Muscle metaboreflex improves O₂ delivery to ischemic active skeletal muscle

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O'Leary, Donal S., Robert A. Augustyniak, Eric J. Ansorge, and Heidi L. Collins. Muscle metaboreflex improves O₂ delivery to ischemic active skeletal muscle. Am. J. Physiol. 276 (Heart Circ. Physiol. 45): H1399–H1403, 1999.—Ischemia of active skeletal muscle elicits a powerful pressor response, termed the muscle metaboreflex. We recently reported that the muscle metaboreflex pressor response acts to partially restore blood flow to the ischemic active skeletal muscle. However, because this reflex is activated by reductions in O₂ delivery rather than blood flow per se, gain of the metaboreflex as analyzed on the basis of blood flow alone may underestimate its true strength if this reflex also acts to increase arterial O₂ content. In conscious dogs chronically instrumented to measure systemic arterial pressure, cardiac output, and hindlimb blood flow, we activated the muscle metaboreflex via graded, partial reductions in hindlimb blood flow during mild (3.2 km/h) and moderate (6.4 km/h, 10% grade) workloads. At rest, during free-flow exercise, and with metaboreflex activation, we analyzed arterial blood samples for Hb concentration and O₂ content and compared muscle metaboreflex gain calculations based on the ability to partially restore flow with those based on the ability to partially restore O₂ delivery (blood flow × arterial O₂ content). During both mild and moderate exercise, metaboreflex activation caused significant increases in arterial Hb concentration and O₂ content. Metaboreflex gain quantified on the ability to partially restore O₂ delivery was significantly greater than that based on restoration of blood flow during both mild and moderate workloads (0.52 ± 0.10 vs. 0.39 ± 0.08, P < 0.05, and 0.61 ± 0.05 vs. 0.46 ± 0.04, P < 0.05, respectively). We conclude that the muscle metaboreflex acts to increase both arterial O₂ content and blood flow to ischemic muscle such that when combined, O₂ delivery is substantially increased and metaboreflex gain is greater when analyzed with a more integrative approach.

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WHEN O₂ delivery to active skeletal muscle is insufficient for the ongoing metabolic demands, metabolites accumulate and activate afferents within the ischemic skeletal muscle. Activation of these afferents elicits a powerful pressor response, termed the muscle metaboreflex. This pressor response is generated via vasoconstriction in nonactive vascular beds (e.g., kidney) and nonischemic active skeletal muscle and via increases in heart rate and ventricular performance that, combined with maintained or increased ventricular filling pressure, cause marked increases in cardiac output (1, 6–12, 17, 18, 23, 24). O'Leary and Sheriff (12) demonstrated in dogs during dynamic exercise that, in response to reductions in hindlimb blood flow by mechanical increases in hindlimb resistance (via graded partial inflation of a vascular occluder on the terminal aorta), the metaboreflex pressor response acts to partially restore blood flow to the ischemic skeletal muscle. Whether this reflex also acts to restore blood flow to ischemic muscle in humans is controversial (3, 16).

In the study by O'Leary and Sheriff (12), the strength or gain of the metaboreflex was analyzed via a closed-loop model quantified by the ability of the pressor response to partially restore blood flow to the ischemic active skeletal muscle. However, in an elegant experiment using dogs, Sheriff et al. (19) dissociated blood flow from O₂ delivery by pretreating the animals with carbon monoxide, which decreased arterial O₂ content. In this setting, the threshold level of hindlimb blood flow for activation of the reflex was shifted to higher blood flows, whereas the relationship between the pressor response and O₂ delivery (arterial O₂ content × blood flow) was unchanged. They therefore concluded that the muscle metaboreflex is activated by reductions in the washout of substances produced in proportion to the deficiency in O₂ delivery, not blood flow per se.

In dogs, the spleen acts as an important red blood cell reservoir. Arterial O₂ content can be increased by activation of sympathetic efferents to the spleen, causing constriction that will increase blood Hb concentration. Indeed, Vatner et al. (22) demonstrated in dogs that during severe exercise hematocrit increased >20% and that this increase was abolished by splenectomy. Interestingly, after splenectomy much greater increases in renal and mesenteric vascular resistances occurred in response to the severe exercise. The afferent mechanism(s) mediating the rise in sympathetic activity to the spleen during exercise is unknown. Potentially, this sympathetic activation during heavy dynamic exercise could be due to the muscle metaboreflex. Several studies from our laboratory and from others (9, 12, 17, 24) have shown in dogs that, whereas during
mild exercise hindlimb perfusion must be reduced below a clear threshold before metaboreflex responses occur, during moderate exercise no apparent metaboreflex threshold exists, e.g., any reduction in hindlimb blood flow elicits reflex responses. Thus, at heavier workloads, the muscle metaboreflex may be tonically active and may contribute to the increase in sympathetic activity.

Whether the muscle metaboreflex acts to increase blood Hb levels has not been investigated. Iasmuch as the metaboreflex pressor response is linked to O₂ delivery rather than blood flow, if even moderate increases in Hb concentration or P O₂, then G O₂ would equal G flow. However, if the muscle metaboreflex acts to increase arterial O₂ content (e.g., via increases in blood Hb concentration or P O₂), then G O₂ would exceed G flow. Experimental procedures. All experiments were performed after the animals had fully recovered from surgery and were active, afebrile, and of good appetite. The animal was brought to the laboratory and allowed to roam freely for ~20 min. The animal was then directed to the treadmill, the blood flow transducers were connected to a flowmeter (Transonic Systems), and the catheters were connected to a pressure transducer (Spectramed 10 E.Z. or Transpac IV, Abbott Laboratories). Heart rate was measured from the CO signal. All data were sampled with a laboratory computer at 1,000 Hz, and beat-by-beat mean values were saved to a hard disk for subsequent analysis.

The animals exercised at mild (3.2 km/h, 0% grade) or moderate (6.4 km/h, 10% grade) workloads. After all variables reached steady state, the terminal aortic vascular occluder was partially inflated to increase terminal aortic vascular resistance. After all variables had again reached steady state (3–6 min), another increase in vascular resistance was induced. At rest, during steady-state free flow exercise, and at steady-state with each level of partial vascular occlusion, arterial blood samples were drawn and blood gases, Hb concentration, and O₂ content were measured using a Radiometer ABL 500 blood gas analyzer interfaced to a Radiometer OSM3 hemoximeter. Analysis of restoration of blood flow and O₂ delivery by the muscle metaboreflex. The objectives of this experiment were to determine 1) whether the muscle metaboreflex elicits increases in arterial Hb concentration and O₂ content and 2) whether the gain of the muscle metaboreflex in terms of the ability to partially restore O₂ delivery to the ischemic skeletal muscle exceeds that based on restoration of blood flow alone. Metaboreflex gain based on the ability to partially restore blood flow was calculated as described by O’Leary and Sheriff (12). Briefly, with each level of partial vascular occlusion, total hindlimb vascular resistance (Rl) is increased. Rl is calculated as (SAP – CVP)/TAQ. This includes both the mechanical (e.g., vascular occluder) and vascular components of the hindlimb vascular resistance. With each level of partial vascular occlusion, the predicted level of TAQ (TAQp) was calculated as TAQp = SAP/Rl, where SAP is the initial level of SAP before each level of occlusion. Thus the closed-loop gain of the muscle metaboreflex in the ability to partially restore blood flow to the ischemic active skeletal muscle (Gflow) can be calculated on the basis of the observed TAQ (TAQo) and TAQp as Gflow = ((TAQo – TAQp)/(TAQo – TAQp j), where TAQo is the initial level of TAQ prior to each step increase in Rl. For example, if the reflex pressor response increased TAQo such that only one-half of the predicted decrease in TAQ occurred, then Gflow = 0.5. An analogous equation was used to calculate the closed-loop gain of the muscle metaboreflex based on the ability to restore O₂ delivery (G O₂). O₂ delivery to the hindlimbs was calculated as arterial O₂ content × TAQ. Thus the predicted and observed levels of O₂ delivery were calculated on the basis of the analogous values of TAQ and the observed levels of arterial O₂ content. The predicted level of O₂ delivery was calculated as the predicted level of TAQ times the O₂ content observed during free-flow exercise (e.g., no occlusion). Thus, if no changes in O₂ content occurred with metaboreflex activation, then G O₂ would equal Gflow. However, if the muscle metaboreflex acts to increase arterial O₂ content (e.g., via increases in blood Hb concentration or P O₂), then G O₂ would exceed Gflow. Statistical analysis. The steady-state levels of all hemodynamic variables were averaged over 1 min. Immediately after this 1-min interval, the blood samples were drawn. At each workload, the predicted versus observed levels of TAQ and O₂
The muscle metaboreflex quantified on the basis of the ability to partially restore O₂ delivery is greater than that based on the restoration of blood flow.

The muscle metaboreflex is elicited by the activation of metaboreceptors within the skeletal muscle (14, 15). A number of putative substances have been implicated in initiating this reflex, including H⁺, lactate, and diprotonated phosphate (4, 5, 13, 20, 21). Using the same animal model as in the present study, Sheriff et al. (19) investigated whether the reflex response to skeletal muscle ischemia was due to insufficient washout of substances that activate metaboreceptors versus insufficient delivery of O₂. They disassociated blood flow from O₂ delivery via decreasing arterial O₂ content with carbon monoxide and found that, based on blood flow, the relationship between the reflex response (e.g., increases in SAP) and blood flow to the ischemic muscle was shifted toward higher flows, whereas the relationship between SAP and O₂ delivery was not affected. They concluded that the muscle metaboreflex is elicited when O₂ delivery falls below a threshold level, thereby causing the accumulation of metabolites due to the lack of sufficient O₂.

Recently, O’Leary and Sheriff (12) quantified the extent to which the muscle metaboreflex can partially restore blood flow to the ischemic muscle as a method of assessing the closed-loop gain of the reflex. They con-

**DISCUSSION**

The major new finding of this study is that in conscious dogs during dynamic exercise metaboreflex activation causes significant increases in arterial Hb concentration and O₂ content such that the gain of the

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**RESULTS**

Table 1 shows the average levels of SAP, HR, CO, TAQ, arterial Po₂, Hb concentration, and O₂ content at rest and during free-flow exercise and the maximum levels observed with metaboreflex activation at both workloads. As shown previously, metaboreflex activation via imposed decreases in TAQ elicited substantial increases in SAP, HR, and CO. These responses were quite similar to those previously reported (10, 11, 17, 18). With the transition from rest to free-flow steady-state exercise, no change in Hb concentration or arterial O₂ content occurred during mild exercise, whereas significant increases in both occurred during moderate exercise. Importantly, metaboreflex activation caused significant increases in arterial Hb concentration and O₂ content over the levels observed during free-flow exercise during both mild and moderate workloads. No significant changes in arterial Po₂ occurred with either the transition from rest to exercise or with metaboreflex activation during either mild or moderate workloads.

Figure 1 shows the average values of metaboreflex gain in terms of the ability to partially restore blood flow (G₉ₒₒ) and O₂ delivery (Gₒₒ) to the ischemic muscle during mild and moderate exercise. During both mild and moderate exercise, Gₒₒ was significantly greater than G₉ₒₒ. There were no significant differences between workloads on the levels of either Gₒₒ or Gₒₒ.
cluded that the muscle metaboreflex acts with a closed-loop gain of ~0.4–0.5, meaning that ~40–50% of the blood flow deficit is corrected by the reflex. That study, like the present study, relied on TAQ as an index of blood flow to skeletal muscle. In dogs, ~85% of iliac blood flow is directed to skeletal muscle at rest (2), and with the large increases in TAQ with even mild dynamic exercise this fraction must increase considerably. Thus the partial restoration of TAQ observed previously (12) is likely directed to the active skeletal muscle. However, Sheriff et al. (19) previously demonstrated that the reflex is activated by reductions in O₂ delivery rather than blood flow per se. In the present study we found that with metaboreflex activation significant increases in arterial Hb concentration and O₂ content occurred such that, when combined with the partial restoration of blood flow, metaboreflex gain calculated in the basis of the ability to restore O₂ delivery was on average one-third greater than that based only on flow during both mild and moderate exercise. It should be noted that O₂ may be underestimated in this experiment due to possible movement of fluid from the interstitium into the capillaries within the ischemic hindlimb because of the fall in hindlimb arterial pressure that occurs with partial vascular occlusion (8, 18, 24). This fluid movement would cause a decrease in Hb concentration rather than the observed increase. In settings in which the metaboreflex may be tonically active without partial vascular occlusion (e.g., severe exercise), O₂ may be even greater.

Our data indicate that the mechanism mediating the rise in arterial O₂ content with metaboreflex activation was the increase in Hb concentration because no change in arterial Po₂ occurred. A likely mechanism for this increase in Hb concentration is metaboreflex-induced constriction of the spleen. In dogs, the spleen acts as a reservoir of red blood cells, and marked increases in hematocrit can occur with splenic constriction. Vatner et al. (22) observed that during severe exercise in dogs hematocrit increased from 40 to 49% and that this increase was abolished by splenectomy. Thus it is likely that with metaboreflex activation sympathetic activity to the spleen is increased. A previous study from our laboratory (17) showed that with metaboreflex activation right atrial pressure is maintained or increased despite the rise in cardiac output that would, by itself, decrease filling pressure, thereby indicating that this reflex increased central blood volume mobilization. Vasoconstriction of the compliant splanchic circulation (which includes the spleen) could cause both increased blood volume mobilization and increased blood Hb concentration. Vatner et al. (22) also observed that during severe exercise after splenectomy much greater vasoconstriction in the mesenteric and renal vascular beds occurred. Inasmuch as the increase in arterial pressure with the exercise was identical before and after splenectomy, this greater sympathoexcitation after splenectomy is likely not a consequence of the arterial baroreflex; rather, it is possible that the delivery of O₂ to the active skeletal muscle was decreased after splenectomy because no increase in hematocrit occurred with exercise. Reduced O₂ delivery to the active skeletal muscle could elicit an increased sympathetic activity via the muscle metaboreflex.

In summary, muscle metaboreflex activation during dynamic exercise elicits a pressor response that acts to partially restore blood flow to the ischemic active skeletal muscle. In addition, this reflex also acts to increase blood Hb concentration and arterial O₂ content. The combination of the increased flow coupled with the increased arterial O₂ content results in a greater metaboreflex gain when analyzed on the basis of the ability to partially restore O₂ delivery than that calculated only on the basis of blood flow. We believe that this integrative approach to analysis of the muscle metaboreflex better reflects the true strength of the reflex in the intact animal.

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