Lipolysis in human adipose tissue during exercise: comparison of microdialysis and a-v measurements

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Lange, Kai Henrik Wiborg, Jeannette Lorentsen, Fredrik Isaksson, Lene Simonsen, Jens Bülow, and Michael Kjaer. Lipolysis in human adipose tissue during exercise: comparison of microdialysis and a-v measurements. J Appl Physiol 92: 1310–1316, 2002; 10.1152/japplphysiol.00690.2001.—Subcutaneous adipose tissue lipolysis was studied in vivo by Fick’s arteriovenous (a-v) principle using either calculated (microdialysis) or directly measured (catheterization) adipose tissue venous glycerol concentration. We compared results during steady-state (rest and prolonged continuous exercise), as well as during non-steady-state (onset of exercise and early exercise) experimental settings. Fourteen healthy women [age: 74 ± 1 (SE) yr] were studied at rest and during 60-min continuous bicycling at 60% of peak O2 uptake. Calculated and measured subcutaneous adipose tissue venous glycerol concentrations increased substantially from rest to exercise but were similar both at rest and during later stages of exercise. In contrast, during the initial ~40 min of exercise, calculated glycerol concentration was significantly lower (~40%) than measured adipose tissue venous glycerol concentration. Despite several methodological limitations inherent to both techniques, the results strongly suggest that microdialysis and catheterization provide similar estimates of subcutaneous adipose tissue lipolysis in steady-state experimental settings like rest and continuous prolonged exercise. However, during shorter periods of exercise (<40 min), the results from the two techniques may differ quantitatively in the studied subjects. Caution should, therefore, be taken when lipolysis is evaluated, based on results obtained by the two techniques under non-steady-state conditions.

catheterization; metabolism; methods; adipose tissue blood flow; arteriovenous measurements

The a-v method and the microdialysis technique have previously been compared in resting, near-steady-state experimental settings in the human subcutaneous abdominal adipose tissue (20, 22). In these two studies, mean measured and mean calculated venous glycerol concentrations were found to be similar. In contrast, a poor correlation between calculated and measured venous glycerol concentration was reported in a recent study in the isolated dog fat pad (21).

Because of the technical simplicity and ease of microdialysis measurements, this method is of potential value to study adipose tissue lipolysis not only in resting conditions, but also during exercise. However, exercise introduces an experimental setting very far from true especially during non-steady-state experimental settings like, for example, exercise.

Major insight into resting adipose tissue physiology has been gained by an a-v method developed by Frayn and colleagues (4, 6, 18) and others (5, 14) to specifically study human adipose tissue. With this method, an abdominal vein that mainly drains the subcutaneous abdominal adipose tissue is catheterized (7). However, technical problems in visualizing and actually catheterizing the vein make this method difficult to use. Another methodology used to study adipose tissue lipolysis is the microdialysis technique (1, 2, 11, 23). With this method, a thin microdialysis fiber is placed in the tissue of interest, e.g., subcutaneous adipose tissue, and hydrophilic substances will exchange over the dialysis membrane. By use of different calibration techniques (11, 13, 19), the interstitial concentration (Ci) of, for example, glycerol can be estimated, and from this the venous concentration of glycerol can be calculated by applying Fick’s law of diffusion for a thin membrane (8). However, both the calibration of the microdialysis fiber and the subsequent calculation of venous metabolite concentration are based on assumptions (e.g., constant diffusion gradient between the microdialysis fiber and the interstitial space, constant Ci) that are violated, especially during non-steady-state experimental settings like, for example, exercise.

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Determination of Lipolysis During Exercise

Methods

Subjects. Fourteen healthy women [age: 74 ± 1 (SE) yr; height: 161 ± 2 cm; body weight: 69.0 ± 2.6 kg; body fat: 41 ± 2%; and \( V_{O2\,peak} \): 1.52 ± 0.05 l/min] were included in the study. Informed consent was obtained according to the declaration of Helsinki 2, and the study protocol was approved by the Ethics Committee for Medical Research in Copenhagen (KF 02-130/97). Before inclusion, the subjects underwent a comprehensive medical evaluation, including history, physical examination, routine blood tests, and an exercise electrocardiogram (ECG). Exclusion criteria were metabolic, cardiac, and malignant disease, anemia, and medication known to interfere with lipid metabolism. Specifically, no hormone replacement therapy was allowed nor were \( \alpha \)- or \( \beta \)-blockers or any antidepressive medication.

Experimental protocol. Methodological details are presented after the experimental protocol section. Subjects were examined in the morning after an overnight fast. A catheter was inserted into a vein draining the subcutaneous abdominal adipose tissue, and another catheter was inserted into a radial artery. Two microdialysis catheters were then placed in the subcutaneous adipose tissue in the right hypogastric region, allowing for determinations of interstitial glycerol concentrations in different local regions. To measure local adipose tissue blood flow (ATBF), a small depot of \( ^{133}\text{Xe} \) was injected into the subcutaneous adipose tissue on the contralateral side, and a scintillation detector was strapped to the skin above the depot. Resting measurements were commenced at least 45 min after injection of the microdialysis catheters, and the subjects rested supine for 60 min at a room temperature of 22–23°C. Immediately after the resting period, the subjects started exercising on an electromagnetically braked cycle ergometer (Ergometrics ER 900 L, Ergoline GmbH, CoKG) in a semisupine position for 60 min. The load, adjusted by 5-W increments, corresponded to 60% of previously determined individual \( V_{O2\,peak} \). Arterial and venous blood samples were taken simultaneously at 30 and 60 min in the resting period and at 5, 15, 30, 45, and 60 min in the exercising period, in which also dialysates were collected from the microdialysis catheters. \( O_2 \) uptake (\( V_{O2} \)) was determined at 30 and 55 min in the resting period and at 15, 30, and 55 min in the exercising period. ECG and heart rate were registered continuously. In six of the subjects, the same experimental procedures were repeated after 12 wk.

Dual-energy X-ray absorptiometry. Body fat was determined by dual-energy X-ray absorptiometry scan. Subjects were scanned in the morning after an overnight fast with the use of a Lunar DPX-iQ scanner (software version 4.6 C; Lunar, Madison, WI).

\( V_{O2\,peak} \) and submaximal \( V_{O2} \). On a separate day, individual \( V_{O2\,peak} \) was determined on an electromagnetically braked biking ergometer. A protocol starting at 15 W and increasing by 15 W every 2 min until exhaustion was used. \( V_{O2} \) and \( CO_2 \) production were measured on an Oxycon system (software version 3.12, Oxycon Champion, Jaeger, Wurzburg, Germany), using a face mask and an external volume sensor in the open-system approach, giving breath-by-breath data. The accuracy of the system was validated by combustion of 99.6% ethanol, and the flowmeter by nitrogen infusion. Before each protocol, the gas analyzers were calibrated using gas mixtures of known composition, and the external volume sensor was calibrated using an external syringe of known volume. ECG and heart rate were registered continuously through chest electrodes connected to a monitor (Athena, Simonsen and Weel, Bagsvaerd, Denmark). \( V_{O2\,peak} \) was chosen as the highest \( V_{O2} \) attained during the test.

In the submaximal experimental protocol, \( V_{O2} \) was measured at the time intervals described previously. According to the measurements of \( V_{O2} \), workload was adjusted by steps of 5 W during exercise. This ensured that a \( V_{O2\,peak} \) corresponding to 60% of individual \( V_{O2\,peak} \) was continuously attained.

Blood flow. Subcutaneous abdominal ATBF was measured by the \( ^{133}\text{Xe} \) washout method (9). \( ^{133}\text{Xe} \) (1 MBq), dissolved in 0.1 ml of sterile, isotonic sodium chloride, was slowly injected into the middle layer of the subcutaneous adipose tissue with a 25-gauge cannula, taking care not to inject any air bubbles. The \( \gamma \)-radiation of the \( ^{133}\text{Xe} \) in the adipose tissue was registered by a portable Sc1 scintillation detector, which was strapped to the skin above the \( ^{133}\text{Xe} \) depot and connected to a multichannel analyzer system (Oakfield Instruments, Oxford, UK). Counts were collected in 30-s periods, starting at least 30 min after the injection. ATBF was calculated as \(-k\cdot\lambda\cdot100\) (ml-100 g \(-1 \cdot \text{min}^{-1}\)), where \( k \) is the rate constant of the monoeponential washout curve, and \( \lambda \) is the tissue-to-blood partition coefficient for \( ^{133}\text{Xe} \). In three subjects, \( \lambda \) was determined from a fat biopsy performed in the area in which the \( ^{133}\text{Xe} \) was injected (3). Mean \( \lambda \) was 10.0 in those subjects (11.1, 10.7, and 8.4), and, accordingly, \( \lambda \) was set to 10 in all subjects. Plasma flow was calculated as \((1 - Hct)\cdot\text{blood flow} \), where Hct is hematocrit. Plasma water flow (Q) was calculated by multiplying plasma flow by 0.94 (15).

Microdialysis. Microdialysis catheters (CMA 60; CMA/Microdialysis, Solna, Sweden; 20-kDa molecular cutoff, outer membrane diameter 0.6 mm; length 30 mm) were inserted in parallel into the periumbilical subcutaneous adipose tissue in the right hypogastric region during local epidural anesthesia. Individual catheters were placed with a vertical spacing of 1.5 cm between each catheter. The catheters were perfused with a perfusate containing 3 mM glucose and 1 mM lactate in isotonic sodium chloride at 3 \( \mu \)l/min, each with a high-precision syringe pump (CMA 100; Carnegie Medicine, Solna, Sweden). With the use of the internal reference technique (19), the in vivo recovery of glycerol and glucose was determined by adding 5 nM [U-\(^{14}\text{C}\)]glycerol (specific activity: 7,400 GBq/mmol; NEN, Boston, MA) and 11 nM [\(^3\text{H}\)]glucose (specific activity: 6,475 GBq/mmol; NEN) to the perfusate. Five microliters of perfusate (duplicate) and dialysate...
(single) were counted in a liquid scintillation counter with appropriate settings for the $^{14}$C and $^{3}$H channels.

**A-V catheterization.** During local analgesia with 1% lidocaine, a catheter (arterial cannula with FloSwitch, 20 gauge, 1.0 mm × 45 mm; Ohmeda, Swindon, UK) was inserted percutaneously into the radial artery of the nondominant arm. The catheter was kept patent by regular flushing with isotonic sodium chloride containing heparin (10 U/ml). With the use of the Seldinger technique and assisted by ultrasound imaging, another catheter (22 gauge, 10 cm; Ohmeda) was inserted into one of the superficial veins on the anterior abdominal wall. The tip was positioned so as not to lie inferior to the inguinal ligament, as described by Frayn et al. (7), and the catheter was kept patent by continuous infusion with isotonic sodium chloride containing heparin (10 IU/ml) at 30 ml/h.

**Calculations.** $C_i$ values were calculated using the internal reference calibration method (19). On the basis of in vitro experiments, it is assumed that the relative recovery (RR) from the interstitial fluid to the perfusate of unlabeled metabolite equals the relative loss (RL) from the perfusate to the interstitial fluid of labeled metabolite. RR was calculated for each individual sample as $RR = \frac{RL}{RL + DSL} = \frac{-C_P}{-C_P + C_D}$, where $C_P$ is disintegrations/minute in the perfusate and $C_D$ is disintegrations/minute in the dialysate. $C_i$ was calculated as $C_i = C_D/RR$, where $C_D$ is dialysate concentration, and the average $C_i$ determined from simultaneous measurements in the two microdialysis fibers was used as an estimate of $C_e$.

**Adipose tissue venous concentrations of glycerol and glucose were calculated using Fick's law of diffusion for a thin membrane:** $J = -PS (C_1 - C_2)$, where $P$ is the membrane permeability of the substrate, $S$ is the membrane surface area, and $C_1$ and $C_2$ are the concentrations on the two sides of the membrane, with $C_1$ being higher than $C_2$. Integrating this equation over the entire length of a capillary gives, according to Intaglia and Johnson (8), $C_v - C_i = C_1 - C_2 = e^{-PSC} + C_2$, where $C_v$ is venous plasma water concentration, $C_i$ is arterial plasma water concentration, $P$ is the permeability surface area product, and $C_1$ is the calculated venous plasma water concentration. The $PS$ was set to 3 ml·100 g$^{-1}$·min$^{-1}$ for glycerol and 2 ml·100 g$^{-1}$·min$^{-1}$ for glucose, as similar-sized molecules are known to have these values (10). It was assumed that the $PS$ remained constant within the range of blood flows recorded (15). Conversion of whole blood glycerol concentrations to plasma water glycerol concentrations and vice versa was accomplished by use of the hematocrit and by assuming a distribution volume of 0.70 for glycerol in the erythrocyte (20). Net glycerol flux across the subcutaneous abdominal adipose tissue was calculated by multiplication of whole blood flow and the a-v concentration differences obtained from either direct measurements or calculated values. To compensate for the differences in sampling procedures (continuous vs. point sampling), the interstitial glycerol and glucose concentrations were assumed to reflect concentration in the middle of the sampling period. Plasma water concentrations were corrected accordingly by linear extrapolation. The dead space volume of the outlet tubing of the microdialysis catheter was 7.4 μl, which, with a perfusion speed of 3 μl/min, equals a time delay of only 2.5 min, which did not have any significant impact on the microdialysis data. Resting values (blood and dialysate) were calculated as the average of the two resting samples taken after 30 and 60 min in the resting period, respectively.

**In vitro microdialysis.** To examine whether glycerol concentrations could be determined accurately by microdialysis when glycerol concentrations were changed instantaneously and substantially, microdialysis was performed in an in vitro experiment. Two microdialysis fibers (CMA 60; CMA/Microdialysis; 20-kDa molecular cut off, outer diameter 0.5 mm; length 30 mm) were placed in a continuously stirred bath containing isotonic saline and glycerol. The temperature of the bath was kept constant at 37°C. Isotonic sodium chloride was perfused through the microdialysis catheters with a perfusion speed of 5 μl/min, and dialysate was collected into sealed vials every 60 s. After an equilibration period (15 min) with a glycerol concentration of ~200 μM, collection of dialysate was commenced. The glycerol concentration in the bath was instantaneously increased from ~200 to ~400 μM after 3 min and from ~400 to ~600 μM after 13 min and decreased from ~600 to ~200 μM after 48 min. RR was determined from corresponding measurements of glycerol concentration in the surrounding fluid and in the dialysate. Glycerol concentrations in the surrounding fluid and in the dialysate were determined as described below.

**Sampling and analytic methods.** Blood was sampled into iced tubes, kept on ice, and centrifuged or precipitated with perchloric acid within 5 min. Plasma D-glucose was determined in duplicate using a YSI 2300 STAT PLUS analyzer (Yellow Springs Instruments, Yellow Springs, OH). Dialysate for determination of glycerol and glucose was sampled into sealed vials (Microvials, CMA/Microdialysis). All samples were stored at ~80°C until further analysis. Blood glycerol was measured in duplicate in neutralized, deproteinized extracts of whole blood (Monarch Plus 750, Instrumentation Laboratory, Lexington, MA). Hematocrit was determined by the microhematocrit method. Dialysate glycerol and glucose were determined spectrophotometrically in duplicate by use of a CMA 600 microdialysis analyzer (CMA/Microdialysis).

**Statistical analysis.** All data are presented as means ± SE. The effects of method (microdialysis or a-v) and time on metabolite concentrations and glycerol release were analyzed by a repeated-measures two-way ANOVA (Graph Pad Prism version 3.00 for Windows 95, GraphPad Software, San Diego, CA). Specific differences were identified by Bonferroni posttests. Student’s paired t-test was used to detect significant changes in blood flow between rest and during exercise. Student’s unpaired t-test was used to detect a significant difference between RR at rest and during exercise. $P < 0.05$ (two-tailed) was considered significant.

**RESULTS**

**Abdominal ATBF.** Mean ATBF was 1.9 ± 0.3 ml·100 g$^{-1}$·min$^{-1}$ at rest and 2.6 ± 0.3 ml·100 g$^{-1}$·min$^{-1}$ during exercise ($n = 14$). This increase was not statistically significant ($P < 0.15$). In the retest experimental protocol ($n = 6$), mean resting ATBF [1.6 ± 0.4 (test) and 1.6 ± 0.4 ml·100 g$^{-1}$·min$^{-1}$ (retest); coefficient of variation: 20%] and mean ATBF during exercise [2.1 ± 0.3 (test) and 2.3 ± 0.3 ml·100 g$^{-1}$·min$^{-1}$ (retest); coefficient of variation: 31%] were similar.

**$V_{O_2}$.** $V_{O_2}$ was 0.21 ± 0.01 l/min at rest and 0.84 ± 0.02 l/min during exercise in the 14 subjects. In the retest experimental protocol ($n = 6$), $V_{O_2}$ was 0.21 ± 0.01 and 0.22 ± 0.02 l/min at rest and 0.92 ± 0.03 and 0.85 ± 0.03 l/min during exercise. $RR$. $RR$ for glycerol was significantly higher during exercise than during rest (0.32 ± 0.01 vs. 0.27 ± 0.01; $P < 0.006$).

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Glycerol concentration and glycerol release. Measured and calculated plasma water glycerol concentrations in the abdominal adipose tissue vein are presented in Fig. 1, A and B. There was a significant effect of both method (P < 0.0001) and time (P < 0.0001), as well as of method × time interaction (P = 0.0069) on venous plasma water glycerol concentration. Post hoc Bonferroni tests revealed significant differences after 2.5 min (P < 0.05), 10 min (P < 0.001), and 37.5 min (P < 0.01) of exercise. A similar pattern was observed for abdominal adipose tissue glycerol release (method: P < 0.0001; time: P < 0.0001; method × time interaction: P = 0.011; post hoc Bonferroni: 2.5 min, P < 0.05; 10 min, P < 0.001; 22.5 min, P < 0.05; Fig. 1C).

The individual calculated values of plasma water glycerol concentrations correlated positively and significantly with the measured values, both in nearly steady-state experimental settings (rest and 52.5 min of exercise) and in non-steady-state experimental settings (2.5, 10, 22.5, and 37.5 min of exercise) (both P < 0.0001). The correlation was better during steady state (R² = 0.70) than during nonsteady state (R² = 0.39) (Fig. 2). Despite this relatively poor correlation, mean measured and calculated plasma water glycerol concentrations showed a highly reproducible time pattern in the six subjects in whom the same experimental protocol was repeated after 12 wk (Fig. 3A).

Glucose concentration. A significant effect of method (P = 0.0011) was detected on plasma water glucose concentration, whereas no effect of time or method × time interaction was revealed (P = 0.16 and P = 0.67, respectively) (Fig. 3B). Calculated adipose tissue venous glucose concentration was, on average, ~95% of the measured value.

In vitro microdialysis. Microdialysis performed in vitro showed that glycerol concentration in the surrounding medium was accurately assessed by this method with virtually no time delay (Fig. 3C). This was true even when the glycerol concentration in the surrounding fluid was increased instantaneously from 200 to 400 μM and from 400 to 600 μM and decreased from 600 to 200 μM.

DISCUSSION

The main finding in the present study is that determination of adipose tissue venous glycerol concentration and release provided similar results at rest, as of both method (P < 0.0001) and time (P < 0.0001), as well as of method × time interaction (P = 0.0069) on venous plasma water glycerol concentration. Post hoc Bonferroni tests revealed significant differences after 2.5 min (P < 0.05), 10 min (P < 0.001), and 37.5 min (P < 0.01) of exercise. A similar pattern was observed for abdominal adipose tissue glycerol release (method: P < 0.0001; time: P < 0.0001; method × time interaction: P = 0.011; post hoc Bonferroni: 2.5 min, P < 0.05; 10 min, P < 0.001; 22.5 min, P < 0.05; Fig. 1C).

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DISCUSSION

The main finding in the present study is that determination of adipose tissue venous glycerol concentration and release provided similar results at rest, as
well as during prolonged, continuous exercise, whether determined by microdialysis or arteriovenous measurements across the adipose tissue. This indicates that the two techniques provide similar estimates of lipolysis when compared in steady-state or quasi-steady-state conditions. In contrast, during non-steady-state conditions (transition from rest to exercise), these methods may yield different results. During non-steady-state conditions, directly measured glycerol concentrations were found to be significantly lower than those determined by microdialysis. This discrepancy was reproduced when the experiment was repeated 12 wk later in six of the subjects.

The reason for the discrepancy between the two methods during non-steady state is unknown. Differences in sampling procedure (continuous sampling in microdialysis vs. point sampling in arteriovenous measurements) do not explain the results, because glycerol concentrations obtained by microdialysis were assumed to reflect the concentrations in the middle of the sampling period. Accordingly, directly measured glycerol concentrations were corrected for linear extrapolation. Similarly, the outlet tubing of the microdialysis catheter represents a dead space volume of 7.4 μl, which, with the present perfusion speed of 3 μl/min, causes a time delay of only 2.5 min in the dialysate. In addition, the data from the in vitro microdialysis experiment clearly demonstrate that the glycerol concentration in the surrounding fluid was accurately assessed with no significant time delay by microdialysis, even when the glycerol concentration was instantaneously and substantially increased or decreased. Therefore, neither diffusion limitation of glycerol through the dialysis membrane nor binding of glycerol to the dialysis probe/outlet tubing can account for the seeming delay in the microdialysis data.

The discrepancy between the two methods during non-steady state may be explained by potential differences in diffusion distance between the sites of intracellular adipocyte lipolysis and the microdialysis fiber or the capillary, respectively. It is known that capillaries are situated in very close contact to the adipocyte (16), and, teleologically, one might expect adipocytes to be polarized, i.e., oriented in such a way that intracellular lipolysis is restricted to areas adjacent to the capillaries. This would minimize the diffusion distance from the adipocyte to the capillary compared with the randomly inserted microdialysis fiber and possibly make feedback regulation of adipose tissue lipolysis more rapid. Although our findings would support such a view, these interpretations are purely speculative. In addition, it has to be acknowledged that the in vivo internal reference method for calibrating the microdialysis fibers is based on a constant concentration gradient of the labeled substance between the interstitial tissue and the fiber (12). This is not the case during non-steady-state lipolysis and may result in an underestimation of the RR, because the RL, which is measured, may actually be less than RR (RL is assumed to equal RR in the calibration). This would, in turn, lead to an underestimation of interstitial and hence calculated venous glycerol concentration. Furthermore, the calculation of venous glycerol concentration is based on microdialysis determinations were found to be significantly lower than directly measured glycerol concentrations in an adipose tissue vein. This phenomenon was clearly reproduced when the experiment was repeated 12 wk later in six of the subjects.

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It also has to be acknowledged that the in vivo internal reference method for calibrating the microdialysis fibers is based on a constant concentration gradient of the labeled substance between the interstitial tissue and the fiber (12). This is not the case during non-steady-state lipolysis and may result in an underestimation of the RR, because the RL, which is measured, may actually be less than RR (RL is assumed to equal RR in the calibration). This would, in turn, lead to an underestimation of interstitial and hence calculated venous glycerol concentration. Furthermore, the calculation of venous glycerol concentration is based on

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*Fig. 2. Correlation between individual calculated (microdialysis) and measured (catheterization) adipose tissue venous glycerol C. A: steady-state (rest and 52.5 min of exercise) experimental settings (calculated value = 1.02 × measured value – 13; P < 0.0001; R² = 0.70). B: non-steady-state (2.5, 10, 22.5, and 37.5 min of exercise) experimental settings (calculated value = 0.51 × measured value + 139; P < 0.0001; R² = 0.39).*
a constant interstitial glycerol concentration, and this requirement is obviously not fulfilled during non-steady-state conditions.

Although presently not studied, it would have been interesting to determine whether, in fact, the adipose tissue interstitial glycerol concentration would have been higher than the adipose tissue venous glycerol concentration when lipolysis was stopped in the period after exercise. In a study by Samra et al. (17), the glycerol concentration in the blood was subsequently raised and lowered from 50 to 250–300 μL/l in a square-wave manner. At the same time, the glycerol concentration was measured in forearm arterialized and venous blood and in microdialysate obtained from the abdominal adipose tissue at 30-min time intervals (17). Although no measurements were performed in a subcutaneous abdominal adipose tissue vein and sampling was less frequent than in the present study, these data suggest a time delay in the microdialysis data, when blood glycerol concentration was both increased and subsequently decreased. The glucose data obtained in the present study are not very useful for elucidating possible mechanisms, because the changes in glucose are very small. They do, however, support previous findings of slightly decreased abdominal adipose tissue interstitial glucose concentrations, compared with measured venous glucose concentrations at rest (12), and extends these findings to also include exercise.

At the onset of exercise, an insignificant decrease in interstitial glycerol concentration was observed. This may merely reflect normal variation in determination of interstitial glycerol concentration. However, it cannot be excluded that movements of the fiber relative to the tissue at the onset of exercise causes an increase in fluid mixing and hence a decrease in the interstitial glycerol concentration.

A major factor in calculating venous metabolite concentration from microdialysis is the PS. From the equation $C_{v,calc} = \left[ (C_i - C_a) \cdot (1 - e^{-PS/Q}) \right] + C_a$, it can be seen that, when $Q$ is in the range of the PS, small changes in both $Q$ and PS will have major impact on $C_{v,calc}$. In our study, the PS for glycerol was set to 3 ml·100 g$^{-1}$·min$^{-1}$. Decreasing the PS to 1.5 ml·100 g$^{-1}$·min$^{-1}$ or increasing it to 5.0 ml·100 g$^{-1}$·min$^{-1}$ (or to infinity) would, however, not change the overall concentration.

**Fig. 3.** A: glycerol C in subcutaneous abdominal adipose tissue venous plasma water measured directly (catheterization) or calculated from microdialysis data. Measurements were performed in 6 subjects at rest and during 60-min continuous cycling at a level corresponding to 60% of peak $O_2$ uptake and repeated in the same subjects with a time interval of 12 wk, denoted by 1 and 2, respectively. Symbols and error bars represent mean values ± SE. Resting values were calculated from pooled resting data. B: glucose C in abdominal adipose interstitial tissue (microdialysis), adipose tissue venous plasma water (catheterization), and calculated (from microdialysis data) adipose tissue venous plasma water. Symbols and error bars represent mean values ± SE; $n = 14$ subjects. See text for further details. C: microdialysis performed in vitro. Glycerol C in the surrounding fluid ($C_{out}$) was instantaneously increased from −200 to −400 μM after 3 min and from −400 to −600 μM after 13 min and decreased from −600 to −200 μM after 48 min. Individual values for the 2 microdialysis fibers (fibers 1 and 2) are presented.
findings during nonsteady state. Inherent in the equation lies also the fact that, if measured \( C_v \) (\( C_{v,\text{measured}} \)), \( C_a \), and \( C_i \) are correctly determined, then \( C_i < C_{v,\text{measured}} \), i.e., \( PS/Q \) cannot be changed to make \( C_{v,\text{calculated}} = C_{v,\text{measured}} \). These factors all indicate that methodological limitations in one or both techniques currently used play a role during nonsteady-state conditions.

In conclusion, the present study demonstrates that microdialysis and arteriovenous methodology provide similar estimates of subcutaneous adipose tissue lipolysis in steady-state experimental settings like rest and prolonged, continuous, constant-load exercise in elderly women. However, during shorter periods of exercise (<40 min), the results from the two techniques may differ quantitatively. This suggests that caution should be taken when comparing data from the two techniques during exercise-induced non-steady-state adipose tissue lipolysis.

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REFERENCES


