Alteration of humoral and peripheral vascular responses during graded exercise in heart failure

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Hammond, Robert L., Robert A. Augustyniak, Noureen F. Rossi, Karen Lapanowski, Joseph C. Dunbar, and Donal S. O'Leary. Alteration of humoral and peripheral vascular responses during graded exercise in heart failure. J Appl Physiol 90: 55–61, 2001.—We hypothesized that performance of exercise during heart failure (HF) would lead to hypoperfusion of active skeletal muscles, causing sympahtoactivation at lower workloads and alteration of the normal hemodynamic and hormonal responses. We measured cardiac output, mean aortic and right atrial pressures, hindlimb and renal blood flow (RBF), arterial plasma norepinephrine (NE), plasma renin activity (PRA), and plasma arginine vasopressin (AVP) in seven dogs during graded treadmill exercises and at rest. In control experiments, sympathoactivation at the higher workloads resulted in increased cardiac performance that matched the increased muscle vascular conductance. There were also increases in NE, PRA, and AVP. Renal vascular conductance decreased during exercise, such that RBF remained at resting levels. After control experiments, HF was induced by rapid ventricular pacing, and the exercise protocols were repeated. At rest in HF, cardiac performance was significantly depressed and caused lower mean arterial pressure, despite increased HR. Neurohumoral activation was evidenced by renal and hindlimb vasoconstriction and by elevated NE, PRA, and AVP levels, but it did not increase at the mildest workload. Beyond mild exercise, sympathoactivation increased, accompanied by progressive renal vasoconstriction, a fall in RBF, and very large increases of NE, PRA, and AVP. As exercise intensity increased, peripheral vasoconstriction increased, causing arterial pressure to rise to near normal levels, despite depressed cardiac output. However, combined with redirection of RBF, this did not correct the perfusion deficit to the hindlimbs. We conclude that, in dogs with HF, the elevated sympathetic activity observed at rest is not exaggerated by mild exercise. However, with heavier workloads, sympahtoactivation begins at lower workloads and becomes progressively exaggerated at higher workloads, thus altering distribution of blood flow.

meeting the perfusion requirements of active muscle as well as the need to supply sustentative flow to the brain, viscera, and all other tissues and to dissipate heat. A primary symptom of cardiac insufficiency is exercise intolerance; in moderate, compensated heart failure (HF), exercise capacity is reduced, and, in profound HF, it is nearly absent. Presumably, this is because the heart, vasculature, and autonomic nervous system are unable to resolve the competing demands of locomotion, organ perfusion, and thermoregulation.

As a normal dog proceeds from rest to dynamic exercise, circulatory adjustments occur in response to increased vascular conductance of the active skeletal muscle. These adjustments include diminution of vagal restraint of heart rate (HR) and a progressive increase in sympathetic outflow to the heart, which results in tachycardia and increased contractility, thus increasing cardiac output (CO) (16). At heavier workloads, secretion of renin, vasopressin, and norepinephrine (3, 9, 27) occurs that may induce vasoconstriction and increase blood pressure. As CO and blood pressure rise with the intensity of exercise, blood flow to the limbs increases (6, 8, 21), renal blood flow (RBF) remains nearly constant by reduction of renal vascular conductance (RVC) (8, 10, 21), and mesenteric blood flow is largely maintained (21) or moderately reduced (6) by adjustments to vascular conductance. Although the conductance of the active muscles is greatly increased, there is also evidence of functional vasoconstriction of the active musculature (13, 14). This limits the vascular conductance of muscle from exceeding cardiac pump capacity, thus preventing a precipitous fall in arterial pressure and circulatory collapse (19).

In the early 1970s, a group of investigators examined the response of dogs to severe exercise, using two major surgical models of cardiac dysfunction: 1) tricuspid valve avulsion with pulmonary artery banding and 2) atrioventricular block (8, 10, 21). A consistent finding in these studies was that, in normal dogs performing severe exercise, blood flow in the mesenteric and renal beds dipped at the onset of exercise and then recovered to normal levels. However, in dogs with cardiac insuf-

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iciency, blood flow to these beds was drastically attenuated during severe exercise. Thus, in extremis, blood flow was presumably shunted from visceral and renal circulation to the active muscles. However, in these studies, neither CO nor responses to mild and moderate workloads were measured.

In the 1980s, Wilson et al. (24) studied responses to graded treadmill exercise in dogs without and with cardiac insufficiency induced by rapid ventricular pacing (24). These experiments yielded important information regarding the cardiovascular and neurohumoral responses during exercise. With respect to measurement of vascular resistances in particular, they noted that, when the large increases in right atrial pressure (RAP) were included in their calculations, the observed changes in systemic and femoral vascular resistances were insignificant, despite a large reduction in CO. However, in the experiments of Wilson et al. (24), the study of regional blood flow was limited to a hindlimb; blood flow in nonmuscular beds was not studied. Furthermore, pacemakers were not disconnected during the exercise bouts, and CO was not continuously monitored. Because HR, stroke volume (SV), and peripheral vascular conductances are determinants of body perfusion, the intermittent collection of CO data, combined with a fixed HR of 260 beats/min, makes interpretation of the relationships among these variables difficult.

In the present study, we examined the systemic, hindlimb, and renal vascular responses and the neurohumoral secretions of chronically instrumented adult dogs at rest and during steady-state treadmill exercise from very mild to heavy workloads. The responses were characterized in good health and then again in HF produced by rapid ventricular pacing. We hypothesized that, on development of HF, muscle blood flow would be lower at rest and during exercise. Also, the mechanisms that normally favor distribution of blood flow to active (and possibly hyperperfused) muscles by limiting blood flow to nonmuscular beds would be elicited at lower workloads and become exaggerated at the higher workloads.

MATERIALS AND METHODS

Seven mongrel dogs (18–25 kg) of either sex were selected for the study, on the basis of successful adaptation to the laboratory environment and treadmill exercise. All aspects of the study complied with Wayne State University guidelines and the National Institutes of Health Guide to the Care and Use of Laboratory Animals.

Surgical Procedures

Instrumentation was implanted in the dogs in three sterile procedures. Animals were anesthetized with intravenous pentobarbital sodium (25 mg/kg body wt) and maintained with 1.5–2.5% isoflurane anesthesia. The right thorax was opened, and an 18- or 20-mm electromagnetic or ultrasonic blood flow probe (Zepeda Instruments or Transonic Systems) was placed around the aortic root to measure CO. Three insulated stranded stainless steel sutures (0 Flexon, Ethicon) were placed on the ventricular apex for subsequent pacing. The pericardium was closed loosely over the heart, and the cables and leads were exteriorized between the scapulae. Through a retroperitoneal incision, an 8- or 10-mm electromagnetic or ultrasonic flow probe (Zepeda Instruments or Transonic Systems) was placed on the terminal aorta to measure hindlimb blood flow (HLBF) after all vessels between the flow probe and the iliac arteries were ligated. A hydraulic occluder (InVivo Metric) was also placed around the aorta, distal to the flow probe, to provide the means to zero the electromagnetic flowmeter signal output during total aortic occlusion. Through a ligated lumbar artery a 20-gauge polyvinyl catheter (Tygon, SS4-HL, Norton) was introduced into the midthoracic aorta to monitor mean aortic pressure (MAP). A pulsed Doppler or ultrasonic blood flow probe (Crystal Biotech or Transonic Systems) was implanted on the left renal artery to measure RBF. A 20-gauge catheter (Tygon, SS4-HL, Norton) was introduced via an external jugular vein to measure RAP and to collect blood samples. Catheters were also inserted in a femoral artery and vein for other, separate studies. All leads and catheters were tunneled subcutaneously to the interscapular site for exit, and the wounds were closed while ligated.

Postoperative pain was controlled by parenteral administration of buprenorphine (0.02–0.04 mg·kg⁻¹·h⁻¹) and acepromazine maleate (0.1 mg·kg⁻¹·h⁻¹) as needed. Antibiotic therapy included prophylactic perioperative administration of cefazolin (500 mg iv) and postoperative treatment with cephalaxin (30 mg/kg body wt, by mouth, 2× per day). Cables and catheters were examined and dressed daily.

Exercise Protocol and Data Collection Methods

After full recovery from the surgeries, the dogs were brought to the laboratory, allowed to acclimate by roaming freely for 10–20 min, and then directed to the treadmill. The catheters were connected to pressure transducers (Spectramed DTX or Statham P-10) set at midheart level, and the blood flow probes were connected to their respective flowmeters (Zepeda SWF-5RD, Crystal Biotech or Transonic T206). HR was computed by tachometry of the CO signal. Data were recorded continuously on a Gould 3800 pen recorder and on two computerized data acquisition systems, for subsequent analysis as beat-by-beat averages and as raw waveforms sampled at 200 samples/s (AT-CEEDAS, Dataq Instruments, Akron, OH).

Experimental protocol. The exercise protocol was performed by each dog in control experiments and after induction of HF after 29 ± 2 days of continuous, rapid ventricular pacing at 225 beats/min (1-ms duration, amplitude of 2–5 V as needed to maintain capture). The pacemaker was disconnected only during the experimental sessions, ~10 min before collection of data. For every session, data were collected continuously after connection to the recording equipment until the end of the session. At standing rest, the dog stood quietly on the treadmill while baseline data were recorded. Arterial blood samples were drawn from the aortic catheters during selected experiments and immediately chilled for later determination of plasma renin activity (PRA) and plasma concentrations of arginine vasopressin (AVP) and norepinephrine (NE) (7, 14). The treadmill was then started at a speed of 3.2 km/h at 0% grade, and the hemodynamic parameters were observed until they remained stable (typically after 3–5 min). Blood samples were drawn again after the parameters stabilized. The same procedures were performed at progressively heavier workloads of 6.4 km/h at 0% grade, 6.4 km/h at 10% grade, and 8 km/h at 15% grade. Although positive verbal encouragement was provided by the laboratory staff, no negative reinforcement techniques were
used, and the exercise session was terminated if the dog could not sustain a steady gait.

**Data reduction and statistical analysis.** After the exercise bouts, the hardcopy and digitized data for each hemodynamic parameter were examined and reduced to the average value for 60 s, sampled at the steady state for each workload, usually just before the withdrawal of blood samples. In some HF runs at the highest workload (8 km/h at 15% grade), it was necessary to sample for shorter periods (~30 s) because the dogs were unable to maintain a steady gait. After validation of each individual experimental run, the data for each dog were averaged to determine the typical individual response for further analysis.

Blood flow data from the electromagnetic probes was corrected for zero drift and extended periods of implantation (1). Total vascular conductance \( TVC = CO / (MAP - RAP) \) and hindlimb vascular conductance \( HLVC = HLBF / (MAP - RAP) \) were calculated. To accommodate the difference of measurement techniques of renal perfusion, RBF was normalized for each dog as a percentage of the resting value observed in control experiments. Renal vascular conductance was calculated \( RVC = RBF / (MAP - RAP) \) and then likewise normalized. Individual averages of the data for each dog were combined to produce group means and standard errors of the means. For MAP, RAP, HR, SV, CO, RBF, and HLBF, statistical analysis consisted of a two-way repeated measures ANOVA, followed by comparisons of individual means by a C-matrix test for simple effects, with statistical significance deemed at \( P < 0.05 \), one-tailed. For PRA, AVP, and NE measurements and the calculated values of TVC, HLVC, and RVC, a Friedman two-way ANOVA for repeated measures was performed, followed by a Wilcoxon signed rank test, with statistical significance deemed at \( P < 0.05 \), one-tailed. Due to technical difficulties, we were unable to collect complete sets of measurements for all dogs; the numbers of measurements for each parameter are indicated in Figs. 1–3.

**RESULTS**

**Hemodynamic Responses**

For all measured parameters (MAP, RAP, HR, SV, CO, RBF, and HLBF), the repeated measures ANOVA indicated a significant interaction between HF and workload. In the control experiments (solid bars, Figs. 1 and 2), with each increase in exercise workload from rest, progressive increases in TVC and HLVC occurred due to muscular vasodilatation, and these were matched by concomitant increases in SV and HR that resulted in greater CO and HLBF. MAP did not differ from rest at 3.2 km/h at 0% grade, but, beyond this workload, MAP rose significantly with each increment of exercise intensity. RAP rose significantly with each increase in workload. RBF was not significantly different from that at rest for all workloads, although there was a tendency to rise during mild exercise. RVC did not change from rest through 6.4 km/h at 0% grade;
however, it fell significantly and progressively at the 6.4 km/h at 10% and 8 km/h at 15% workloads.

In HF, SV was significantly depressed at rest and during exercise at all workloads, and, despite significant tachycardia that was sustained through 6.4 km/h at 10% grade, this resulted in significantly lower CO (3.2 ± 0.26 vs. 2.6 ± 0.14 l/min). At 8 km/h at 15% grade, HR was slightly, but not significantly, higher than control, but CO was still significantly lower. MAP was significantly lower at rest than control (91 ± 3.7 vs. 82 ± 2.6 mmHg) and, as in the control experiments, did not differ from rest at 3.2 km/h at 0% grade. At 6.4 km/h at 0% grade and beyond, MAP was approximately the same as the control values at each workload. RAP was significantly elevated at rest and rose markedly at every workload. TVC tended to be somewhat lower than control at rest and during mild exercise, and this difference became statistically significant at the two highest workloads. HLBF was significantly lower at rest and during exercise, and HLVC was significantly lower than control values at rest and during exercise, until 8 km/h at 15% grade, when it was slightly lower than the control value. RBF was 50% of the control value at rest and fell steadily with each increment in exercise intensity, with significant drops at 6.4 km/h at 10% and 8 km/h at 15% grade. RVC fell insignificantly in transition from rest to 3.2 km/h at 0% grade and fell steadily beyond this workload, with significant reductions from rest occurring at 6.4 km/h at 10% and at 8 km/h at 15% grade.

**Neuroendocrine Responses**

In control experiments, PRA and NE were at basal levels at rest and rose insignificantly with exercise at 3.2 km/h at 0% grade (Fig. 3). However, PRA and NE rose progressively and significantly with each increment in workload beyond 3.2 km/h at 0% grade. AVP rose slightly but significantly from rest during exercise at 3.2 km/h at 0% grade, with progressive and significant increases occurring at each successive workload.

In HF experiments, PRA and NE were significantly elevated at rest with respect to the control values and increased slightly but not significantly with mild exercise at 3.2 km/h at 0% grade. The increases in PRA and NE did, however, approach statistical significance (P =
Responses at Rest

In the control experiments, the HR, aortic pressures, and the basal levels of PRA, AVP, and NE indicate that the dogs were well socialized to the environment. In HF, the condition of the animals was as previously reported (2, 23), with tachycardia and elevated RAP and depression of CO and MAP. However, TVC did not fall significantly, despite the vasoconstriction that occurred in the kidneys and hindlimbs, which decreased the blood flow to these areas. Other evidence of sympathoactivation included elevated plasma NE and PRA, as well as a small but statistically significant rise in AVP. It is unclear whether the observed increase in AVP (1.7 ± 0.5 pg/ml) raised blood pressure appreciably, inasmuch that this does not occur because of baroreflex buffering in normal dogs (5, 11). However, in HF, where hypotension exists and the baroreflexes are impaired, this concentration may have some minor pressor effect (11). Moreover, this elevation in AVP may have significant effects on the regulation of water balance in the longer term (12).

Responses to Exercise in the Control Studies

With the assumption of exercise in control experiments, the rise in muscle conductance by vasodilation and the muscle pump led to an increase in TVC at every workload, and this was matched by increases in ventricular performance (i.e., HR and SV) that increased CO. Beyond the mildest workload, this increase in CO was sufficient to raise MAP. RAP rose moderately at every workload as well, which was likely due to the muscle pump in conjunction with sympathetically mediated vasoconstriction and venoconstriction at higher workloads, thus increasing cardiac filling pressures during high contraction rates and enabling the rise in CO (17, 20). Also indicative of increased sympathetic activity at the higher workloads were progressive increases in release of renin and NE, as well as AVP, with substantial increases occurring at 8 km/h at 15% grade. Our results also confirm the work of previous investigators (8, 10, 21), in that, as CO and MAP rose during exercise, RVC decreased such that RBF was largely unaffected, with a slight rise during mild exercise followed by a slight fall to near the resting value with heavy exercise.

Responses to Exercise in the Heart Failure Studies

During exercise in HF conditions, CO was depressed because SV was diminished despite markedly elevated RAP, and the augmentation of SV during exercise by increased ventricular contractility was blunted. This was the principal factor in the attenuation of CO at every workload in HF despite tachycardia. In normal dogs, White et al. (22) showed that tachycardia alone does not increase CO. Whereas we did not directly explore this phenomenon, in this study, the observed tachycardia did not fully compensate for the SV deficit and the decrease of inotropic responsiveness.

The vascular and neurohumoral responses to exercise in HF show that further sympathoactivation does occur at a lower workload but in a graded manner, as in normal dogs. It does not appear that the sympathoactivation evident at rest was especially exacerbated by exercise at 3.2 km/h at 0% grade. As TVC rose secondary to the rise in vascular conductance of the activated muscles, the attenuated rise in CO did not significantly raise MAP beyond the resting value, as in the control experiments. As HLVC rose and HLBF increased, the muscle pump likely increased venous return to raise RAP. In addition, there were only minor, insignificant increases in NE, PRA, and AVP, and RBF fell only slightly from rest. Thus, on the basis of these observations, we conclude that there was no large increase in sympathetic tone at this workload. However, work performed at 6.4 km/h at 0% grade appears to be beyond the threshold for a substantial increase of sympathetic tone, in that there were marked increases in NE, PRA, and AVP that were similar to those observed at the heaviest workload under control conditions. Furthermore, MAP rose until it was approximately the same as control, despite a pronounced attenuation in the rise of CO. Also, TVC was approximately the same as control, but the modest reduction seen does not imply that further regional vasoconstriction did not occur. It may be that the rise in MAP that resulted from the attenuated rise in CO was also due, in part, to diversion of blood flow from the active muscles, as shown by the decrease in HLVC. However, only a minor diversion of RBF occurred at this workload. Furthermore, the maintenance of a near normal TVC at this workload resulted from a decrease in the arteriovenous pressure gradient, which was proportional to the fall in CO. The marked rise in RAP that accompanied the rise in MAP likely resulted from increased venous return from the active muscles via the muscle pump and from regional vasoconstriction and venoconstriction (i.e., a reduction in blood volume in the periphery).

At the two heaviest workloads, there was apparent exaggerated sympathoactivation in HF. TVC fell significantly, indicating that the rise in MAP to near normal levels was due to peripheral vasoconstriction as well as the attenuated rise in CO. The rise in MAP may
have also been due, in part, to the very large increases in neurohumoral secretions, which may have had direct pressor effects. At the concentrations observed in this study, the direct pressor effect of AVP has been previously demonstrated (4). Wilson et al. (25) studied the vascular response of isolated hindlimbs in anesthetized dogs with pacing-induced HF and high spinal sympathetic block to norepinephrine infusions. They demonstrated a 28-mmHg rise in hindlimb perfusion pressure and a 131% increase in vascular resistance under constant blood flow rate conditions, with an NE infusion rate that produced a venous plasma concentration comparable to that observed at the second highest workload in the present experiment. Thus, in the present study, the increase in MAP and the attenuation of HLVC during exercise may have been partially due to this direct vasoconstrictive effect. The data also indicate that blood flow at these workloads was progressively shunted away from the kidneys in rough proportion to the workload. Although the decrease in HLVC persisted for all but the highest workload, substantial increases in HLBF did occur. Concurrently, the conductance of blood through the renal beds fell from ~50% to ~30% of resting control values, resulting in progressive decreases in RBF. At the highest workload, the difference in HLVC between the control and HF states was insignificant, but RVC and RBF fell to ~20% of the control resting values. Indeed, in two animals, RBF fell to virtually zero at the highest workload. These data clearly indicate that the pattern of vasoconstriction was altered and, presumably, that the blood flow was redirected from the renal vascular beds to the active muscles in HF.

Perspectives

The origins and mechanisms responsible for the sympathoactivation observed in HF are not well characterized. At rest, there is little or no stimulus for sympathetic activation via the muscle metaboreflex (MMR). Thus it is likely that the sympathoactivation at rest resulted from baroreflex activation or other mechanisms.

Studies from this and other laboratories have shown that, in normal dogs, exercise at 6.4 km/h at 10% grade approximates the threshold of elicitation of sympathoactivating activity due to MMR activation (15, 17, 26). Below this workload, alterations in cardiac and vascular performance during exercise are thought to result chiefly from the influence of central command and the resetting of the arterial baroreflexes (16). In a recent study (7) of HF, we demonstrated that substantial reductions of HLBF were required to elicit the MMR at 3.2 km/h at 0% grade, indicating that the MMR was quiescent at this workload in HF. Thus it is likely that the sympathoactivation observed at 3.2 km/h at 0% grade in the present study also resulted from baroreflex activation and other mechanisms. However, at 6.4 km/h at 0% grade and beyond, MAP was only slightly lower than that observed in the control experiments and presented a relatively small stimulus for the arterial baroreflexes, although pulse pressure was also diminished due to the fall in SV. However, the deficit in HLBF to the exercising limbs in the present experiment likely created a substantial stimulus for metaboreflex activation, and indeed, the blood flow to the hindlimbs at 6.4 kph at 10% grade in HF was below the threshold of MMR activation in our previous study (7). Also, in the aforementioned study, we demonstrated that MMR activation by ischemia produced by partial aortic occlusion at 6.4 km/h at 10% grade resulted in a redirection of blood flow away from the kidneys in both the normal and HF states. Thus we propose that, at this degree of HF, mechanisms other than the MMR modulate cardiac and vascular performance at rest and during very mild exercise, but, at higher workloads, the MMR likely exerts a powerful influence on circulatory dynamics, leading to massive sympathoactivation with marked secretion of NE, PRA, and AVP. Moreover, because the gain of the arterial baroreflex is decreased in HF (28), we may further speculate that the normal restraining influence of the baroreflex on MMR was attenuated (18).

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REFERENCES

11. Montani JP, Liard JF, Schoun J, and Mohring J. Hemodynamic effects of exogenous and endogenous vasopressin at low


