Skeletal Muscle Reflex in Heart Failure Patients
Role of Hydrogen

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Background—An important role of the increased stimulation of skeletal muscle ergoreceptors (intramuscular afferents sensitive to products of muscle work) in the genesis of symptoms of exertion intolerance in chronic heart failure (CHF) has been proposed. With the use of selective infusions and dietary manipulation methods, we sought to identify the role of H\(^+\), K\(^+\), lactate, and peripheral hemodynamics on ergoreflex overactivation.

Methods and Results—Ten stable CHF patients (aged 67.9±2.5 years, peak oxygen uptake 16.3±1.2 mL·kg\(^{-1}\)·min\(^{-1}\)) and 10 age-matched and sex-matched healthy subjects were studied. The ergoreflex contribution to ventilation was assessed by post-handgrip regional circulatory occlusion (PH-RCO) and computed as the difference in ventilation between PH-RCO and a control run without PH-RCO. This test was performed on 6 separate occasions. On each occasion a different chemical was infused (insulin, sodium nitroprusside, sodium bicarbonate, dopamine, or saline) or a 36-hour glucose-free diet was undertaken before the test. During all stages of the protocol, the local muscular blood effluent concentrations of H\(^+\), K\(^+\), glucose, and lactate were assessed. An ergoreflex effect on the ventilatory response was seen in patients (versus control subjects) during the saline infusions (6.7±2.3 L/min versus −0.1±0.5 L/min, \(P<0.01\)). The only intervention to significantly lower the ergoreflex was sodium bicarbonate (0.4±0.3 L/min versus −0.2±0.4 L/min in control subjects, \(P=\)NS; versus saline \(P<0.05\)), which also reduced H\(^+\) concentration during exercise (47.4±1.3 versus 50.0±1.4 mmol/L on saline, \(P<0.05\)).

Conclusion—A reduction of the H\(^+\) concentration by infusion of sodium bicarbonate abolishes the increased ergoreceptor activity in CHF, suggesting a role of H\(^+\) in ergoreflex activation, either directly or indirectly. (Circulation. 2003;107:300-306.)

Key Words: heart failure ▪ muscles ▪ nervous system, autonomic ▪ reflex ▪ ventilation

The ergoreflex consists of a brain stem autonomic and ventilatory response activated by signals from the periphery, such as free nerve ending group III and IV muscle afferents.\(^1\) It has been hypothesized that muscle metabolic abnormalities and/or circulatory insufficiencies of chronic heart failure (CHF) cause local overproduction and accumulation of muscle metabolic products, which trigger the ergoreceptors.\(^2,3\) Our lab and others have confirmed this hypothesis by demonstrating that the muscle ergoreflexes of the forearm muscles,\(^4\) the small muscles used in dorsiflexing the foot,\(^5\) and the muscles of the quadriceps\(^6\) cause an enhanced ventilatory response in CHF. An overactivation of the muscle ergoreflex during exercise has been shown to be closely related to patient symptoms and to the ventilatory response to exercise, and a role of this reflex in contributing to exercise intolerance in CHF has been proposed.\(^4\)

The triggering mediators responsible for the ergoreflex stimulation and their involvement in the abnormal exercise responses in CHF remain to be established. Different metabolic and humoral products have been studied in animals\(^7\),\(^12\) and in humans,\(^13\),\(^15\) but few investigators have investigated factors specifically involved in the ergoreflex overactivation of CHF patients.\(^16\),\(^17\) One (or more) of these mediators may be involved as a link between alterations in muscle metabolism and/or hemodynamics present in CHF and the ventilatory response to exercise, via activation of muscle reflex.

We have investigated the role of the putative triggers in muscle ergoreflex activation. The identification of the biochemical factors contributing to this effect could lead to the development of treatments aimed specifically at reducing ergoreflex overactivation, which in turn may improve symptom control in CHF patients.

Methods

There are two possible ways to investigate this issue. One is based on measuring the reflex after interventions aiming at reducing the
TABLE 1. Clinical Characteristics and Baseline Values of the Study Population

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concentration or activity of a putative trigger. An alternative approach is to measure the reflex after interventions aiming at enhancing the putative factor.

On the basis of our own6,6 and others’ experiences,15,19 we have chosen the first approach to assess the role of extracellular hydrogen ion (H⁺), potassium (K⁺) (by sodium-bicarbonate and insulin-dextrose infusions), glucose metabolism (ie, glycogen-free diet), and peripheral vasodilatation (dopamine infusion). The second method has been selected to address the issue of role of vasodilatation on the reflex by infusing sodium nitroprusside.

Study Population

Ten patients with stable CHF due to ischemic heart disease or idiopathic dilated cardiomyopathy were compared with 10 age-matched healthy control subjects (Table 1). All patients were consecutively recruited from the outpatient Heart Failure Clinic at our institution, whereas control subjects were recruited from “The 316 Club,” which is comprised of ex-members of the executive of British Aerospace at Stevenage (England, UK). No subject from the control group had clinical signs or past history of heart or pulmonary diseases.

All CHF patients were symptomatic on exercise and limited by breathlessness or muscle fatigue. The study was approved by the local ethics committee and conformed to the Declaration of Helsinki. All subjects gave written informed consent.

Protocol

Each subject preliminarily underwent a clinical screening and a routine cardiopulmonary exercise (CPX) test to determine the subject’s exercise capacity and to gain familiarization with the laboratory environment. The CPX test was performed with a maximal symptom-limited, modified Bruce protocol (commencing at stage 0: 1.0 mph at 5.0% gradient) on a Marquette Case 15 treadmill.

Ventilatory data were recorded throughout the CPX and the ergoreceptor tests. Subjects breathed air through a mouthpiece and wore a nose clip, and ventilation variables were measured continuously.

All subjects then returned for 3 subsequent visits, 3 to 4 days apart, to perform the 6 ergoreflex tests (2 on each visit). The order of the interventions was randomized, with the exception of the glycogen-free diet test, which was always performed as the first test of the day and was followed by a normal meal.

All experimental sessions were performed in a temperature-controlled, air-conditioned room. The subjects were asked to avoid strenuous physical activity for 24 hours before each test and to refrain from eating, smoking, or consuming caffeine for 3 hours before the study. The test was preceded by 30 minutes in a quiet environment.

Ergoreceptor Test

A maximal voluntary handgrip contraction was measured as the greatest of the peak forces produced by three brief maximal handgrip contractions preliminarily performed before the test.

Each ergoreflex test consisted of a 5-minute resting period followed by 2 exercise sessions that were performed in a random order: (1) a 5-minute session of rhythmic handgrip achieved by squeezing the balloon of a sphygmomanometer (30 squeezes/min) at 50% of the predetermined maximal capacity; and (2) the same protocol followed by 3 minutes of blood-flow stasis (post-handgrip regional circulatory occlusion, PH-RCO) in the exercising arm induced by inflation of an upper-arm biceps tourniquet to 30 mmHg above systolic pressure at the beginning of recovery. This protocol has been shown to isolate the activity of the ergoreflex after exercise by “freezing” the chemical status of the muscle at peak exercise.6,6

For the calculation of the ergoreceptor activity, the changes in ventilation (L/min) between the second and third minute of PH-RCO recovery and the second- and third-minute control recovery without PH-RCO with regard to the corresponding mean baseline values were measured. The difference between these two changes was computed as a measure of the reflex activity.6 Figure 1 is a schematic representation of the methodology of the ergoreflex measurement.

Interventions

On each visit, the ergoreflex tests were preceded by the insertion of two venous lines: one in the nonexercising arm antecubital vein for infusion and one in the opposite exercising arm antecubital vein for blood sampling. In each subject, the same arm, the same infusion vein, and the same draining vein were used for all the tests. Before each test, one of the following interventions was performed:

- Saline (50 mL 0.9% NaCl, 60 mL per hour) as a control infusion;
- Sodium bicarbonate (4.2%, 1 mL·kg⁻¹·h⁻¹) to lower H⁺19;
- Dopamine (5 μg·kg⁻¹·min⁻¹) to increase cardiac output and increase peripheral vascular resistance;18
- Insulin infusion (10 U of Human Actrapid in 50 mL of 50% dextrose before the test) followed by dextrose (50 mL of 50% dextrose 50 mL per hour throughout the test) to reduce K⁺ concentration;19
- Sodium nitroprusside (0.5 μg·kg⁻¹·min⁻¹ in 50 mL 5% dextrose) to lower vascular resistance and to increase blood flow within the exercising districts;18
- Glycogen-free diet: The subjects were advised to adhere to a glycogen-free diet for 36 hours before the visit, to lower body lactate.20 The subjects were given a list of food substances to avoid. This regimen also included a low-glucose diet, inasmuch as subjects were also asked to not add any sugar to their daily diet. On this visit, only a single venous line was inserted in an antecubital vein in the exercising arm for blood sampling.

All the infusions started 15 minutes before the exercise and continued throughout the tests.

Data

Ventilatory Data

Ventilation (V̇E) and respiratory rate (RR) were measured continuously on-line with a calibrated heated pneumotachograph, whereas
oxygen uptake ($V_{O2}$) and carbon dioxide production ($V_{CO2}$) were measured breath-by-breath with a respiratory mass spectrometer (Amis, Innovision).

On the CPX test, peak $V_{O2}$ was computed as the average $V_{O2}$ values measured during the last 30 seconds of the exercise, whereas the slope of the $V_{E}/V_{CO2}$ ratio was calculated throughout the test as index of the ventilatory response.\textsuperscript{21}

**Blood Tests**

Blood was taken from the antecubital vein in the exercising arm during the last minute of each phase of the ergoreceptor test (last minutes of the resting phase, exercise handgrip, PH-RCO, and control recovery phases) and immediately placed on ice for subsequent analysis.

The blood concentrations of the following metabolites were assessed: $H^+$, $K^+$, and glucose by Bayer Rapidlab 800/865 analyzer (Bayer, H\textsuperscript{\textregistered}, reference value 35.50 to 44.70 mmol/L; $K^+$ reference value 3.50 to 5.30 mmol/L; glucose reference value 3.70 to 5.20 mmol/L); and lactate by lactate oxidase to catalyze the oxidation of lactate to monitoring oxygen consumption with an oxygen electrode with the Analox LM3 Analyser (Analox Instruments). The reference range in our laboratory is 0.6 to 2.5 mmol/L (coefficient of variation <6% at 2.3 mmol/L). $H^+$ and $K^+$ were assessed during all interventions. Glucose was assessed during the saline infusion and during the glycogen-free diet intervention. Lactate was assessed during the infusions of saline, sodium bicarbonate, dopamine, and the glycogen-free diet intervention.

**Heart Rate**

Heart rate was continuously monitored by standard ECG leads.

**Statistical Analysis**

The concentration of the measured metabolites, the ventilatory variables, and the ergoreflex contribution to ventilatory variables are presented as mean±SEM.

For both the ventilatory ergoreflex activity and the blood concentrations of metabolites, we compared the effect of each intervention versus the control effect of the saline infusion. Changes in these variables during the interventions were analyzed by repeated-measures ANOVA, and all pairwise comparisons were performed with the use of Dunn’s test. The changes in the blood metabolite concentrations from rest to exercise were analyzed by a paired t test. For the comparison of ergoreflex contribution to ventilatory variables between the control subjects and patients with CHF, an unpaired t test was used. If data did not follow a normal distribution, repeated-measures ANOVA on ranks, Wilcoxon test, and Mann-Whitney rank sum test were performed, respectively. A probability value <0.05 was considered significant.

**Results**

All subjects successfully completed the CPX and each of the 6 ergoreceptor tests. No adverse events occurred.

**Cardiopulmonary Exercise Test**

The patients demonstrated an impaired exercise tolerance (lower peak $V_{O2}$) and abnormally elevated ventilatory response to exercise (higher $V_{E}/V_{CO2}$ ratio) in comparison with control subjects (Table 1).

**Handgrip Exercise Test and Ergoreflex Test**

Equivalent levels of exercise were performed on each occasion of the 6 ergoreceptor tests. The average peak $V_{O2}$ was 0.40±0.02 L/min during the first exercise runs and 0.40±0.02 L/min during the second runs in CHF patients (P=NS) and 0.45±0.01 L/min during the first runs and 0.47±0.02 L/min during the second runs in control subjects (P=NS, P<0.05 CHF versus control subjects).

**Control Saline Infusion**

In both CHF and control subjects, exercise significantly increased blood concentrations of all measured metabolites with the exception of glucose concentrations in the CHF patients. Higher lactate and $K^+$ concentrations in the local effluent at rest and during exercise were detected in the patients compared with control subjects. Exercise $H^+$ was higher in patients than in controls (Table 2).

All ventilatory variables significantly increased on exercise in both study groups; heart rate also rose, but not significantly. RR was significantly more elevated in patients than in control subjects both at rest and on exercise (Table 3).

The contribution of the ergoreflex to ventilation was significantly increased in the CHF patients compared with control subjects: 6.7±2.3 L/min versus −0.1±0.5 L/min, P<0.01 (Figure 2).

**Effect of Interventions**

During interventions, exercise significantly increased blood concentrations of the measured variables in both study groups, with the exception of glucose concentration in CHF. Sodium bicarbonate infusion resulted in a decrease in the $H^+$ concentration in the venous effluent during exercise in
patients compared with saline; control subjects showed lower K⁺ concentration compared with CHF patients.

Dopamine lowered resting lactate concentrations in CHF patients compared with saline; control subjects showed lower K⁺ concentrations than did CHF patients. Insulin infusion led to a reduction in K⁺ concentration at rest and during exercise compared with saline infusion in both groups of subjects. Glycogen-free diet decreased resting and exercise serum lactate concentrations in patients compared with saline infusion in both groups of subjects. Glucose concentrations showed nonsignificant reductions in both groups of subjects (Table 2).

RR was consistently more elevated in patients than in control subjects during all infusions. However no intervention induced any significant effect on the ventilatory variables, with the exception of the glycogen-free diet regimen, which lowered VeCO₂, VeO₂, and VCO₂ in control subjects. Sodium nitroprusside infusion was the only intervention that significantly increased heart rate in both groups of subjects on exercise (Table 3).

The infusion of sodium bicarbonate virtually abolished the ergoreflex contribution to the ventilatory response in patients with CHF. None of the remaining interventions had a significant effect on the ergoreflex contribution to the ventilatory response. Instead, no intervention had any significant effect on the ergoreflex in control subjects (Figure 2).

Figure 3 represents the differences in responses in ventilation during saline infusion control, saline infusion with PH-RCO, and bicarbonate infusion with PH-RCO tests in a representative CHF patient.

Discussion

Background

Muscle fatigue and breathlessness are major symptoms of CHF and relate to a poor prognosis: Objectively, patients show reduced exercise tolerance and an increased ventilatory response to exercise, the mechanisms of which are still not completely understood.21 This clinical syndrome is characterized by abnormalities in skeletal muscle metabolism due to structural and functional changes, such as modifications in fiber type and decreased activity of oxidative enzymes.22,23 A neural link between peripheral abnormalities and reduced exercise tolerance has been postulated: the ergoreflex.4 The ergoreceptors are chemosensitive nerve endings located in the interstitium of working muscles responsible for the activation of the sympathetic drive, cardiovascular, ventilatory, and neuroendocrine responses to exercise.1

Controversies exist about the exact triggers of this muscle reflex. In experimental and human physiological studies, several putative metabolic and humoral triggers have been investigated.8–16 In particular, chemical products of muscle metabolism, such as increased production of lactic acid,7 H⁺,8,24 or increased blood release of K⁺,19,25 have been investigated. Alterations in peripheral vessel conductance have also been suggested.26

Even less is known about the putative factors activating the ergoreflex in CHF. The possibility that humoral factors with vasodilatory properties such as endogenous adenosine,16 prostaglandins, or bradykinin17 may be the potential trigger for the ergoreflex has been recently proposed.
TABLE 3.  Ventilatory Variables and Heart Rate at Rest and on Exercise During Interventions in Heart Failure Patients and
Control Subjects

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<th>Patients</th>
<th>Control</th>
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<td>V_{O_2} L/min</td>
<td>V_{CO_2} L/min</td>
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<td>Rest</td>
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<td>Rest</td>
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<td>0.29 ± 0.02</td>
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<td>0.40 ± 0.03†</td>
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<td>19.50 ± 1.50°</td>
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<td>77.80 ± 2.40</td>
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Values are mean ± SEM.
*P<0.05 vs control subjects; †P<0.05 vs respective rest; ‡P<0.05 vs respective saline.

Present Study
In the first instance, this study confirms that the ergoreceptor contribution to the ventilatory response to exercise was significantly increased in the patients with CHF compared with control subjects during control conditions (ie, saline infusion). This was associated with elevated H⁺ levels on exercise and increased lactate concentration, both at rest and during exercise, in keeping with the well-known observation of altered muscle metabolism present in CHF with early anaerobic metabolism on exercise.22,23 Moreover, elevated K⁺ blood levels were detected in the CHF group, which supports the important role of this ion in the abnormal ventilatory response to exercise in this syndrome.19

The major novel finding of our study is that a reduction of the serum H⁺ concentration by infusion of sodium bicarbonate completely abolishes the increased ergoreceptor activity in CHF patients; a crucial role of H⁺ ion in modulating the activity of the ergoreflex is suggested. In fact, during PH-RCO, the muscles are bathed in intracellular fluid, maintained in the same state as immediately after exercise, and this small difference in H⁺ ion concentration may contribute to the increased ergoreflex response observed in CHF patients. During exercise, the absolute difference in H⁺ concentration...
between patients and controls is small but still significant. It is not a novel concept that even small differences in H\(^+\) ion may explain the large changes in ventilation detected on exercise.\(^{27}\) We cannot exclude the possibility that incomplete diffusion of protons meant that the differences we detected were smaller than the true difference in pH at the receptor site within the muscle.

Thus, the increased ergoreceptor response in CHF can be interpreted as a reflection of increased proton production, reflecting a decreased aerobic capacity, particularly on exercise. In reality, the generation of H\(^+\) is associated with anaerobic exercise and is commonly attributed to the formation of organic acids, primarily lactic acid.\(^{28}\) However, in the present study, the reduction in lactic acid levels obtained by the glycogen-free regimen did not affect either H\(^+\) ion blood concentration or the ergoreceptor response, thus suggesting that lactic acid formation is not the major determinant of the activation of the muscle reflex. Other factors have been advocated as more important contributors to proton formation during anaerobic exercise, and in particular the hydrolysis of adenosine triphosphate (ATP) to adenosine diphosphate and Pi: This reaction is providing the energy for the muscle contraction. When the level of muscle work determines a rate of cytosolic ATP hydrolysis exceeding the rate at which the mitochondria can remove and/or utilize the products of this reaction, protons can accumulate.\(^{29}\)

Whatever the mechanism, either lactate production or hydrolysis of ATP, during heavy exercise cellular acidification occurs, which triggers the ergoreflex. This reflex exerts a protective feedback action favoring exercising muscle metabolism in conditions of elevated energetic demands by increasing the ventilatory drive, blood pressure, sympathetic activation, and vasoconstriction of the nonexercising beds.\(^1\)

Our findings do not prove that H\(^+\) is the sole and direct trigger of the muscle reflex. From our experiments and the experiments of other groups, we cannot exclude the possibility that products of muscle metabolism, facilitated by intracellular acidification, might activate such afferents. H\(^+\) ion may be responsible for the secondary formation of a direct trigger, or a different mediator may affect the sensitivity of the afferent fibers of the ergoreflex loop themselves, which consequently become more sensitive to H\(^+\) in CHF. The possibility exists that humoral factors with vasodilatory properties, the production of which is increased in conditions of intense exercise or muscle acidosis such as adenosine,\(^{16}\) prostaglandins, or bradykinin,\(^{17}\) act as potential stimulants of the ergoreflex in CHF patients, as has been recently proposed. Animal studies have suggested a triggering role of diprotonated phosphate, also considered mediator of muscle cell fatigue and vasodilatation.\(^7\)

The action of these vasodilatory substances is protective against tissue ischemia. In vasoconstrictive states such as heart failure, these substances serve as counterregulatory mechanisms to the potent vasoconstrictor sodium-retentive hormones, such as angiotensin, vasopressin II, and the sympathetic nervous system.

Although sodium bicarbonate infusion mainly exerts an antacid role, the possibility that muscle metabolic effects of this intervention (for example, tissue alkalization, which may improve skeletal muscle glycolytic ATP production and lactate efflux) might be involved in the reducing ergoreflex activity cannot be a priori excluded.\(^{30}\) However, both H\(^+\) and lactate concentration from the contracting muscle to the blood were \(\approx 5\%\) and 15\%, respectively, greater in the control saline trial in CHF patients. This contradicts greater efflux of lactate from contracting muscle during exercise sodium bicarbonate trial.

A potential role of extracellular K\(^+\) as a trigger of the muscle reflex, previously proposed,\(^{19}\) could not be supported by our findings, because K\(^+\) level reductions during exercise by insulin infusion failed to affect the reflex.

Interventions to improve muscular blood supply in our study failed to decrease metabolite levels and the ergoreceptor response, which indicates that blood flow may not be the limiting factor of reflex activity. Furthermore, anaerobic metabolism was not reduced by an improvement in blood supply to the muscle, which is in keeping with the well-established concepts that oxygen delivery is not the sole limiting factor of aerobic capacity in these patients.\(^{23}\)

**Limitations**

The lack of measurement of blood pressure during the intervention aiming at assessing the role of changes in peripheral hemodynamics is an important limitation of the study. Unfortunately, the presence of too many artifacts during the continuous noninvasive monitoring of blood pressure prevented accurate measurements, particularly during the exercise tests.

The detection of occasional elevated blood glucose levels in our subjects in the absence of a past history of impaired glucose tolerance might indicate that not all subjects adhered perfectly to the commitment to refrain from eating for 3 hours before the study. However, this did not preclude the glycogen-free diet significantly reducing lactate levels in both study groups.

**Conclusion**

An important role for H\(^+\) concentration in the blood returning from the exercising muscle in stimulating peripheral chemical afferents confirms the key role of muscle abnormalities because of anaerobic metabolism in contributing to the abnormal responses to exercise in CHF population. The results of this study do not support the concept that H\(^+\) is the sole muscle ergoreflex stimulant. It is likely that a number of other substances, including prostaglandins, bradykinin, and adenosine, the production of which is promoted by muscle acidification, could contribute either directly or indirectly. The relationship between these metabolic changes and the activation of the muscle ergoreceptors still warrants further investigations.

**Acknowledgments**

Adam Scott and Dr Piepoli are supported by The Wellcome Trust, Dr Wensel is supported by the Ernst Schering Research Foundation, and Dr Coats is supported by the Viscount Royston Trust.

**References**


Skeletal Muscle Reflex in Heart Failure Patients: Role of Hydrogen
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*Circulation*. 2003;107:300-306; originally published online December 23, 2002;
doi: 10.1161/01.CIR.0000042704.37387.29
*Circulation* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2002 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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