip in both HTR patients and control subjects (ANOVA, \( P > 0.5 \)).

**Dynamic Exercise**

See Figs. 1, 2, and 4. Three minutes of dynamic handgrip increased MSNA, V˙E, BP, and HR (ANOVA, all \( P < 0.001 \)). The percent increase in MSNA above baseline levels was more important in the HTRs (167 ± 46%) than in the control subjects (107 ± 11%; ANOVA, \( P = 0.001 \)). MSNA, expressed as bursts per 100 heartbeats, was also higher in the HTRs (70 ± 8 bursts/100 heartbeats) than in the controls (58 ± 12 bursts/100 heartbeats; \( P = 0.03 \)). V˙E (9.2 ± 1.2 l/min in the HTR and 9 ± 1.2 l/min in the control subjects) was comparable in the two groups (ANOVA \( P = 0.9 \)). Mean arterial BP (120 ±
11 mmHg in the HTR and 109 ± 6 mmHg in the control subjects) did not differ (ANOVA, \( P = 0.2 \)) between groups, and neither did the increase in HR (92 ± 13 beats/min in the HTR and 69 ± 7 beats/min in the control, ANOVA, \( P = 0.53 \)).

During the local circulatory arrest after dynamic handgrip, MSNA, expressed as percent increase of baseline values, returned to baseline values and did not differ in the HTRs and in the control subjects (ANOVA; \( P = 0.5 \)). \( \dot{V}_e \) remained increased (ANOVA, \( P < 0.0001 \)) with larger values in the patients than in the control subjects (8.8 ± 1.3 vs. 8.1 ± 1 l/min; ANOVA; \( P < 0.04 \)). BP also remained elevated above baseline values (ANOVA; \( P < 0.05 \)), with no differences between groups (ANOVA; \( P > 0.05 \)). HR returned to baseline levels during the local circulatory arrest after handgrip, with no difference between HTR patients and control subjects (ANOVA; \( P > 0.5 \)).

Isocapnic Hypoxia

See Fig. 1. Three minutes of isocapnic hypoxia decreased arterial saturation and increased MSNA, \( \dot{V}_e \), and HR in both groups (ANOVA, all \( P < 0.001 \)) but did not modify MBP (ANOVA, \( P = 0.4 \)).

The \( \dot{V}_e \) responses to the 3 min of hypoxia were more marked in the HTR (103 ± 1.3 l/min) than in the control subjects (9 ± 1.2 l/min; ANOVA, \( P = 0.04 \)), despite identical reductions in arterial oxygen saturation (80 ± 6% in the HTR and 82 ± 5% in the control subjects, ANOVA, \( P = 0.7 \)). The enhanced ventilatory response to hypoxia was also paralleled by a larger increase in MSNA in the HTRs (167 ± 40% in the HTR and 132 ± 23% in the control subjects, ANOVA, \( P = 0.04 \)). HR remained higher in HTRs (93 ± 12 beats/min) than in the control subjects (77 ± 11 beats/min, ANOVA, \( P = 0.001 \)), as the magnitude of the increase in HR did not differ between the patients and the controls during hypoxia (\( P > 0.05 \)).

Cardiopulmonary Exercise Testing

HTRs and controls achieved the same respiratory exchange ratio, but HTRs had a lower peak \( \dot{V}O_2 \), a lower peak workload, a lower anaerobic threshold, lower peak \( \dot{V}e \), lower peak oxygen pulse, and higher ventilatory equivalents for \( \dot{V}O_2 \) and \( \dot{V}CO_2 \) (Table 2, all \( P < 0.02 \)). HTRs had a larger ventilatory response to exercise, as shown by a steeper linear regression slope of \( \dot{V}e \) to \( \dot{V}CO_2 \) during exercise than the controls (40 ± 5 vs. 31 ± 3 l/mmHg, respectively, \( P = 0.007 \)).

At peak exercise, the HR in the HTRs is lower than that in healthy controls (133 ± 11 beats/min in the HTRs vs. 165 ± 12 beats/min in the control, \( P < 0.05 \)). Mean arterial BP (109 ± 9 mmHg in the HTRs and 115 ± 8 mmHg in the control subjects) did not differ (\( P = 0.4 \)) between groups.

The \( \dot{V}O_2/\dot{V}T \) at the work load of 20 W did not differ between controls and HTRs (0.21 vs. 0.22, \( P = 0.45 \)). No difference

### Table 2. Comparison of exercise test variables in HTRs and the control group

<table>
<thead>
<tr>
<th></th>
<th>HTRs</th>
<th>Controls</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>RER</td>
<td>1.3±0.1</td>
<td>1.3±0.1</td>
<td>0.2</td>
</tr>
<tr>
<td>Peak workload, W</td>
<td>102±30</td>
<td>224±25</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Peak ( \dot{V}O_2 ), ml·kg(^{-1})·min(^{-1})</td>
<td>19.5±2.5</td>
<td>34.1±6.8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>AT, ml·kg(^{-1})·min(^{-1})</td>
<td>0.95±0.1</td>
<td>1.52±0.1</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Peak ( \dot{V}e ), l/min</td>
<td>77±13</td>
<td>99±6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Peak ( \dot{O}_2 ) pulse, ml/beat</td>
<td>11.6±3.2</td>
<td>15.4±2.8</td>
<td>0.002</td>
</tr>
<tr>
<td>( \dot{V}e/\dot{V}O_2 )</td>
<td>53.3±11.5</td>
<td>38.6±5.2</td>
<td>0.002</td>
</tr>
<tr>
<td>( \dot{V}e/\dot{V}CO_2 )</td>
<td>25.8±0.52</td>
<td>35.7±3.3</td>
<td>&lt;0.007</td>
</tr>
<tr>
<td>HR at peak exercise, beats/min</td>
<td>133±11</td>
<td>165±12</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>MBP at peak exercise, mmHg</td>
<td>109±9</td>
<td>115±8</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Values are means ± SD. Comparison is shown of exercise test variables in heart transplant recipients (HTRs) and control subjects. RER, respiratory exchange ratio; peak \( \dot{V}O_2 \), peak oxygen uptake; AT, anaerobic threshold; \( \dot{V}e/\dot{V}O_2 \) peak \( \dot{V}e \) over peak oxygen consumption; \( \dot{V}e/\dot{V}CO_2 \), peak \( \dot{V}e \) over peak carbon dioxide production.
was observed in the Vds/Vt at peak load between the two groups (0.11 vs. 0.13, \( P = 0.25 \)).

Abnormal Metaboreflex Control and Cardiopulmonary Exercise Testing

See Fig. 5. In HTRs, only exaggerated metaboreflex activity, as assessed by the larger increase in MSNA during the local circulatory arrest after isometric exercise, was related to the steeper linear regression slope of \( V\dot{E} \) to \( V\dot{CO}_2 \) \((r = +0.8; P < 0.02)\) and the lower peak \( V\dot{E} \) \((r = +0.81; P < 0.015)\) during cardiopulmonary exercise.

The ventilatory responses to the local circulatory arrest after isometric exercise were not related to the cardiopulmonary testing in the HTRs. No correlation was found in the control group between metaboreflex activity and cardiopulmonary exercise testing.

Abnormal Chemosensitivity and Cardiopulmonary Exercise Testing

The lower peak ventilation during ergospirometry was related to the enhanced peripheral chemosensitivity during hypoxia, expressed as the ratio between the rise in ventilation and the reduction in oxygen saturation during the 3 min of isocapnic hypoxia \((r = +0.91; P < 0.005)\). Changes in MSNA during hypoxia were not related to the cardiopulmonary exercise testing parameters. No similar relations were observed in the control group.

Abnormal Metaboreflex vs. Chemoreflex Control and Cardiopulmonary Exercise Testing

A stepwise regression analysis, using metaboreflex control (characterized by the MSNA response to post-handgrip circulatory arrest) and chemoreflex sensitivity (expressed as the rise in ventilation and percent reduction in oxygen saturation) as independent variables, and peak \( V\dot{E} \) as a dependent variable, revealed that only exaggerated chemoreceptor activity was an independent predictor of the lower peak \( V\dot{E} \) in the HTRs \((r = +0.91; P < 0.005)\).

Effects of Transplantation Time

Time after transplantation was not related to metaboreflex control, chemoreflex responses to hypoxia, and exercise variables in the HTRs (all \( P > 0.1 \)).

DISCUSSION

The main new finding of our study is that muscle metaboreceptor activity is increased and is related to exercise intolerance in HTRs. To our knowledge, this is the first study to assess metaboreflex control after cardiac transplantation. Furthermore, the present results confirm our laboratory’s previous report (2) that HTRs have increased peripheral chemoreceptor sensitivity.

In our study, HTRs had a higher resting MSNA than matched control subjects.

Heart transplantation initially decreases the MSNA in relation to the heart failure state, with values tending to return to normal within 1 yr after the surgical procedure (33), although not permanently (34, 42). This elevated MSNA after heart transplantation is thought to be related to cyclosporine therapy (34) and increases with time after transplantation (42). This higher MSNA at rest could also be attributed in part to a higher chemoreflex drive in HTRs (4), because peripheral chemoreflex deactivation by hyperoxia decreases MSNA and mean BP after cardiac transplantation (5). The contribution of peripheral chemoreceptors to MSNA is also directly related to the time from heart transplantation (4). Effects of cardiac transplantation on metaboreflex control have not been reported previously. Isometric handgrip increased MSNA in both HTRs and control subjects, but this increase was larger in the HTRs. This finding was observed very consistently in our study during isometric and dynamic exercise, as well as during the post-handgrip circulatory arrest. We attribute this elevation in MSNA to abnormal muscle metaboreceptor regulation after cardiac transplantation.

Rhythmic handgrip, in contrast, did not increase MSNA in our control subjects, presumably because the intermittent muscle relaxation permits rapid washout of any ischemic metabolites that are generated (44). However, it has been shown that, if washout is interrupted by occlusion of forearm venous return, 30% rhythmic handgrip does elicit a significant increase in MSNA (44). Rhythmic handgrip increased MSNA in HTRs...
in our study, in contrast to the reference group. This observation reveals that low levels of rhythmic handgrip exercises, which are insufficient to elicit sympathetic excitation in healthy subjects, are able to trigger the muscle metaboreflex in HTRs. This may also reflect increased mechanoreflex activity, which has been consistently observed in heart failure patients (22, 38). Thus sympathoexcitatory reflexes arising from exercising muscle are stimulated at a lower threshold in HTRs than in control subjects. This finding has also been observed in heart failure patients, in whom 2 min of isometric handgrip at 30% MVC increased MSNA but failed to do so in control subjects (25). Our observations in HTRs cannot be attributed to left ventricular dysfunction, because all of our patients had a normal left ventricular ejection fraction.

Muscle metaboreflex mechanism may contribute to the hyperpnea of the static muscular contractions, but its involvement varies in function of contraction intensity and the amount of active muscle mass involved, resulting in a greater amount of metabolite production (11, 35). Ventilation remained increased, with larger values in the patients than the controls, during the local circulatory arrests after both isometric and dynamic handgrips. Both groups achieved, however, identical MVCs. Thus our study highlights that increased muscle reflex activity, besides work intensity and muscle mass, is also an important determinant of the ventilatory response for a given exercise.

Skeletal muscle metabolism is altered in advanced heart failure (21), and blood flow to small and large muscle groups is reduced at rest and during dynamic exercise (6, 16, 30, 46). Heart transplantation does not normalize oxidative muscle metabolism (2, 15), which may elicit early muscle ischemia and subsequent excessive sympathetic activation (1) as a result of marked metaboreflex activation. This occurrence may further alter muscle oxidative metabolism through excessive vasoconstriction and ischemia (18, 36, 41).

The HTRs presented a lower peak $\dot{V}O_2$ compared with control subjects. Identical $\dot{V}R_0/\dot{V}R_t$ values at rest and at peak exercise allowed us to exclude that uneven ventilation-perfusion relationships accounted for differences in exercise tolerance in both groups. The lower peak $\dot{V}O_2$ we observed after heart transplantation is in accordance with the persisting exercise intolerance after transplantation and can be attributed to chronotropic reserve impairments and slower adjustments in cardiac output to exercise, as well as an impaired vasodilatory response to exercise (3, 7, 37).

Another important new finding of our study is that exercise intolerance, assessed by the increased $\dot{VE}/\dot{V}CO_2$ slope, and reduced peak ventilation are related to the exaggerated metaboreflex activity during exercise in HTRs. This is in keeping with previous observations that muscle metaboreceptors play a role in the regulation of ventilation during exercise (8). The ventilatory component of the metaboreflex has also been shown to be an important predictor of peak $\dot{V}O_2$ and $\dot{VE}/\dot{V}CO_2$ in patients with chronic heart failure (28). Together, these findings are in keeping with the notion of a neural link between impaired exercise capacity, exertional dyspnea, and augmented metaboreflex activity in these patients (27, 28). Metaboreflex activity to arm exercise has been correlated with indexes of exercise limitation during leg exercise, suggesting the presence of a generalized abnormality of the skeletal muscles in patients with heart failure (28); however, there were no recordings of MSNA in that study (28).

Enhanced peripheral chemoreflex sensitivity was also related to the reduced peak ventilation in our HTRs. This finding is in agreement with our laboratory’s previous study and with the observation that peripheral chemoreceptors play an important role in the control of ventilation during exercise (4). Both increased metaboreflex and chemoreflex activity are important in abnormal exercise intolerance in HTRs. However, in a stepwise regression analysis, increased chemoreflex, but not metaboreflex activity, accounted for the lower peak ventilation during exercise.

**Study Limitations**

Whether immunosuppressive therapy contributed to the abnormalities we observed is not known. This is important because immunosuppressive therapy reduces cardiac and skeletal muscle oxidative capacities (2).

In contrast to our findings, however, metaboreceptor sensitivity is decreased in essential untreated hypertensive patients (31). Whether hypertension, almost always accompanying heart transplantation, contributed to our findings is thus unclear. Last, we can also not exclude that the treatment of hypertension affected our results. In mitigation, however, antihypertensive therapy withdrawal may have created another cause of exercise limitation, because of uncontrolled rises in exercise BP (17).

There is also controversy (26) on the contribution of heightened metaboreceptor (25, 27, 28, 40) and mechanoreceptor (22, 38) stimulation in heart failure. Because new mechanisms may be responsible for exercise reflex dysregulation in the HTRs, our study may, however, provide only limited insights in the abnormal muscle regulation that preexisted transplantation. Last, our patients disclosed a large time lag after cardiac transplantation. However, time after transplantation was not related to metaboreflex control, chemoreflex responses to hypoxia, and exercise variables in the HTRs.

In conclusion, our study demonstrates that heart transplantation does not normalize muscle metaboreceptor activity. Moreover, both increased metaboreflex and chemoreflex control are related to exercise intolerance in HTRs.

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