Low blood flow at onset of moderate-intensity exercise does not limit muscle oxygen uptake

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It has been suggested that blood flow determines muscle oxygen uptake in the transition from rest to exercise (39). Hughson et al. (20) studied intermittent static handgrip exercise and found that both blood flow and VO2 of the forearm increased more rapidly at the onset of exercise with the arm below compared with above heart level. On the other hand, there is good evidence to support that a limited extraction of oxygen by the contracting muscle cells causes the delay in oxygen utilization in the initial phase of exercise (3, 24, 25, 33, 41). In a study using an isolated in situ canine gastrocnemius muscle preparation, Grassi et al. (12, 13) observed that elevated oxygen diffusion or oxygen delivery did not change oxygen uptake in the initial phase of electrically induced muscle contractions, and muscle PO2 in rat skeletal muscle has been shown to be only moderately reduced in the first phase of exercise (4, 5). Furthermore, these observations are supported by findings in humans where the difference between muscle oxygen delivery and VO2 is greatest at the onset of exercise, and it becomes reduced to a constant level after ~15 s of exercise (3, 24, 25), indicating that oxygen supply is in excess of demand in the first phase of dynamic exercise and that oxygen delivery is not limiting VO2 of the contracting muscles. The possibility cannot be excluded, however, that a nonmaximal oxygen extraction by the contracting muscle in the initial phase of exercise is due to an inefficient flow distribution, i.e., hyperperfusion in areas of the muscle that were inactive (2). Thus it is still unclear whether oxygen availability to contracting muscles influences muscle VO2 at onset of dynamic exercise. One way to study this issue would be to reduce blood flow to the contracting muscle at onset of exercise. Pharmacological inhibition of nitric oxide synthase (NOS) and cyclooxygenase (COX), which leads to a reduction in the formation of nitric oxide (NO) and prostanoids, have been shown to reduce muscle blood flow during steady-state exercise (6, 31, 32). On the basis of the assumption that this effect on blood flow also exists in the initial phase of exercise, infusion of inhibitors of NOS and COX would be a useful model to study the effect of reduced blood flow on muscle oxygen uptake.

Therefore, the aim of the present study was to examine whether infusion of inhibitors of NOS and COX could lead to a reduction in muscle blood flow and influence the VO2 response in the exercising muscles in the initial phase of exercise in humans. Femoral arterial blood flow as well as arterial and venous oxygen content was frequently measured in subjects performing one-legged knee-extensor exercise with and without inhibition of NOS and COX. It was hypothesized that infusion of inhibitors of NOS and COX would reduce muscle blood flow.

Since Krogh and Lindhard (23) first showed that in the transition from rest to work in humans there is a certain transient state during which the pulmonary oxygen uptake (VO2) increases, a high number of studies on oxygen uptake in the initial phase of exercise have been performed. Nevertheless, the biochemical and physiological mechanisms responsible for the oxygen uptake at the onset of exercise are not fully understood (33). It is still unclear whether oxygen delivery limits muscle oxygen utilization at onset of exercise (2, 14). There appear to be conditions in which oxygen availability can reduce whole body oxygen utilization. Altered oxygen availability to the working muscles has been shown to cause a change in VO2 kinetics under conditions such as hypoxia (9, 26) or β-blockade (18). Furthermore, MacDonald et al. (28) observed that leg blood flow (LBF) and pulmonary oxygen uptake rose at a slower rate when knee extensor/flexor exercise was performed in the supine position compared with an upright position. In contrast, Williamson et al. (41) found that lower body negative pressure, leading to a significant reduction in
that a reduction in muscle blood flow and oxygen delivery in the initial phase of exercise would not affect muscle VO\textsubscript{2}.

**METHODS**

**Subjects.** Seven moderately trained male subjects with a mean ± SD age of 25 ± 4 yr, body weight of 79 ± 8 kg, height of 183 ± 6 cm, and VO\textsubscript{2,\text{max}} relative to body mass of 46.6 ± 5.7 ml·min\textsuperscript{-1}·kg\textsuperscript{-1} participated in the study. The purpose, nature, and potential risks were explained to the subjects before they gave their informed, written consent to participate in the study. The study was approved by the Ethics Committee of Copenhagen and Frederiksberg (KF 11289201) and conducted in accordance with the guidelines of the Declaration of Helsinki. The subjects were informed to abstain from caffeine, alcohol, and exercise for 24 h prior to the experiment.

**Experimental protocol.** Prior to the experiment the subjects visited the laboratory on two occasions. On the first visit the subjects were screened to ensure that they had a body mass index below 25, were normotensive, were nonsmokers, and were not taking any medications. The subjects also completed a session to become accustomed to the knee-extensor model (1) and to ensure that there was no inadvertent muscle activity during the passive movement of the leg as well as to make sure that the transition from passive movement of the leg to steady-state exercise was similar between trials. After being accustomed to the model, the subjects performed an incremental knee-extensor exercise test that consisted of 2 min at 15 W, after which the load was increased by 10 W every 2 min until exhaustion. Exhaustion was defined as the moment where the kinking frequency was below 55 rpm and the corresponding power output was defined as the maximal aerobic power output (W\textsubscript{max}). On the second visit to laboratory the subjects completed another exercise session to ensure that they were fully accustomed to the knee-extensor exercise.

On the day of the experiment the subjects arrived at the laboratory at 8:30 AM after a light breakfast. After 30 min in the supine position, subjects were fitted with catheters in the femoral artery and vein of the experimental leg and femoral artery of the nonexperimental leg under local anesthesia (lidocaine, 20 mg/ml). After 30 min of rest, the subject’s leg was moved passively for 1 min followed by 3.5 min of upright one-legged knee-extensor exercise (24 ± 1 W, i.e., 30% of W\textsubscript{max}) under two conditions: without (control; Con) or with infusion of indomethacin (Indo; COX inhibitor) + G-monomethyl-L-arginine (L-NMMA; NOS inhibitor) into the femoral artery [double blockade (DB)] to inhibit the formation of prostanooids and NO. The trials were separated by 30 min of rest, and due to the potential long-term effect of Indo and L-NMMA the control trial was performed first. Saline, Indo (150 mg·min\textsuperscript{-1}·kg mass\textsuperscript{-1}; Confortid, Alphapharma), and L-NMMA (4 mg·min\textsuperscript{-1}·kg mass\textsuperscript{-1}; Clinalfa) were infused into the femoral artery for 4 min prior to and during the 1 min of passive movement of the leg (loading dose) and were then infused at a rate of 50 mg·min\textsuperscript{-1}·kg mass\textsuperscript{-1} (Indo) and 2 mg·min\textsuperscript{-1}·kg mass\textsuperscript{-1} (L-NMMA) during the 1.5 min of exercise (maintenance dose). Blood samples (1–5 ml) were drawn simultaneously from the femoral artery of the nonexperimental leg and from the femoral vein of the experimental leg at rest, during the passive movement of the leg (immediately prior to exercise), and after 5, 10, 15, 20, 30, 90, and 210 s of exercise. LBF was measured at the same time as blood samples were drawn.

Data acquisition and analyses. LBF was measured with ultrasound Doppler (Vivid 7, GE Healthcare) as described previously (34). Heart rate (beats/min) was obtained from electrocardiogram, and arterial pressure (mmHg) was monitored with transducers positioned at the level of the heart (Pressure Monitoring Kit, Baxter, Deerfield, IL). Arterial and venous blood samples were immediately analyzed for PO\textsubscript{2}, PCO\textsubscript{2}, pH, oxygen saturation, and hemoglobin (ABL725, Radiometer, Copenhagen, Denmark). Leg mass of the experimental leg was calculated from the whole-body dual-energy X-ray absorptiometry scan-

**RESULTS**

**Cardiovascular responses.** At rest, LBF was 43 ± 10% lower (P < 0.05) in DB compared with Con (Fig. 1). LBF was 65 ± 6, 51 ± 8, 46 ± 7, 51 ± 6, 37 ± 10, 30 ± 7, 24 ± 5, and 25 ± 6% lower (P < 0.05) in DB compared with Con during passive movement of the leg and after 5, 10, 15, 20, 30, 90, and 210 s of exercise, respectively. The increase in leg and estimated m. quadriceps muscle blood flow during exercise was not different between Con and DB.

Preexercise mean arterial pressure (MAP) was ~94 mmHg and the end exercise value was ~112 mmHg. MAP was not different between Con and DB at any time.

**Leg oxygen uptake.** Arterial oxygen content was the same between Con and DB at rest, during passive movement of the leg, and after 5, 10, and 15 s of exercise but was lower (P < 0.05) in DB from 20 s to end of exercise (~20 s: 203 ± 2 vs. 199 ± 4 ml/l) (Fig. 2A). At rest, venous oxygen content was similar between Con and DB but was lower (P < 0.05) during passive movement of the leg and exercise (~5 s: 70 ± 5 vs. 142 ± 6 ml/l) in DB compared with Con.

At rest, leg oxygen extraction [arteriovenous (a-v) O\textsubscript{2} difference] was higher (P < 0.05) in DB compared with Con (103 ± 6 vs. 87 ± 7 ml/l) and remained higher during passive move-
ment of the leg and during exercise (5 s: 127 ± 3 vs. 56 ± 4 ml/l) (Fig. 2B). Estimated m. quadriceps oxygen extraction at rest was higher (P < 0.05) in DB compared with Con (103 ± 6 vs. 87 ± 7 ml/l) and remained higher to 30 s (5s: 140 ± 5 vs. 64 ± 5 ml/l) (Fig. 2C).

Leg oxygen uptake (Fig. 3A) was similar between Con and DB at rest, during passive movement of the leg, and during exercise. Similarly, estimated m. quadriceps oxygen uptake (Fig. 3B) was the same between Con and DB at rest, during passive movement of the leg, and during exercise. The leg and estimated m. quadriceps oxygen uptake at rest was not different from the oxygen uptake during the passive movement of the leg in both Con and DB.

At rest, leg oxygen delivery was similar between Con and DB, but was lower (P < 0.05) during passive movement of the leg in DB compared with Con. Throughout exercise leg oxygen delivery was 52 ± 7, 47 ± 6, 52 ± 5, 39 ± 10, 33 ± 6, 27 ± 5, and 26 ± 5% lower (P < 0.05) after 5, 10, 15, 20, 30, 90, and 210 s, respectively, in DB compared with Con. The difference between leg oxygen delivery and leg oxygen uptake was the same between Con and DB at rest but was lower (P < 0.05) during passive movement of the leg and exercise (5 s: 262 ± 39 vs. 59 ± 12 ml/min) in DB compared with Con (Fig. 4).

Blood gases and pH. Femoral arterial PO2 was above 100 mmHg throughout exercise with no difference between Con and DB (Fig. 5A). Femoral venous PO2 was similar between Con and DB at rest, but was lower (P < 0.05) in DB during passive movement of the leg and exercise (5 s: 39 ± 2 vs. 23 ± 1 mmHg).

Femoral arterial PCO2 remained at ~42 mmHg throughout exercise with no difference between Con and DB. Femoral venous PCO2 was the same between Con and DB at any time with start- and end-exercise values of ~52 and ~65 mmHg, respectively.

Femoral arterial pH remained at ~7.39 throughout exercise with no difference between Con and DB. No difference in femoral venous pH was detected between Con and DB with start- and end-exercise values of ~7.36 and ~7.30, respectively.

**DISCUSSION**

The major finding of the present study was that a marked reduction (~30–50%) in blood flow and oxygen delivery in
the initial phase of exercise did not affect leg \( \dot{V}O_2 \), suggesting that oxygen uptake in the initial phase of moderate-intensity exercise is not limited by oxygen delivery.

Leg blood flow and estimated quadriceps blood flow was reduced during the initial phase of exercise, allowing an examination of the effect of lowered muscle blood flow on muscle respiration at the onset of exercise in humans. It clearly shows that lowered oxygen delivery does not affect muscle oxygen uptake. In support of this finding, the difference between leg oxygen delivery and leg oxygen uptake in the first 20 s of exercise in Con was higher than after 180 s (Fig. 4), indicating that oxygen supply is in excess of demand in the initial phase of dynamic exercise. This finding is in accordance with a number of other knee-extensor studies (3, 24, 25) and it may be that a spatial and temporal heterogeneity of blood flow within the contracting muscle have occurred within the first phase of exercise as observed in animal studies (16). Then as exercise progresses respiration in the active fibers is elevated and blood flow is regulated to supply the active areas of the contracting muscle, leading to a closer match between oxygen delivery and oxygen uptake (29).

In DB the leg (a-v) \( O_2 \) difference was high prior to exercise, compensating for the lower blood flow, and remained high in the initial phase of exercise, leading to the same leg and quadriceps oxygen uptake as in Con (Fig. 3). Furthermore, the difference between oxygen delivery and oxygen uptake was much less than in Con, and an overshoot of oxygen delivery in the initial phase was not observed. It may be that a significant vasoconstriction already occurred in all the muscles of the leg before exercise and that the elevated blood flow during exercise was mainly directed to the active parts of the quadriceps muscle. Nevertheless, despite the restricted blood flow the quadriceps muscle oxygen uptake was as high as during the control condition. It should be noted that NO has been demonstrated to inhibit mitochondrial respiration by binding to the oxygen-binding site at cytochrome c oxidase in the electron transport chain (7). Furthermore, inhibition of NOS by the L-arginine analog nitro-L-arginine methyl ester (L-NAME) has been shown to speed the phase II pulmonary \( \dot{V}O_2 \) kinetics during moderate-intensity exercise in Thoroughbred horses and humans (21, 22). Therefore, it could be speculated that any possible effect of the reduced blood flow on muscle oxygen uptake in the present study was compensated by an elevated respiratory rate due to the inhibition of NOS. However, the infusion of L-NAME in the study by Jones et al. (21) had significant systemic effects and, because pulmonary measurements were performed, it is unclear to what extent the oxygen uptake of the contracting muscles were affected. In addition, despite the difference in the phase II time constant, the pulmonary oxygen appeared to be similar in the first phase of exercise in the study by Jones et al (21).

There are conditions where a reduction in oxygen delivery appears to slow muscle oxygen kinetics. A reduction of the local arterial perfusion pressure via supine exercise (19, 28) and lowering the blood flow to an exercising forearm by positioning the arm above heart level has resulted in lowered muscle oxygen uptake (20). A number of studies using pulmo-
nary measurements have also identified conditions in which oxygen uptake is reduced. However, caution should be taken when using pulmonary oxygen measurements to express muscle oxygen kinetics at onset of exercise, especially when muscle oxygen delivery is manipulated. Although an agreement between the phase II oxygen kinetics at the pulmonary and muscle level was obtained in a recent study, it was observed that the active muscles contributed to the phase I pulmonary oxygen kinetics (25). Furthermore, Essfeld et al. (10) have suggested based on modeling that the relationship between pulmonary and muscle VO₂ kinetics is sensitive to manipulations in muscle blood flow. Nevertheless, inspiring air with a reduced oxygen content (10–14%; 9, 26) and lowering cardiac output via β-blockade (18) have been shown to be associated with slower pulmonary VO₂ kinetics in the transition from rest to exercise. Apparently, there is a limit to how much oxygen delivery can be reduced without having an effect on muscle oxygen uptake (33), but the finding in the present study suggests that under normal conditions blood flow is not limiting for muscle oxygen uptake in the first phase of exercise. In accordance, Williamson et al. (41) observed that lower body pressure, leading to a significant reduction in leg blood flow during exercise, did not change pulmonary oxygen kinetics. It has been observed that hyperoxia (>60% inspired oxygen) accelerates the pulmonary VO₂ kinetic response during cycling exercise at work rates above, but not below, the ventilatory threshold (26, 27), suggesting that an enhanced oxygen delivery at high-intensity submaximal exercise can elevate oxygen uptake. The work rate used in the present study did lead to a steady state of oxygen uptake after 90 s, and it cannot be excluded that the VO₂ response in DB would have been reduced if the work rate used was higher.

Another interesting finding in the present study was that a pharmacological blockade of COX and NOS reduced blood flow in the initial phase of exercise. We have previously shown that single inhibition of COX (31) or NOS (35) does not lead to a reduction in blood flow during steady-state exercise. Conversely, when both enzyme systems are inhibited simultaneously blood flow is significantly reduced (6, 31, 32), which may reflect that there is a redundancy in these two systems, i.e., an impaired synthesis of one of these compounds is compensated by the synthesis of the other. The reduced blood flow during DB in the initial phase of exercise in the present study may therefore be related to the inhibition of both NOS and COX, suggesting that NO and vasodilator prostanoids are essential for exercise hyperemia in the initial phase of exercise.

The fact that the increase in blood flow was not completely attenuated in the initial phase of exercise with DB does indicate that other vasodilator mechanisms (34) and a widening of the arteriovenous pressure gradient generated by the skeletal muscle pump (38) also contribute to exercise hyperemia in this phase. It should be noted that NO may not only be enzymatically derived from NOS but may also originate from red blood cells (30) and from nitrite reduced to NO by deoxygenated hemoglobin (8), and it is also unlikely that the efficacies of the inhibitors used in the present study were 100% (11). Therefore, the role of prostanoids and NO may be even greater than observed in the present study.

Perspectives and significance. The present findings provide strong evidence for blood flow and oxygen delivery not being limiting for oxygen uptake of the contracting muscles at the onset of moderate-intensity exercise in humans. Future studies using higher work rates are warranted because the increased energy expenditure would mandate a higher muscle respiration whereby muscle blood flow and oxygen delivery may be limiting muscle oxygen uptake. In addition, to study the individual role of NO and prostanoids studies of blood flow and oxygen uptake in the initial phase of exercise with single inhibition of NOS or COX are also needed.
GRANTS

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DISCLOSURES

No conflicts of interest are declared by the author(s).

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