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Novel system for real-time ex vivo lactate monitoring in human whole blood[1]

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Abstract

The objective of the study was to evaluate the performance of an amperometric enzyme based lactate sensor and to investigate the possibility of replacing a double lumen catheter based blood withdrawal system with a heparin coated single lumen system. The inner lumen of a double lumen catheter which was placed in a peripheral vein was perfused with heparin solution. The outer lumen was used to collect heparinized blood samples at a defined flow rate. The single lumen system was attached to a heparinized catheter which was also placed in a peripheral vein. The undiluted blood samples were collected at a specified flow rate. A sensor flow chamber incorporating an amperometric thin-film lactate microbiosensor was placed in the sampling line for real-time lactate monitoring. Plasma lactate concentrations were measured during frequently performed hyperlactatemia bicycle ergometer experiments in six healthy volunteers (age 25.8 $\pm$ 2.8 years, BMI 22.7 $\pm$ 1 kg/m^2). Additionally, plasma lactate was measured in real-time using the lactate sensors. The first three experiments were performed with a double lumen based catheter system whereas the following three experiments were performed with a heparin coated catheter system. The correlation coefficients of sensor readings and laboratory analyzer results in all six experiments were between 0.93 and 0.99, respectively ($P < 0.001$). The miniaturized lactate sensors showed a linear range up to 25 mmol/l lactate concentration and 95% response times $<$ 30 s in undiluted serum. During the experiments maximum lactate concentrations of 14 mmol/l were achieved. Improvements of system performance using heparin coated catheter systems could be shown. The overall SD of the sensor readings compared to laboratory results using three double lumen catheter based systems was 0.91 mmol/l whereas the SD using three heparin coated systems was 0.65 mmol/l. In summary, real-time monitoring of lactate in human whole blood is feasible with such a device and can be improved by using heparin coated catheter systems.

Keywords: Blood lactate monitoring; Heparin coating; Biosensor-array

1. Introduction

Wherever hormonal or metabolic profiles in clinical routine or research investigations are required, intermittent phlebotomy followed by discrete probe analysis are the favored means. Hence, the need for skilled medical staff to obtain the requested blood samples, the possibility of probe handling errors and the delay times of laboratory results are the limiting factors for studies in which repeated blood samples are needed. Continuous monitoring of hormonal or metabolic changes could be advantageous where permanent control is desired, e.g. in intensive care units (ICU) or during major operations (open heart surgery). In these conditions, where the vital functions like blood pressure, ECG, core temperature, and oxygen saturation are monitored continuously, a device comprising a biosensor-array including a lactate sensor as a powerful indicator for the oxygen supply of the patient and for therapy control would be of great

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benefit. When invasively monitoring critically ill patients the amount of withdrawn blood samples has to be kept minimal. In addition sterility and biocompatibility are substantial prerequisites for the clinical acceptance of such a system. Moreover, such a device should meet a set of requirements like robustness, accuracy, portability (bed side as well as ambulatory), safety, real-time capability and simple use in clinical setup. Avoiding systemic heparinization of ICU patients is one of the most important issues for the acceptance of a monitoring system.

The objective of this study was twofold: First, to evaluate the performance of an amperometric enzyme based lactate sensor during hyperlactatemia bicycle ergometer exercise; Second, to investigate the possibility of replacing double lumen catheter based systems with heparin coated single lumen catheter systems.

2. Subjects, materials and methods

2.1. Blood sampling system

2.1.1. Blood withdrawal system based on a double lumen catheter

This blood withdrawal method based on a double lumen catheter for extra corporeal blood heparinization has been described previously (Trajanoski et al., 1996). However, some slight differences compared to the double lumen catheter system were achieved. A more precise peristaltic pump (Minipuls MP3, Gilson, Cedex, France) and a different catheter length were used in this study.

A catheter (Vasofix®, 18 gauge × 33 mm, B. Braun Melsungen AG, Melsungen, Germany) was placed in a peripheral vein of the forearm in antegrade position. Heparin, dissolved in saline solution, was infused at a rate of 5.5 units/min (Novo Nordisk, Bagvaerd, Denmark) through the inner lumen of the double lumen catheter. The outer lumen of the double lumen catheter was clamped in a peristaltic pump whose speed was set 150 μl/min. A sensor flow chamber incorporating an amperometric thin-film lactate/glucose sensor array was connected in series to the outlet of the tubing.

2.1.2. Blood withdrawal system based on a heparin coated single lumen system

The principle of the sampling system is given in Fig. 1. The blood sampling part is based on a heparin coated single lumen catheter system called ConFlo® (CBAS®, Carmeda, Stockholm, Sweden) for the prevention of blood coagulation. A catheter (Vialon®, 20 gauge × 25 mm, Beckton & Dickinson, Rutherford, U.S.A.) was placed in a peripheral vein of the forearm in antegrade position. After removing the steel cannula, the coated tubing system was connected to the catheter. The distal end of the tubing set was clamped in a peristaltic pump (Minipuls MP3, Gilson, Cedex, France), whose speed was set 322 μl/min. Since the biosensor-array was not heparin coated, a heparin/saline solution had to be added to the blood immediately preceeding the sensor flow chamber. Therefore, the tubing set was connected to a luer adapter of a Y-connector which was attached to the sensor flow chamber incorporating a thin-film microbiosensor array for the measurement of glucose and lactate. A bag containing heparin solution (5000U Novo Nordisk, Bagsvaerd, Denmark) mixed with an acetate buffered saline solution (10 ml ELO-MEL isoton, Leopold Pharma, Graz, Austria) was connected to a tubing set which was also clamped in the same peristaltic pump (Minipuls MP3, Gilson, Cedex, France). The high precision of the pump ensured a constant dilution of the blood with the saline solution. The outlet of the tubing set containing heparin/saline was also attached to the Y-connector. The dilution of the blood samples with the heparin solution was considered in the calculations of measured data. The outlet of the sensor flow cell was attached to a tubing system which served for the supply of vacuum tubes.

2.2. Miniaturized thin-film lactate sensor and sensor electronics

A microbiosensor-array was produced on a glass carrier by means of thin-film technology which has been described previously (Urban et al., 1992, 1994; Jobst et al., 1996, 1997). The internal volume of the sensor flow cell was 1 mm^3. The lactate sensor is based on oxidation of lactate oxidase (LOD) resulting in the production of hydrogen peroxide which is subsequently oxidized to oxygen, hydrogen ions and electric current flow. Thus the electric current flow is proportional to analyte concentration.

One thin-film device consisted of four 0.25 mm^2 platinum working electrodes, a Ag/AgCl reference electrode made on a 0.3 mm thick glass carrier and a gold counter electrode. The platinum electrodes were covered by a semipermeable membrane on which the enzymes were immobilized. A catalase membrane (5 μm) prevented cross-talking, reduced the flow sensitivity and additionally, reduced H_2O_2 escape. The catalase membrane was separated from the oxidase membrane by a enzyme-free diffusion limiting spacer membrane (pHEMA). The working electrodes were polarized to + 500 mV vs the Ag/AgCl reference electrode. A four-channel potentiostat (POT 4, Institut für Microsystemtechnik, Freiburg, Germany), which was operated in a three electrode mode, produced the current to voltage signal conversion. In order to minimize cross sensitivity a working electrode comprising no enzymes (empty electrode) was used to perform differential measurements. The four-

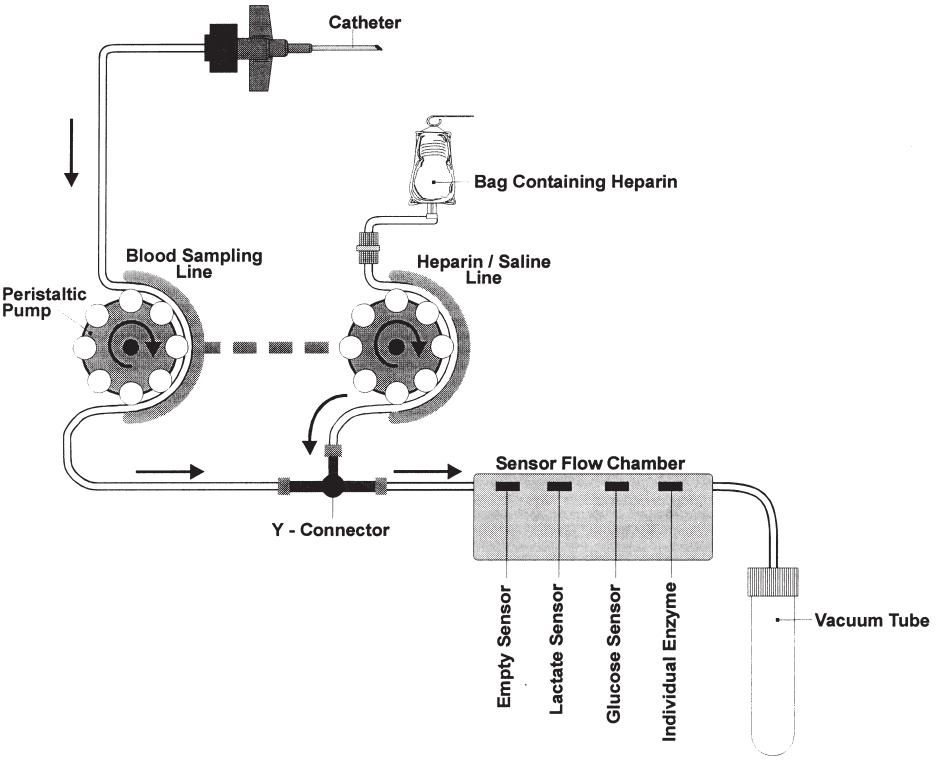

Fig. 1. Heparin coated single lumen catheter system (ConFlo®, Carmeda AB, Stockholm, Sweden). A two headed peristaltic pump was used for the blood sampling line and for the heparin/saline solution. The outlet of the tubing systems were attached to vacuum tubes. The sensor array included four pads incorporating individual enzymes which could be produced on the chip (e.g. glutamate), in this case the "individual enzyme" was a second "empty electrode".

channel potentiostat (lactate-, empty-, glucose-, empty-electrode) was linked to a data acquisition system which performed the signal subtraction after the analogue/digital (AD) converter.

2.3. Data acquisition system

The data acquisition system consisted of a notebook computer, a 12 bit analogue/digital data acquisition PC-Card (DAQ-Card 700, National Instruments Inc., Austin, TX, U.S.A.) and a data acquisition software based on LabVIEW® 4.0 (National Instruments Inc., Austin, TX, U.S.A.). Signals were sampled at 0.1 Hz acquisition rate and filtered by a second order low pass Butterworth filter with a cut-off frequency 0.02 Hz. The program created a Microsoft Excel® worksheet file (Microsoft Inc., Redmond, WA, U.S.A.) and displayed the data on the display in real-time.

2.4. Heparin coating

The Carmeda® BioActive Surface (CBAS®, Carmeda AB, Stockholm, Sweden) as an End-Point Attached Heparin has first been described by Larm et al. (1983). Briefly, a thermoplastic elastomer USP class VI (C-FLEX®, Consolidated Polymer Technologies Inc., Largo, FL, U.S.A.) was coated by means of end-point attachment of heparin, where heparin is partially degraded and the fragments then coupled via their reducing terminal units. In this process, an aldehyde of each heparin molecule is covalently bound to the artificial surface (Hoffmann et al., 1983). So the remainder of the heparin molecule including the active binding sites stays free. This specific method of attachment imitates the orientation of heparan sulfate on the plasma membrane of endothelial cells (Fig. 2). The basic principle of this surface is to bind antithrombin III, which is an inhibitor of the coagulation cascade. Since the binding sites of the end-point attached heparin molecule are free, the

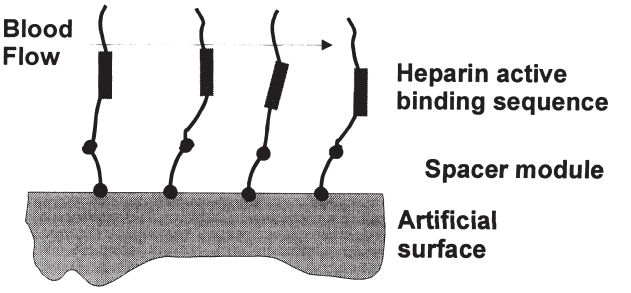

Fig. 2. Principle of a heparin coated artificial surface mimicking the natural orientation of heparan sulfate on the plasma membrane of endothelial cells. A spacer molecule imitating heparan sulfate (Hoffmann et al., 1983; Larm et al., 1983) keeps the active binding sites of the heparin molecule free.

antithrombin III molecules can be bound to heparin at the active sites. In this state, anthithrombin III is able to bind thrombin (Factor IIa) forming harmless inactive complexes. The inactivated complexes are released from the immobilized heparin and can be swept away from the site by the flowing blood. The immobilized heparin molecules serve as catalysts because they are not consumed by the reaction. A recent study by Yii et al. (1996) describes improved hemocompatibility by enhancing platelet and neutrophil survival, decreasing their activation and limiting the activation of the coagulation and complement cascades through this kind of heparin coating. A successful approach which avoids clotting and enables fast sensor response using a heparin coated enzyme surface of a needle-type biosensor was recently published (Yang et al., 1997).

The catalytic activity of the coating was 33 pmol/cm^2. This means that the heparin surface could bind around 33 pmol antithrombin III/cm^2. The surface and its properties have been described in more detail previously by Larm et al. (1989) and Lindhout et al. (1995). The heparin coated catheter system was sterilized in a GFS S2000 sterilizer (Münchner Medizin Mechanik, Planegg, Germany) with ETO (ethylene-oxide) at 55°C and 3 h gas time.

2.5. Subjects and protocols

Six healthy male volunteers (age 25.8 ± 2.8 years, body mass index (BMI) 22.7 ± 1 kg/m^2, means ± SD) were subjected to incremental bicycle ergometer exercise tests. The study was approved by the local ethical committee and written consent was given by all participants. The experiments were performed two hours postprandially following a carbohydrate enriched meal. ECG and blood pressure were measured throughout the entire experiment. A catheter (18 gauge × 45 mm, B. Braun Melsungen, Melsungen, Germany) was placed into an antecubital vein of the left forearm for drawing blood samples manually. The continuity of the cannula was provided with a controlled saline infusion. Contralaterally, the catheter system of the lactate monitoring system was placed into an antecubital vein. During the experiments No. 1–3, double lumen catheter systems (18 gauge × 33 mm, B. Braun Melsungen, Melsungen, Germany) were used, whereas during the experiments No. 4–6, single lumen heparin coated catheter systems (20 gauge × 25 mm, Beckton & Dickinson, Rutherford, U.S.A.) were applied. After insertion of the catheters, the ECG measurements were started and the blood withdrawal system was switched from calibration solution to blood. A resting period of at least half an hour was followed by an incremental bicycle ergometer test which was started at 50 W work with 25 W increments every two minutes and continued up to exhaustion of the subject. After achieving peak values of lactate, the experiments were continued until resting lactate concentrations of approximately 2 mmol/l were reached. The subjects were pedaling at 0 W work for recovery of pulse and blood pressure for 5 min and remained seated until the end of the experiment.

Blood samples were drawn manually prior to the start of the exercise tests and at the end of each work performance level. After having achieved the peak values of lactate samples were drawn manually in 10 min. intervals. The blood samples were collected in pre-chilled 3 ml lithium–heparin lithium–lodoacetate vacuum tubes (Beckton Dickinson Vacutainer Systems Eur., Meylan Cedex, France) and were subsequently centrifuged. The plasma probes were kept in ice for laboratory analysis performed immediately after the experiments.

2.6. Laboratory analysis, calibration of the sensors, data analysis and statistics

Manually drawn plasma samples were analyzed using lactate oxidase (Cobas Integra, Hoffmann La Roche, Basel, Switzerland). Results of the measurements are given in mmol/l lactate concentrations. For calibration of the monitoring system, initially two point calibrations were performed at 2 and 10 mmol/l lactate concentrations in two different acetate buffered aqueous solutions.

The results were analyzed using the linear regression analysis performed by the method of least squares. The relationship of the sensor data with the results of laboratory measurements of the manually drawn samples were estimated with Pearson's correlation coefficient. A method for assessing agreement between two methods of clinical measurement, published (Bland and Altman, 1989), was applied for the analysis of sensor values compared to lactate concentrations obtained from manually drawn samples. The results of this method are given as means (sensor values minus reference values), standard deviations (SD) and as standard error of the mean (SEM). Additionally, the percentage of sensor signals that were within the ± 10, ± 20, ± 30, ± 40, and over 40% range of the reference values were calculated. All calculations were made on a personal computer using Microcal™ Origin 4.10 (Microcal Software Inc., Northampton, MA, U.S.A.).

3. Results

In total, the continuous sensor readings were compared to 129 accurately timed lactate concentrations obtained from manually drawn blood samples. Plasma lactate values up to 14.05 mmol/l were obtained during the bicycle ergometer experiments. The miniaturized lactate sensors showed a linear range up to 25 mmol/l lactate concentration and 95% response times < 30 s in

undiluted serum (Jobst et al., 1997) which is demonstrated in Fig. 4. The lactate sensors typically showed electric base currents at 1 nA and sensitivities of 6 nA/mmol lactate.

Fig. 3(a) shows the regression analysis obtained from experiments using double lumen catheter based systems (Exp. No. 1–3, $n = 55$). Sensor readings and laboratory analyses of these experiments correlated between 0.93 and 0.98 (Table 1, $P < 0.001$). Mean of the residuals of all three experiments ($n = 55$) was − 0.35 mmol/l, the maximum and minimum were 3.85 and − 2.51 mmol/l respectively, SD was 1.47 mmol/l.

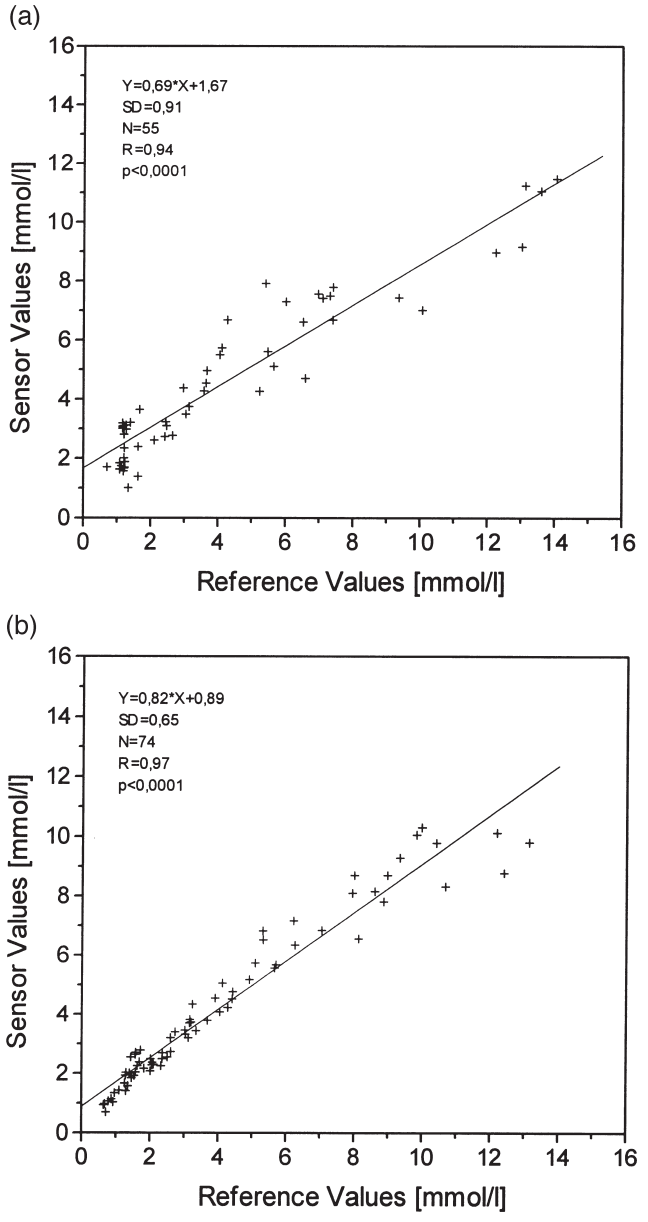

Fig. 3. Comparison between lactate sensors and the reference method. The regression line, the correlation coefficient r, the linear regression equation $y = ax + b$ and the number of estimations N are shown ($P < 0.0001$). (a) Shows the results of three experiments using double lumen catheter systems whereas in (b) the results of three experiments using heparin coated catheter systems are demonstrated.

The results from the experiments based on heparin coated catheter systems are given in Fig. 3(b). Correlation coefficients between 0.94 and 0.99 ($P < 0.001$) for sensor readings and laboratory results were obtained (Table 1). The overall results of residuals of the experiments using heparin coated catheter systems (Exp. No. 4–6, $n = 74$) were: mean = − 0.16 mmol/l, max and min (3.62 mmol/l and − 1.52 mmol/l), SD = 0.88 mmol/l.

The original results of a single experiment based on a heparin coated catheter system are demonstrated in Fig. 5. The box plot in Fig. 6 compares the distribution of the percentages of deviation from the reference values of the two setups (double lumen vs. heparin coating). Detailed results of the individual experiments are given in Table 1. Deviations of sensor readings compared to laboratory analyses are given as percentages in Table 2.

4. Discussion

A real-time ex vivo lactate monitor would be advantageous in a variety of clinical situations where lactic acidosis is likely to occur such as hypoxia, impaired oxygen delivery to tissues (anemia), poor tissue perfusion (low flow states) and sepsis. Furthermore, it could be of value in sports physiology. In this study a laboratory prototype for monitoring plasma lactate in human whole blood which meets clinical requirements is presented.

This prototype was used in two different operating modes concerning the prevention of blood coagulation, first based on a double lumen catheter for extracorporeal blood heparinization (Hakanson et al., 1993; Brückel et al., 1990; Trajanoski et al., 1996) and second based on a heparin coated tubing system. The dilution of the samples due to heparinization with heparin solution was considered in the calculations. In all six experiments sensors of the same batch were used. The results of this study allow the following conclusions:

First, considering the sensor performance, the evaluation data of lactate measurements show excellent accuracy of the presented system. The design of the sensors allowed flows between 0.24 and 350 μl/min (Jobst et al., 1996). The considerable high flow of 322 μl/min was used to obtain the requested amount of blood after the sensor (two minute intervals) for further analyses to confirm the calculated dilution with heparin solution. The 95% rise time was < 30 s (Fig. 4) and flow dependencies were negligible (Jobst et al., 1997). Regarding the slight deviations of the mean of the residuals in Table 1, the sensor performance can be considered as excellent. The percentages of deviation (mean, 85.2% within ± 30%) during the experiments with heparin coated catheter systems confirm this finding.

Table 1
Parameters of linear regression and method of residuals between sensor signals and manually drawn lactate concentrations

Parameters of linear regression:	Exp. No. 1	Exp. No. 2	Exp. No. 3	Exp. No. 4	Exp. No. 5	Exp. No. 6
n	16	17	22	21	24	29
Correlation coefficient *r*†	0.98	0.93	0.96	0.99	0.94	0.97
Intercept *b* (mmol/l)	0.45	1.16	2.7	0.42	0.56	1.33
Slope *a*	0.96	0.71	0.59	0.95	0.98	0.73
Method of residuals:						
SD (mmol/l)	0.46	0.8	2.22	0.59	0.44	1.17
SEM (mmol/l)	0.11	0.19	0.47	0.13	0.09	0.22
Mean (mmol/l)	− 0.31	− 0.36	− 0.37	− 0.21	− 0.51	0.17

*Relationship between the lactate concentration (x) and the sensor signal (y) is assumed to have the form: $y = ax + b$, whereas a is the slope and b is the intercept. †$P < 0.001$.

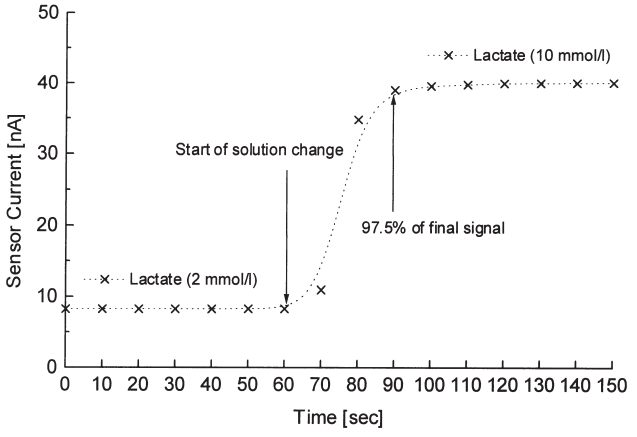

Fig. 4. Calibration curve at a solution flow of 150 μl/min. The 95% rise time is less than 30 seconds. Linear measuring ranges of these biosensors are up to 25 mM lactate in undiluted serum (Jobst et al., 1997).

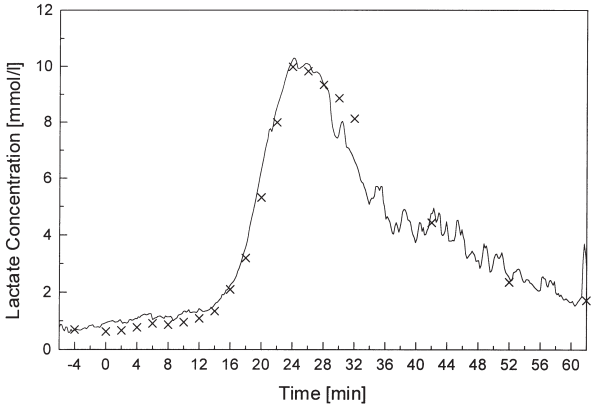

Fig. 5. Lactate concentrations measured with a lactate sensor (line) compared to results of manually withdrawn samples (reference values, crosses) obtained during an incremental bicycle ergometer test. Results from a single experiment using a heparin coated single lumen catheter system.

In the second part of this discussion some consideration about the kind of catheter system have to be made. The coated tubing system provided more accurate measurements than the double lumen system. This is probably due to the fact that the dilution of blood during continuous administration in a double lumen catheter is not constant. Furthermore this study demonstrates that a heparin coating based catheter and tubing system provides a reliable and simple use monitoring system. The evaluation data of lactate measurements showed excellent accuracy of the presented system. Erroneous measurements due to dilution problems using double lumen based catheter and tubing systems could be reduced with single lumen heparin coated systems. Hence, Fig. 6 demonstrates the differences of the two methods. Variations of the differences of sensor signals and reference values are greater using double lumen catheter systems. Major improvements for such setups are due to heparin coated catheter systems.

Comparatively, the heparin coated single lumen catheter and tubing system can be described as a “safe and easy to use” system. Furthermore, difficulties with sterile handling of solutions like heparin/saline can be avoided. A series of publications confirm improved biocompatibility (Matheve, 1996; Li-Chien, 1996) and reliability (Yii et al., 1996; Yang et al., 1997) of heparin coated systems.

Since heparin coating allows flow operation down to 16 μl/min the required blood amount and the loss of blood cells and proteins can be reduced significantly. Although only lactate sensors were used in this study, such biosensor-arrays allow the determination of other substances like glucose (Trajanoski et al., 1996), pH (Sheppard et al., 1995), pO_2,pCo_2, glutamate and glutamine. If measurements in intensive care units (ICUs) are desired, flows of < 50 μl/min will be desirable to reduce the amount of withdrawn blood to allow operations of the system up to 24 h or more. Further experiments with

Table 2
Deviations of sensor signals from manually drawn lactate concentrations given as percentage of measurements within a defined range of the reference values

Range of reference values (%)	± 10%	± 20%	± 30%	± 40%	> ± 40%
Exps 1–3 Double lumen	16.4%	32.8%	49.2%	63.7%	36.3%
Exps 4–6 Heparin coated	39.2%	64.9%	85.2%	94.6%	5.4%

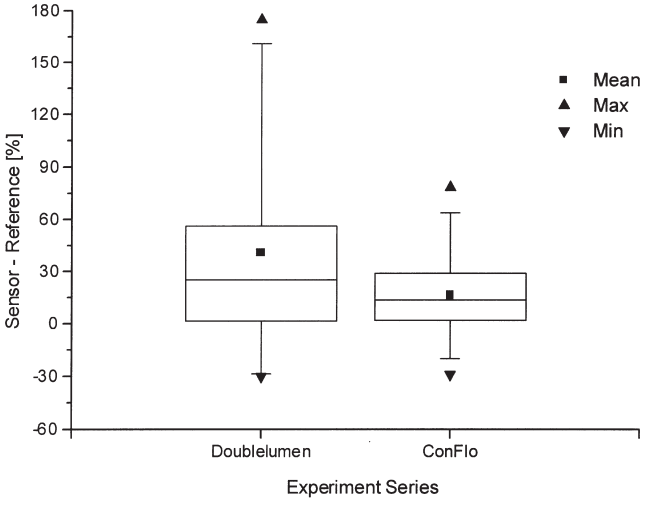

Fig. 6. Deviations of sensor signals from reference values given as percentage (double lumen system vs heparin coated system) demonstrate improved accuracy using heparin coated catheter systems (ConFlo®). Mean, minimum, maximum, the median value, the 25 and 75 percentiles are shown.

low flow rates should be performed for evaluation of the sensors for intensive care application. The non specific interference of the enzyme LOD was recorded with the "empty electrode" used as a "dummy electrode" and was subtracted from the lactate sensor signal. Interference with uric acid was not evaluated in the presented study and should be an objective future experiments. Interference with paracetamol had been performed previously (Jobst et al., 1996) where increased sensor readings had been observed only at toxic levels of 2 mm/l.

In conclusion, this study demonstrates the feasibility of a system for real-time ex vivo lactate monitoring. If temperature and pO_2 measurements were added to a system of this kind, the accuracy and reliability could be improved. Moreover, a heparin coated tubing system improves the non-thrombogenic properties, the biocompatibility of the catheter system and the results of the sensor measurements. The presented device could be a valuable tool in routine applications (intensive care units (ICU), surgery, sports medicine) as well as in research investigations, where real-time monitoring is required.

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