Understanding exercise-induced hyperemia: central and peripheral hemodynamic responses to passive limb movement in heart transplant recipients

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Using second-by-second temporal analysis of femoral blood flow, cardiac output (CO), heart rate (HR), and stroke volume (SV), our group (30) recently reported that passive leg extension resulted in an HR-driven increase in CO. This contrasts with previous research (14, 51) that either reported or assumed no change in CO with this passive exercise model. Indeed, we suggested that the increase in HR and the resulting rise in CO are a primary initiator of movement-induced hyperemia (30). Additionally, we observed that a significant increase in CO occurred at the onset of passive exercise even when blood flow to the exercising leg was prevented via cuff occlusion, revealing that an increase in leg blood flow is not obligatory for an increase in CO. Although these data further support a distinct contribution of both peripheral and central hemodynamic factors to exercise-induced hyperemia, the failure to manipulate the CO response still leaves one unable to clearly separate the two.

Heart transplant (HTx) results in heart denervation, a unique circumstance where both afferent and efferent cardiac control mechanisms are interrupted. It is well documented that heart transplantation limits the central hemodynamic response to exercise (19, 22, 38), providing a model through which it is possible to separate the contributions of central and peripheral mechanisms to passive movement-induced blood flow. Additionally, exercise tolerance of HTx recipients is reduced compared with healthy individuals (19, 35), which is particularly true in the first years following transplantation (17, 41). Therefore, a better understanding of the challenges to be overcome as the recipients transition from rest to exercise could in turn lead to an identification of rehabilitative approaches that might result in a faster recovery of exercise tolerance after HTx and an improved quality of life.

Consequently, we sought to use HTx recipients as a model devoid of cardiac innervation to further delineate central and peripheral contributions to exercise-induced hyperemia. Using passive leg movement in both healthy and HTx recipients, we hypothesized that 1) in HTx recipients, passive limb movement will not increase HR and CO as it does in healthy, age-matched controls, and 2) as a CO increase is an important contributor to exercise-induced hyperemia, movement-induced increases in leg blood flow will be significantly attenuated in the HTx recipients.

METHODS

Subjects

Nine HTx recipients (<2 yr post-HTx) and nine healthy controls were recruited in the HTx clinic at the University of Utah and the Salt
Table 1. Subject characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Control</th>
<th>HTx</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/Female</td>
<td>8/1</td>
<td>8/1</td>
</tr>
<tr>
<td>Age, yr</td>
<td>58 ± 5</td>
<td>56 ± 4</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>85 ± 4</td>
<td>85 ± 10</td>
</tr>
<tr>
<td>Height, cm</td>
<td>178 ± 2</td>
<td>177 ± 7</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>27 ± 1</td>
<td>24 ± 3</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>129 ± 5</td>
<td>127 ± 8</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg</td>
<td>75 ± 3</td>
<td>78 ± 7</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>93 ± 2</td>
<td>107 ± 12</td>
</tr>
<tr>
<td>Cholesterol, mg/dl</td>
<td>187 ± 13</td>
<td>145 ± 15</td>
</tr>
<tr>
<td>HDL, mg/dl</td>
<td>50 ± 5</td>
<td>39 ± 3</td>
</tr>
<tr>
<td>LDL, mg/dl</td>
<td>122 ± 12</td>
<td>86 ± 12</td>
</tr>
<tr>
<td>Triglycerides, mg/dl</td>
<td>97 ± 17</td>
<td>132 ± 18</td>
</tr>
<tr>
<td>Hemoglobin, g/dl</td>
<td>15 ± 0.3</td>
<td>12 ± 0.5 *</td>
</tr>
<tr>
<td>WBC, K/µL</td>
<td>5.5 ± 0.5</td>
<td>5.5 ± 0.7</td>
</tr>
<tr>
<td>Neutrophil, K/µL</td>
<td>3.3 ± 0.5</td>
<td>4.5 ± 0.7</td>
</tr>
<tr>
<td>Lymphocyte, K/µL</td>
<td>1.6 ± 0.1</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td>Monocyte, K/µL</td>
<td>0.4 ± 0.0</td>
<td>0.4 ± 0.1</td>
</tr>
</tbody>
</table>

*Values are means ± SE. HTx, heart transplant; HDL, high-density lipoprotein; LDL, low-density lipoprotein; WBC, white blood cells. *Significantly different from control.

Lake City Veterans Affairs Medical Center. Additional subject characteristics and the therapy of the HTx recipients at the time of the study are reported in Tables 1 and 2. The protocol was approved by and written informed consent was obtained according to the Institutional Review Board of the University of Utah and the Salt Lake City Veterans Affairs Medical Center. All studies were performed on the same day in a thermoneutral environment. Subjects reported to the laboratory in the fasted state and had not performed any exercise within the past 24 h.

Brachial Artery Flow-Mediated Dilation and Reactive Hyperemia Protocol

Subjects rested supine for ~20 min, and a blood pressure cuff was placed on the upper right arm proximal to the elbow but distal to the placement of the ultrasound Doppler probe on the brachial artery. Baseline measurements were obtained, and the arm cuff was inflated to a suprasystolic pressure (>250 mmHg) for 5 min. Measurements of brachial artery diameter and blood velocity were collected continuously for 2 min following cuff deflation.

Passive Exercise Protocol

Subjects again rested supine for ~20 min before the start of the data collection and remained in this position throughout the entire protocol. The protocol consisted of a 60-s resting baseline data acquisition followed by a 3-min bout of passive leg extension. Before the start of baseline and passive movement, a cuff was placed distal to the knee on the passive leg and inflated to 250 mmHg, eliminating blood flow to the lower leg for the entire 3 min. This was done to eliminate fluctuations in blood flow to the lower leg as a consequence of changing gravitational and centrifugal forces on the lower leg throughout the movement. Initial pilot work revealed a minimal effect of either cuffing or not cuffing the control leg in the same manner. Therefore, a lower leg cuff on the control leg was not applied in this study. Passive exercise was achieved by a member of the research team moving the subject’s lower leg through a range of motion, defined by 90° and 180° knee joint angles, at a rate of 1 Hz (throughout the protocol, the control leg remained fully extended and supported). Real-time feedback to the investigator was provided by a position sensor to ensure a consistent range of motion, and a metronome was used to maintain the cadence. Before the start and throughout the protocol, the subjects were encouraged to remain passive and resist any urge to assist with leg movement. To avoid a startle reflex and active resistance to the passive movement, the subjects were made aware that passive movement would take place, but, to minimize the chance of an anticipatory response, they were not informed of exactly when this movement would initiate.

Measurements

Arterial blood flow and blood velocity measurements and analyses. Measurements of arterial blood velocity and vessel diameter were performed in both the brachial artery flow-mediated dilation (FMD) and passive exercise protocols with Logic 7 and Logic e ultrasound systems (General Electric Medical Systems, Milwaukee, WI). The Logic 7 and Logic e were equipped with linear array transducers operating at an imaging frequency of 14 and 12 MHz, respectively. Vessel diameter was determined at a perpendicular angle along the central axis of the scanned area. Blood velocity was obtained using the same transducers with a Doppler frequency of 5 MHz. All blood velocity measurements were obtained with the probe appropriately positioned to maintain an insonation angle of 60° or less. The sample volume was maximized according to vessel size and was centered within the vessel based on real-time ultrasound visualization. Arterial diameter was measured, and mean velocity (Vmean) values [angle-corrected and intensity-weighted area under the curve (AUC)] were then automatically calculated using commercially available software (Logic 7 and Logic e). Using arterial diameter and Vmean, the blood flow in the brachial and femoral vessels was calculated as follows: Blood flow = Vmeanπ(vessel diameter/2)² × 60, where blood flow is in milliliters per minute.

Flow-mediated dilation. Relative and absolute FMD were calculated as the percent change and the absolute Δ, respectively, from resting artery diameter to the largest diameter achieved during the 120 s of postinflation imaging. All ultrasound vessel lumen diameter measurements were evaluated during end diastole (corresponding to an R wave documented by the simultaneous ECG signal) (Logic 7). An analysis of the diameters was performed using off-line automatic edge-detection Brachial Analyzer software (Medical Imaging Applications, Coralville, IA), which is described in detail elsewhere (33a).

Shear rate and reactive hyperemia calculation. Shear stress is believed to be the mechanism that stimulates the vascular endothelium and results in subsequent vasodilation (7). Since blood viscosity was not measured, shear rate, an adequate surrogate measure (4, 36), was calculated using the following equation: Shear rate (in s⁻¹) = 8·Vmean i(vessel diameter/2)² (37). To normalize the vasodilation for shear rate, FMD was divided by the cumulative shear rate (%Δdiameter/s⁻¹) (37).

Table 2. Characteristics pertinent to the HTx recipient group

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>HTx</th>
</tr>
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<tbody>
<tr>
<td>Diagnosis (nonischemic cardiomyopathy)</td>
<td>4/9</td>
</tr>
<tr>
<td>Diagnosis (ischemic cardiomyopathy)</td>
<td>5/9</td>
</tr>
<tr>
<td>Time post-HTx, months ± SE</td>
<td>9 ± 2</td>
</tr>
<tr>
<td>Rejection episodes</td>
<td>1/9</td>
</tr>
<tr>
<td>Left ventricular ejection fraction, %</td>
<td>65.3 ± 1.8</td>
</tr>
<tr>
<td>Medications, number of all cases</td>
<td></td>
</tr>
<tr>
<td>Cyclosporine</td>
<td>1/9</td>
</tr>
<tr>
<td>Tacrolimus</td>
<td>7/9</td>
</tr>
<tr>
<td>Azathioprine</td>
<td>3/9</td>
</tr>
<tr>
<td>Mycophenolic acid</td>
<td>5/9</td>
</tr>
<tr>
<td>Prednisone</td>
<td>5/9</td>
</tr>
<tr>
<td>β-Blockers</td>
<td>1/9</td>
</tr>
</tbody>
</table>
Central variables. HR, SV, CO, and mean arterial pressure (MAP) were determined with a Finometer (Finapres Medical Systems, Amsterdam, The Netherlands). SV was calculated using the Modelflow method that includes age, sex, height, and weight in its algorithm (Beatscope version 1.1; Finapres Medical Systems) (5) and has been shown to accurately track CO during a variety of experimental protocols including exercise (9, 10, 44, 44a, 46). CO was then calculated as the product of HR and SV. Vascular conductance within each leg was calculated as leg blood flow/MAP.

Data acquisition. Throughout the entire protocol, HR, SV, CO, MAP, ECG, and knee joint angle signals underwent analog-to-digital conversion and were simultaneously acquired (200 Hz) using commercially available data acquisition software (AcqKnowledge, Biopac Systems). In addition, this data acquisition software also acquired (10,000 Hz) the audio anterograde and retrograde signals from both Doppler ultrasound systems to serve as a qualitative indicator of blood velocity changes and to ensure an accurate temporal alignment of blood velocity measurements obtained from these systems and the other signals collected (i.e., finometer and goniometer) (30).

Statistical Analyses

Statistics were performed using commercially available software (SPSS, v. 17.0, Chicago, IL). An independent t-test (α < 0.05) was used to compare FMD and RH in HTx recipients and controls. As both central and peripheral responses were transient, only data from the first 40 s of passive movement were compared, and before analysis all data were smoothed using a rolling 3-s average. A paired t-test (α <
was used to compare whether individual maximal relative changes in CO, HR, SV, and blood flow differed from baseline within each group, and an independent t-test (α < 0.05) was used to identify differences between healthy controls and HTx recipients. A paired t-test (α < 0.05) was also used to compare control leg blood flow responses from the passively moved leg within each group. All group data are expressed as means ± SE.

RESULTS

Peripheral Responses to Passive Exercise

The peripheral blood flow responses to passive movement over time are illustrated in Fig. 1, A and B, and all maximum changes from baseline are represented in Fig. 2. Baseline blood flows were different between HTx recipients (218 ± 29 ml/min; 237 ± 65 ml/min) and controls (259 ± 41 ml/min; 154 ± 29 ml/min) in the passive and control leg, respectively. Following the onset of passive movement, the maximum blood flow achieved in the passively moved leg increased significantly above baseline in both HTx recipients (397 ± 53 ml/min, Δ85%) and controls (623 ± 78 ml/min, Δ156%) although attenuated in the HTx recipients (Fig. 2). In the HTx recipients, the percent change from baseline (Fig. 2) in the passively moved leg was not significantly different from the control leg, unlike the control group where there was a significant difference between legs. The transient increase in femoral blood flow, as assessed by the AUC during the first 40 s of passive limb movement, resulted in a smaller blood volume entering the leg in the HTx recipients (24 ± 8 ml) compared with the controls (93 ± 25 ml).

Central Responses to Passive Exercise

The central responses to passive movement over time are illustrated in Fig. 1, C–F. Baseline values for CO and SV were not significantly different between the HTx recipients and controls; however, there were baseline differences in HR (P = 0.001) and MAP (P = 0.05). All maximum changes from baseline are represented in Fig. 2. The HTx recipients displayed a significant increase in CO (0.4 ± 0.1 l/min, Δ5%) although greatly attenuated, compared with the controls (1.0 ± 0.2 l/min, Δ18%). In the HTx recipients, there was an absence of a significant increase in HR (0 ± 0 beats/min, Δ0%). Therefore, the CO response was solely dependent on increased SV (5 ± 1 ml, Δ5%). These central responses in the HTx recipients were accompanied by a significant decrease in MAP (—5 ± 1 mmHg, Δ—5%). In contrast, the controls who significantly increased HR (6 ± 2 beats/min, Δ11%) as well as SV (5 ± 2 ml, Δ7%) exhibited an increase in MAP (7 ± 1 mmHg, Δ7%) (Fig. 2). The maximal changes in vascular conductance in the passive leg were greater in the controls (3 ± 1 ml·min⁻¹·mmHg⁻¹, Δ156%) compared with the HTx recipients (2 ± 0.3 ml·min⁻¹·mmHg⁻¹, Δ87%).

Vascular Function: FMD and RH

There was no significant difference in peripheral vascular function between the HTx recipients and controls as assessed by FMD (Fig. 3). This was the case whether FMD was expressed in traditional terms (%diameter change) (controls, 6 ± 1%; and HTx recipients, 8 ± 2%) or normalized for shear rate (FMD/shear rate) (controls, 0.1 ± 0.2; and HTx recipients, 0.2 ± 0.06). Resting brachial artery blood flows did not differ between controls and HTx recipients (110 ± 15 and 90 ± 11 ml/min, respectively) (Fig. 4). Similarly, RH, both in terms of peak (controls, 805 ± 64 ml/min; and HTx recipients, 680 ± 64 ml/min) and AUC (controls, 627 ± 69 ml; and HTx recipients, 564 ± 58 ml), was not different between groups (Fig. 4).

DISCUSSION

In this study, we sought to determine the central and peripheral contributions to movement-induced hyperemia in response to passive movement by comparing humans with a denervated heart (HTx) to intact controls. With this approach, we observed a fourfold reduction in the transient increase in femoral blood volume entering the leg in response to passive limb movement in the HTx recipients compared with controls. This attenuated hyperemic response to movement in the HTx recipients was not likely due to diminished peripheral vascular function, as measured by brachial artery FMD and RH, implicating a differing central hemodynamic response as the source of disparity in leg blood flow. These findings highlight the key role of the reflex increases in HR and the associated rise in CO response as an important mechanism that contributes to movement-induced hyperemia in humans.

Central and Peripheral Responses to Passive Limb Movement

Almost instantaneously upon the initiation of leg movement in the control subjects, there was a significant increase in HR (Fig. 1), which was likely due to the stimulation of peripheral muscle and joint afferent reflexes (1, 2, 32) and subsequent vagal inhibition (33). This HR increase contributed to the
increase in CO (≈18%) in the control subjects. The timing and magnitude of both these responses was similar to recent results published by our group (30), which suggested that an HR-driven increase in CO is a primary initiator of movement-induced hyperemia. Despite baseline values that were not statistically different, there was no HR response to passive limb movement in the HTx group, which confirmed the appropriateness of using HTx recipients as a model devoid of a chronotropic response (Fig. 1). Interestingly, HTx recipients also exhibited a significant increase in CO (≈5%) with passive limb movement. When compared with that of the normal controls, this CO response was reduced in magnitude and the timing of the CO increase was delayed by 11 to 12 s. This delay related to the fact that the CO increase in HTx patients was solely dependent on an increase in SV rather than HR.

Previous studies have suggested that an increase in limb blood flow and the resultant rise in venous return could be the predominant mechanism responsible for an increase in CO with passive limb movement (14). In the present study, there was no difference in the magnitude of SV increases between the controls and the HTx recipients and, as already noted, the SV response was similarly delayed in both groups, perhaps indicative of the transit time for the increased blood volume to travel through the passively moved leg (3) and then return back to the heart. Although long-established hemodynamic principles demand that an increase in venous return is required to sustain an increase in CO, the temporal relationships between SV, HR, and CO documented in this study suggest that in the first few seconds of limb movement, there may be a mismatch between CO and venous return, such that the initial increase in CO can be attributed to the almost instantaneous increase in HR. This observation is emphasized here by the lack of an HR response and a subsequently delayed and attenuated CO increase in the HTx recipients (Fig. 1).

Upon initiation, passive leg movement consistently results in an increase in femoral blood flow (14, 30, 51). As hypothesized, hyperemia in the passively moved leg was significantly attenuated by approximately fourfold in the HTx recipients (Figs. 1 and 2), and much of the observed difference in maximum blood flow between the controls (≈156%) and the HTx recipients (≈85%) could be attributed to the lack of an HR increase and the concomitantly diminished CO response. The differing hyperemic response between these groups further supports the previous conclusions from our group (30) that an HR-driven increase in CO is an essential component of the hyperemic response at the onset of leg movement and that limb vasodilation and the subsequent venous return are not solely responsible for the increased CO in the first few seconds following limb movement.

**MAP and Vascular Conductance**

During passive leg movement, there was an increase in MAP (≈7 mmHg) in the control subjects, an expected effect of an increase in CO and the concomitant change in vascular conductance. However, this intuitive finding is in contrast with our previous study (30), which reported a small decrease in MAP (≈3 mmHg) upon initiation of passive leg movement. This discrepancy may be a result of age-related differences in vascular function and/or structure (6, 28, 31, 48) and baroreceptor reflexes that previously minimized this potential for a rise in blood pressure with an increase in CO as the present control group was older (≈58 yr) compared with the group in our previous study (≈33 yr). Thus perhaps baroreflex control of HR in response to limb movement plays a more important role in young rather than older subjects. In contrast, MAP in the HTx recipients exhibited a comparable magnitude of increase compared with the controls, which is consistent with the previously noted chronotropic insensitivity of these patients.

![Fig. 3. Brachial artery flow-mediated dilation (FMD) following 5-min cuff occlusion expressed as a percent change from precuff baseline (A) and after normalizing FMD for shear rate (B) in HTx recipients and controls. Values are means ± SE. There were no significant differences.](http://ajpheart.physiology.org/)

![Fig. 4. Resting brachial artery blood flow and reactive hyperemia following cuff occlusion in healthy controls and HTx recipients.](http://ajpheart.physiology.org/)
change to the current age-matched controls but in the opposite direction (≈5 mmHg). This was most likely a consequence of a drop in peripheral resistance by mechanically induced vessel dilation (8, 24) and FMD (25, 26, 34), allowing blood flow to increase to the passively moved limb but without the same magnitude of CO increase documented in the controls to either raise or successfully maintain MAP. Despite the contrasting and significant changes in MAP, vascular conductance was qualitatively very similar to blood flow with the maximal change being far greater in the controls compared with the HTx recipients (Fig. 2). Thus the difference in hyperemia observed between the two groups could not be explained solely by changes in MAP.

Peripheral Vascular Function

Differences in the hyperemic response to leg movement between the HTx recipients and controls could conceivably be attributed to differences in peripheral vascular function as a result of the prior disease state, independent of central cardiac differences. Peripheral vascular dysfunction is known to occur in chronic heart failure (11, 12, 20, 21). However, somewhat surprisingly, there is evidence that endothelial function improves within the first 12 mo following transplantation (27, 39). In agreement with this concept the peripheral vascular function of the current HTx recipients, as assessed by brachial artery FMD and RH, was not attenuated (Figs. 3 and 4). This further supports that the limited femoral blood flow response in this group was a result of the lack of an HR response and not peripheral vascular dysfunction. Although FMD and RH are just two of the possible measures of vascular function, these observations of vascular normalcy also support the use of the HTx patient model to elucidate the importance of HR and CO on movement-induced hyperemia.

Clinical Perspective

Following HTx, recipients exhibit substantial exercise intolerance (19, 35), particularly in the initial years following the procedure (17, 41). Many studies have demonstrated an association between exercise intolerance and chronotropic incompetence (15, 19, 38, 40). The HTx recipients in this study also demonstrated a lack of an HR response and an attenuated hyperemia at the initiation of passive limb movement. Although still controversial, there is accumulating evidence suggesting some sympathetic reinnervation occurs in HTx recipients based on a more normal chronotropic exercise response (15, 40), evidence of coronary norepinephrine spillover (43, 39). In agreement with this concept the peripheral vascular function of the current HTx recipients, as assessed by brachial artery FMD and RH, was not attenuated (Figs. 3 and 4). This further supports that the limited femoral blood flow response in this group was a result of the lack of an HR response and not peripheral vascular dysfunction. Although FMD and RH are just two of the possible measures of vascular function, these observations of vascular normalcy also support the use of the HTx patient model to elucidate the importance of HR and CO on movement-induced hyperemia.

Experimental Considerations

Although many subject characteristics and vascular function of the HTx recipients were not different from the controls, it must still be acknowledged that, in addition to the main reason for studying this population (a lack of cardiac innervation), the HTx recipients all previously had heart failure. Thus the recognized skeletal muscle dysfunction associated with this pathology may have influenced the results of this study; however, this issue was likely minimized by the fact that this study employed passive exercise which does not require a significant skeletal muscle metabolic response. An additional limitation of this study is the extrapolation of peripheral vascular function from the upper to the lower limbs, where the response to passive movement was actually assessed.

Summary

With the use of the denervated HTx patient model, these data provide evidence that an elevation in HR and the subsequent CO increase are an important contributor to the hyperemic response following the onset of passive limb movement. The attenuated blood flow response in the HTx recipients cannot easily be explained by differences in peripheral vascular function, as FMD and RH were not attenuated in the HTx recipients. These findings highlight the key role of the reflex increases in HR and the associated rise in CO response as an important mechanism for movement-induced hyperemia in humans.

ACKNOWLEDGMENTS

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GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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