Gas exchange and cardiovascular kinetics with different exercise protocols in heart transplant recipients

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Grassi, Bruno, Claudio Marconi, Michael Meyer, Michel Rieu, and Paolo Cerretelli. Gas exchange and cardiovascular kinetics with different exercise protocols in heart transplant recipients. J. Appl. Physiol. 82(6): 1952–1957, 1997.—Metabolic and cardiovascular adjustments to various submaximal exercises were evaluated in 82 heart transplant recipients (HTR) and in 35 control subjects (C). HTR were tested 21.5 ± 25.3 (SD) mo (range 1.0–137.1 mo) posttransplantation. Three protocols were used: protocol A consisted of 5 min of rectangular 50-W load repeated twice, 5 min apart [5 min rest, 5 min 50 W (Ex 1), 5 min recovery, 5 min 50 W (Ex 2)]; protocol B consisted of 5 min of rectangular load at 25, 50, or 75 W; protocol C consisted of 15 min of rectangular load at 25 W. Breath-by-breath pulmonary ventilation (Ve), O2 uptake (V̇O2), and CO2 output (V̇CO2) were determined. During protocol A, beat-by-beat cardiac output (Q̇) was estimated by impedance cardiography. The half times (t1/2) of the on- and off-kinetics of the variables were calculated. In all protocols, t1/2 values for V̇O2 on-, Ve on-, and V̇CO2 on-kinetics were higher (i.e., the kinetics were slower) in HTR than in C, independently of workload and of the time posttransplantation. Also, t1/2 Q on-was higher in HTR than in C. In protocol A, no significant difference of t1/2 V̇O2 on-was observed in HTR between Ex 1 (48 ± 9 s) and Ex 2 (46 ± 8 s), whereas t1/2 Q on-was higher during Ex 1 (55 ± 24 s) than during Ex 2 (47 ± 15 s). In all protocols and for all variables, the t1/2 off-values were higher in HTR than in C. In protocol C, no differences of steady-state Ve, V̇O2, and V̇CO2 were observed in both groups between 5, 10, and 15 min of exercise. We conclude that 1) in HTR, a “priming” exercise, while effective in speeding up the adjustment of convective O2 flow to muscle fibers during a second on-transition, did not affect the V̇O2 on-kinetics, suggesting that the slower V̇O2 on-kinetics in HTR was attributable to peripheral (muscular) factors; 2) the dissociation between Q on- and V̇O2 on-kinetics in HTR indicates that an inertia of muscle metabolic machinery is the main factor dictating the V̇O2 on-kinetics; and 3) the V̇O2 off-kinetics was slower in HTR than in C, indicating a greater alactic O2 deficit in HTR and, therefore, a sluggish muscle V̇O2 adjustment.

heart denervation; oxygen uptake kinetics; exercise transients

IT HAS BEEN KNOWN FOR SEVERAL YEARS that, with a rectangular increase in workload (on-transition), heart transplant recipients (HTR) show a sluggish heart rate (HR) adjustment (on-kinetics), presumably attributable to surgical denervation of the heart (23). More recently, Cerretelli et al. (5, 6) and Grassi et al. (12) showed that the slower HR on-kinetics of HTR was associated with a slower adjustment of pulmonary ventilation (Ve), O2 uptake (V̇O2), and CO2 output (V̇CO2). In HTR, also the on-kinetics of cardiac output (Q̇) was found to be somewhat slower than in control subjects, despite the finding of a powerful Frank-Starling mechanism at the very onset of work (6). Cerretelli et al. (6) concluded that in HTR the slower Q on-kinetics, by affecting the rate of adjustment of O2 delivery to the exercising muscles, could be, at least in part, responsible for the slower gas-exchange on-kinetics. On the other hand, Sinoway et al. (25) observed in HTR an impairment of the vasodilatory response to exercise, which could also be responsible for the slower gas-exchange readjustment. On the basis of all these observations, Meyer et al. (21) reasoned that, if the cardiovascular system could be “primed” by a preceding constant-load exercise, a subsequent rest-to-work transition carried out shortly after the first (i.e., in the presence of HR, Q, and blood catecholamine levels presumably higher than in normal resting conditions and of muscle vasodilation) should be characterized by a faster V̇O2 on-kinetics.

A somewhat faster V̇O2 on-kinetics was indeed observed by Paterson et al. (22) during the second of two rectangular workload steps, separated by a 6-min interval. These authors examined a limited number of HTR (n = 6), all tested shortly (1.9–2.5 mo) after transplantation. This did not allow the authors to evaluate any effect on the investigated variables of the time elapsed after surgery, which could represent an important factor, particularly in the light of recent indications of the possibility of reinnervation of the transplanted heart (17, 34), or of heart β2-adrenoceptors upregulation and increased sensitivity to epinephrine as a function of time after transplantation (26). Moreover, to confirm the efficacy of the priming exercise on the cardiovascular system, at least an indirect determination of Q on-kinetics would be needed, which would lend weight to the speculations about the rate of O2 delivery to the exercising muscles. Therefore, the present study was carried out on a large number of HTR (n = 82), covering a large time interval (between 1 and 137 mo) after surgery, thus allowing an analysis of the V̇O2 on-kinetics as a function of the time posttransplantation. The sequential exercises protocol was different from that of Paterson et al. (22) to circumvent some methodological limitations of that study (see also Discussion), and an indirect estimate of beat-by-beat Q was
also obtained. In addition to the main working hypothesis, the following questions were addressed: 1) Are the VE, VO₂, and VCO₂ on-kinetics in HTR related to the intensity of exercise, as in normal subjects? 2) Are the VE, VO₂, and VCO₂ off-kinetics (e.g., during a rectangular work-to-rest transition) in HTR different than in control subjects? 3) Do HTR reach a steady state for gas exchange during exercises lasting only 5 min? Thus, in the present study, in addition to the sequential exercise protocol, HTR performed also rectangular 5-min exercises at different workloads, as well as a rectangular exercise lasting for 15 min, and both on- and off-kinetics of ventilation and gas exchange were analyzed.

**METHODS AND EXPERIMENTAL PROCEDURE**

Subjects. The study was conducted on a total of 82 HTR (70 men, 12 women) and 35 (29 men, 6 women) healthy untrained control subjects (C). All subjects gave informed consent to participate in the study, which was approved by the ethical committees of the institutions involved. HTR were from the Department of Cardiovascular Surgery, Hôpital de la Pitié-Salpêtrière, University of Paris VI (France); of Respiratory Physiopathology, Niguarda Hospital, Milano (Italy); and from the Centro di Riabilitazione, Fondazione Clinica del Lavoro, University of Pavia, Tradate (Italy). The experiments were carried out at each patient's institution. Operators, equipment, experimental setup, and protocols were identical in all cases. Some physical characteristics of the subjects, pretransplant diagnosis, and time elapsed between surgery and the present investigation are presented in Table 1. When the measurements were performed, HTR were considered healthy and free from signs and symptoms of rejection as evidenced by standard clinical and laboratory examinations. Some HTR had resumed after surgery some light physical activity (e.g., walking, cycling, gardening, household duties) but none was engaged in regular physical rehabilitation programs. Pharmacological treatment included standard immunosuppressive therapy (cyclosporine A, prednisone) and other conventional drugs. No patients were treated with β-blockers.

Exercise protocols. Experiments were conducted in the morning after a light meal. The subjects were allowed enough time to familiarize with the investigators and with the experimental setup and were carefully instructed about the experimental procedure. The subjects were familiarized with the experimental protocol by means of short preliminary practice runs. The subjects were highly motivated, and their collaboration was excellent.

After sitting for a few minutes at rest on an electrically braked bicycle ergometer (Cardioline STS 3), the subjects performed one of the three exercise protocols described below. Some subjects performed more than one protocol. In such cases, enough time was allowed between protocols for the investigated variables to resume resting values. In protocol A, the subjects repeated a rectangular 50-W load twice, 5 min apart and then 5 min rest (Rec 1), 5 min 50 W (Ex 1), 5-min recovery (Rec 1), 5 min 50 W (Ex 2), 5-min recovery (Rec 2). In protocol B, a 5-min rectangular load (25, and/or 50, and/or 75 W, depending on the subject) was performed, followed by a 5-min recovery. Protocol C consisted in a 15-min rectangular load at 25 W. During all exercises, the subjects were invited to keep a constant pedaling frequency of ~60 revolutions/min. The pedaling rate was digitally displayed to the subject. Care was taken that, at exercise onset, the pedaling rate was reached in 3–4 s. All recovery periods were passive.

Methods. A computerized O₂-CO₂ analyzer-flowmeter combination (SensorMedics MMC 4400tc) was utilized for breath-by-breath assessment of tidal volume (VT, in BTPS), VE (in BTPS), and gas exchange (VO₂, VCO₂; in STPD). VT and VE were calculated by integration of the flow tracings recorded at the mouth of the subject by a low-resistance turbine flowmeter. Volume calibration was performed before each experiment by means of a 3-liter syringe, at three different flow rates. Respiratory frequency (fr) was calculated as V̇E/VT. VO₂ and VCO₂ were determined by continuously monitoring PO₂ and PCO₂ at the mouth of the subject throughout the respiratory cycle and from established mass balance equations. Calibration of the O₂ and CO₂ analyzers was performed before each experiment by utilizing gas mixtures of known composition.

HR was determined from the electrocardiogram, which was continuously monitored throughout the tests. Beat-by-beat values of stroke volume (SV) and Q were estimated noninvasively by impedance cardiography. Cardiograms were obtained by means of an impedance device designed at the Department of Biomedical Engineering of the University of Stuttgart (Germany). A constant current of 4 mA at a frequency of 100 kHz was introduced by two disposable self-adhesive electrodes. Two separated electrodes were used to measure changes of voltage within the segment under consideration. The four-spot electrode array was placed according to the scheme by Kubicek et al. (19). Baseline thoracic impedance (Z₀), changes of impedance (dZ/dt), and maximum of impedance derivative (dZ/dt_{max}) were automatically derived along with estimates of the prejection period, left-ventricular ejection time (LVET), and HR. SV was calculated according to the formula of Kubicek et al. (19), with the known distance (L) between the inner electrodes and the resistivity (ρ) of blood at 100 kHz

$$SV = \rho \cdot \frac{L}{Z_0} \cdot \frac{dZ}{dt_{max}} \cdot LVET$$

During protocol A, blood lactate concentration ([La]b) was determined by an immunoenzymatic method (Kontron 640 lactate analyzer) in 20 µl of arterialized capillary blood obtained from a preheated earlobe at rest, during the last 30–45 s of each exercise, and at different times during the recovery periods. During protocol C, arterialized blood per-

<table>
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<tr>
<th>Table 1. Some physical characteristics of subjects, pretransplant diagnosis, and time elapsed between surgery and the present study</th>
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<tbody>
<tr>
<td>Gender</td>
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<tr>
<td>HTR (n = 82)</td>
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<tr>
<td>Range</td>
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<tr>
<td>C (n = 35)</td>
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<tr>
<td>Range</td>
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</table>

Values are means ± SD; n, no. of subjects. HTR, heart transplant recipients; C, controls; M, males; F, females; CCM, congestive cardiomyopathy; IHD, ischemic heart disease; RHD, rheumatic heart disease; O, others; U, unknown.
and, therefore, in these cases, the \( t_{1/2} \) was taken when the 50% mark was reached again during the ensuing phase II (see e.g., the second on-transition of Fig. 1); in the rare occasions in which phase I was not followed by a drop, \( t_{1/2} \) was taken along a line extrapolated between the value at the beginning of phase II and the value at time 0 (i.e., just before exercise onset). By following this procedure, \( t_{1/2} \) was in all cases calculated on the basis of the phase II response, but the time elapsed during phase I was also taken into consideration. The same procedures were applied to the off-transition. Before the calculation of \( t_{1/2} \), a "smoothing" of the curves was obtained by calculating a five-breath moving average. A typical breath-by-breath \( \dot{V}_{O_2} \) vs. time tracing obtained in a HTR during protocol A, together with the calculated moving average and the \( t_{1/2} \) on- and off-marks, is shown in Fig. 1.

Whereas HTR performed only one repetition of each protocol, most of the control subjects performed multiple (2–5) repetitions of protocols A and B. Breath-by-breath data obtained in each repetition were superimposed for each subject, and the moving average and the \( t_{1/2} \) were calculated as described above.

Statistics. Data were expressed as means \( \pm \) SD. To check the statistical significance of differences between two means, paired or unpaired Student's t-test (two-sided) was performed as indicated. To check the statistical significance of differences between more than two means, a one-way analysis of variance was performed. A Tukey's test was utilized to discriminate where significant differences occurred. Regression analysis and analysis of variance were performed as indicated. The level of significance was set at \( P = 0.05 \).

RESULTS

Protocol A. Steady-state values during the sequential 50-W rectangular loads are presented in Table 2. Both at rest and at 50 W, \( V_E \) was higher in HTR than in C subjects, as a consequence of a higher fr. In HTR, the hyperventilation was responsible for the observed higher gas-exchange ratio, higher end-tidal \( P_{O_2} \) (\( P_{ETO_2} \)) and lower end-tidal \( P_{CO_2} \) (\( P_{ETCO_2} \)) as compared with C. Whereas at rest hyperventilation in HTR might be attributable to emotional factors, during exercise it is more likely a reflection of a lower exercise capacity. No significant \( \dot{V}_{O_2} \) differences were observed, both at rest and during exercise, between HTR and C groups. HR was higher in HTR than in C both at rest (as a typical manifestation of heart denervation) and during exercise. The HR increase from rest to steady-state exercise was about the same ( – 20 beats/min) in the two groups. The higher HR in HTR at Rest 1 was compensated by a

![Fig. 1. Breath-by-breath \( \dot{V}_{O_2} \) values in a typical heart transplant recipient (HTR) during sequential exercises protocol. Calculated moving average is also shown (solid line), together with half time (\( t_{1/2} \)) on- and \( t_{1/2} \) off-marks. See text for further details.](image)

Table 2. Steady-state values of respiratory, cardiovascular, and metabolic variables during the sequential 50-W exercises

<table>
<thead>
<tr>
<th>V(_E), l/min</th>
<th>V(_T), liters</th>
<th>( f_r), breaths/min</th>
<th>( \dot{V}_{O_2}), l/min</th>
<th>( \dot{V}_{CO_2}), l/min</th>
<th>R</th>
<th>( P_{ETO_2}), Torr</th>
<th>( P_{ETCO_2}), Torr</th>
<th>HR, beats/min</th>
<th>SV, ml</th>
<th>( Q), l/min</th>
<th>( [\text{La}]_b), mM</th>
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<tbody>
<tr>
<td>HTR (n = 29)</td>
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<tr>
<td>Rest 1</td>
<td>10.6 ± 2.4†</td>
<td>0.59 ± 0.14†</td>
<td>18 ± 4</td>
<td>0.27 ± 0.06</td>
<td>0.24 ± 0.05</td>
<td>0.89 ± 0.10†</td>
<td>13 ± 3</td>
<td>34 ± 3</td>
<td>100 ± 11</td>
<td>72 ± 24</td>
<td>7.2 ± 2.3</td>
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<tr>
<td>Ex 1</td>
<td>34.5 ± 6.8†</td>
<td>1.48 ± 0.27</td>
<td>24 ± 5</td>
<td>1.00 ± 0.10</td>
<td>1.07 ± 0.13†</td>
<td>1.08 ± 0.15</td>
<td>116 ± 7</td>
<td>37 ± 4</td>
<td>120 ± 10</td>
<td>131 ± 29</td>
<td>15.7 ± 4.9†</td>
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<tr>
<td>Rest 2</td>
<td>13.5 ± 3.2†</td>
<td>0.73 ± 0.22†</td>
<td>19 ± 5</td>
<td>0.31 ± 0.06†</td>
<td>0.33 ± 0.07†</td>
<td>1.06 ± 0.12†</td>
<td>119 ± 5</td>
<td>33 ± 3</td>
<td>119 ± 9†</td>
<td>80 ± 24†</td>
<td>8.9 ± 2.6†</td>
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<tr>
<td>Ex 2</td>
<td>35.5 ± 7.9†</td>
<td>1.47 ± 0.32</td>
<td>25 ± 6</td>
<td>1.02 ± 0.10</td>
<td>1.07 ± 0.12†</td>
<td>1.05 ± 0.12†</td>
<td>117 ± 7</td>
<td>36 ± 4</td>
<td>121 ± 10</td>
<td>128 ± 31</td>
<td>15.5 ± 4.4†</td>
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<td>C (n = 14)</td>
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<tr>
<td>Rest 1</td>
<td>8.5 ± 1.7</td>
<td>0.72 ± 0.23</td>
<td>13 ± 4</td>
<td>0.29 ± 0.05</td>
<td>0.23 ± 0.04</td>
<td>0.82 ± 0.07</td>
<td>106 ± 6</td>
<td>38 ± 3</td>
<td>73 ± 8</td>
<td>95 ± 14</td>
<td>6.9 ± 1.5</td>
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<tr>
<td>Ex 1</td>
<td>22.9 ± 2.2</td>
<td>1.34 ± 0.34</td>
<td>18 ± 5</td>
<td>1.00 ± 0.06</td>
<td>0.87 ± 0.06</td>
<td>0.87 ± 0.05</td>
<td>102 ± 4</td>
<td>44 ± 3</td>
<td>94 ± 11</td>
<td>137 ± 19</td>
<td>12.9 ± 3.4</td>
</tr>
<tr>
<td>Rest 2</td>
<td>9.6 ± 1.8</td>
<td>0.75 ± 0.30</td>
<td>14 ± 4</td>
<td>0.28 ± 0.04</td>
<td>0.26 ± 0.04†</td>
<td>0.91 ± 0.09†</td>
<td>111 ± 6</td>
<td>37 ± 4</td>
<td>85 ± 8</td>
<td>91 ± 14</td>
<td>7.7 ± 1.9</td>
</tr>
<tr>
<td>Ex 2</td>
<td>24.3 ± 2.4</td>
<td>1.26 ± 0.25</td>
<td>20 ± 4</td>
<td>1.00 ± 0.06</td>
<td>0.89 ± 0.06</td>
<td>0.88 ± 0.07</td>
<td>104 ± 4</td>
<td>43 ± 3</td>
<td>98 ± 12</td>
<td>131 ± 22</td>
<td>12.8 ± 4.0</td>
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</table>

Values are means \( \pm \) SD. \( V_E \), pulmonary ventilation; \( V_T \), tidal volume; \( f_r \), respiratory frequency; \( \dot{V}_{O_2} \), \( \dot{V}_{CO_2} \), oxygen; \( R \), gas-exchange ratio; \( P_{ETO_2} \), end-tidal pressure of \( O_2 \); \( P_{ETCO_2} \), end-tidal pressure of \( CO_2 \); HR, heart rate; SV, stroke volume; \( Q \), cardiac output; \( [\text{La}]_b \), blood lactate concentration. \( [\text{La}]_b \) values were obtained during last 30–45 s of each resting or exercise period. See text for group description. *Significantly different from Rest 1; †significantly different from corresponding value in C.
lower SV, so that the Q values were not significantly different in the two groups. On the other hand, during both Ex 1 and Ex 2, the higher HR in HTR compared with C, in the presence of only slightly lower SV values, determined higher Q in the first group. In HTR the priming exercise elicited significantly higher Q during Rest 2 (i.e., the last minute of Rest 1) than during Rest 1, whereas no significant difference was observed between Ex 1 and Ex 2. In C, priming exercise determined only a slightly higher (no significant difference) Q at Rest 1 compared with Rest 1. Blood lactate concentration ([La]b) values in HTR were moderately elevated during Ex 1 and Ex 2, compared with normal resting values, and they were higher during Rest 1 compared with Rest 1. [La]b values in C were not affected by exercise.

The t1/2 on- and off-values determined for VE, VO2, and VCO2 during the sequential 50-W exercises are shown for HTR and C in Fig. 2. For both Ex 1 and Ex 2, the values of t1/2 on- and t1/2 off- were for all investigated variables significantly higher (i.e., the kinetics were slower) in HTR than in C. No significant differences were observed, for both HTR and C, between the t1/2 VO2 on- and the t1/2 VO2 off-values, whereas for VE and VCO2 the t1/2 on-values were higher than the corresponding t1/2 off-values. No significant differences were observed for any of the investigated values, between the values obtained during Ex 1 and Ex 2, for both HTR and C.

The t1/2 on- and off-kinetics of Q during the sequential 50-W exercises are shown for HTR and C in Fig. 3. The t1/2 Q on-values during Ex 1 were significantly higher in HTR compared with C, whereas no significant difference between the two groups was observed during Ex 2. Such absence of a significant difference can be attributed both to faster Q on-kinetics in HTR (at the limit of statistical significance, P = 0.05), and to slightly slower (no significant difference) Q on-kinetics in C, during Ex 2 compared with Ex 1. The t1/2 Q off-values were slightly higher in HTR than in C, during both Rest 1 and Rest 2, even though a statistically significant difference was observed only for Rest 2. In both groups, t1/2 Q on-values were not significantly different from t1/2 VO2 on- and t1/2 Q off-values were not significantly different from t1/2 VO2 off-values.

Protocol B. Steady-state values obtained in HTR and C at rest and during the 5-min rectangular exercises at different workloads are presented in Table 3. The hyperventilation described above (see protocol A), in HTR compared with C, was confirmed by this set of data as well, both at rest and during exercises at the indicated loads. Steady-state VO2 values are plotted as a function of workload in Fig. 4. No differences were observed between HTR and C at rest and at 25 or 50 W, whereas VO2 values were slightly but significantly higher in HTR at 75 W.

The calculated t1/2 for the on- and off-responses of VE, VO2, and VCO2, at the three investigated workloads, are shown in Fig. 5A (t1/2 on-) and 5B (t1/2 off-). With regard to the t1/2 on-values, for all variables and at all workloads the values were significantly higher in HTR than in C. The t1/2 on-values in C were progressively higher with increasing workload, whereas such behavior was not observed in HTR (there were no significant differences, in this group, among the VE, VO2, and VCO2 values obtained at 25, 50, or 75 W). The differences between HTR and C became, therefore, progressively
smaller at heavier workloads, although they were statistically significant at all workloads. Also, for $t_{1/2}$ off, the values in HTR were significantly higher than those in C, for all variables and at all workloads, with the exception of $V_\dot{E}$ and $V_C0_2$ at 75 W, in which cases the differences did not reach statistical significance. At all workloads, no significant differences were observed, for both HTR and C, between the $t_{1/2}$ $V_\dot{O}_2$ on- and the $t_{1/2}$ $V_\dot{O}_2$ off-values.

The $t_{1/2}$ $V_\dot{O}_2$ on- and off-values obtained in HTR at 25 and 50 W are plotted as a function of the time elapsed between surgery and the tests in Fig. 6, A and B, respectively. Both $t_{1/2}$ $V_\dot{O}_2$ on- and off-values did not bear a significant relationship to the time elapsed after transplantation. This analysis was not performed for the 75-W exercises, as a consequence of the relatively small number of HTR subjects who carried out the exercise at this load.

Protocol C. Steady-state values at rest and at 5, 10, and 15 min of the 15-min 25-W rectangular exercise are shown in Fig. 7. Also, for this protocol, $V_\dot{E}$, $f_r$, gas-exchange ratio, and $P_ET_{O_2}$ were higher, whereas $P_ET_{CO_2}$ was lower, in HTR than in C. Both in HTR and in C, no differences were observed for any of the investigated variables between the values obtained at 5, 10, and 15 min of exercise. In HTR, $S_AO_2$ was not affected by exercise (98 ± 2% at rest, 98 ± 1% at 5-min exercise, 98 ± 2% at 10-min exercise, 98 ± 1% at 15-min exercise).

**DISCUSSION**

Gas-exchange and cardiovascular on-kinetics during the sequential exercises protocol. HTR show, with a rectangular increase in workload (on-transition), a slower $V_\dot{O}_2$ adjustment to a higher steady state (5, 6, 12, 22). This slower $V_\dot{O}_2$ on-kinetics has been attributed, at
least in part, to the slower HR (5, 23) and Q (6) on-kinetics, as a consequence of the denervation of the transplanted heart. Alternative explanations could be an impairment of the vasodilatatory response to exercise (25), muscle deterioration attributable to physical deconditioning, chronic corticosteroid (15) or cyclosporine A (20) therapy, or to chronic bioenergetic abnormalities of skeletal muscle associated with the congestive heart failure preceding the transplantation (27). On such premises, Meyer et al. (21) and, subsequently, Paterson et al. (22) hypothesized that, if the factors responsible for the slower VO₂ on-kinetics in HTR were, indeed, related to a limitation in the rate of adjustment of O₂ delivery to the exercising muscles, a priming of the cardiovascular system obtained by a preceding exercise would determine, on a second on-transition carried out a few minutes later, a faster VO₂ on-kinetics. Before the second on-transition was performed, HTR would in fact present higher HR (5, 23) and, presumably, also higher Q and muscle blood flow, higher blood catecholamine levels (5), and a more favorable situation as far as blood flow distribution to the exercising muscles, compared with the scenario immediately preceding the first on-transition. Moreover, lactic acid accumulation in blood during the recovery after the first exercise would likely determine by itself vasodilation and an increased blood flow at the muscle level, together with a rightward shift of the hemoglobin-O₂.

Fig. 6. Individual values of t VO₂ on (A, left) and t VO₂ off (B, right) calculated in HTR for the 25-W and 50-W exercises, expressed as a function of time elapsed between transplantation and tests. Linear regression lines (solid) and 95% confidence intervals lines (broken) are also shown. See text for further details.

Fig. 7. Average (±SD) steady-state values of VE, tidal volume (VT), respiratory frequency (f), VO₂, VCO₂, gas exchange ratio (R), end-tidal pressures of O₂ (PETO₂) and CO₂ (PETCO₂), and heart rate (HR) at rest and at 5, 10, and 15 min of exercise at 25 W. See text for further details. * Statistically significant difference.
dissociation curve, thereby favoring \( \text{O}_2 \) delivery to muscle fibers at the onset of the second exercise (29).

The validity of this hypothesis was not confirmed by the results of the present study, which showed in HTR no differences in \( t_{1/2} \) \( \text{Vo}_2 \) on between the first and the second on-transition of the sequential exercises (see Fig. 2). To interpret these results correctly, the question should be raised whether the priming exercise was indeed effective in determining in HTR more favorable conditions with regard to the rate of adjustment of \( \text{O}_2 \) delivery to muscle fibers. The measurements carried out in the present study allow at least a partial answer to such question. Indeed, during the 30-s resting period preceding the second on-transition (Rest 2), HR and Q values were significantly higher compared with the homologous period before the first on-transition (Rest 1) (see Table 2). The Q on-kinetics were faster during Ex 2 compared with Ex 1 (see Fig. 3). No data are available for blood catecholamines or muscle blood flow, which, however, should have been higher during Rest 2 compared with Rest 1, for the reasons discussed above. \([\text{La}]_b\) values were moderately but significantly higher during Rest 2 compared with Rest 1. Even a low degree of metabolic acidosis should determine some vasodilatation, some increase in blood flow, and some degree of rightward shift of the hemoglobin-O\( _2 \) dissociation curve, favoring \( \text{O}_2 \) delivery to muscle fibers. Five minutes of recovery were allowed between the two exercises, so that blood, tissue, and lung \( \text{O}_2 \) stores, which might have been depleted during the first exercise (a depletion could influence the following \( \text{Vo}_2 \) on-kinetics) were presumably fully reestablished when the second exercise was carried out (10).

Considering the very particular set of subjects, all measurements of the present study were supposed to be noninvasive; thus, muscle blood flow, arterial \( \text{Po}_2 \) \( (\text{Pa}_o) \), and arterial \( \text{O}_2 \) concentration \( (\text{Ca}_o) \) could not be determined. Inferences on convective \( \text{O}_2 \) delivery to muscles must, therefore, rely on two assumptions: 1) the time course of blood flow to the active muscles could be reasonably estimated on the basis of Q time course; 2) no significant arterial desaturation occurred during exercise. The first assumption holds if perfusion to active skeletal muscles accounts for most of the increase in Q at the on-transition. Although this would appear to be reasonable, it could not be directly tested. In this context, however, it may be noteworthy that previous work by our group showed a rapid adjustment (not different from that of controls) of muscle blood flow at exercise onset in a limited number of HTR (6). As far as the second assumption, whereas it appears obvious for the control subjects, it might be slightly more controversial in HTR. Indeed, whereas Degre et al. (9) showed no significant decreases in \( \text{Pa}_o \) during exercise in HTR, Braith et al. (4) showed some reduction in \( \text{Pa}_o \) (to \( \sim 85-92 \) Torr) during submaximal exercises in \( \sim 50\% \) of their HTR. In any case, even if it occurred in some of the HTR of the present study, a \( \text{Pa}_o \) reduction similar to that described above would not significantly influence \( \text{Ca}_o \) (the important variable when dealing with \( \text{O}_2 \) delivery), since \( \text{Pa}_o \) would still be on the flat portion of the \( \text{O}_2 \)-hemoglobin dissociation curve. In some HTR of the present study, arterialized \( \text{Sa}_o \) was, indeed, monitored during the tests by earlobe pulse oximetry, and no \( \text{Sa}_o \) decreases were observed. Moreover, even if some \( \text{Ca}_o \) decrease occurred during Ex 1, it appears reasonable to assume that during the following recovery \( \text{Ca}_o \) would resume its resting values, so that at the onset of Ex 2 \( \text{Ca}_o \) would be the same as at the onset of Ex 1. Therefore, the most important aspect of the present study would be from this point of view, flawless.

From the above considerations, it would appear reasonable to conclude that the priming exercise was effective in determining more favorable conditions for the adjustment of \( \text{O}_2 \) delivery to the increased metabolic demand. Despite this, the \( \text{Vo}_2 \) on-kinetics was unchanged. Thus it would appear that the slower \( \text{Vo}_2 \) on-kinetics in HTR was not attributable to a slower adjustment of convective \( \text{O}_2 \) transfer, confirming what was previously hypothesized by our group (5, 6, 12). Other factors, likely involving muscle metabolism (e.g., chronic deconditioning, effects of chronic corticosteroid (15) or cyclosporine A (20) therapy, or chronic biochemical abnormalities attributable to the congestive heart failure preceding the transplantation (27)) would appear to play a determinant role. Considered in broader terms from the standpoint of metabolic control mechanisms, the dissociation between the rate of adjustment of \( Q \) and \( \text{O}_2 \) delivery (faster during Ex 2 compared with Ex 1) and the rate of adjustment of \( \text{Vo}_2 \) (unchanged during Ex 2 compared with Ex 1) in HTR appears in favor of the hypothesis that the \( \text{Vo}_2 \) on-kinetics in humans are mainly determined by an inertia of the intramuscular oxidative machinery (7, 31) and not by the rate of \( \text{O}_2 \) delivery to the exercising muscles (16). This conclusion, which, on the basis of the present results can, of course, be drawn only for the transition from rest to an exercise presumably lower than the lactate threshold, appears in agreement with recent studies on muscle metabolism carried out in exercising humans by nuclear magnetic resonance spectroscopy (3) as well as with studies analyzing the \( \text{Vo}_2 \) on-kinetics in the human quadriceps muscle (13).

The results of the present study do not confirm the data obtained by Paterson et al. (22) by utilizing a similar protocol of sequential exercises. Indeed, these authors described faster \( \text{Vo}_2 \) on-kinetics in HTR during the second on-transition, with time constant (\( t \)) values that were not different from those of control subjects. The on-transition studied by Paterson et al. (22) was from unloaded pedaling to a workload lower than the subjects' anaerobic threshold. Considering the limited exercise capacity of HTR, the amplitude of the \( \text{Vo}_2 \) response was, therefore, very small (change in \( \text{Vo}_2 \) above the baseline \( \sim 0.21 \) l/min), with a signal-to-noise ratio, during breath-by-breath analysis of gas exchange, which was inevitably rather poor. The limited amplitudes of the \( \text{Vo}_2 \) response, together with the small number of investigated subjects (\( n = 6 \)), was such that the estimated 95% confidence intervals of the \( \pm \) data obtained by Paterson et al. (22) were, by their own
admission, “wide” (40 s). Moreover, by utilizing an unloaded pedaling baseline, the cardiovascular system was somewhat primed even before the first on-transition, making the difference between the two sequential transitions rather small.

The priming exercise did not affect $t_{1/2} \bar{V}_O_2$ on- in C subjects as well. This is not surprising, considered that in this group Q values during Rest 2 were only slightly higher (no significant difference) than during Rest 1, and the $t_{1/2} Q$ on- during Ex 2 were slightly higher (no significant difference) than during Ex 1. Moreover, no $[\text{La}]_b$ changes were described in C during this protocol, so that the acidosis-induced effects described above for HTR could not occur. Finally, no significant increases in blood catecholamine levels should occur in normal subjects at this low workload (2). Thus the priming exercise could not significantly affect the rate of adjustment of $O_2$ delivery to muscle fibers in C. The results of the present study, as far as C subjects are concerned, confirm those obtained by Gerbino et al. (11), according to whom in normal subjects a prior sublact酸 threshold exercise is not effective in speeding up $V_2O_2$ kinetics during a second on-transition.

In the present study, the $VCO_2$ and $VE$ on-kinetics were significantly slower than the $V_2O_2$ on-kinetics, as previously shown in normal subjects (28) as well as in HTR (5, 6) and in heart and lung transplant recipients (12). The slower $VCO_2$ on-kinetics, compared with $V_2O_2$ on-, are presumably attributable to the CO2 storage capacity of body tissues, whereas the mechanisms responsible for the tight coupling of the $VCO_2$ and $VE$ on-kinetics are not firmly established (30). In the present study, the $VE$ on- and $VCO_2$ on-kinetics were significantly slower in HTR compared with C, confirming previous results by our group (5, 6, 12). On the basis of the present data, no inferences can be made as far as any cause-effect relationships between the delayed gas-exchange and ventilatory kinetics. It seems reasonable to hypothesize, however, that a delayed $VCO_2$ on-kinetics would follow the delayed $V_2O_2$ on-kinetics and that a delayed $VE$ on-kinetics would then strictly follow the delayed $VCO_2$ on-kinetics. HTR slightly hyper-ventilated at rest, compared with C (see Tables 2 and 3). The influence of such modest hyperventilation on the $VE$ on-kinetics was presumably negligible.

Gas-exchange on-kinetics as a function of workload and of time posttransplantation. The results of the present study showed in C an increase in $t_{1/2} \bar{V}_O_2$ on- (i.e., a slower $V_2O_2$ on-kinetics) with increasing workload (see Fig. 5), even if the latter was moderate, i.e., was presumably lower than the lactic threshold. These data confirm previous observations by Cerretelli et al. (8) and di Prampero et al. (10). The slowing of the $V_2O_2$ on-kinetics with increasing exercise intensity, even in the moderate-exercise domain, was associated by the above-mentioned authors with a transient (“early”) lactate increase occurring during the first minutes of exercise, before the attainment of a steady state for $V_2O_2$. The present results, on the other hand, do not confirm those by Whipp and Ward (32), according to whom, for moderate-intensity exercise, the $t_{1/2} \bar{V}_O_2$ on-response is relatively independent of work rate. No insights to explain these discrepancies can be obtained from the present study. In any case, the slowing of the on-kinetics with increasing workload was not observed in HTR. No clear-cut explanation can be offered for this finding, which appears rather surprising, considering that in some HTR the higher investigated workloads might have corresponded to (or even exceeded) the lactate threshold and that there is substantial agreement among authors that at or above this threshold the $V_2O_2$ on-kinetics is significantly slower than during moderate exercise. A possible explanation is that in HTR intrinsic metabolic factors at the muscle level impose a slow kinetics already at low workloads. An alternative partial explanation may lie in the fact that the method utilized in the present study to calculate the $t_{1/2} \bar{V}_O_2$ on- does not take into account the presence of a “slow component” of the $V_2O_2$ on-kinetics (31), which occurred in some HTR at 75 W, as also manifested by the steady-state $V_2O_2$ values (see below).

At all investigated workloads (i.e., 25, 50, and 75 W), the ventilatory and gas-exchange on-kinetics were significantly slower in HTR than in C subjects. Slower on-kinetics in HTR were also described when HTR and C were examined at the same relative (with respect to their $V_2O_2max$ workload (12). The slower on-kinetics in HTR appear, therefore, to be independent of workload, either absolute or relative.

No statistically significant relationship was observed, either at 25 or at 50 W, between the $t_{1/2} \bar{V}_O_2$ on-response and the time elapsed between the test and the heart transplantation, although a trend toward slightly slower $t_{1/2}$ with time was observed. This observation appears of some interest, considering the wide range (between 1 and 137 mo) of time elapsed after surgery encompassed by the present study and recent observations by some authors indicating the possibility of some kind of reinnervation of the transplanted heart (17, 34) or of heart $\beta_2$-adenoreceptors upregulation and increased sensitivity to epinephrine as a function of time after transplantation (26). On the basis of the present results, it can be concluded that, even if some reinnervation or increased sensitivity to catecholamines occurs with time, it does not appear to have any significant effect on the $V_2O_2$ on-kinetics, which remain significantly slower in HTR compared with C subjects even more than 10 yr after surgery. The slightly faster $V_2O_2$ on-kinetics as a function of time after surgery might, in fact, be well accounted for by some improvement of the muscle function of HTR.

Off-transients. During the recovery after a moderate-intensity exercise, an analysis of the $V_2O_2$ time course in normal subjects identifies a fast, workload-independent exponential component with a $t_{1/2}$ of 25–30 s, associated with the resynthesis of muscle phosphocreatine and the replenishment of the $O_2$ stores of the body (8, 10). This fast component is followed by a second, slow exponential component ($t_{1/2}$ of several minutes), which has been attributed to the repayment of the fraction of the $O_2$ deficit associated with early lactate accumulation (8, 10). Because, in the present study, the $t_{1/2} \bar{V}_O_2$ off-values were calculated over the first 4–5 min
of recovery, the obtained values mainly reflect the fast ("alactic") component mentioned above. The fact that the $t_{50}\ V_{O2}$ off-values were significantly higher in HTR than in C indicates that in the former group the alactic $O_2$ deficit was greater than in C, thereby reinforcing the notion of a sluggish $V_{O2}$ adjustment at the muscle level (on the assumption of an equal contribution of $O_2$ stores to the $O_2$ deficit in the two groups). In the present study, $t_{50}\ V_{O2}$ off-values were, in both groups, independent of workload, confirming previous observations by others in normal subjects (8).

In HTR, the slower $V_{O2}$ off-kinetics, as compared with C, were associated with slower $VCO_2$ off- and $VE$ off-kinetics. As discussed above for the on-response, on the basis of the present data, no inference can be made as to any cause-effect relationships between the delayed gas-exchange and ventilatory kinetics. Also for the off-phase, however, it seems reasonable to hypothesize that a delayed $VCO_2$ kinetics would follow the delayed $V_{O2}$ kinetics and that a delayed $VE$ kinetics would then strictly follow the delayed $VCO_2$ kinetics.

Steady-state values during prolonged exercise. As mentioned above, with a rectangular increase in workload, HR follows in HTR a time course that is distinctly different from that of healthy subjects (5, 23). Indeed, after an initial delay during which HR does not increase appreciably, an almost linear increase as a function of the time of exercise is observed, and a new steady state is not reached before the fourth or fifth minute of exercise, and sometimes even later (5, 23). Thus it might be questioned whether during a standard 5-min rectangular exercise protocol HTR do, indeed, reach a "steady state" as far as the main cardiovascular, ventilatory, and gas-exchange parameters are concerned. Therefore, a prolonged (15-min) rectangular exercise protocol was carried out in the present study. The workload chosen for this protocol was low (25 W) to prevent the subjects from developing signs of fatigue, which could influence the investigated variables. The obtained results indicate that, even in the presence of HR values that kept slightly (although statistically not significantly) increasing from the fifth to the fifteenth minute of exercise, no changes were observed for the ventilatory and gas-exchange parameters between the fifth, the tenth, and the fifteenth minute of exercise (see Fig. 7). Therefore, after 5 min of moderate-intensity exercises, HTR appear in a situation of steady state with regard to $VE$, $V_{O2}$, and $VCO_2$, indicating that also in HTR the ventilatory and gas-exchange kinetics can be reliably evaluated by standard means and protocols. More caution is probably needed for the cardiovascular parameters.

Steady-state $V_{O2}$ values were substantially the same in HTR and in C subjects at rest and at 25 and 50 W (see Fig. 4). This indicates that despite the pharmacological treatment the mechanical efficiency of exercise was unaltered in HTR, confirming previous observations (5, 6). However, $V_{O2}$ values were slightly but significantly higher in HTR than in C subjects at 75 W. This presumably indicates that at this load in HTR the "slow component" of $V_{O2}$ kinetics was superimposed on the $V_{O2}$ time course for moderate exercise (31). Such slow component could, indeed, be responsible for the higher $V_{O2}$ values that were attained in HTR between the fourth and the fifth minute of exercise at 75 W, compared with those expected according to an extrapolation of the $V_{O2}$ vs. workload relationship obtained at lower workloads. Some caution is, therefore, warranted in the analysis of the gas-exchange kinetics in HTR at 75 W.

Methodological considerations. Only two techniques can provide noninvasive estimates of $Q$ or $SV$ on a beat-by-beat basis (i.e., with the time resolution necessary for kinetics studies): impedance cardiography and Doppler ultrasound. Both techniques are indirect, as a result of the underlying principles, and thus in this respect there is not a clear advantage of one over the other. Indeed, there are common problems for both techniques, which may not be relevant for measurements at rest but which may become important during exercise, mainly as a consequence of movements of the chest and the upper body. As far as impedance cardiography is concerned, artifacts may derive from the movement of the electrodes, whereas for the Doppler ultrasound technique placement and stability of the Doppler probe becomes increasingly difficult at higher workloads. $Q$ measurements in the present study were, therefore, mainly restricted to moderate exercise, in which movements of the upper part of the body are limited. In any case, the reliability of thoracic impedance as an indirect index of $Q$, even during exercise, has been supported by several studies [see e.g., Hatcher and Srb (14) and Kobayashi et al. (18)]. Moreover, what really mattered for the present study were the relative changes (compared with the resting baseline) of $Q$, the absolute changes being much less important. Indeed, the obtained results would have been essentially the same if the changes in thoracic $Z$, rather than the calculated $SV$, were utilized as an indirect index of $Q$.

Breath-by-breath determinations of $VE$, as well as beat-by-beat estimations of $Q$, are inherently noisy. To improve accuracy and precision of kinetic analysis of these parameters, several repetitions of the same experiment, with subsequent superimposition of the results, are usually recommended. In the present study, practical reasons mainly related to the rigid schedules of the hospital wards in which the experiments were conducted prevented us from examining each patient for longer than ~45–60 min. In most cases, it was therefore impossible for the patients to perform several repetitions of the experimental protocols. On the other hand, in the present study, we could examine a particularly elevated number (82) of HTR, thereby reducing intersubject variability. Multiple (2–5) repetitions of the rectangular exercises were, however, performed by some of the C subjects. The same practical reasons mentioned above did not allow determination of $V_{O2\ max}$ in every HTR. An incremental exercise protocol to determine $V_{O2\ max}$ was, however, carried out by a limited number ($n = 8$) of our HTR, and the obtained results (maximal workload $97 \pm 25$ W, $V_{O2\ max} 1.60 \pm 0.36$ l/min, peak HR 128 ± 17 beats/min) appear fully in agreement with those obtained by previous
The authors are grateful to all the patients and control subjects who willingly collaborated in this study. The authors are also indebted to Prof. C. Cabrol, Prof. B. Carù, and Prof. I. Brambilla for the clinical supervision of the patients during the experiments, and to A. Colombini and M. Pellegrini for technical assistance.

This work was partially supported by funds provided by the National Research Council of Italy, Special Target Biotechnology and Bioinstrumentation; by Grants no. 32-28719.90 and 32-40397.94 of the Fonds National Suisse for Scientific Research; and by the Carlos and Elisie de Reuter Foundation, Geneva, Switzerland.

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Received 12 June 1996; accepted in final form 4 March 1997.