Metabolic response in different muscle types to reduced blood flow during exercise in perfused rat hindlimb

PAUL M. WALKER, JAN-PETER IDSTRÖM, TORE SCHRERSTEN AND ANN-CHRISTIN BYLUND-FELLENIUS

Surgical Metabolic Research Laboratory, Department of Surgery I, Sahlgrenska Sjukhuset, University of Göteborg, Göteborg, Sweden

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Summary

1. In peripheral arterial insufficiency, leg blood flow during exercise is reduced. The aim of this study was to investigate the metabolic response in different muscle types during exercise at reduced versus normal exercise blood flow.

2. A modified rat hindlimb perfusion model was used. Muscle metabolites and distribution of labelled microspheres were analysed in the soleus and the gastrocnemius muscles during exercise induced by sciatic nerve stimulation.

3. Blood flow distribution between the soleus and the gastrocnemius muscles (per unit weight) was 1.7:1 at rest, and this ratio did not change significantly during exercise at reduced flow.

4. There was a more pronounced decrease in the [phosphocreatine], the [glycogen] and the [ATP]/[ADP] ratio as well as a more pronounced increase in the [lactate] and the [lactate]/[pyruvate] ratio in the gastrocnemius muscle during exercise at reduced blood flow as compared with values obtained at normal exercise flow. In the soleus muscle the difference between the two conditions was confined to an increased [lactate]/[pyruvate] ratio.

5. The results show that a muscle composed mainly of fast-twitch fibres with a high glycolytic and low oxidative capacity is much more susceptible to a reduced exercise flow than a muscle composed of slow-twitch, oxidative fibres. It is suggested that claudicating pain is related to these metabolic changes and it is concluded that pain most probably originates in type II fibres.

Key words: blood flow, exercise, gastrocnemius, intermittent claudication, muscle fibres, soleus.

Introduction

The performance of skeletal muscle during exercise is to a large extent dependent on sufficient blood flow. Thus, when blood flow is reduced physical performance is limited. The characteristic symptom of ischaemic pain in arterial insufficiency called 'intermittent claudication' is confined to the calf muscles and sets a definite limit to the muscular performance [1-3]. The immediate cause for the development of the pain is not known, nor is it known how various muscle-fibre types respond to a reduced exercise blood flow.

The aim of the present study was to investigate the metabolic response in the rat soleus and gastrocnemius muscles as well as the blood flow distribution between these muscles during exercise at normal and reduced blood flow. For this purpose a modified rat hindlimb perfusion model was used [4]. Muscle metabolites reflecting the energy and the redox state were analysed in the muscle tissue after exercise induced by sciatic nerve stimulation. Blood flow distribution between the muscles was evaluated by injection of labelled microspheres [5].

Materials and methods

Chemicals. Glucose, pyruvate and other reagents were manufactured by the Sigma Corp. (St...
Inorganic compounds were manufactured by Merck (Darmstadt, West Germany). Bovine albumin (fraction V) was obtained from Johnson and Company AB (Malmö, Sweden). Pig insulin (lot no. S-834098) was from Nova Kemi AB (Farsta, Sweden) and labelled with 125I. Inorganic compounds were from New England Nuclear Corp. (Boston, MA, U.S.A.).

Animals. Sprague-Dawley rats (n = 24), weighing 235-260 g, from Anticimex (Stockholm, Sweden) were used. The rats were fed with Purina chow ad libitum and remained in normal cages until the experiments were performed.

Perfusion technique. The modified hindlimb perfusion technique has been presented in detail previously [4], and, therefore, a summary will be given here.

Operative procedure. The rats were anaesthetized by interperitoneal injection of Nembutal, 30 mg/kg (Packo Company ACO, Sweden). The left hindlimb was skinned and a small electrode was placed around the sciatic nerve. The tendons of the gastrocnemius, plantaris and soleus muscles were isolated at the ankle, tied together, cut free from the heel and attached to a small metal ring connected to a force displacement transducer. The femoral artery and vein were dissected free and cannulated in the groin. After injection of 0-2 ml of sodium chloride solution (154 mmol/l: saline), containing heparin concentration of 1 mg/l, the arterial cannula was immediately connected to the arterial inflow. The animal was then moved into the thermostatically controlled cabinet (37°C) and killed by an intracardiac injection of Nembutal.

Muscle stimulation. Exercise was induced by electric stimulation of the sciatic nerve. Square wave impulses, at a frequency of 4 Hz and a duration of 0-3 ms, from a Grass ST-5 stimulator were used. The amplitude of the muscle contractions was recorded by a force displacement transducer attached to the muscles. In each rat the resting tension of the muscles was adjusted with a micrometer screw to achieve the maximal contraction amplitude. During the exercise period the voltage was adjusted to maintain this amplitude as constant as possible (2-0-3-0 V).

Perfusion medium. The perfusate consisted of Krebs–Henseleit high bicarbonate buffer [6], pH 7-4, bovine albumin (fraction V), 40 g/l, and aged, rejuvenated [4] human erythrocytes to a haemoglobin concentration of 120 g/l. The pH was adjusted to 7-40 immediately before the perfusion was started. Glucose was added to a concentration of 5-5 mmol/l. Pyruvate was added to a concentration of 0-08 mmol/l, which gave an initial lactate/pyruvate ratio of approximately 15. The insulin concentration used was 0-1 unit/l. In some experiments phenolamine (Regitin, Ciba-Geigy) was added to the perfusate to a final concentration of 1 mg/l.

Perfusion system. From the reservoir, which was maintained at +37°C, the perfusate was pumped through a semipermeable tube (Silitastic Brand, Dow Corning Corp., Midland, MI, U.S.A.) contained in a glass bottle, gassed with oxygen/carbon dioxide (95:5). The oxygenated medium was returned to the reservoir. Through a second channel in the pump (Multiperpex Pump 2115, LBK Beckman, Stockholm, Sweden) the medium was pumped through an infusion filter into the rat hindlimb. The arterial pressure was monitored just before the arterial inflow to the rat. The venous outflow drained to a separate container, which allowed collection of venous blood samples and blood flow determinations by timed collection of 1 ml of blood. The perfusable was not recycled.

Metabolite analyses. Blood samples for determination of glucose and pyruvate were deproteinized and neutralized as described previously [4]. Glucose was determined with a Boehringer Mannheim Kit and lactate and pyruvate by conventional enzymatic methods as described by Lowry & Passoneau [7]. Blood samples for analysis of \( P_O_2 \), \( P_CO_2 \) and oxygen saturation were kept in glass syringes on ice and analysed by means of an Astrup Automatic Blood Gas Analyzer (Radiometer Company, Copenhagen, Denmark). The oxygen consumption was calculated from the known \( P_O_2 \), oxygen saturation, haemoglobin concentration and blood flow. The frozen muscle samples were freeze-dried at -20°C, stored at -80°C and extracted as described previously [4] for determination of metabolite concentrations (ATP, ADP, AMP, phosphocreatine, lactate and pyruvate). The metabolites were analysed fluorimetrically by enzymatic methods as described by Lowry & Passoneau [7]. Glycogen was determined in a portion of the freeze-dried muscle as described by Hultman [8].

Microsphere analyses. The microsphere technique was adapted from studies by Reneman & Verheyen [5]. The fresh muscle biopsies were weighed and the radioactivity (c.p.m./g of muscle) was counted in a gamma-counter (Palle Medico Technique Company, Copenhagen, Denmark). The values were corrected for the ‘spill over’ from the \( ^{103}Ru \) to the \( ^{14}Ce \) channel.

Statistical analyses. The non-parametric Mann–Whitney U-test was used to compare two independent samples [9].

Experimental procedure. The experimental procedure is summarized in Fig. 1. Perfusion at a...
blood flow of 0.5 ml min\(^{-1}\) g\(^{-1}\) of muscle was performed for 20 min at rest. The first 10 min was considered as an equilibration period, after which the actual experiment was started. The 10 min resting period was followed by 10 min of contractions (exercise) induced by sciatic nerve stimulation. At the start of the exercise period the blood flow was increased to approximately 1.1 ml min\(^{-1}\) g\(^{-1}\) of muscle, in the rats designated "normal blood flow", and decreased to approximately 0.3 ml min\(^{-1}\) g\(^{-1}\) of muscle in the rats designated "reduced blood flow". Twelve rats were perfused, six with a reduced blood flow and six with a normal blood flow. Arterial and venous blood samples for determination of glucose, lactate and oxygen were taken at 5 min intervals during the resting as well as the exercising period. At the completion of the 10 min exercise period, samples of the soleus muscle and the lateral portion of the gastrocnemius muscle were clamp-frozen with tongs cooled in liquid nitrogen. Control samples (at rest) were obtained from a separate group of rats. All muscle samples were immediately frozen in liquid nitrogen and analysed for the concentrations of ATP, ADP, AMP, phosphocreatine, lactate, pyruvate and glycogen.

To study the blood flow distribution between the soleus and gastrocnemius muscles at rest and at reduced exercise flow, labelled microspheres (15 µm diameter) were injected in the arterial inflow in six separate rats. Microspheres labelled with \(^{141}\)Ce (10 µCi), dispersed in 0.5 ml of saline, were injected into the arterial inflow after 7 min of perfusion at rest. Microspheres labelled with \(^{198}\)Ru (10 µCi) were injected in the same way after 7 min of exercise at reduced blood flow. The total injection time was about 1 min. No rise in the arterial pressure was observed after these injections. At the end of the exercise period, muscle samples from the soleus and the gastrocnemius were taken for determination of radioactivity.

Phentolamine was added (final concentration 1 mg/l) to ensure adequate muscle perfusion [10–12]. To evaluate the effect of this drug on the blood flow distribution and the metabolic state of the muscle tissue, six rats were perfused at rest and at reduced exercise flow without addition of phentolamine to the perfusate. Blood samples for determination of oxygen consumption and muscle biopsies for determination of muscle metabolites and radioactivity were taken as described above.

### Results

Glucose uptake, lactate release and oxygen consumption during perfusion at rest, at normal exercise flow and at reduced exercise flow are shown in Table 1. Glucose uptake, lactate release and oxygen consumption were significantly higher during exercise at both normal and reduced blood flow compared with the values at rest. Because of the fourfold difference in the blood flow, the delivery of glucose as well as of oxygen was correspondingly reduced. In spite of this glucose uptake was the same during exercise at both flow rates, and oxygen consumption was significantly lower during exercise at reduced flow.

The amplitude of the muscle contractions at the end of the 10 min exercise period was 82.2 ± 3.7% of the initial amplitude at the normal flow rate, whereas the corresponding figure at the reduced flow rate was 69.2 ± 4.0% (P < 0.1). The total amount of contractile work, calculated as the area under the curve, did not, however,

<table>
<thead>
<tr>
<th>Blood flow (ml min(^{-1}) g(^{-1}) of muscle)</th>
<th>Glucose uptake (µmol h(^{-1}) g(^{-1}) of muscle)</th>
<th>Lactate release (µmol h(^{-1}) g(^{-1}) of muscle)</th>
<th>Oxygen consumption (µmol h(^{-1}) g(^{-1}) of muscle)</th>
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<tbody>
<tr>
<td>(1) Rest, normal blood flow (n = 12) 0.53 ± 0.02</td>
<td>12.7 ± 0.6 2.90 ± 0.82 20.5 ± 1.1</td>
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<td>(2) Exercise, normal blood flow (n = 6) 1.07 ± 0.12</td>
<td>24.9 ± 2.9 39.3 ± 8.8 60.6 ± 7.0</td>
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<tr>
<td>(3) Exercise, reduced blood flow (n = 6) 0.29 ± 0.01</td>
<td>22.3 ± 2.0 44.9 ± 8.3 39.3 ± 2.9</td>
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<td>(2) vs (3) P &lt; 0.001</td>
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TABLE 2. Metabolite levels in soleus muscles from control rats at rest, and from rat hindlimbs perfused at normal and reduced blood flow during exercise induced by sciatic nerve stimulation

Mean values ± sem are shown. Abbreviations: PCr, phosphocreatine; L, lactate; P, pyruvate; N.S., not significant.

<table>
<thead>
<tr>
<th>ATP</th>
<th>ADP</th>
<th>AMP</th>
<th>PCr</th>
<th>L</th>
<th>P</th>
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<td>(μmol/g dry wt.)</td>
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(1) Controls, rest
(n = 12)
20.4 ±0.7 2.1 ±0.1 0.3 ±0.0 60.4 ±2.8 5.5 ±0.7 0.3 ±0.1 147 ±0.3 20.3 ±12 ±2.0 9.7 ±9.4

(2) Exercise, normal blood flow (n = 6)
21.1 ±0.8 2.0 ±0.2 0.3 ±0.0 49.6 ±7.2 14.5 ±3.2 0.8 ±0.0 130 ±17 ±17 ±3.2 ±1.3

(3) Exercise, reduced blood flow (n = 6)
23.2 ±0.7 2.2 ±0.1 0.3 ±0.0 54.7 ±4.1 13.1 ±1.7 0.4 ±0.1 135 ±7 ±4.6 ±12 ±0.8

N.S. N.S. N.S. N.S. <0.02 <0.001 N.S. N.S. N.S. N.S. N.S.

N.S. N.S. N.S. N.S. <0.001 N.S. N.S. N.S. <0.005 N.S. N.S.

N.S. N.S. N.S. N.S. <0.001 N.S. N.S. N.S. <0.001 <0.005 N.S.

TABLE 3. Metabolite levels in gastrocnemius muscles from control rats at rest, and from rat hindlimbs perfused at normal and reduced blood flow during exercise induced by sciatic nerve stimulation

Mean values ± sem are shown. Abbreviations: PCr, phosphocreatine; L, lactate; P, pyruvate; N.S., not significant.

<table>
<thead>
<tr>
<th>ATP</th>
<th>ADP</th>
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<th>PCr</th>
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(1) Controls, rest
(n = 12)
28.8 ±0.6 2.1 ±0.1 0.2 ±0.0 94.1 ±4.8 6.4 ±0.7 0.3 ±0.0 145 ±0.3 10 ±10 ±3.7 ±0.6

(2) Exercise, normal blood flow (n = 6)
23.7 ±1.9 1.8 ±0.2 0.4 ±0.1 62.1 ±10.9 ±8.2 ±0.2 ±28 ±15.0 ±15.0 ±2.3

(3) Exercise, reduced blood flow (n = 6)
23.1 ±0.8 2.5 ±0.2 0.5 ±0.1 43.1 ±8.9 ±24 ±0.1 ±15 ±124 ±1.5 ±1.5

N.S. N.S. N.S. N.S. <0.02 <0.02 N.S. N.S. N.S. N.S. N.S.

N.S. N.S. N.S. N.S. <0.002 <0.001 N.S. N.S. <0.001 <0.005 <0.05 N.S.

N.S.

Differ significantly between the groups at the normal versus the reduced exercise flow (2.45 ± 0.12 and 2.37 ± 0.12 respectively).

The metabolite levels at rest and after exercise are shown in Tables 2 and 3 for the soleus and the gastrocnemius muscles respectively. During exercise at normal blood flow a significant increase in [lactate] and [pyruvate] was observed in the soleus as well as in the gastrocnemius muscles. A significant decrease in the [ATP]/[ADP] ratio was also observed. Except for a significant increase in the [lactate]/[pyruvate] ratio no further changes were observed in the soleus muscle at reduced exercise flow as compared with normal exercise flow. In the gastrocnemius muscle, however, exercise at reduced blood flow caused more pronounced changes in the muscle metabolites. Thus, a significant decrease in the [phosphocreatine] as well as in the [glycogen] and the [ATP]/[ADP] ratio was found. There was also a marked increase in the [lactate] and the [lactate]/[pyruvate] ratio. Injection of labelled microspheres showed that the soleus muscle was supplied with about twice as much blood per unit weight than the gastrocnemius muscle (ratio 1.7:1) at rest. This ratio was not significantly changed during exercise at reduced blood flow.

Oxygen consumption and the blood flow distribution between the soleus and the gastrocnemius muscles were not significantly changed by omitting phentolamine from the perfusate. No significant changes in metabolites were found either, except for a somewhat more pronounced decrease in the [phosphocreatine] in the soleus muscle at the end of exercise at reduced blood flow in the absence of phentolamine.

Discussion

The perfusion technique used in the present study has previously been described and characterized [4]. Thus, it was demonstrated that adequate
metabolite levels were maintained in the skeletal muscle tissue during perfusion at rest at a blood flow of 0.5 ml min$^{-1}$ g$^{-1}$ of muscle and during muscle stimulation (4 Hz) at a blood flow of 1.1 ml min$^{-1}$ g$^{-1}$ of muscle. These flow rates were consequently chosen as normal levels for the present investigation. To study the metabolic consequences of exercise at reduced blood flow, one-quarter of the normal exercise flow rate was chosen. This particular flow rate was chosen to mimic the situation in patients with intermittent claudication, who often have a blood flow as low as 10–25% of the normal during exercise [1–3].

Phentolamine is an $\alpha$-adrenoceptor blocker, which is used to eliminate vasoconstriction of the vascular bed, thereby giving an optimal perfusion of the muscle tissue [10–12]. A comparison of circulatory and metabolic changes after perfusion with and without phentolamine in the perfusate revealed no major differences, showing that the vasodilatation obtained after the animal was killed is enough to obtain optimal perfusion of the muscle tissue. The blood flow distribution will under these circumstances reflect merely the vascularization of the muscle, i.e. the capillary density. The similar blood flow distribution between the soleus and the gastrocnemius muscles, at rest and at reduced exercise flow, confirms this conclusion. When the external flow rate is reduced a proportional decrease of the blood flow to all the muscles perfused can therefore be expected.

A 40% reduction of the oxygen consumption in the leg was obtained at reduced exercise flow compared with that at normal exercise flow. This shows that the oxygen delivery at the lower flow rate was too low to maintain the oxidative metabolic rate in the leg. The muscle work-recordings show, however, that the amount of contractile work was the same under both conditions.

Glucose uptake in the leg increased during exercise by the same amount at both flow rates. Thus, in spite of a 75% reduction in glucose delivery, glucose uptake was maintained at the reduced blood flow. We have previously found the glucose uptake to be proportional to the glucose delivery at insulin concentrations of both 0.1 unit/l and 10 units/l [4]. A maintained glucose uptake in spite of a reduced blood flow as found here, therefore suggests an additional stimulus for the glucose uptake. A prime candidate for this is a decreased energy state in the muscle tissue. Indeed, we previously observed significant correlations between the glucose uptake and the tissue [ATP]/[ADP] ratio, [lactate]/[pyruvate] ratio and [phosphocreatine] [4].

Lactate release increased during exercise, but no additional increase was noticed at reduced exercise flow. An increased lactate release might have been expected with the reduced flow, since the oxygen uptake was decreased while the contraction intensity was maintained. Accordingly, an increased lactate concentration in the gastrocnemius muscle was found. If lactate release from rat muscle is saturated at a concentration of 4–5 mmol/kg wet weight, as reported for human muscle [13], this would explain why no further release of lactate occurs when the lactate concentration in the gastrocnemius muscle increased from approximately 5 to 20 mmol/kg wet weight when the exercise flow was reduced.

A marked difference in the metabolic response to reduced exercise flow was observed between the soleus and the gastrocnemius muscles. Thus, reduction of the blood flow during exercise caused no changes in the soleus muscle in addition to the exercise per se. In the gastrocnemius muscle, on the other hand, exercise at reduced blood flow caused a further decrease in [phosphocreatine] and the [ATP]/[ADP] ratio and substantial increases in [lactate] and the [lactate]/[pyruvate] ratio, as compared with values in exercise at normal blood flow. These different responses can be explained by the different characteristics of the two muscle types. The lateral portion of gastrocnemius is composed of 95% fast-twitch fibres, of which two-thirds have a high glycolytic, low oxidative capacity (FG), and one-third has a high glycolytic, high oxidative capacity (FOG) [14]. The soleus muscle on the other hand is composed of 84% slow-twitch fibres with an intermediate oxidative capacity (SO) [14]. The soleus muscle is supplied by approximately twice as many capillaries and has a resting blood flow twice as high as that of the gastrocnemius muscle [15]. The high blood flow to the soleus supplies this muscle with oxygen in excess of the demands both at rest and during exercise. The maintained metabolic state in this muscle during exercise at reduced blood flow suggests that oxygen delivery was sufficient for the metabolic demands at this blood flow as well.

The distribution of blood flow between the two muscles was the same at reduced exercise flow as at rest. This excludes the possibility of a redistribution of the blood flow in favour of the soleus muscle, as an explanation of the maintained metabolic state in this muscle. The gastrocnemius muscle thus also received about 50% of the blood flow to the soleus at the reduced exercise flow. This means that the gastrocnemius muscle does not have the same margin of oxygen
delivery as the soleus muscle, which explains the more pronounced changes in the former in response to the reduced blood flow. The increased lactate production, together with the increased glycogen breakdown, demonstrate that the gastrocnemius muscle relies to a large extent on anaerobic glycolysis and that glycogen is an important substrate in this condition.

In a recent study [1] we investigated the metabolic changes in the gastrocnemius muscle of patients with intermittent claudication, during acute leg exercise. We found a decrease in the [ATP], the [phosphocreatine] and the [ATP]/[ADP] ratio, and an increase in the [lactate]/[pyruvate] ratio. Thus the changes in metabolites in this muscle were qualitatively the same in the exercising rat hindlimb with an artificially reduced blood flow and in vivo in the patient performing exercise simulating walking.

The nomenclature of human skeletal muscle fibre types is different from that of rat muscle. The human fibre types are usually designated type I and type II, where the former is slow-twitch and has a high oxidative capacity and the latter is fast-twitch and has a high glycolytic capacity [16]. Type II fibres can be further divided into the subgroups A and B, differing mainly in their oxidative capacity. During submaximal low-intensity exercise in man, mainly type I fibres are thought to be recruited [17], but at higher intensities recruitment of type II as well as type I fibres has been shown [18, 19]. Accordingly, in response to repeated exercise, biochemical and morphological changes have been shown in both fibre types [20, 21], in agreement with the findings of adaptive changes in SO, FG as well as FOG populations in five mammals.

In patients with peripheral arterial insufficiency adaptive changes similar to those observed with repeated exercise have been shown, i.e. increased activities of oxidative enzymes [1, 24], increased mitochondrial content in both type I and type II fibres [25] and an increased capillary density mainly in type II fibres [26]. These findings suggest that both fibre types are recruited during the patients' daily physical activity and that the adaptive changes are most prominent in the type II fibres.

The present study shows that a muscle composed of fast-twitch fibres is much more susceptible to a reduced exercise flow than is a muscle composed of slow-twitch fibres. Owing to the less dense capillary supply of the former, oxygen delivery will always be lower at a certain blood flow and will sooner reach a critical level when the blood flow is reduced. The subsequent increase in the redox state, as shown in the [lactate]/[pyruvate] ratio, and the decrease in the [ATP]/[ADP] ratio will further accelerate the glycogen breakdown and the lactate production. The low blood flow will also impair the washout of waste products and thereby aggravate lactate accumulation. It is suggested that the pain of claudication is directly related to these metabolic changes and that the prime candidates for the location of this pain are the type II fibres.

Acknowledgments

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References


