Vascular endothelial dysfunction is a systemic pathology that impairs the health of both conduit and resistance vessels. Impaired endothelium-dependent vasodilation has been associated with various cardiovascular diseases, including hypertension, coronary artery disease, and heart failure. Importantly, impaired endothelium-dependent vasodilation may also precede the development of these cardiovascular diseases. Endothelial dysfunction has, therefore, been proposed as a prognostic marker in both healthy individuals and patients with cardiovascular disease. Noninvasive tests, such as flow-mediated dilation (FMD) and the measurement of reactive hyperemia (RH), have become common research tools used to assess and quantify disturbances in vascular function. However, the real impact of disease progression and surgical intervention on vascular function across the continuum from health, heart failure (HF), and heart transplantation (HTx) has not been well characterized.

HF patients have chronically elevated peripheral vasoconstriction, the result of elevated sympathetic nervous system activity, as well as a concomitant dysfunction of the peripheral vasculature. The latter appears to be the consequence of an attenuated l-arginine-NO pathway and has been, at least partially, attributed to the increased destruction of NO by free radicals. Indeed, previous studies have revealed that elevated levels of free radicals, particularly superoxide, contribute to decreased NO bioavailability and the subsequent attenuation in endothelial function. Antioxidant supplementation has previously restored endothelial function in healthy aged individuals, as well as HF patients, presumably by improving NO bioavailability. HTx restores many of the hemodynamic abnormalities characteristic of advanced HF. However, whether HTx also results in normalization of endothelial function remains controversial.

Accordingly, using FMD and RH to assess vascular function and an acute oral antioxidant cocktail (AOC) to examine the role of free radicals, this cross-sectional study sought to provide greater insight into vascular function in HF patients with reduced ejection fraction (HFrEF) and at several time points beyond HTx. We hypothesized that, when compared with controls, vascular function would be blunted in HFrEF patients, would be comparable within...
Table 1. Subject Characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Controls</th>
<th>HFrEF (&lt;3 y)</th>
<th>HTx (5–10 y)</th>
<th>HTx (&gt;14 y)</th>
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<td>14</td>
<td>12</td>
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<td>77±4</td>
<td>83±3</td>
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<td>93±5†</td>
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<td>146±12</td>
<td>138±11</td>
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</tr>
<tr>
<td>Hemoglobin, g/dL</td>
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<td>15±0.4</td>
</tr>
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<td>Hematocrit, %</td>
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</tbody>
</table>

Data are mean±SE. HDL indicates high-density lipoprotein; LDL, low-density lipoprotein; RBC, red blood cells; WBC, white blood cells; HFrEF, heart failure patients with reduced ejection fraction; HTx, heart transplantation.

*Data are significantly different from control.
†Data are significantly different from HFrEF.
‡Data are significantly different from HTx (>14 y).

Methods

Subjects

A total of 61 subjects (12 healthy age-matched controls, 14 New York Heart Association class II and III HFrEF patients, and 35 HTx recipients (<3 years post-HTx, 5–10 years post-HTx, and >14 years post-HTx) were recruited in the HF and HTx clinics at the University of Utah and the Salt Lake City Veterans Affairs Medical Center and the controls by word of mouth. 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 of the studies were performed in a thermoneutral environment ±3 days apart to allow for washout of the oral antioxidants. Subjects reported to the laboratory in the fasted state and without caffeine for the past 24 hours, and, if antioxidants and/or a multivitamin were part of a subject’s daily routine, they were asked to refrain from such use for ±3 days before testing.

FMD and RH Measurements

Details of the FMD procedure have been described previously25 and were performed in accordance with current recommendations.26 Briefly, a blood pressure cuff was placed on the right arm proximal to the elbow and distal to the placement of the ultrasound Doppler probe on the brachial artery. The brachial artery was insonated approximately midway between the antecubital and axillary regions, and measurements of brachial artery diameter and blood velocity (Vmean) measurements were obtained continuously at rest and for 2 minutes after cuff deflation (Logiq 7, GE Medical Systems, Milwaukee, WI).

Analyses

Vvean was automatically calculated using commercially available software (Logiq 7). End-diastolic, ECG R-wave gated images were collected via video output from the Logiq 7 for offline analysis of brachial artery vasodilation using automated edge-detection software (Medical Imaging Applications, Coralville, IA). FMD was quantified as the maximal percentage of change in brachial artery diameter after cuff release. Shear rate was calculated as follows: shear rate (s⁻¹)=8Vmeandiameter. Blood flow was calculated as follows: blood flow = Vmean (arterial diameter/2)²×60. For both shear rate and blood flow, cumulative area under the curve values were integrated with the trapezoidal rule and calculated as follows: ∑[y[x(i+1)−x(i)]+1/2[y(i+1)+y(i)][x(i+1)−x(i)]]. RH was quantified as cumulative brachial artery blood flow for 2 minutes (area under the curve) after cuff occlusion. Normalized FMD was calculated by dividing FMD (percentage) by the cumulative shear rate area under the curve until the time of peak brachial artery vasodilation.

Antioxidant Supplementation

Subjects received either the AOC or placebo in a balanced, single blind design for the subject’s 2 visits. The supplements were administered 90 and 60 minutes before the FMD protocol. A split-dosing was used to improve absorbance and distribution of the antioxidants. The first antioxidant dose included vitamin E (200 IU), vitamin C (500 mg), and α-lipoic acid (300 mg), and the subsequent dose included vitamin E (400 IU), vitamin C (500 mg), and α-lipoic acid (300 mg). The placebo microcrystalline cellulose capsules, of similar taste, color, and appearance, were also consumed in 2 equivalently timed doses. The efficacy of this AOC to reduce plasma free radical concentration has been established previously using ex vivo spin trapping and electron paramagnetic resonance spectroscopy.27

Assays

In both the placebo and antioxidant trials, blood samples were obtained from the antecubital vein before FMD testing (1 hour after...
Flow-Mediated Dilation

Baseline vascular function, as measured by placebo (PL) FMD, was not different among the healthy age-matched controls (6.8±1.9%), recent <3-year post-HTx group (8.1±1.2%), and the 5- to 10-year post-HTx group (5.5±1.0%; Figure 1). In contrast, PL FMD was lower in the HFrEF patients (4.5±0.7%) and in the >14-year post-HTx recipients (2.9±0.8%) compared with the controls. The HFrEF PL FMD was also significantly different from the <3-year post-HTx recipients. There was a significant negative correlation between time after HTx and PL FMD (r = −0.52; Figure 2). When the HFrEF and HTx patient groups were broken down according to either current (HFrEF) or pre-HTx ischemic or nonischemic disease etiology, there were no significant within-group differences for PL FMD; however, the overall trends in vascular function remained the same (Figure 3). The antioxidant intervention increased FMD by 35% in the controls (PL, 6.8±1.9%; AOC, 9.2±1.0%) and by 55% in the >14-year post-HTx recipients (PL, 2.9±0.8%; AOC, 4.5±1.3%). The AOC had no measurable vascular effect in any of the other patient groups. When the patient groups were separated by disease etiology, the >14-year post-HTx ischemic group was the only group that exhibited a significant increase in FMD after the AOC (PL, 2.2±0.6%; AOC, 3.4±0.7%; Table 3). Shear rate was not different between groups and, therefore, was not used to normalize FMD.

Resting Blood Flow and RH

Resting blood flow did not differ among healthy controls, HFrEF, and HTx recipients (Figure 4A). Similarly, RH, both

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**Table 2. Characteristics Pertinent to the Heart Failure and HTx Recipient Groups**

<table>
<thead>
<tr>
<th>Variable</th>
<th>HFrEF</th>
<th>HTx (&lt;3 y)</th>
<th>HTx (5–10 y)</th>
<th>HTx (&gt;14 y)</th>
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<td>12</td>
<td>12</td>
<td>11</td>
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<td>7/12</td>
<td>6/12</td>
<td>6/11</td>
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<td>5/12</td>
<td>6/12</td>
<td>5/11</td>
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<td>Time post-HTx, mo±SE</td>
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<td>13±4 (~1 y)</td>
<td>85±7 (~7 y)</td>
<td>226±12 (~19 y)</td>
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<td>1/12</td>
<td>2/12</td>
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<tr>
<td>Left ventricular ejection fraction, %</td>
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<td>3/12</td>
<td>6/11</td>
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<td><strong>Medications, No. of all cases</strong></td>
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<td>2/12</td>
<td>4/12</td>
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<td>3/12</td>
<td>5/12</td>
<td>3/11</td>
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<tr>
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<td>5/12</td>
<td>3/12</td>
<td>4/11</td>
</tr>
</tbody>
</table>

Data are mean±SE. ACE indicates angiotensin-converting enzyme; HFrEF, heart failure patients with reduced ejection fraction; HTx, heart transplantation; NA, not applicable.

*Data were significantly different from HFrEF.
when examined as peak flow (control, 742 ± 77 mL/min; HFrEF, 707 ± 104 mL/min; <3 years post-HTx, 77 mL/min; 5–10 years post HTx, 845 ± 97 mL/min; and >14 years post HTx, 858 ± 97 mL/min) and area under the curve (control, 561 ± 65 mL; HFrEF, 625 ± 87 mL; <3 years post-HTx, 673 ± 82 mL; 5–10 years post-HTx, 728 ± 82 mL; and >14 years post-HTx, 589 ± 71 mL), was not different between groups (Figure 4A). After antioxidant administration, there was no significant change in RH evaluated either as peak flow (control, 724 ± 89 mL/min; HFrEF, 705 ± 98 mL/min; <3 years post-HTx, 781 ± 88 mL/min; 5–10 years post-HTx, 796 ± 92 mL/min; and >14 years post-HTx, 760 ± 72 mL/min) or area under the curve (control, 549 ± 85 mL; HFrEF, 578 ± 83 mL/min; <3 years post-HTx, 651 ± 75 mL; 5–10 years post-HTx, 696 ± 115 mL; and >14 years post-HTx, 548 ± 62 mL; Figure 3B).

Assays

At baseline (PL) plasma ascorbate levels were significantly higher in the healthy controls compared with all of the other patient groups (Figure 5A). Two hours after AOC administration, there was a significant increase in plasma ascorbate concentration in all of the subject groups (Figure 5A). Baseline (PL) TBARS did not differ significantly between groups (Figure 5B), but after administration of the AOC, there was a trend for a reduction in TBARS in the healthy controls and in the HTx recipients. There were no significant differences in superoxide dismutase and catalase activity between the study groups, and no significant changes in these values were observed after AOC administration. There were no significant differences in baseline endothelin-1 values between groups, although levels tended to be lower in the control group compared with the patient groups, and there was evidence of an increased concentration in the >14-year post-HTx group (control, 1.5 ± 0.2 pg/mL; HFrEF, 1.8 ± 0.1 pg/mL; <3 years post-HTx, 1.9 ± 0.2 pg/mL; 5–10 years post-HTx, 1.7 ± 0.2 pg/mL; and >14 years post-HTx, 2.1 ± 0.2 pg/mL). Baseline nitrite values were significantly higher in the HTx recipient groups compared with both the

![Figure 1](image1.png)

*Figure 1. A, Brachial artery flow-mediated vasodilation (FMD) with placebo and antioxidant supplementation after 5-minute cuff occlusion expressed as a percentage of change from precuff baseline in healthy controls, heart failure patients with reduced ejection fraction (HFrEF), and 3 groups of heart transplantation (HTx) recipients. †Significantly different from placebo. ‡Significantly different from controls. *Significantly different from HTx (<3 years). B, Brachial artery FMD expressed as absolute change in diameter from precuff baseline in healthy controls, HFrEF patients, and 3 groups of HTx recipients. †Significantly different from controls. ‡Significantly different from HTx (<3 years). Values are mean±SE.

![Figure 2](image2.png)

*Figure 2. Time after heart transplantation (HTx) and its influence on flow-mediated vasodilation (FMD; percentage of diameter change).
controls and the HFrEF patients (Figure 6A), with no measurable effect of the AOC. Baseline nitrate values were significantly higher in all of the patient groups compared with the controls (Figure 6B), which tended to be reduced by the AOC.

**Discussion**

This study, with a cross-sectional design, sought to determine vascular function and the role of oxidative stress in healthy controls, patients with HFrEF, and comprehensively with time beyond HTx. Using FMD to assess endothelial-dependent vascular function, compared with controls, we documented reduced vasodilatory capacity in the HFrEF patients and comparable vascular function in the early HTx recipients. However, vascular function was lower in the other HTx recipient groups to such a point where those who were the furthest time beyond transplantation (>14 years post HTx) exhibited vascular function similar to, if not more compromised than, the HFrEF patients. Interestingly, unlike the other patient groups, the acute ingestion of the AOC significantly increase FMD by 55% in these >14-year post-HTx recipients, suggesting that free radicals, and the associated decrease in NO bioavailability, are largely responsible for endothelial dysfunction in this group. RH, an index of microvascular function, was not different across the groups and did not change after AOC administration, highlighting the differing physiology and pathophysiology assessed by FMD and RH. Also of significant importance to the interpretation of these data is the fact that these cross-sectional observations were not confounded by aging, because the controls and all of the patient groups were of similar age. These findings not only highlight the transient nature of vascular function across the continuum from HF to HTx but also reveal the significant deterioration in endothelium-dependent vasodilation in HTx recipients, regardless of the initial disease etiology, who are 1 to 2 decades beyond transplantation. This ultimate decline, as with the controls, appears to be a consequence of a free radically mediated reduction in NO bioavailability.

**Flow-Mediated Vasodilation in HFrEF and HTx Recipients**

It is well accepted that peripheral endothelial function is impaired in patients with HF, as a consequence of an attenuated cardiac output, reduced levels of physical activity, elevated peripheral vasoconstriction, and neurohormonal activation. Data from the current study are in agreement with this dogma, because FMD, a reliable noninvasive measure of endothelial function, was significantly blunted in HFrEF patients compared with the healthy, age-matched controls (Figure 1). Vascular function appears to be relatively normal in the first few years after HTx, as the <3-year post-HTx recipients were similar to the controls. Both Kubo et al. and Roig et al. documented a normalization of endothelial function within the first year after HTx, which is most likely a result of an improved cardiac output with the donor heart in place and a reduction in sympathetic nervous system activity, both of which would increase NO bioavailability. However, endothelial function in HTx recipients, as a whole, is still somewhat controversial.

To our knowledge, this is the first vascular function study to include healthy age-matched controls, HFrEF patients, and a comprehensive cross-sectional group of HTx recipients at 3 discrete time points after transplantation, particularly a group that was >14 years post-HTx. With this approach, using FMD, we clearly documented relatively normal endothelial-dependent vasodilation after HTx that gradually declines to a level similar to that of HFrEF patients. This took place despite normal left ventricular ejection fraction in all 3 of the HTx groups examined. It is important to note that this decrease in FMD is not a consequence of age-related changes in vascular function, as all of the groups were similar in terms of age.
Although certainly not the main goal of the current study because of limited numbers of available subjects in the groups long after HTx, these data do afford the opportunity to examine vascular function in HFrEF patients and HTx recipients over time according to ischemic and nonischemic disease etiology. Previous work has suggested that endothelium-dependent vasodilation, as measured by FMD, is attenuated to a greater degree in HF patients\textsuperscript{34} and HTx recipients\textsuperscript{32} with ischemic cardiomyopathy compared with those with nonischemic cardiomyopathy. However, the study by Klosinska et al\textsuperscript{34} also documented that, although the vascular function in the nonischemic HF patients was significantly higher than those with ischemic cardiomyopathy, it was still significantly lower than the age-matched healthy controls. In the current study, although the ischemic HTx recipients in each group tended to have lower FMD values in comparison with those with nonischemic cardiomyopathy, there were no significant differences in PL FMD between disease etiologies in any of the patient groups (Figure 3). Interestingly, when only examining the patients with ischemic cardiomyopathy, FMD in both the 5 to 10 years post-HTx and >14 years post-HTx groups was significantly lower than that in the group <3 years post-HTx, and FMD in the 5 to 10 years post-HTx was comparable to the ischemic HFrEF patients. These data suggest the decrease in vascular function with time after surgery occurs more rapidly in those with ischemic cardiomyopathy. However, despite this additional group delineation based on disease etiology, the overall trend for declining vascular function in all of the HTx recipients with the passage of time remained essentially unchanged.

**Figure 4.** Resting brachial artery blood flow and reactive hyperemia (RH) with placebo and antioxidant supplementation after 5-minute cuff occlusion in healthy controls, heart failure patients with reduced ejection fraction (HFrEF), and 3 groups of heart transplantation (HTx) recipients. There were no significant differences at baseline, peak RH, or area under the curve (AUC) for either placebo (A) or antioxidant (B).

**Figure 5.** Quantitative assessment of antioxidant efficacy in healthy controls, heart failure patients with reduced ejection fraction (HFrEF), and 3 groups of heart transplantation (HTx) recipients. A, Plasma ascorbate. B, Thiobarbituric acid reactive substances (TBARS). Values are mean±SE. *Significantly different from controls. †Significantly different from placebo.
Although HTx improves central hemodynamics, an event likely to be favorable to vascular function, there are also a number of factors that may actually exert negative effects on the vasculature. These include ischemia,35 preservation and reperfusion effects at the time of transplant,36,37 impaired cardiovascular and pulmonary responses to physical activity,38,39 infections such as cytomegalovirus in the immunosuppressed patient,40 and other effects of chronic immunosuppression and complex long-term pharmacological therapy after transplant.41 Specifically, most HTx recipients are treated with calcineurin inhibitors, cyclosporine or tacrolimus.42 Previous studies have revealed that cyclosporine may result in endothelial dysfunction,43,44,50 because it has direct cytotoxic effects on the endothelium,43 impairs endothelium-derived relaxing factor release,44 and increases endothelin production.45 In addition, cyclosporine has been linked to increased sympathetic tone, new-onset hypertension, and peripheral vasocostriction in HTx recipients.46 These effects may, at least in part, be mediated by free radicals.22 Our data provide further support for the idea that long-term cyclosporine exposure contributes to endothelial dysfunction, because ≈75% of the >14-year post-HTx recipients were currently taking cyclosporine (Table 2), and there was a tendency for endothelin levels to be increased in this group.

**RH in HFrEF and HTx Recipients**

In contrast to FMD, the RH response represents both endothelial-dependent and -independent vasodilation of the microvasculature.47,48 We hypothesized that RH would follow a similar pattern to the FMD measurements, such that, when compared with controls, RH would be reduced in HFrEF patients, improve immediately after HTx, and progressively decline thereafter. In fact, resting limb blood flow and RH, both in terms of peak and area under the curve, were not different between groups (Figure 4). Thus, in this study, there was no evidence of microvascular dysfunction in the controls, HFrEF patients, or HTx recipients. These results are in contrast to many of the HF animal model studies that suggest that microcirculatory function is impaired,49,50 but the overall body of literature related to endothelial-independent vascular function in HF patients is equivocal.34,37,51 In addition, there is convincing evidence that endothelial-independent vasodilation is not attenuated in HTx recipients.20,21 Again, it should be noted that microvascular function (ie, RH) does not necessarily track conduit vessel endothelial function, which makes the divergent FMD and RH results not so surprising and suggests that further studies are necessary to better examine the changes in blood flow distribution in patients with HF.

**Free Radicals and Vascular Function in HFrEF and HTx Recipients**

We hypothesized that the administration of an acute AOC would attenuate the circulating levels of free radicals and improve vascular function. In this study, administration of the AOC did not alter FMD or RH response compared with placebo in the HFrEF patients or the HTx recipients who were <3 years and 5 to 10 years after HTx. However, the AOC increased FMD by 35% in the controls and by 55% in the group of HTx recipients furthest beyond transplant (>14 years; Figure 1). This finding suggests that, in these patients with markedly decreased FMD and the longest immunosuppressant use, the attenuation in vascular function can be improved by decreasing free radical concentration.

As direct measurement of NO bioavailability in humans is often not feasible vasomotor function (eg, FMD) or NO-related compounds are often measured as surrogates. Recognizing that such NO-related compounds vary in their biological activity, concentration, and compartmentalization among plasma, blood, and other tissues,52 we measured plasma nitrates and nitrites in the current study. Interestingly, plasma nitrates were not different between the controls and patients with HF but were significantly increased in all of the HTx recipients (Figure 6A). Also, despite the nitrates being elevated in all of the HTx groups, there was a significant decline in those that who were 5 to 10 years and >14 years post-HTx in a pattern similar to the FMD results. Plasma nitrites tended to be elevated in all of the patient groups compared with the
controls (Figure 6B), with the >14-year post-HTx group exhibiting significantly greater levels than the controls. There was also a tendency for a reduction in nitrates after AOC consumption in all of the patient groups (Figure 6B). Elevated plasma nitrates have been reported previously in HF patients, and the higher values in the HTx recipients could be the result of poor renal function and greater oxidative stress in these individuals.

Despite the confirmation of an AOC-induced increase in circulating antioxidant capacity in all of the subject groups by documenting an increase in plasma vitamin C levels (Figure 5A), there were no significant changes in TBARS, a marker of total oxidative stress (Figure 5C). However, there was an overall trend for TBARS to decrease in the control and HTx groups, but it is likely that the sensitivity of this assay was not adequate to detect acute changes in oxidative stress in the current study. Such conclusions about the TBARS assay are supported by Silvestro et al., who reported that, in patients with intermittent claudication, there was no relationship between TBARS and FMD after vitamin C infusion, suggesting that the TBARS assay is unable to accurately reflect acute changes occurring within the vasculature.

**Experimental Considerations**

It should be noted that the present study used a cross-sectional experimental design; however, although this approach comes with limitations, it was necessary to include a wide range of HTx recipients, some of whom were >20 years post-HTx. We also acknowledge that, although we matched the groups for age and many other factors, including etiology of HF, we did not control for variations in pharmacological therapies across these groups, which may have influenced our findings. In addition, HTx recipients who were 3 to 5 years post-HTx and 10 to 14 years post-HTx were specifically not recruited and, therefore, not studied; however, given our hypothesis that vascular function gradually declines as time post-HTx increases, this was necessary to differentiate our groups.

**Perspectives**

HTx is an accepted therapy that results in improved quality of life and dramatically better survival in patients with severe HF. Although survival after HTx has been improving over the past 2 decades, most of this has been a result of decreased mortality in the first posttransplant year. Approaches to improve long-term survival after HTx are, therefore, needed. This study extends the current understanding of the long-term effects of HTx on vascular function. These findings may help guide new approaches aimed at maintaining vascular health after HTx.

**Conclusions**

This study has documented that endothelial-dependent vasodilation, determined by FMD, is reduced in HFrEF patients, and by using a comprehensive, cross-sectional approach this study has revealed normal vascular function soon after HTx followed by a gradual decline to a level similar to HFrEF patients in the years beyond transplant regardless of previous disease etiology. Interestingly, the attenuated vascular function in HTx recipients 1 to 2 decades after transplantation is most likely related to decreased NO bioavailability, because an acute dosage of oral antioxidants, and a likely decrease in free radicals, significantly improves FMD in these subjects.

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**Disclosures**

None.

**References**


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**Novelty and Significance**

**What Is New?**
- There is relatively little information regarding peripheral artery endothelial function in HFrEF patients and HTx recipients at various time points after surgery.
- Endothelial-dependent vasodilation, determined by FMD, is reduced in HFrEF patients and is restored to normal soon after HTx, followed by a gradual decline to a level similar to HFrEF patients in the years beyond transplant, regardless of previous disease etiology.
- The attenuated vascular function in HTx recipients 1 to 2 decades after transplantation is most likely related to decreased NO bioavailability, because an acute dosage of oral antioxidants, and a subsequent decrease in free radicals, significantly improves FMD in these subjects.

**What Is Relevant?**
- Impaired endothelium-dependent vasodilation has been associated with various cardiovascular diseases, including hypertension, coronary artery disease, and even heart failure.
- Importantly, impaired endothelium-dependent vasodilation may also precede the development of these cardiovascular diseases.
- Hypertension is common in HTx recipients, therefore, determining levels of vascular function, and the role of NO may be beneficial in better understanding the mechanisms contributing to the abnormal pressures often seen in this patient population.

**Summary**
This cross-sectional study reveals that, compared with controls, vascular function is blunted in HFrEF patients and is similar soon after HTx, but decreases with greater time beyond HTx, with free radicals implicated in this progression.
Vascular Function and the Role of Oxidative Stress in Heart Failure, Heart Transplant, and Beyond
Melissa A.H. Witman, Anette S. Fjeldstad, John McDaniel, Stephen J. Ives, Jia Zhao, Zachary Barrett-O'Keefe, Jose N. Nativi, Josef Stehlik, D. Walter Wray and Russell S. Richardson

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