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Continuous measurement of subcutaneous lactate concentration during exercise by combining open-flow microperfusion and thin-film lactate sensors

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Abstract

The present study was carried out to investigate in vivo in healthy humans the method of open-flow microperfusion for monitoring of the subcutaneous (s.c.) lactate concentration during rest and cycle ergometer exercise. Using open-flow microperfusion, a perforated double lumen catheter with an inflow and an outflow connection is inserted into the s.c. adipose tissue and perfused with a sterile, isotonic, ionfree fluid. Due to the low flow rate, the fluid partially equilibrates with the surrounding tissue. The equilibrated perfusate passes a sensor flow chamber where the substance of interest and the rate of recovery (i.e. the ratio of sampled concentration to interstitial concentration) are continuously monitored. Within this study, the method was evaluated in four healthy volunteers during cycle ergometer exercise. The relative increase of the lactate concentration was approximately a third in the s.c. tissue compared to the capillary blood and the peak time was delayed on average by 10 min. The correlation coefficient between blood and s.c. tissue lactate concentration ranged from $r = 0.41$ to $r = 0.90$ ($n = 29$) in the individual experiments. The combination of open-flow microperfusion and lactate and conductivity sensors enables on-line monitoring of the s.c. lactate concentration without in vivo calibration during steady-state and cycle ergometer exercise.

Keywords: Biosensor; Exercise; Lactate; Microperfusion; Monitoring

1. Introduction

Assessment of tissue metabolism in free living animals and men is a goal long sought by scientists of various disciplines. A commonly used method is the measurement of arterio-venous differences (Avogaro et al., 1996; Frayn et al., 1989). However, this method involves several assumptions like selective venous drainage and specific blood flow determination and may not reflect the concentrations at the cellular level. Therefore, numerous techniques like capillary ultrafiltration (Linhares & Kissinger, 1992), wick technique (Fischer et al., 1987) or microdialysis (De Boer et al., 1994a, b; Hagström et al., 1990; Hickner et al., 1992; Jansson et al., 1994, 1996; Korf et al., 1993; Rosdahl et al., 1993) have been developed to achieve direct access to different body compartments. The development of the microdialysis technique enabled the measurement of relative dynamic changes of substances in brain, subcutaneous (s.c.) adipose tissue or muscle. However, measurement of absolute concentrations in peripheral tissues still requires time consuming calibration procedures and the assumption of a constant recovery.

In a recent study we developed the method of open-flow microperfusion for the measurement of the s.c. extracellular glucose concentration during hypo- and hyperglycemia (Skrabal et al., 1995; Trajanoski et al., 1997). A perforated double lumen catheter with an inflow and an outflow connection is inserted into the s.c. adipose tissue and perfused with a sterile, isotonic, ion-

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free fluid. The fluid partially equilibrates with the surrounding tissue and passes a sensor flow chamber where the substance of interest and the rate of recovery are continuously monitored. The main advantage of the method of open-flow microperfusion is that the absolute concentration of the substance of interest can be calculated on line and without any time consuming in vivo calibration procedure.

The purpose of this study was to evaluate the technique for on-line monitoring of the lactate concentration in the s.c. adipose tissue. Lactate is an exclusive anaerobic metabolite of glucose. Body compartments like skeletal muscle, the splanchnic area, erythrocytes, brain and skin are thought to produce this metabolite. De Boer et al. (1994b) evaluated the microdialysis technique for off-line measurement of relative changes of lactate during cycle ergometer exercise. However, using microdialysis the measurement of absolute lactate concentrations during dynamic events in the s.c. tissue was not possible so far. The present study was carried out to investigate in vivo in healthy humans if the method of open-flow microperfusion permitted a stable monitoring of the absolute s.c. lactate concentration during rest and cycle ergometer exercise.

2. Materials and methods

2.1. Subjects

Four healthy male volunteers participated in the study. They were all moderately well-trained students and researchers of similar age (30 ± 3, mean ± SD) and body mass index (22.8 ± 0.47 kg m^{-2}, mean ± SD). The subjects were told to eat a regular lunch between 12.00 and 12.30 h and did not ingest anything thereafter. The experiments started at 15.00 h and lasted 3–4 h. The subjects were investigated while sitting on a cycle ergometer at room temperature of 20–22°C. A 24 gauge double lumen catheter was inserted into the right abdominal region. A detailed description of the study was given to each subject and their written consent was obtained.

2.2. Monitoring device

A schematic illustration of the monitoring device is given in Fig. 1. A conventional intravenous cannula (24 gauge × 19 mm, 0.6 mm diameter; Neoflon, Viggo AB, Helsingborg, Sweden) was used as the outer lumen of the double lumen catheter. The cannula was perforated with 24 holes (0.2 mm diameter) using an Excimer Laser (Krypton–Fluorine, 280 nm, 600 mJ, LPX205i; Lambda Physik GmbH, Göttingen, Germany). The perforated cannula was inserted into the s.c. tissue of the right abdominal region by the use of a steel mandrin. The steel mandrin was removed thereafter and replaced by the inner lumen (steel tube, length 16 mm, inner diameter 0.1 mm, outer diameter 0.2 mm; Goodfellow, Cambridge, UK) of the double lumen catheter. The inner lumen was connected to the cannula by a male Luer Lock connector. The catheter was continuously perfused with a sterile, isotonic, ionfree solution (275.mmol l^{-1} mannitol in aqueous solution; Leopold, Graz, Austria) at constant flow rate of 1.2 μl min^{-1}. To achieve a constant flow of the perfusate, the same roller pump (Gilson, Minipuls 3, Villiers-le-Bel, France) was used for the influx and the efflux of the double lumen catheter (Fig. 1). The perfusion medium entered the double lumen catheter through the inner lumen and flowed towards the perforations of the outer lumen in the space between the inner and the outer lumen of the catheter in a retrograde direction to the outlet of the cannula. Due to the perforations of the outer lumen of the catheter, the perfusion fluid partially equilibrated with the s.c. tissue fluid. The flow rate was chosen as a compromise between small delay times due to the tubing system and a long period of equilibration between the interstitial fluid and the perfusion fluid (Skrabal et al., 1995). From the outlet of the double lumen catheter, the partially equilibrated perfusion fluid was transported to the sensor flow chamber. Tubing made of polyvinylchloride (inner diameter 0.25 mm, outer diameter 2.32 mm; Labokron, Sinsheim, Germany) was used for the transport of the perfusion fluid. Due to the low sample volume and the low concentration in the sampled fluid, a measurement of the lactate concentration in the collected perfusate using the reference method was not possible.

2.3. Sensor flow chamber

Two sensor flow chambers arranged in series were used in this study. One flow chamber contained a conductivity sensor (impedance measurement at 50 kHz) and a temperature sensor (NTC-resistor) (Skrabal et al., 1995). The other flow chamber contained electrochemical glucose and lactate sensors (Jobst et al., 1996). A scheme of the sensors with an integrated flow cell is given in Fig. 2. The sensors are based on the amperometrical measurement of the electrochemical oxidation of hydrogenperoxide (H_2O_2) which is produced by the membrane enclosed enzyme lactate oxidase (LOD). The sensors were manufactured on a glass carrier by means of thin-film technology. The electrode structure consisted of four working electrodes (area 0.25 mm^2 each) and an Ag/AgCl reference electrode. The used sensor chip was assembled with a printed circuit board comprising a gold counter electrode and a 200 μm thick seal to form the flow chamber. The internal volume of the chamber was 1 mm^3. The sensor flow chamber and the sensor electronics were mounted on a device near the roller pump. The device was connected to an IBM com-

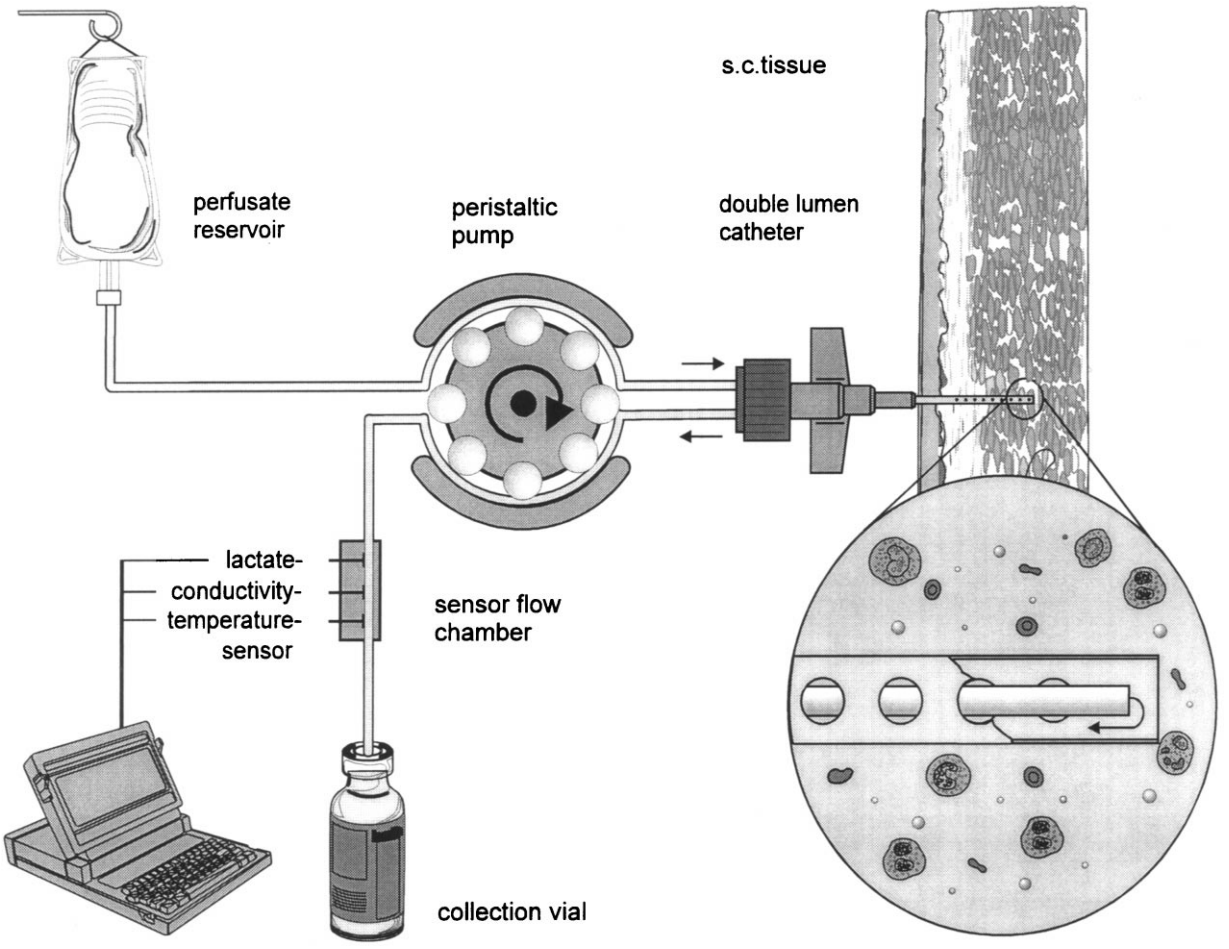

Fig. 1. Schematic representation of the monitoring device. The flow of the fluids is indicated by arrows. The double lumen catheter was inserted into the s.c. adipose tissue. The catheter was continuously perfused with sterile, isotonic perfusate by the use of a peristaltic pump. From the outlet of the catheter the s.c. tissue fluid was transported to the sensor flow chamber and to the collection vial. A notebook computer was used for on-line data processing and visualization.

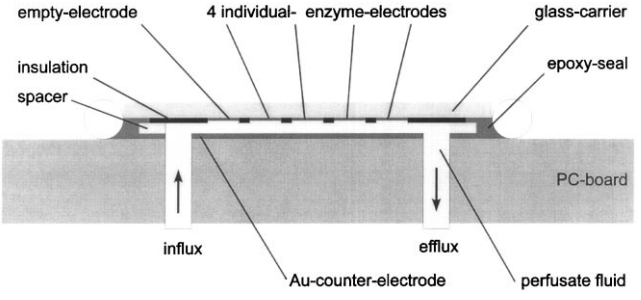

Fig. 2. Scheme of the enzyme sensors with an integrated flow cell. The sensors were produced on a glass carrier by means of thin film technology. The electrode structure consisted of four working electrodes (area 0.25 mm^2 each). The used sensor chip was assembled with a printed circuit board comprising a gold counter electrode and a 200 μm thick seal to form the flow chamber.

patible portable computer. The sensor signals were sampled at a frequency of 1 kHz using a DAQ-Card 700 (National Instruments, Austin, TX, USA). The graphical programming language LabVIEW® (National Instruments) was used to enable on-line data processing and visualization. All measured and processed data were stored in intervals of 10 s during the whole study and calibration period. Statistical analysis was accomplished by Micro Cal Origin™ (technical graphics and data analysis; Microcal™ Software, Inc., Northhampton, MA, USA) software.

2.4. Calibration procedure

The sensors were calibrated in vitro before and after each experiment using a two-point calibration procedure. The calibration solutions contained 0.14 mmol l^{-1} lactate and 1.91 mmol l^{-1} lactate, respectively, and the conductivity of the solutions was 0.149 S m^{-1} and 1.28 S m^{-1}, respectively. The calibration procedure was performed at the same flow rate used during the experiments. The values of the lactate concentration and the conductivities of the calibration solutions were chosen in order to take into account equilibration of the interstitial fluid with the perfusate measured in previous experiments (Skrabal et al., 1995; Trajanoski et al., 1997).

The rate of recovery between the perfusate and the interstitial fluid was determined on-line as the ratio between the measured conductivity in the sampled fluid and the conductivity of the s.c. tissue fluid (1.28 S m^{-1}) assumed to be constant (Trajanoski et al., 1997) (Fig. 3). The absolute concentration of the lactate concentration in the s.c. adipose tissue was calculated as the ratio between the measured lactate concentration in the sampled fluid and the rate of recovery.

2.5. Blood lactate measurement

Fifteen minutes after embrocating the earlobe with a paste, increasing circulation (Finalgon; Thomae GmbH, Biberach, Germany), the ear was cleaned with alcohol. After earlobe puncture 20 μl of blood were sampled in heparinized micro capillaries. Each capillary was sampled in an Eppendorf® probe-ampoule filled with 1 ml Eppendorf system solution. The capillary blood lactate concentrations were measured immediatly subsequent to each experiment using an Eppendorf EBIO 6666 lactate analyser (Eppendorf Netheler Hinz GmbH, Hamburg, Germany).

2.6. Protocol

The subjects were seated on the cycle ergometer 2 h postprandially. A 24 gauge double lumen catheter was inserted into the s.c. tissue of the right abdominal region and was then perfused at a constant flow rate of 1.2 $\pm$ 0.04 μl min^{-1} (mean $\pm$ SD). The on-line measurement started after a period of approximately 1 h in order to take into account the acute trauma caused by the insertion of the catheter. The subjects were investigated similar to a recent published protocol (De Boer et al., 1994b): each exercise started with a 6 min warm-up period at 50 W. The exercise was increased by 50 W every 3 min up to 150 W and thereafter by 25 W every 3 min until exhaustion. The exercise was followed by a diminished work load of 50 W and 12 min duration and 30 min of rest. Capillary blood probes were withdrawn every 3 min during the whole study period.

2.7. Statistical analysis

Since the physiological condition of the subjects was different, the duration of the exercise varied among the subjects. Therefore, in order to allow comparison of the

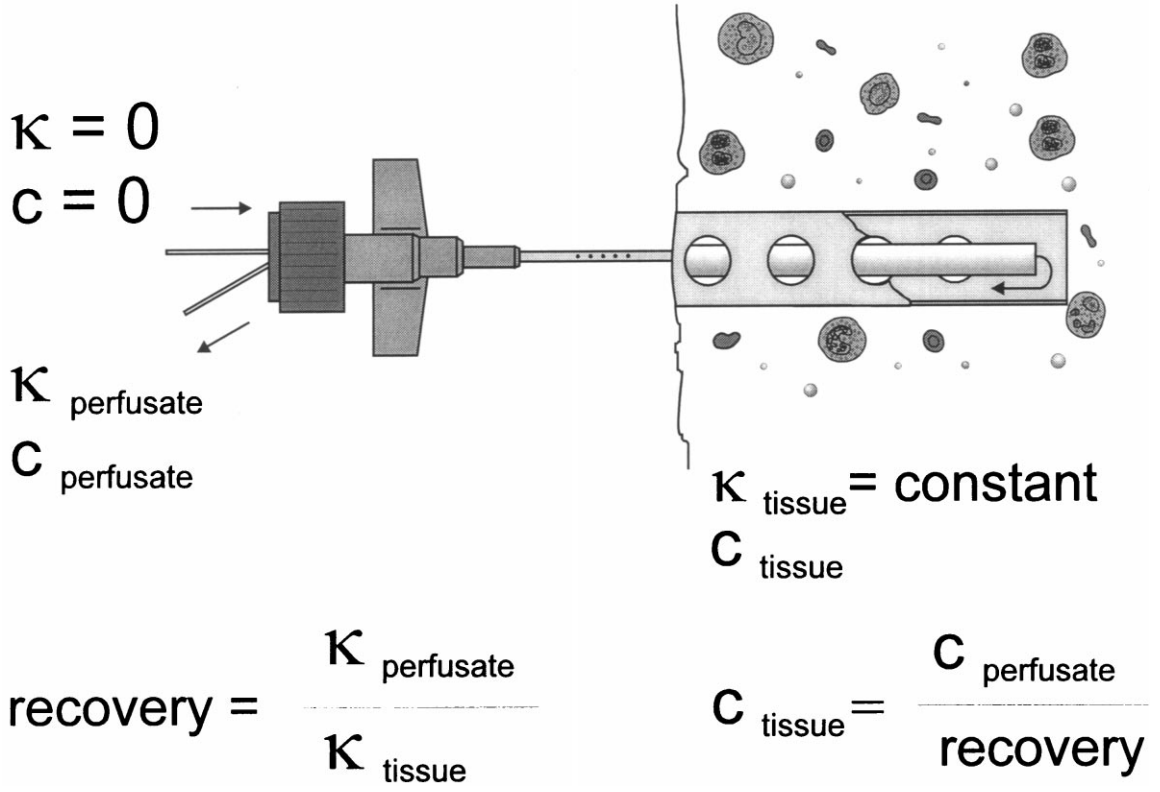

Fig. 3. Illustration for the estimation of the s.c. tissue lactate concentration. The rate of recovery between the ion- and lactate-free perfusate and the s.c. tissue fluid was calculated as the ratio between the measured conductivity in the sampled fluid ($\kappa_{perfusate}$) and the conductivity of the s.c. tissue fluid (κ_{tissue}). The s.c. tissue lactate concentration (c_{tissue}) was calculated as the ratio between the measured lactate concentration in the sampled fluid ($c_{perfusate}$) and the rate of recovery.

individual experiments, each exercise period from beginning till the end of the incremental exercise was divided into 10 sections (De Boer et al., 1994b). Since capillary blood lactate concentrations were obtained every 3 min, they were related to the defined sections by linear interpolation, whereas the continuously monitored tissue concentrations were related directly to the defined sections.

The results were corrected for the system-dependent time delay (tubing length, flow rate) between s.c. tissue and lactate sensors. Results in the text, figures and tables are presented as mean ± SEM unless otherwise indicated. Significance tests were performed by paired Student's *t*-test ($p < 0.05$).

3. Results

Fig. 4 shows the time course of the mean capillary blood and s.c. tissue lactate concentration (upper panel), the lactate concentration of the sampled fluid (middle panel) and the continuously monitored rate of recovery (lower panel) during the incremental cycle ergometer exercise. Values on the *y*-axis are expressed as absolute values of the capillary blood and the calculated s.c. tissue lactate concentration, respectively. Both capillary blood and s.c. tissue lactate concentrations increased during the exercise period and declined thereafter. The s.c. tissue lactate concentration reached its maximum 10 ± 1.7 min (mean ± SEM) later compared to the capillary blood lactate concentration. During steady state (2 h postprandially) the calculated s.c. lactate concentration was 176 ± 42% (mean ± SEM) of the capillary blood lactate concentration. Peak concentrations in the s.c. tissue reached 49 ± 9% (mean ± SEM) of the capillary blood. Due to these results the relative increase of the s.c. tissue lactate concentration was 3.2 times smaller compared to the capillary blood lactate concentration. The correlation coefficient between s.c. and blood lactate concentrations ranged from $r = 0.41$ to $r = 0.90$ ($n = 29$) in the individual experiments. The lactate concentrations measured in the sampled fluid ranged from 0.06 mmol l^{-1} to 2.28 mmol l^{-1}. The recovery was calculated as the percentage of the conductivity of the sampled fluid to the absolute conductivity of the s.c. tissue fluid (1.28 S m^{-1}). At the flow rate of 1.2 μl min^{-1} the recovery ranged from 6.5 to 46.9%. The within-subject variation for the recovery was 21.2 ± 3.6% (mean ± SEM) (calculated from the mean coefficients of variation of the individual experiments). The interindividual coefficient of variation was 64.9% (calculated from the mean recoveries of the individual experiments). The mean temperature in the sampled fluid was 27.6 ± 1.9°C (mean ± SD) and the within-subject variation was 0.8%. The lactate sensors were strictly linear within the interesting range between 0.2 and 2.5 mmol l^{-1} ($r = 0.99$, $n = 7$). The mean base current and the sensitivity of the lactate sensors were 1.05 ± 0.42 nA (mean ± SEM) and 2.89 ± 1.3 nA mmol l^{-1} (mean ± SEM), respectively. The lactate sensors exhibit a linear response range up to 25 mmol l^{-1} lactate concentration in combination with a temperature coefficient of 2.8%/K (Jobst et al., 1996).

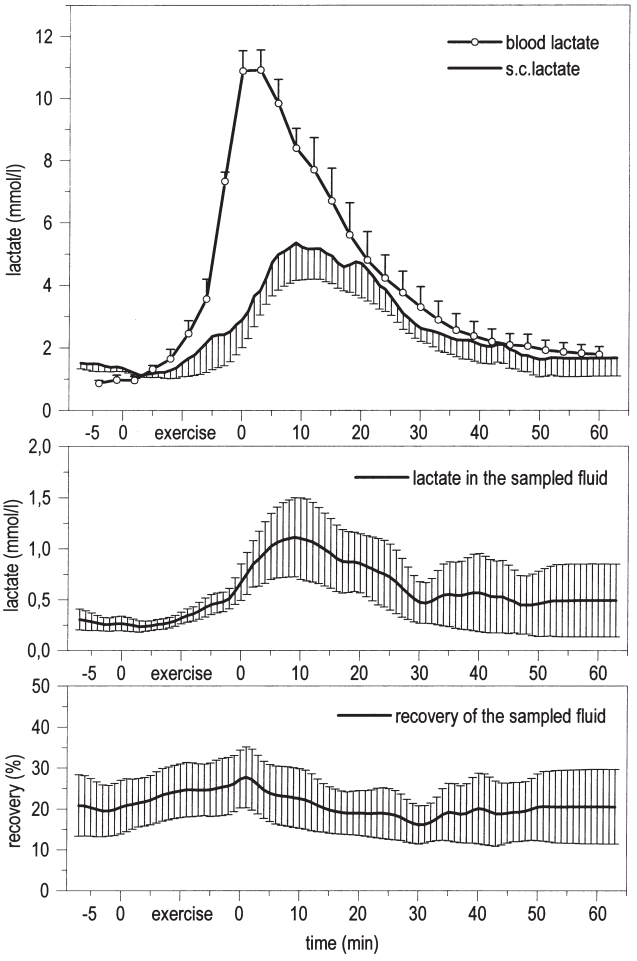

Fig. 4. Lactate concentrations (mean ± SEM) of capillary blood and on-line measured s.c. adipose tissue (upper panel), lactate concentration (mean ± SEM) of the sampled fluid (middle panel) and recovery (mean ± SEM) of the sampled fluid (lower panel) during incremental cycle ergometer exercise.

4. Discussion

The present results demonstrate that open-flow microperfusion allows monitoring of the lactate concentration in subcutaneous (s.c.) adipose tissue both at rest and during cycle ergometer exercise and that the technique is useful in detecting physiological responses. The lactate concentration measured in the subcutaneous adipose tissue was observed for a period of 1 h before the start of the exercise. Similar steady state values were reached at the end of the exercise period. In contrast to the subcutaneous lactate concentration the capillary blood lactate concentration was not in a steady state at

the end of the observation period. With special respect to the subjects it was not possible to monitor the lactate concentration for more than 1 h after the exercise. During steady state (2 h postprandially) the s.c. lactate levels were significantly higher ($p < 0.05$) than the blood lactate concentrations. Various study groups showed that s.c. tissue is a source of in vivo lactate production after glucose ingestion (Frayn et al., 1989; Hagström et al., 1990; Jansson et al., 1990, 1994) and that the s.c. lactate concentration is higher than the blood lactate concentration (Jansson et al., 1990, 1994; Rosdahl et al., 1993) but the true physiological concentration of lactate in the s.c. adipose tissue still remains unclear. In their study, Hagström et al. (1990) measured the s.c. lactate concentration to be 90% of the plasma lactate concentration after overnight fast, but 85% above the basal level 3 h post-prandially. In both studies Jansson et al. (1990, 1994) postulated the interstitial lactate levels to be significantly higher than blood lactate levels. Using microdialysis at a perfusion rate of 0.5 μl min^{-1} Rosdahl et al. (1993) measured a higher adipose tissue lactate concentration compared to blood but probably equal to the plasma concentration, not taking into account the rate of recovery. In our study the absolute s.c. lactate concentration was 176% of the capillary blood lactate concentration during steady state 2 h post-prandially. The observed s.c. lactate dynamics of the present study are in accordance with the findings of De Boer et al. (1994b), who performed a similar protocol using s.c. microdialysis. In their study the relative increase of the lactate concentration was three times smaller in dialysate compared to that in plasma and the moment of increase in lactate concentration was 4 min later in dialysate than in blood. In the present study the relative increase was approximately a third in the s.c. tissue compared to capillary blood and peak concentrations were 10 min delayed in the s.c. tissue. Although the results of the present in-vivo study are similar to the published adipose tissue lactate kinetics during rest and cycle ergometer exercise (De Boer et al., 1994b) the data have to be interpreted carefully as long as two underlying assumptions have been made for the calculation of the absolute lactate concentration in the s.c. adipose tissue. The assumption of a constant conductivity in the interstitial fluid has been made likely by the measurement of the conductivity of ultrafiltrated plasma and by theoretical considerations (Trajanoski et al., 1997). The calculation of the absolute lactate concentration of the s.c. tissue using the sum of the ion concentrations (i.e. the conductivity) for the calculation of the rate of recovery demands a second assumption, i.e. that there is the same equilibration for both the ions and the lactate molecules respectively, at a defined flow rate. Even if the presented results are physiologically plausible and in agreement with the results of other study groups (De Boer et al., 1994b; Jansson et al., 1990, 1994), further confirmatory studies will be necessary. One suggestion could be the use of different calibration techniques (Lönnroth & Strindberg, 1995).

In the present experiments some within-subject variation of the rate of recovery (n.s.) was observed. This could possibly be due to changes in local blood flow affecting the exchange of substances with the sampled fluid. The high interindividual variability of the rate of recovery might be caused by different sites of the insertion of the catheter, i.e. by different distances between the catheter and a local blood vessel. Hickner et al. (1992) developed a method for the measurement of adipose tissue blood flow based on the clearance of ethanol from microdialysis probe into the tissue. In their study, Rosdahl et al. (1993) observed a within-subject variation for the ethanol outflow/inflow ratio in adipose tissue of 21.6% at a flow rate of 1 μl min^{-1}. These results and the present data demonstrate the importance of having a reliable indicator for the rate of recovery of the substance being monitored.

During cycle ergometer exercise, the calculated s.c. lactate concentration did not show a relation to the capillary blood lactate concentration, being strong enough to serve as an alternative to blood sampling. The s.c. lactate concentration measured in our experiments showed a delayed and lower peak and a smaller increase than the capillary blood lactate concentration. This might be explained by the fact that the s.c. adipose tissue in humans must be considered as an own-body compartment with its own dynamic processes and can therefore not be used as a substitute for lactate measurement in blood.

The data presented indicate that the used lactate sensors are strictly linear even at a low range of concentration and in combination with open-flow microperfusion a novel application field for this sensor technology is opened. The open-flow microperfusion technique has already been applied in continuous monitoring of glucose (Trajanoski et al., 1997), but in addition to these studies where the subjects were investigated in supine position the present study demonstrates that open-flow microperfusion and thin-film lactate sensors permit a stable monitoring of the subcutaneous lactate concentration even during extensive body movement caused by cycle ergometer exercise. Moreover, continuous monitoring of the absolute subcutaneous lactate concentration during cycle ergometer exercise was not possible so far.

It can be concluded that the combination of open-flow microperfusion and lactate and conductivity sensors enables on-line monitoring of the s.c. lactate concentration without in vivo calibration during steady-state and cycle ergometer exercise which should provide valuable insight into the dynamics of s.c. lactate metabolism.

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References

Avogaro, A., Toffolo, G., Miola, M., Valerio, A., Tiengo, A., Cobelli, C., Del Prato, S., 1996. Intracellular lactate- and pyruvate-interconversion rates are increased in muscle tissue of non-insulin dependent diabetic individuals. J. Clin. Invest. 1, 108–115.

De Boer, J., Korf, J., Plijter-Groendijk, H., 1994a. In vivo monitoring of lactate and glucose with microdialysis and enzyme reactors in intensive care medicine. Int. J. Art. Org. 17, 163–170.

De Boer, J., Plijter-Groendijk, H., Visser, K.R., Mook, G.A., Korf, J., 1994b. Continuous monitoring of lactate during exercise in humans using subcutaneous and transcutaneous microdialysis. Eur. J. Appl. Physiol. 69, 281–286.

Fischer, U., Ertle, R., Abel, P., Rebrin, K., Brunstein, E., Hahn Von Dorsche, H., Freyse, E.J., 1987. Assessment of subcutaneous glucose concentration: validation of the wick technique as a reference for implanted electrochemical sensors in normal and diabetic dogs. Diabetologia 30, 940–945.

Frayn, K.N., Coppack, S.W., Humphreys, S.M., Whyte, P.L., 1989. Metabolic characteristics of human adipose tissue in vivo. Clin. Sci. 76, 509–516.

Hagström, E., Arner, P., Ungerstedt, U., Bolinder, J., 1990. Subcutaneous adipose tissue: a source of lactate production after glucose ingestion in humans. Am. J. Physiol. 258, E888–E893.

Hickner, R.C., Rosdahl, H., Borg, I., Ungerstedt, U., Jorfeldt, L., Henriksson, J., 1992. The ethanol technique of monitoring local blood flow changes in rat skeletal muscle: implications for microdialysis. Acta Physiol. Scand. 146, 87–97.

Jansson, P.A., Krogstad, A.L., Lönnroth, P., 1996. Microdialysis measurements in skin: evidence for significant lactate release in healthy humans. Am. J. Physiol. 271, 138–142.

Jansson, P.-A., Larsson, A., Smith, U., Lönnroth, P., 1994. Lactate release from the subcutaneous tissue in lean and obese men. J. Clin. Invest. 93, 240–246.

Jansson, P.A., Smith, U., Lönnroth, P., 1990. Evidence for lactate production by human adipose tissue in vivo. Diabetologia 33, 253–256.

Jobst, G., Moser, I., Varaham, M., Svasek, P., Aschauer, E., Trajanoski, Z., Wach, P., Kotanko, P., Skrabal, F., Urban, G., 1996. Thin-film microbiosensors for glucose-lactate monitoring. Anal. Chem. 68, 3173–3179.

Korf, J., De Boer, J., Baarsma, R., Venema, K., Okken, A., 1993. Monitoring of glucose and lactate using microdialysis: applications in neonates and rat brain. Dev. Neurosci. 15, 240–246.

Linhares, M.C., Kissinger, P.T., 1992. Capillary ultrafiltration: in vivo sampling probes for small molecules. Anal. Chem. 64, 2831–2835.

Lönnroth, P., Strindberg, L., 1995. Validation of the 'internal reference technique' for calibrating microdialysis catheters in situ. Acta Physiol. Scand. 153, 375–380.

Rosdahl, H., Ungerstedt, U., Jorfeldt, L., Henriksson, J., 1993. Interstitial glucose and lactate balance in human skeletal muscle and adipose tissue studied by microdialysis. J. Physiol. 471, 637–657.

Skrabal, F., Trajanoski, Z., Kontschieder, H., Kotanko, P., Wach, P., 1995. Portable system for on-line continuous ex vivo monitoring of subcutaneous tissue glucose using open tissue perfusion. Med. Biol. Eng. Comp. 33, 231–234.

Trajanoski, Z., Brunner, G.A., Schaupp, L., Ellmerer, M., Wach, P., Pieber, T.R., Kotanko, P., Skrabal, F., 1997. Open-flow microperfusion of subcutaneous adipose tissue for on-line continuous ex vivo measurement of glucose concentration. Diabetes Care 20, 1114–1121.