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Original Article

Lactate concentrations in human skeletal muscle biopsy, microdialysate and venous blood during dynamic exercise under blood flow restriction

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Abstract The intramuscular microdialysate lactate concentration during dynamic exercise with various degrees of blood flow restriction and its relation to lactate concentration in skeletal muscle biopsy and venous blood were studied. Nine healthy males performed three one-legged knee extension exercises (Ex 1–3). Blood flow was restricted stepwise by applying supra-atmospheric pressure over the working leg. Microdialysate mean (range) lactate concentrations at the end of the exercise periods were 3.2 (0.5–6.6), 4.4 (1.1–9.8) and 7.9 (1.1–11.6) $mmol \cdot l^{-1}$ during unrestricted, moderately restricted and severely restricted blood flow respectively. There was a significant correlation between microdialysate and venous lactate concentrations at the end of all three exercise periods. Microdialysate lactate concentration correlated significantly to skeletal muscle biopsy lactate concentration at the end of Ex 1. In conclusion, microdialysate lactate concentration in the

working muscle increased step-wise with increasing blood flow restriction. It showed a better correlation to venous than to muscle biopsy lactate, which is possibly partly explained by the characteristics of diffusion between body compartments and differences in time resolution between the methods used.

Keywords Human · Ischaemia · Lactate · Microdialysis · Muscle biopsy

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Introduction

It is very useful to be able to measure the metabolic status in human skeletal muscle. Apart from its worth in exercise physiology, it could prove to be important in the evaluation of patients with peripheral arterial occlusive disease, since grading of blood flow restriction in these patients is often a major problem. The diagnostic methods currently used are not specific or sensitive enough to accurately identify patients with limb-threatening ischaemia. The identification of metabolic changes preceding tissue necrosis in ischaemic limbs could be a means of improving both knowledge of the pathophysiology of limb ischaemia and patient selection for reconstructive surgery.

In patients with intermittent claudication, lactate in muscle biopsy samples has been shown to increase during exercise, in parallel with a reduced oxygen partial pressure in the tissue [1]. In acute limb ischaemia lactate levels were found to be elevated and of prognostic importance for limb survival [2]. Increased lactate release in venous blood from the symptomatic limb has been shown to occur in patients with rest pain or ischaemic ulcers, while patients with claudication were found to have normal levels [3]. Thus, lactate can be an important marker for ischaemia – i.e. an insufficient blood flow to meet the metabolic demand of the tissue – and determination of tissue lactate levels could prove to be a useful aid in identifying patients with ischaemia and grading the severity of the blood flow restriction.

The introduction of the microdialysis technique has enabled us to continuously sample fluid with minimal invasiveness, and to analyse substances from the interstitial space in, for example, the brain, adipose tissue, heart and skeletal muscle in animals and humans [4]. This technique could be an attractive option in patients with critical limb ischaemia, as wound healing in these patients is impaired and the muscle biopsy technique therefore carries a risk.

An experimental model designed to mimic the blood flow restriction encountered in patients with peripheral arterial occlusive disease has been described previously [5]. In this model muscle ischaemia is induced by the application of positive pressure over a working leg. Muscle biopsy lactate and venous lactate have been shown to correlate to the degree of blood flow restriction applied [6, 7].

There is no published study, to our knowledge, in which the microdialysate concentration of lactate at various degrees of blood flow restriction has been investigated and compared to lactate in other body compartments in dynamically contracting human skeletal muscle.

The aims of the present study were to investigate the effect of various degrees of experimentally restricted leg blood flow on microdialysate lactate concentration and the relationships between microdialysate, skeletal muscle and venous lactate concentrations under the same conditions.

Materials and methods

Subjects

Nine healthy male volunteers took part in the present study. Mean (range) age, height, weight and number of physical exercise hours/week were 24 (22–27) years, 182 (169–195) cm, 74 (63–90) kg and 3 (0–5) h. The study was approved by the Ethics Committee of the Karolinska Institute. The experimental protocol was explained to all subjects and their consent was obtained before inclusion.

Exercise model

We used the model introduced by Eiken and Bjurstedt [5] in which it is possible to reduce blood flow during one-legged bicycle exercise in a controlled fashion by applying supra-atmospheric pressure over the lower body. With 50 mmHg applied, exercise blood flow is reduced by 15–20% [7]. The subjects performed one-legged knee-extension exercise with the lower part of the body inserted in a pressure chamber, which was sealed at the level of the groin. The work load was selected individually during a familiarization exercise, aiming at a constant work load that would lead to exhaustion at the end of the experiment when blood flow was restricted. This work load [mean (range) 14 (10–20) W] was then used for all exercise periods in each individual.

Study protocol

Two different experiments were performed. All nine subjects participated in the blood flow restriction experiment (R), where external application of pressure – 30 and 50 mm Hg – over the working leg restricted blood flow during part of the experimental session [7]. In this way exercise was performed at three different levels of blood flow (see below). Five of the subjects also took part in a control experiment with non-restricted blood flow (NR).

Each experiment started with the insertion of two microdialysis catheters into the vastus lateralis muscle of the working leg. The subjects rested for 1 h and before the onset of exercise they were positioned in the chamber opening. Three 15-min exercise periods (Ex 1–3) were performed. Ex 1 was carried out under normal atmospheric pressure. In the R-experiment 30 and 50 mmHg supra-atmospheric pressure, respectively, was applied over the working leg during Ex 2 and Ex 3.

In the NR-experiment, normal atmospheric pressure was applied throughout the experiment including the exercise periods (Ex 1–3). The subjects rested for 10 min – under normal atmospheric pressure – between the exercise periods.

Microdialysate samples were collected every 5 min.

In the R-experiment four muscle biopsy samples were taken from the vastus lateralis muscle: immediately (5–10 s) after each exercise period and 10 min after the last exercise period.

Microdialysis

The microdialysis technique is based on the principle that a perfusion fluid is equilibrated with the interstitial fluid by diffusion through a semi-permeable membrane. The microdialysis catheter, placed inside a steel cannula, is inserted percutaneously. Withdrawal of the steel cannula leaves the microdialysis catheter in the tissue. A perfusion fluid is pumped at low speed by a precision pump through the catheter under an outer semi-permeable cylindrical membrane. At the far end of the catheter a small opening allows the fluid – the microdialysate – to escape into the inner impermeable plastic tube through which it leaves the catheter to be collected in fractions in vials. For a detailed description of the technique see [4].

In the present study we used a catheter with diameter 0.5 mm and membrane length 30 mm (CMA 60, CMA, Solna, Sweden). The perfusion fluid had the following composition: Na^+ 147 mM, K^+ 4 mM, Ca^{2+} 2.3 mM, Cl^- 156 mM; osmolality 290 mosmol·kg^{-1}. Flow rate was 2 µl·min^{-1} and the pump used was a CMA 107, (CMA, Solna, Sweden).

Two microdialysis catheters were placed in the vastus lateralis muscle 2–3 cm apart, 10 cm proximal to the knee joint space at a 45° angle to the surface of the skin with the tip proximally. Insertion was performed with the knee joint flexed midway between maximum extension and the flexion angles used in the experiment. Withdrawal of the catheter was first performed with the leg still attached to the exercise equipment. Early in the experimental series one catheter broke during withdrawal. This led to a slight modification of the withdrawal procedure such that – before catheter removal – the working leg was first detached from the exercise equipment in order to assure full relaxation of the muscle. After this, the withdrawal procedure was problem-free. Approximately 5% of the catheters suffered membrane damage, probably due to trauma from muscle motion leading to blood contamination of the microdialysate and/or arrest of flow. These samples were not analysed. Microdialysis samples were stored at –20°C and subsequently analysed using the CMA 600 Microdialysis Analyser (CMA, Solna, Sweden) for glucose, lactate, glycerol and urea. The CMA 600 utilizes enzymatic reagents and colorimetric detection for all four substances simultaneously.

The mean concentration in the two vials corresponding to the last 5 min of pre-exercise rest, Ex 1–3 and post-exercise periods was used for statistical comparisons. The calculated time delay from catheter to vial was 1 min at the flow rate used and this was compensated for when timing the collection of the microdialysate.

Muscle biopsy samples

The percutaneous needle biopsy technique was used to obtain samples from the vastus lateralis muscle of the working leg [8]. Biopsy samples were taken from a position 2–6 cm proximal to the tips of the microdialysate catheters. The biopsy samples were frozen in isopentane precooled with liquid nitrogen and stored at –70°C until later analysis. Muscle lactate was analysed in neutralized perchloric acid muscle extract by a fluorometric enzymatic method [9]. The biopsy results from one of the nine subjects were not included in the statistical analysis because of technical errors in the analysis.

Blood samples

Blood was drawn from a vein in the ante-cubital fossa at the end of the pre-exercise rest period, at the end of Ex 1–3 and 10 min post-exercise in the R-experiment. Blood lactate concentration was analysed in neutralized perchloric acid extracts of whole blood by a fluorometric enzymatic method [9].

Statistics

Unless otherwise stated, values in the text are means ±range. For statistical comparisons, the mean of the microdialysate concentrations from the two simultaneously sampled vials was calculated, see above for detailed description. A two-factor analysis of variance was applied to compare differences between the R- and the NR-experiments (n=5). The interaction term was accepted as statistically significant at $P<0.05$ and in the subsequent contrast analysis Bonferroni correction was used. Corrected α-level for repeated comparisons was set at $\alpha=0.05/2$. A one-way analysis of variance was applied to compare differences between the three degrees of blood flow restriction in microdialysate lactate (n=9) and muscle biopsy lactate (n=8) concentrations. For post-hoc comparisons of means, Scheffés test was applied. Linear regression was applied to analyse the relationship between the concentrations of muscle biopsy lactate, microdialysate lactate and venous blood lactate simple.

Results

Microdialysate metabolite concentrations before exercise

The 1-h equilibration period was adequate to achieve a stable concentration of lactate (Fig. 1). The concentrations in the microdialysate of all metabolites were generally higher during exercise than during rest (Figs. 1, 2A–C).

Fig. 1. Microdialysate lactate concentrations (mmol·l^{-1}) in 5-min fractions during the experiment including exercise with blood flow restriction (R-experiment, n=9, ■) and the control experiment with non-restricted blood flow (NR-experiment, n=5, ▲). Before, in between and after the three 15-min one-legged knee-extension exercise periods (Ex 1–3, *shadedboxes*) the subjects were resting. In the R-experiment, normal atmospheric pressure, 30 and 50 mmHg supra-atmospheric pressures were applied over the working leg during the Ex 1, Ex 2 and Ex 3 periods, respectively, and ↑ denotes time points when muscle biopsy samples were taken. In the NR-experiment, normal atmospheric pressure was applied during all three exercise periods. Values are means ±SE

Fig. 2. Microdialysate glucose (**A**), glycerol (**B**) and urea (**C**) concentrations in 5-min fractions during the experiment including exercise with blood flow restriction (R-experiment, n=9, ■) and the control experiment with non-restricted blood flow (NR-experiment, n=5, ▲). Values are means ±SE. For explanation of other symbols, see Fig. 1

Microdialysate lactate concentration

In the R-experiment, the mean (range) microdialysate lactate concentrations were 3.2 (0.5–6.6), 4.4 (1.1–9.8) and 7.9 (1.1–11.6) mmol·l^{-1} at the end of Ex 1, Ex 2 and Ex 3, respectively (Fig. 1). One-way ANOVA showed a general difference between the exercise periods (P<0.001), i.e. a higher lactate concentration the more blood flow was restricted.

In the five subjects that performed both experiments the increase in microdialysate lactate concentration in the R-experiment was significantly greater than the corresponding change in the NR-experiment (P=0.004, Fig. 1). The increase from Ex 1 to Ex 2 in microdialysate lactate concentration in the R-experiment was significantly greater than the corresponding change in the NR-experiment (P=0.01). The difference between the R- and the NR-experiment in lactate concentration increase between Ex 2 and Ex 3 was almost significant (P=0.05).

Microdialysate glucose, urea and glycerol concentrations

During exercise, the microdialysate concentrations of glucose, glycerol and urea (Fig. 2A–C) were higher than during rest but there was no general difference between the R- and the NR-experiments for any of these substances.

Muscle biopsy lactate

In the R-experiment, the mean (range) muscle lactate concentrations were 21 (7–48), 31 (13–67), and 48 (8–86) mmol·kg^{-1} dry muscle at the end of Ex 1, Ex 2, and Ex 3 respectively. One-way ANOVA showed a general difference between time points (P=0.033), and the post-hoc comparisons showed that the muscle lactate concentration was greater at the end of Ex 3 than at the end of Ex 1 (P=0.04).

Ante-cubital vein lactate

In the R-experiment the mean (range) lactate concentration in a vein in the ante-cubital fossa was 1.3 (0.8–2.1), 2.0 (1.2–4.1), 2.6 (1.3–5.8), 3.6 (1.4–6.1) and 3.4 (1.4–5.2) $mmol \cdot l^{-1}$ at the end of the pre-exercise rest period, Ex 1, Ex 2, Ex 3 and post-exercise period, respectively.

Correlations between microdialysate, ante-cubital vein and muscle biopsy lactate concentrations

There was a significant correlation between microdialysate and venous lactate concentrations at the end of all three exercise periods and 10 min post-exercise (Fig. 3A–D). The microdialysate lactate concentration correlated significantly to mixed muscle lactate concentration at the end of Ex 1 (Fig. 4A). At the end of exercise periods 2 and 3 and 10 min post-exercise there were tendencies to significant correlations (Fig. 4B–D). Muscle biopsy lactate and venous lactate concentrations correlated at the end of Ex 1 and 10 min post-exercise (Fig. 5A–D).

Fig. 3. Correlations by simple linear regression between individual microdialysate and venous blood lactate concentrations at the end of exercise periods 1 (**A**), 2 (**B**), 3 (**C**) and post exercise (**D**). Each *symbol* refers to the same individual in all diagrams (Figs. 3, 4 and 5). The correlation coefficient (*r*) and corresponding *P* value is given in each diagram

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Fig. 4. Correlations by simple linear regression between individual microdialysate and muscle biopsy lactate concentrations at the end of exercise periods 1 (**A**), 2 (**B**), 3 (**C**) and post exercise (**D**). Each *symbol* refers to the same individual in all diagrams (Figs. 3, 4 and 5). The correlation coefficient (*r*) and corresponding *P* value is given in each diagram

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Fig. 5. Correlations by simple linear regression between individual venous blood and muscle biopsy lactate concentrations at the end of exercise periods 1 (**A**), 2 (**B**), 3 (**C**) and post exercise (**D**). Each *symbol* refers to the same individual in all diagrams (Figs. 3, 4 and 5). The correlation coefficient (*r*) and corresponding *P* value is given in each diagram

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Comparisons between microdialysate levels from the two catheters

There is biological as well as methodological variation between the concentrations in the two vials from each subject. This can be expressed as the observed mean relative difference between the concentrations from the two concomitant measurements in relation to the average concentrations. The values were as follows: lactate 8%, glucose 7%, glycerol 24% and urea 13%. The average absolute differences and the corresponding 95% confidence interval between the concentrations from the two concomitant measurements in relation to the average concentrations were as follows – lactate: 1.0 (0.6–1.4), glucose: 0.69 (0.51–0.86), urea: 0.90 (0.47–1.34) $mmol \cdot l^{-1}$ and glycerol: 24 (17–31) $\mu mol \cdot l^{-1}$. To find out whether the difference between the two catheters always had the same sign, lactate levels were studied separately at the end of the equilibration period and at the four time points when the four biopsy samples were taken, i.e. a total of 45 observations. Values from both catheters were obtained at 38 instances, signifying dysfunction of one of the catheters at seven occasions. Thirty-three of the observed differences had the same sign, including all the measurements carried out during the exercise period. Data were obtained from at least one of the catheters from all subjects at all time points.

Discussion

The main finding from the present study is that the intramuscular microdialysate lactate concentration increases with stepwise increments in the degree of blood flow restriction applied. Also, the microdialysate lactate concentrations correlate strongly to lactate concentrations in venous blood and tend to correlate to lactate concentrations in muscle biopsy samples.

The hypothesis that microdialysate lactate concentrations increase with stepwise increments in blood flow restriction was based on previous studies in which ante-cubital vein [5] and femoral vein [7] lactate concentrations were found to increase with increasing degrees of blood flow restriction, and this was confirmed in the present study.

Leg muscle microdialysate and ante-cubital vein lactate concentrations correlated at all time points during and after exercise. This is probably due to rapid diffusion of lactate from the interstitial space to the venous blood during exercise and during increased blood flow post-exercise. These findings lend support to the use of the microdialysate lactate concentration as an indicator of changes in interstitial lactate concentrations in lactate-producing tissues, such as in dynamically

exercising or ischaemic limbs. In patients with peripheral arterial occlusive disease where blood flow is low, changes in lactate concentrations are likely to be found earlier in muscle than in venous blood.

The microdialysate lactate concentration only tended to correlate to muscle biopsy lactate concentration, and there may be several explanations for this. The validity of the correlations may be compromised by the fact that the biopsy was taken once while the microdialysate was sampled over a longer period of time – in the present study 5 min. Also, the biopsy sample was not taken during exercise, but immediately after it, when the intramuscular lactate concentration might no longer be in a steady state. Moreover, there may be some time delay in the flux of lactate from the production site in the skeletal muscle cytosol to the interstitium. Finally, although biopsy samples were obtained from the same muscle and as close as possible to the site of the microdialysis catheters, regional differences in lactate concentration could have occurred. This could also partly account for the difference between the two microdialysis catheters.

Microdialysis has been utilized previously for muscle metabolic studies at rest, where 20 min of total ischaemia by the use of a tourniquet approximately doubled the microdialysate lactate concentration [10], also during static exercise where microdialysate lactate concentrations increased with increasing contraction force [11] and under perioperative ischaemic conditions in skin flaps [12]. The technique has also recently been used in dynamically exercising muscle where it was shown that microdialysate lactate and glucose recovery, i.e. the concentration of the metabolites in the microdialysate in relation to the true concentrations in the tissue surrounding the catheter, was increased during muscle contractions [13]. In accordance with these data we found in the present study a marked elevation during exercise in microdialysate concentrations of all substances studied compared to concentrations at rest (Figs. 1, 2A–C). This is a well-known phenomenon, which is due to the fact that the increased blood flow makes more glucose, lactate, glycerol and urea available for removal by the microdialysis catheter [11]. For glucose, urea and glycerol there were no differences between the R- and the NR-experiments. The lactate concentrations, however, were significantly higher when blood flow was restricted. The additional microdialysate lactate concentration increase during blood flow restriction probably reflects a true increase in the interstitial lactate concentration. There are several possible explanations for the increased lactate concentration during ischaemic exercise: increased lactate production and release from muscle cells as well as decreased removal of lactate resulting from reduced blood flow. In the NR-experiment lactate tended to decrease during Ex 2 and 3 compared to Ex 1, consistent with previous reports on a

decreasing lactate concentration in muscle biopsy samples with increasing exercise duration at a sub-maximal work load.

It should be noted that microdialysate lactate levels failed to return to baseline values between exercise periods (Fig. 1). This possibly reflects an accumulation of lactate during the experiment, which might have influenced the lactate levels reached at subsequent measurement times. However, the correlation between microdialysate and venous lactate concentrations remains strong throughout the experiment, suggesting that this is a physiological event rather than a methodological problem.

In conclusion, this experiment shows that graded blood flow restriction in healthy males at a given exercise work load leads to a "dose-dependent" increase in the skeletal muscle microdialysate lactate concentration, which correlates to lactate concentrations in ante-cubital vein blood and tends to correlate to lactate levels in skeletal muscle biopsy samples. Based on the findings from this study, in which the experimental restriction of blood flow was used to induce blood flow reduction in a controlled fashion, the use of microdialysis to assess the degree of ischaemia in patients with peripheral vascular disease could be an attractive alternative to the more invasive biopsy technique and the probably less sensitive measurements of venous blood.

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