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Lactate Metabolism of Subcutaneous Adipose Tissue Studied by Open Flow Microperfusion

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ABSTRACT

Open flow microperfusion and a novel calibration technique (ionic reference technique) were evaluated for the frequent measurement of the absolute lactate concentration in sc adipose tissue. Furthermore, the influence of the plasma insulin concentration on the lactate concentration of sc adipose tissue was investigated during hyperglycemia. Sixteen lean healthy young men participated in the studies. In the postabsorbtive state the mean sc lactate concentrations were 1.29 and 1.36 mmol/L for the ionic reference technique and the no net flux protocol, respectively (not significant, $P > 0.05$). The simultaneously measured arterialized plasma lactate concentration was significantly lower at 0.77 mmol/L ($P < 0.05$). Both the sc lactate concentration (1.8 ± 0.33 mmol/L) and the plasma lactate concentration (0.96 ± 0.03 mmol/L) were significantly elevated during a hyperinsulinemic euglycemic clamp experiment. During a hyperglycemic clamp experiment the sc lactate concentration reached a significantly elevated plateau (2.15 ± 0.27 mmol/L) that was not influenced by the increasing plasma insulin concentration. It is concluded that 1) open flow microperfusion combined with the ionic reference technique enables frequent measurement of the sc lactate concentration; 2) sc adipose tissue is a significant source of lactate release in the postabsorbtive state as well as during hyperinsulinemic clamp conditions; and 3) insulin concentrations greater than 180 pmol/L have no further influence on adipocyte stimulation of sc adipose tissue with respect to lactate release. (*J Clin Endocrinol Metab* **83:** 4394–4401, 1998)

LACTATE is known to be a major regulatory substrate in carbohydrate metabolism. Since it was shown that adipocytes of sc adipose tissue are a significant source of lactate release, interest focused on the measurement of lactate kinetics in peripheral tissues. In these studies tissue metabolism was monitored using two different techniques. Numerous investigators (1–8) established the microdialysis technique for the measurement of lactate concentration in interstitial fluid of sc adipose tissue or muscle during various metabolic interventions. Other investigators have used the measurement of arterio-venous differences for estimation of tissue metabolism and have applied this technique to the study of skeletal muscle and forearm tissues in man (9, 10). Furthermore, Coppack *et al.* (11) and Frayn *et al.* (12) investigated arterio-venous differences across sc adipose tissue by cannulation of the superficial epigastric or superficial circumflex iliac vein. Using both microdialysis and the measurement of arterio-venous differences, it was reported that lactate is produced by sc adipose tissue in the postabsorbtive state as well as during hyperinsulinemia (5, 8, 11). In a recent study Henry *et al.* (8) found that adipocytes take up glucose to produce and release lactate and that this process is stimulated by the insulin concentration. Henry *et al.* suggested that the increased fat mass and/or hyperinsulinemia of obese patients may contribute to increase whole body lactate production.

In recent studies a novel technique, called open flow microperfusion (13), was developed for continuous extracorporeal monitoring of sc adipose glucose concentrations during metabolic events such as hyper- or hypoglycemia (14) or lactate concentrations during extensive cycle ergometer exercise (15) using a novel thin film sensor technology. In contrast to microdialysis, in which a sterile barrier prevents direct contact with the interstitial compartment, open flow microperfusion allows direct access to the interstitial compartment of sc adipose tissue and therefore may produce further insight into tissue metabolism. Using open flow microperfusion a perforated double lumen catheter is set into sc adipose tissue and perfused with isotonic ion-free perfusate. Via the perforations of the catheter, the perfusate partially equilibrates with the surrounding tissue fluid. A simple calibration technique (ionic reference technique) allows estimation of the absolute concentrations of the interstitial compartment of sc adipose tissue (13–15).

The first objective of this study was the validation of the ionic reference technique for the frequent monitoring of absolute lactate concentrations in the interstitial fluid of sc adipose tissue. We measured the sc lactate concentration in the postabsorbtive state using an established calibration protocol (no net flux protocol) (7, 16) and the ionic reference technique.

The second objective was to test whether the method of open flow microperfusion is appropriate for the frequent monitoring of dynamic processes in sc adipose tissue. We

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performed a hyperinsulinemic euglycemic clamp experiment to confirm the recent findings of a significantly elevated interstitial lactate concentration during hyperinsulinemia.

Our third objective was to investigate the influence of the insulin concentration on the lactate concentration of sc adipose tissue during hyperglycemia. Using a hyperglycemic clamp experiment without somatostatin infusion, we tested whether the sc lactate concentration increases in parallel with the physiological plasma insulin response or is uninfluenced by the increasing insulin concentration.

Subjects and Methods

Subjects

Sixteen lean healthy young men participated in this study. None of them used any medication for 2 months before and during the study. All subjects were informed about possible risks and gave their volunteered consents. The study was approved by the local ethics committee of the University of Graz. The subjects were subdivided into different groups for different study protocols (Table 1); they were of similar age (mean ± SD, 25.4 ± 3.1 yr) and body mass index (mean ± SD, 23.6 ± 1.9 kg/m^{-2}). All subjects were investigated at 0800 h after an overnight fast while they were in the supine position at a room temperature of 21 C. In all protocols a retrograde cannula was inserted into a dorsal hand vein, and the hand was placed in a thermoregulated box (55 C) to obtain arterialized blood samples (17).

Sampling device

A schematic representation of the sampling device is given in Fig. 1. The system consists of a perfusate reservoir, a perforated double lumen catheter, a peristaltic pump, and vials collecting the perfusate.

A conventional iv cannula (18 gauge; 50 × 1.2 mm diameter; Angiocath, Becton Dickinson Inc., Sandy, UT) was perforated with 120 holes (0.5 mm diameter) using an Excimer Laser (krypton fluorine; 280 nm; 600 mJ; LPX 205i, Lambda Physik GesmbH, Gottingen, Germany). After local skin anesthesia (Novanest purum 2%, Gebro Broschek, Fieberbrunn, Austria), the perforated cannula was inserted into the sc adipose tissue of the abdominal region by the use of a steel mandrin. The steel mandrin was removed thereafter and replaced by an inner tubing made of Teflon (od, 0.76 mm; id, 0.3 mm; PTFE tubing, Cole-Parmer Instrument Co., Vernon Hills, IL). Figure 2 shows a photoprint of the double lumen catheter. The double lumen catheter was perfused with 5% mannitol in aqueous solution (protocols II, III, and IV) or with Ringer's solution or mannitol (protocol I). A peristaltic pump (Minipuls 3, Gilson, Villiers-le-Bel, France) was used for applying a negative pressure to the system, drawing the perfusate out of the reservoir bag and pulling it through the double lumen catheter as indicated by the *arrows* in Fig. 1.

Via the perforations of the outer lumen of the double lumen catheter, the perfusion fluid had the opportunity to partially equilibrate with the surrounding tissue fluid by diffusion and/or convective transport. The partially equilibrated perfusate was transfered to the collecting vials (PCR soft tubes; 0.2 mL; Biozym Diagnostik GesmbH, Oldendorf, Germany), which were changed manually in intervals as described in the respective protocol sections. The system was perfused at a constant flow rate of 2 μL/min in all protocols. The average flow rate was checked by weighing the collected samples. To prevent evaporation of the sampled fluid, the collecting vials were covered with Parafilm (Parafilm M, American National CONTM, Greenwich, CT) and cooled by ice. The samples were stored at −70 C and analyzed subsequent to each experimental study. To avoid contamination of the samples due to the acute trauma caused by catheter insertion, each experiment started after an initial equilibration period of 60 min.

Calculating absolute concentrations in sc adipose tissue (ionic reference technique)

As mentioned in the description of the sampling device, the perfusate partially equilibrates with the surrounding tissue fluid. In previous investigations (14, 15), the conductivity, which is proportional to the sum of the ion concentrations, of the interstitial fluid of sc adipose tissue was assumed to be constant, and the conductivity of the sampled fluid was measured as an indicator of the rate of recovery of interstitial fluid in the perfusate. In the present study the sodium concentration of the perfusate was used to calculate the recovery rate.

The recovery of the sc tissue fluid in the perfusate was calculated as the ratio of the sodium concentration in the sampled fluid to the sodium concentration of the interstitial fluid of sc adipose tissue (140 mmol/L (18, 19)). The interstitial sodium concentration was assumed to be constant during the experiment (14, 20). Absolute lactate concentrations of the sc adipose tissue were calculated as the ratio of the lactate concentration measured in the sampled fluid to the rate of recovery.

Protocol I (no net flux protocol)

Two double lumen catheters were inserted into the abdominal region of sc adipose tissue of six subjects (Table 1) to perform a no net flux calibration protocol. One catheter was perfused with 5% mannitol in aqueous solution (Mayrhofer Pharmazeutika GesmbH, Linz, Austria) the other catheter was perfused with Ringer's solution (Ringer Lösung, Leopold Pharma, Graz, Austria; 147.2 mmol/L Na, 155.7 mmol/L Cl, 4.00 mmol/L K, and 2.25 mmol/L Ca; 309 mosmol/L). The subjects were fasting throughout the experiment. The no net flux protocol is based on the principle that the substance of interest is added to the perfusate in different concentrations, and the point of no net change of the substance in the sampled fluid compared with the concentration in the perfusate is calculated by linear regression analysis. The estimate represents the absolute interstitial fluid concentration of the substance of interest (7, 16). Within this study, we added four different concentrations of L-lactate to the perfusates (0.6, 1.2, 1.8, and 2.4 mmol/L) and included a fifth perfusion step where no lactate was added to the perfusate. Catheters were perfused in randomized order in every experiment. For each concentration a initial equilibration period of 30 min was added.

Protocol II (postabsorbtive state)

In protocol II, five subjects (Table 1) were investigated in the postabsorbtive state for a period of 6 h. One double lumen catheter was set into the sc adipose tissue of the right abdominal region and was perfused with 5% mannitol in aqueous solution. After an initial equilibration period of 1 h, the experiment was started. Interstitial fluid samples were obtained every 30 min and were analyzed for lactate and sodium concentrations.

Protocol III (euglycemic hyperinsulinemic clamp)

Five subjects (Table 1) participated in this 6-h protocol. An iv cannula for continuous glucose and insulin infusion was set into a cubital vein opposite the forearm from which arterialized blood samples were withdrawn. After a basal period of 90 min, a continuous insulin infusion (40 IU/mL; Actrapid HM, Novo Nordisk A/S, Bagsvaerd, Denmark) was

TABLE 1. Subject characteristics

Experimental study	BMI (kg/m^{-2})	Age (yr)	Fasting plasma glucose (mmol/L)	Fasting plasma lactate (mmol/L)	n
Protocol I (no net flux)	23.3 ± 1.2	25.8 ± 4.3	5.2 ± 0.2	0.61 ± 0.03	6
Protocol II (postabsorptive state)	23.2 ± 1.7	27.0 ± 4.2	5.4 ± 0.2	0.77 ± 0.07	5
Protocol III (euglycemic hyperins. clamp)	24.4 ± 2.9	25.2 ± 3.0	5.0 ± 0.1	0.85 ± 0.13	5
Protocol IV (hyperglycemic clamp)	23.9 ± 1.1	24.0 ± 1.1	5.2 ± 0.1	0.76 ± 0.11	6

Values are the mean ± SD, BMI, Body mass index.

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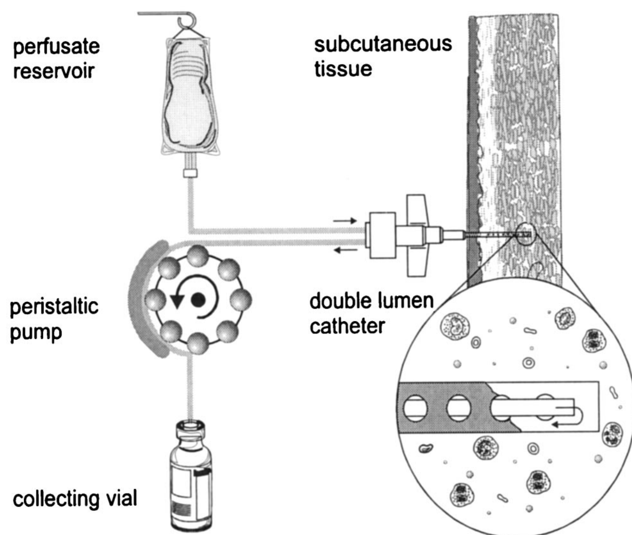

FIG. 1. Schematic representation of the sampling device. The flow of the fluids is indicated by *arrows*. The double lumen catheter (18 gauge; 50 × 1.2 mm diameter) is inserted into the sc adipose tissue of the abdominal region.

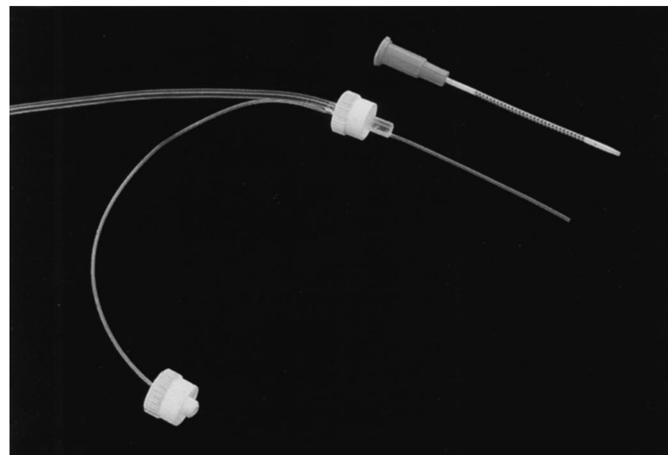

FIG. 2. Perforated double lumen catheter (perforated iv cannula (18 gauge; 50 × 1.2 mm diameter) and inner lumen made of PTFE Teflon tubing (od, 0.76 mm; id, 0.3 mm).

started for a period of 3 h. Insulin was infused at a fixed rate (1 mU/kg·min) during the clamp period. The arterialized plasma glucose concentration was clamped at euglycemic values (5 mmol/L) by variable glucose infusion (21). After the clamp period of 3 h, insulin infusion was stopped, and variable glucose infusion was continued to maintain euglycemia for another 90 min. Arterialized plasma glucose was measured in duplicate at intervals of 5 min; arterialized plasma samples were withdrawn every 30 min and analyzed for lactate and insulin concentrations. Subcutaneous tissue fluid was sampled at intervals of 30 min and analyzed for lactate and sodium concentrations.

Protocol IV (hyperglycemic clamp)

A hyperglycemic clamp experiment at the physiological insulin response was performed in six subjects (Table 1). One double lumen catheter was set into the sc adipose tissue of the right abdominal region and was perfused with 5% of mannitol in aqueous solution at a constant flow rate of 2 μL/min. An iv cannula was set into a cubital vein for continuous glucose infusion (Glucose 20% Leopold, Leopold Pharma, Graz, Austria) at the forearm contralateral to the arm from which arterialized blood samples were withdrawn. After a 1-h initial equilibration period and 2 h of measurement under basal conditions, the hyperglycemic clamp was started with a bolus infusion of 20% glucose (300 mg/kg). Thereafter, the plasma glucose concentration was clamped at 10 mmol/L for a period of 3 h by continuous variable infusion of 20% glucose (21). Arterialized plasma glucose was measured in duplicate at intervals of 5 min throughout the clamp period. Arterialized plasma samples were withdrawn every 30 min and analyzed for lactate, insulin, and lactate dehydrogenase (LDH) concentrations. Interstitial fluid samples were obtained every 30 min and were analyzed for lactate, sodium, and LDH concentrations. Additionally, for the first hour after catheter insertion, interstitial fluid was sampled at intervals of 15 min and analyzed for LDH concentration.

Chemical analysis

Plasma and interstitial lactate and LDH concentrations were determined photometrically using a Cobas Integra Laboratory Analyzer (Cobas Integra, Hoffmann-La Roche, Basel, Switzerland). Using an external lactate standard of 0.8 and 0.2 mmol/L, coefficients of variation of 0.6% and 1.7% (n = 10) were determined. The interstitial sodium concentration was analyzed by a flame photometer (IL 943, Instrumentation Laboratory S.p.A., Milan, Italy). Coefficients of variation of 1.2% and 1.5% for sodium concentrations of 70.0 and 35.0 mmol/L were measured, respectively. Plasma glucose was measured enzymatically using a Beckman Glucose Analyzer II (Beckman Instruments, Fullerton, CA). Plasma insulin was measured using a commercial RIA kit (human insulin-specific RIA kit, Linco Research, Inc., St. Charles, MO).

Statistical analysis

Analysis of the no net flux protocol was performed adopting linear regression analysis of the mean value of each concentration step. Student's paired *t* test was used when different time points or different catheters in the same subject were compared, and Student's independent *t* test was applied when concentrations between study groups were compared. All statistical analyses and diagrams were performed using Micro Cal Origin (technical graphics and data analysis, Microcal Software, Inc., Northhampton, MA). Unless otherwise indicated, results in the text, figures, and tables are the mean ± SEM.

Results

Protocols I and II (evaluation studies)

Mean regression lines for the no net flux protocol are given in Fig. 3A. The lactate concentrations estimated for the point of no net flux were 1.36 mmol/L (r = 0.99; n = 6) and 1.50 mmol/L (r = 0.98; n = 6) for 5% of mannitol in aqueous solution and Ringer's solution, respectively. Individual sc lactate concentrations (mannitol *vs.* Ringer's) were not significantly different ($P > 0.05$). The simultaneously measured mean arterialized plasma lactate concentration was significantly lower (0.61 ± 0.03 mmol/L; $P < 0.05$) than the estimated absolute sc tissue lactate concentration.

The lactate concentration in sc adipose tissue calculated by the ionic reference technique was 1.29 ± 0.16 mmol/L (mean ± SEM; n = 5; Fig. 3B). A small, but not significant, increase ($P > 0.05$) in the concentration was observed during the 6-h period (Fig. 3B). The absolute lactate concentration of protocol II was not significantly different ($P > 0.05$) from the absolute sc concentration as estimated by protocol I. The recovery measured by the ionic reference technique was 56.2 ± 7.9% (mean ± SEM; n = 5; Fig. 6). The arterialized plasma concentrations of glucose and lactate did not change from basal levels and were 5.4 ± 0.2 and 0.77 ± 0.07 mmol/L (mean ± SEM), respectively.

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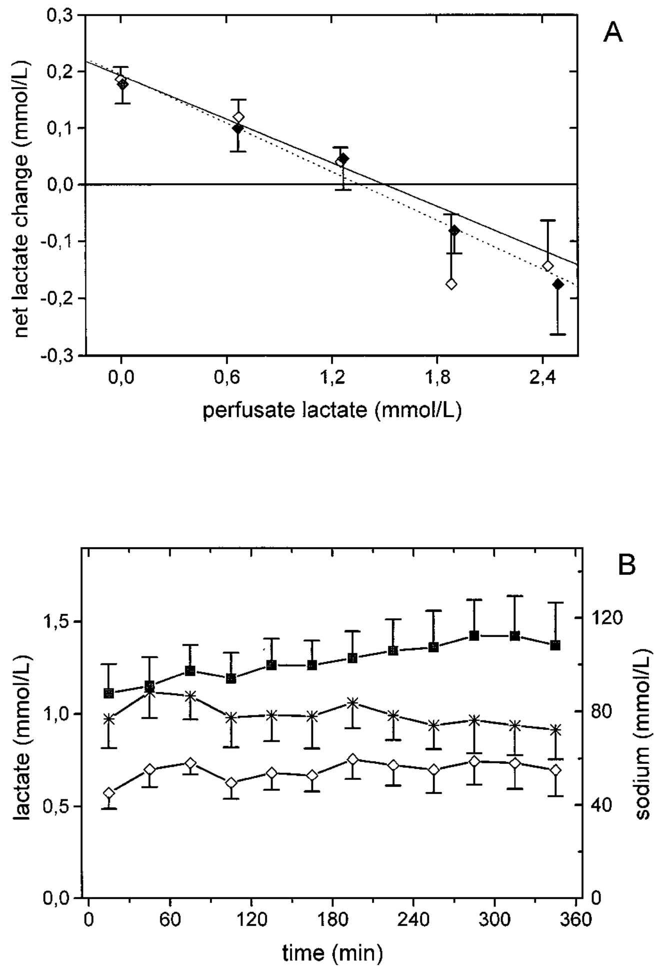

FIG. 3. The data of the no net flux calibration procedure are depicted in A. The lactate concentrations are expressed as the difference between sampled fluid and perfusate on the *y*-axis and are plotted against the concentrations of the perfusate on the *x*-axis. Data are plotted for 5% mannitol (*closed diamonds*) and Ringer's solution (*open diamonds*) at five different perfusate concentrations of lactate. Regression lines are plotted for 5% mannitol (*dotted line*) and Ringer's solution (*straight line*), respectively. B depicts the absolute lactate concentration (*closed squares*) and the lactate concentration of the sampled fluid (*open diamonds*) on the *left y*-axis and the sodium concentration of the sampled fluid (*stars*) on the *right y*-axis in the postabsorptive state in six healthy volunteers. All data are given as the mean ± SEM.

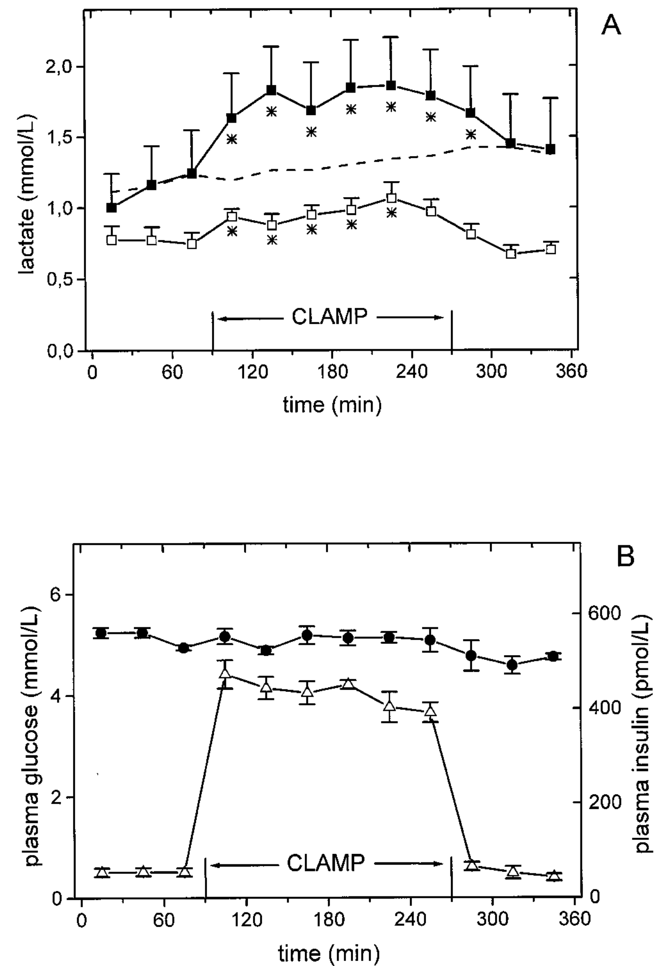

FIG. 4. Adipose tissue (*closed squares*) and arterialized plasma (*open squares*) lactate concentrations during basal conditions (0–90 min), euglycemic hyperinsulinemic clamp conditions (90–270 min), and again basal conditions (270–360 min) are shown in A. The absolute interstitial lactate concentration of protocol II (Fig. 3B) is redundantly depicted in A (*dotted line*). The clamp period is indicated by the *arrows*. B shows the arterialized plasma glucose (*closed circles*, *right y*-axis) and insulin (*open triangles*, *right y*-axis) concentrations during the study period. All data are given as the mean ± SEM (n = 5); *, $P < 0.05$ *vs.* baseline.

Protocol III (euglycemic hyperinsulinemic clamp)

The results of the euglycemic hyperinsulinemic clamp are given in Fig. 4. During basal conditions the absolute sc lactate concentration calculated by the ionic reference technique was 1.14 ± 0.27 mmol/L (mean ± SEM; n = 5). The concentration was not significantly different ($P > 0.05$) from absolute concentrations estimated with protocols I and II. The sc lactate concentration was significantly elevated during the clamp (1.8 ± 0.33 mmol/L (mean ± SEM; $P < 0.05$) and reached basal values 30 min after the clamp (Fig. 4A). The mean recovery measured by the ionic reference technique was 43.5 ± 8.2% (mean ± SEM; Fig. 6). The basal arterialized plasma lactate concentration was 0.74 ± 0.02 mmol/L (mean ± SEM). A significant increase ($P < 0.05$) in the plasma lactate concentration was observed during the clamp period (Fig. 4A). The sc lactate concentration was significantly higher than the arterialized plasma lactate concentration throughout the study ($P < 0.05$). The time courses for plasma glucose (5.01 ± 0.08 mmol/L) and plasma insulin (basal, 54.6 ± 0.36 pmol/L; clamp, 432.6 ± 12.6 pmol/L) concentrations are depicted in Fig. 4B.

Protocol IV (hyperglycemic clamp)

The results for the hyperglycemic clamp study are presented in Fig. 5. The basal sc lactate concentration calculated by the ionic reference technique was 1.28 ± 0.10 mmol/L (mean ± SEM; n = 6) and was not significantly different ($P >$ 0.05) from the absolute lactate concentration of protocols I, II, and III. Both the sc lactate concentration (2.15 ± 0.27 mmol/L; $P < 0.05$) and the arterialized plasma lactate con-

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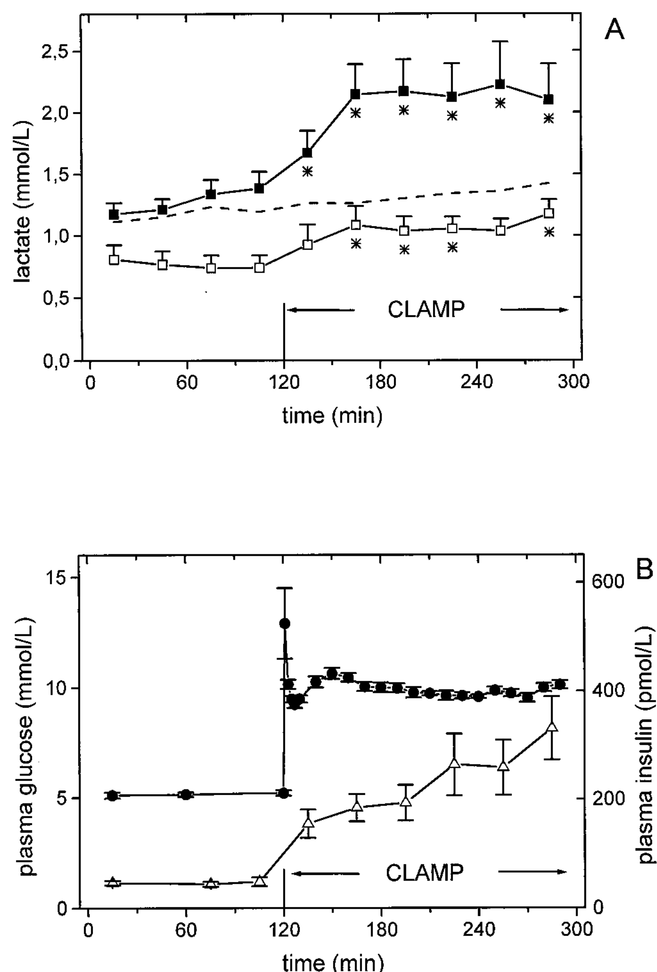

FIG. 5. Adipose tissue (*closed squares*) and arterialized plasma (*open squares*) lactate concentrations in the postabsorbtive state and during hyperglycemic clamp conditions at a physiological insulin response are depicted in A. The absolute interstitial lactate concentration of protocol II (Fig. 3B) is redundantly depicted in A (*dotted line*). The plasma glucose concentration (B, *closed circles*, *left* y-axis) was clamped at 10 mmol/L for a period of 3 h. The insulin concentration (B, *open triangles*) is shown on the *right* y-axis. The clamp period is indicated by the *arrows*. All data are given as the mean ± SEM (n = 6); *, $P < 0.05$ *vs.* baseline.

centration (basal, 0.76 ± 0.11 mmol/L; clamp, 1.08 ± 0.11 mmol/L; $P < 0.05$) were significantly elevated during the hyperglycemic clamp period. The lactate concentration was significantly higher in sc adipose tissue than in arterialized plasma throughout the study ($P < 0.05$). A mean recovery of 38.1 ± 9.7% (mean ± SEM) between the interstitial fluid and the perfusate fluid was measured in this study (Fig. 6). Basal plasma glucose and insulin concentrations in this protocol were 5.15 ± 0.12 and 46.2 ± 4.8 pmol/L (mean ± SEM). Plasma glucose was clamped at 10.01 ± 0.12 mmol/L (mean ± SEM) for a period of 3 h (Fig. 5B). Arterialized plasma LDH levels were stable throughout the observation period (72.9 ± 16.7 U/L). LDH levels in the sampled fluid of sc adipose tissue were high in the first sample after catheter insertion (69.5 ± 14.6 U/L), but fell to significantly ($P < 0.05$) lower levels for the rest of the study period (25.7 ± 1.5 U/L; Fig. 7).

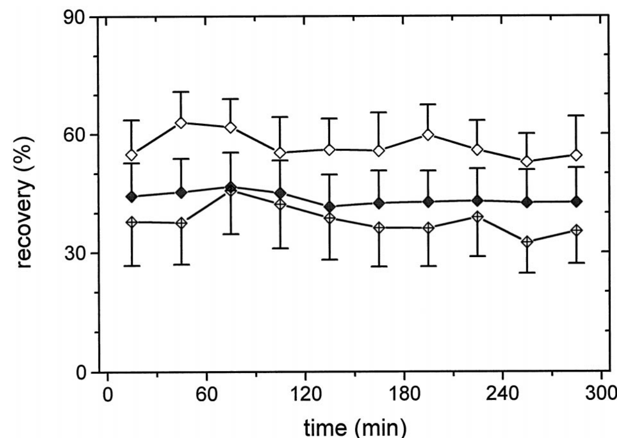

FIG. 6. Sodium recovery rates in the sampled fluid of protocol II (*open symbol*), protocol III (*closed symbol*), and protocol IV (*cross inside open symbol*) as a percentage of the interstitial sodium concentration (140 mmol/L). The clamp period of protocols III and IV started at 120 min. All data are given as the mean ± SEM.

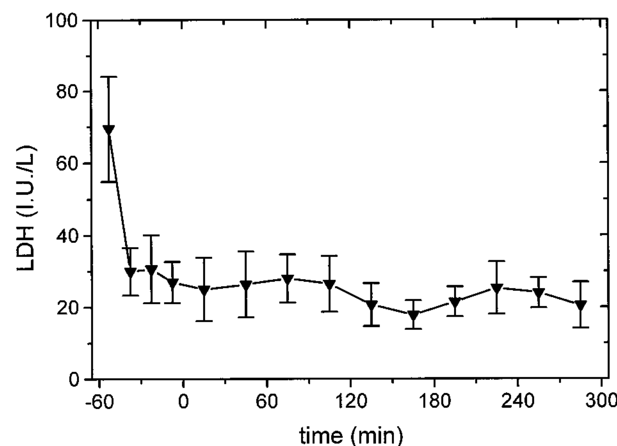

FIG. 7. LDH concentrations of the sampled fluid measured during protocol IV. LDH levels in the sampled fluid of sc adipose tissue were high in the first sample after catheter insertion, but fell to significantly lower levels for the rest of the study period ($P < 0.05$). All data are given as the mean ± SEM (n = 6).

Discussion

In the present study the open flow microperfusion technique was evaluated for the frequent measurement of absolute lactate concentrations in interstitial fluid of sc adipose tissue. The results indicate that plasma insulin concentrations greater than 180 pmol/L cause no additional effect on adipocyte stimulation with respect to lactate release. Furthermore, our findings corroborate the significant findings of study groups using microdialysis or the measurement of arterio-venous differences, *i.e.* that adipocytes of sc adipose tissue are a significant source of lactate release in both the postabsorbtive state as well as during hyperinsulinemic conditions (5, 6, 11) and that this process is stimulated by the insulin concentration (8).

We have recently shown that combining open flow microperfusion, glucose sensor, and conductivity measurement enables a continuous extracorporal monitoring of sc adipose tissue glucose concentration (13, 14). Moreover, the possi-

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bility of on-line monitoring of the sc lactate concentration during basal conditions and extensive cycle ergometer exercise was demonstrated with the same technique (15). However, in these two studies the sampled fluid was analyzed on-line by means of newly developed sensor techniques, and perfusate was not sampled for analysis using well established laboratory analyzers. In the present study we collected the sampled perfusate fluid and used high precision laboratory analyzers (see *Analysis and statistics* section).

In contrast to microdialysis, open flow microperfusion allows a free exchange of substances between the tissue and the perfusate fluid. The concentration of a substance in fluid sampled by open flow microperfusion depends on various technical parameters such as the flow rate of the perfusate, the area of exchange of the double lumen catheter, the chemical composition of the perfusate, and various physiological characteristics of the tissue, such as temperature, microvascular transport rate, size of the adipocytes, or specific tissue blood flow. Although technical parameters can be kept constant, physiological parameters can only be considered to some extent. Therefore, the recovery varies between the subjects as well as within the subjects as shown previously (13–15). The ionic reference technique is a newly developed calibration method accounting for these influences on recovery. In protocol I we used a well established calibration protocol (no net flux protocol) (7, 16) for evaluation of the ionic reference technique. The no net flux protocol was performed using two different perfusate solutions to investigate a possible influence of mannitol on equilibration processes in the double lumen catheter. The estimated absolute concentration measured with the more physiological Ringer's solution was not significantly different from the concentration obtained with 5% mannitol as perfusate ($P > 0.05$). Therefore, one may conclude that the use of mannitol as an ion-free perfusate, which is necessary for the ionic reference technique, has no significant influence on local equilibration processes relating to lactate measurement.

In protocol II it was shown that the combination of open flow microperfusion and the ionic reference technique allows a frequent and stable measurement of the sc lactate concentration in the postprandial state for a period of at least 6 h. Furthermore, as depicted in Figs. 4A and 5A, we used the results of protocol II to corroborate the significant findings of protocols III and IV. The ionic reference technique is based on the assumption that the sodium concentration in the interstitial fluid of sc adipose tissue is known and constant. The ionic strength, the osmolarity, and consequently the sodium concentration of the interstitial fluid are closely regulated by antidiuretic hormones and the kidney. Under normal circumstances, the osmolarity is fairly constant and varies only slightly from day to day (14, 18–20). Even pathophysiological influences on the interstitial ion status result in minor effects on the accuracy of the ionic reference technique, as discussed in an earlier report of Trajanoski *et al.* (14). Therefore, it may be concluded that variations in the interstitial sodium concentration are negligible and do not significantly influence the accuracy of the ionic reference technique.

The mean recoveries of the individual protocols (protocols II, III, and IV) were stable throughout the experimental study periods. However, different mean recoveries of the individual protocols were observed, as indicated in Fig. 6. As mentioned above, the concentration of a substance in the fluid sampled by open flow microperfusion depends on various technical parameters as well as on physiological characteristics of the tissue. These effects and the fact that only five or six subjects of a large population were investigated in the protocols of the present study may lead to variations in the recoveries in the individual protocols. However, independent of the degree of equilibration (recovery) of a substrate in the perfusate, basal lactate concentrations of the interstitial fluid of sc adipose tissue estimated by the ionic reference technique were very similar in all protocols. In three independent studies (protocols II, III, and IV), the interstitial lactate concentration calculated by the ionic reference technique was not significantly different from the lactate concentration estimated by the no net flux protocol. This indicates that open flow microperfusion combined with the ionic reference technique is applicable for the measurement of absolute lactate concentrations in sc adipose tissue.

Measurement of postprandial lactate concentrations in sc adipose tissue of healthy humans was performed by various investigators (1–3, 5, 6, 22). In all studies, the sc lactate concentration was significantly higher (range, 1.01–2.02 mmol/L) than the lactate concentration in arterialized plasma. Using open flow microperfusion, we have also measured a comparable and significantly increased sc lactate concentration compared with arterialized plasma values. It remains unknown whether the high sc lactate values represent true physiological values or are the result of tissue trauma and/or inflammation due to the insertion of microdialysis or double lumen catheters. Open flow microperfusion catheters and microdialysis probes are similar in size and flexibility; therefore, it may be assumed that even the effects of a probe insertion into the tissue are comparable. Several investigators attempted to clarify questions of tissue trauma and measured substances such as adenosine (23), pyruvate (1), LDH (24), ATP (25, 26), and potassium (27) or the glucose/lactate ratio (28) in the sampled fluid. Evidence for tissue trauma was found only for approximately the first hour after insertion of microdialysis catheters. In protocol IV of the present study we measured the LDH concentration in the sampled tissue fluid as well as in arterialized plasma, because LDH is known to be elevated during ischemia and cell destruction (18, 24, 29). In arterialized plasma, LDH concentrations were constant throughout the experiment. In the sampled interstitial fluid, LDH levels were significantly elevated during the first 15 min after catheter insertion compared with those during the remainder of the experimental period (Fig. 7). Inflammation is another long acting process after tissue injury. Signs of inflammation are the liberation of large quantities of histamine, bradykinin, serotonin, and other substances. These, especially histamine, increase the local tissue blood flow. Mediators of inflammation have not yet been measured using open flow microperfusion. However, no visible sign of inflammation (*e.g.* erythema due to a local blood flow increase) occurred in the different protocols of the present study. Furthermore, measurement of the sodium concentration in the sampled fluid did not indicate any sign of instability of the method, as the sodium concentration was stable throughout the study period in all protocols (Fig.

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6); this might be an indication for a stable tissue blood flow around the catheter. Therefore, it is concluded that the double lumen catheter as constructed for the open flow microperfusion technique is appropriate for human studies, causing only minor and transient damage to sc adipose tissue.

Coppack *et al.* (11) used the measurement of arterio-venous differences for the evaluation of adipose tissue metabolism. This method does not give direct access to a specific tissue region, but has the advantage that the investigated tissue region is not traumatically influenced. From their studies Coppack *et al.* reported that lactate is produced by sc adipose tissue in the postabsorbtive state as well as during hyperinsulinemia. Therefore, there remains little doubt from all experimental protocols that sc adipose tissue is a significant source of lactate production in the postabsorbtive state.

In protocol III the influence of hyperinsulinemia on lactate release of sc adipose tissue was investigated. Corresponding with previous microdialysis studies (5, 6, 8) and the measurement of arterio-venous differences (11), we observed that hyperinsulinemia at euglycemic values results in a significant increase in lactate in both sc adipose tissue and arterialized plasma (Fig. 4A). Furthermore, in this study for the first time the decrease in the sc lactate concentration to basal levels after the clamp period was monitored.

To investigate the influence of the insulin concentration on the sc lactate concentration during hyperglycemia, we performed a hyperglycemic clamp experiment at the physiological insulin response. We monitored the sc lactate concentration and the arterialized plasma lactate concentration for a clamp period of 3 h (protocol IV). Figure 5A depicts a significantly elevated lactate concentration in the sc adipose tissue 30 min after the start of the clamp compared with the basal concentration. These results indicate that the combination of hyperinsulinemia and hyperglycemia results in a significantly elevated lactate production by sc adipose tissue. The increase in the sc lactate concentration during the clamp period was similar in protocol III (58% of the basal level during hyperinsulinemia) and protocol IV (68% of the basal level during hyperinsulinemia and hyperglycemia); therefore, it is concluded that the increase in lactate was primarily stimulated by the insulin concentration, as it was shown in the study of Henry *et al.* (8) using the microdialysis technique. During the hyperglycemic clamp of the present study, the sc lactate concentration reached an early plateau 30 min after the start of the clamp, which was not altered by continuously increasing insulin concentrations (Fig. 4). This finding leads to the assumption that at most a 4-fold increase in the plasma insulin concentration is necessary to stimulate adipocytes for maximum lactate release. Further increasing insulin concentrations has no additional effect on adipocyte stimulation with respect to lactate formation, as the data indicate. The findings of the present study suggest that maximal levels of adipose lactate concentrations at submaximal insulin concentrations might accelerate the effects of increased fat mass in human obesity. This increased lactate production driven by moderately elevated insulin levels, as seen in obesity, may contribute to increased liver gluconeogenesis and, in consequence, lead to impaired glucose tolerance associated with human obesity (1).

The determination of quantitative amounts of lactate release by the sc adipose tissue depot was not feasible in the present study because the extraction fraction of lactate and the specific tissue blood flow were not measured (30). However, expressing differences in arterialized to interstitial lactate concentrations as qualitative changes in the local lactate production in sc adipose tissue is still valid, as the increase in the interstitial lactate concentration observed during hyperinsulinemia would underestimate the actual lactate production due to a possible tissue-specific blood flow increase. The effects of hyperinsulinemia on adipose tissue blood flow changes remain unclear, as contrary results have been obtained. In various studies using the microdialysis technique it was shown that hyperinsulinemia has no effect on adipose tissue blood flow *in vivo* (5, 31–34), whereas Jannson *et al.* (1) and Henry *et al.* (8) found an increase in adipose tissue blood flow during hyperinsulinemic conditions using the same technique. In the present study the sodium concentration was used as an endogenous marker and remained constant during the change from basal to hyperinsulinemic conditions of protocols III and IV. This finding might be an additional evidence that hyperinsulinemia has no effect on tissue blood flow around the catheter. However, as the blood flow was not directly measured in the present study, a possible influence of insulin on adipose tissue blood flow cannot be ruled out.

In summary, it was shown that the novel technique of open flow microperfusion combined with the ionic reference technique allows the measurement of absolute lactate concentrations in sc adipose tissue. Furthermore, it could be substantiated that adipocytes in sc adipose tissue are a significant source of lactate release both in the postabsorbtive state as well as during hyperinsulinemic conditions. It is concluded that at the most a 4-fold increase in the plasma insulin concentration is necessary to stimulate adipocytes for maximum lactate release.

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