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Regional differences in interstitial glycerol concentration in subcutaneous adipose tissue of women

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Hickner, R. C., J. S. Fisher, and W. M. Kohrt. Regional differences in interstitial glycerol concentration in subcutaneous adipose tissue of women. *Am. J. Physiol.* 273 (*Endocrinol. Metab.* 36): E1033–E1038, 1997.—The aims of this study were to 1) compare two methods of determining interstitial glycerol concentration in subcutaneous adipose tissue (AT) and 2) determine whether there are regional differences in interstitial glycerol concentration in subcutaneous AT of nonobese, premenopausal women. Microdialysis probes were inserted under local anesthesia into the abdominal (2 probes) and femoral (1 probe) subcutaneous AT in each subject ($n = 5$) and perfused with a Ringer solution containing 2.5 mM glucose and glycerol in concentrations ranging from 0 to 900 μM . Microdialysis probe relative recoveries and interstitial glycerol concentrations were determined by the no-net-flux method (NNF) and the internal reference method (IR) with the use of [^{13}C]glycerol. Microdialysis probe relative recoveries were $57.4 \pm 3.6\%$ by NNF and $61.2 \pm 10.1\%$ by IR in femoral AT [$P =$ not significant (NS)] and were $55.2 \pm 6.0\%$ by NNF and $66.6 \pm 4.2\%$ by IR in abdominal AT ($P =$ NS). The calculated interstitial glycerol concentrations determined by NNF and IR were 236.4 ± 42.7 and $241.1 \pm 39.6 \mu\text{M}$ ($P =$ NS) in femoral AT and 151.4 ± 29.7 and $129.4 \pm 18.7 \mu\text{M}$ in abdominal AT (NNF vs. IR, $P =$ NS; femoral vs. abdominal, $P < 0.05$). It can be concluded that the interstitial glycerol concentration in the femoral AT of nonobese, premenopausal females is $\sim 240 \mu\text{M}$ and is higher than in abdominal AT (140 μM). Furthermore, the use of a stable isotope of glycerol as an internal reference is suitable for determining interstitial glycerol concentrations in subcutaneous adipose tissue in humans at rest.

lipolysis; microcirculation; microdialysis

THERE HAS BEEN CONSIDERABLE interest in recent years concerning the true interstitial glycerol concentration within subcutaneous adipose tissue of humans. Regional differences in interstitial glycerol concentration are reflective of lipolytic activity of the adipocytes, lipoprotein lipase activity, and blood flow around the adipocytes. Furthermore, differential lipolytic rates of the adipocytes in femoral and abdominal sites may result in regional accumulation of adipose tissue. Using microdialysis in obese men, Jansson et al. (11, 12) found that the interstitial glycerol concentration was higher in abdominal than femoral subcutaneous adipose tissue and suggested that this was evidence of an increased rate of lipolysis in the abdominal depot (11). To the best of our knowledge, regional differences in

subcutaneous adipose tissue interstitial glycerol concentration have not been investigated in women. Thus, to gain insight into potential mechanisms for the gender-related differences in regional fat accumulation, one purpose of this study was to measure interstitial glycerol concentration in femoral and abdominal adipose tissue of women.

Under most experimental conditions, the dialysate glycerol concentration is not a direct measure of interstitial glycerol concentration; the latter must be derived from calibration procedures employed during microdialysis. Using the no-net-flux (NNF) microdialysis calibration method, most investigators (1, 2, 11, 12) have found that the interstitial glycerol concentration in subcutaneous adipose tissue of young, lean men and women is $\sim 200 \mu\text{M}$. In rats, the interstitial concentration derived from the NNF method correlated well with values derived from the internal reference, extrapolation to zero flow, and very low flow methods (13, 18). A disadvantage of the NNF method is that it requires ~ 5 h to conduct. The addition of a radioisotope of the molecule investigated as an internal reference allows for sample-to-sample determination of interstitial concentrations, thereby requiring much less time than other calibration methods. The addition of a stable isotope to the perfusion medium would eliminate the need for a radioisotope, thereby increasing the practicality of the internal reference (IR) method in humans. The second purpose of this investigation was therefore to compare in humans a stable isotope-labeled IR method with the well-established NNF method of microdialysis calibration for the determination of interstitial glycerol concentrations. To determine whether any differences between the methods *in vivo* would also be apparent under more carefully controlled experimental conditions, additional *in vitro* studies were also conducted.

METHODS

In Vivo Studies

Subjects. Five healthy women (24.4 ± 2.25 yr, 168.1 ± 3.4 cm, 61.8 ± 4.6 kg, 21.8 ± 1.3 body mass index) participated in the study after giving their informed consent. None of the subjects had any prior history of diabetes or cardiovascular disease, and none was taking any medications at the time of study. The study was approved by the Human Studies Committee at Washington University School of Medicine.

Microdialysis. Microdialysis probes were DL-3 microdialysis probes from Bioanalytical Systems (West Lafayette, IN). The probes consisted of inlet extension tubing (30-cm length) and outlet extension tubing (15-cm length) separated by a polyacrylonitrile dialysis membrane (3 cm in length, 0.25 mm ID, 0.35 mm OD). Microdialysis probes were sterilized for 20 min in 80% ethanol and soaked for a minimum of 16 h overnight in 5% ethanol while perfused with Ringer solution to remove the glycerol coating that was placed on the dialysis membrane during the manufacturing process. The absence of measurable glycerol on the microdialysis membrane was verified before in vitro and in vivo experiments. Microdialysis probes were perfused at 2.0 $\mu\text{l}/\text{min}$ for all in vivo studies with a Ringer solution containing 2.5 mM glucose and glycerol (0–900 μM in randomized order). A large difference between perfusate and interstitial concentrations can theoretically result in a systematic build-up or drainage of interstitial metabolites from the area around the dialysis membrane (15, 18). Glucose (2.5 mM) was therefore added to the perfusate to minimize the drainage of glucose from the interstitial fluid. Furthermore, the order of perfusate glycerol concentrations was randomized to avoid any systematic effect of perfusate glycerol concentration on interstitial fluid glycerol concentration. No significant net loss or gain of perfusate fluid occurred over the dialysis membrane during the microdialysis process, as verified by weighing of dialysate samples.

Subject preparation and protocol. Subjects reported to the laboratory after an overnight fast. Microdialysis probes were inserted under sterile technique and local anesthesia into the abdominal (2 probes: bilaterally ~4 cm lateral to the umbilicus) and femoral (1 probe: midhigh) subcutaneous adipose tissue in each subject. This was accomplished by inserting a 14-gauge catheter through the skin ~0.5 cm, advancing it through the subcutaneous adipose tissue parallel to the skin ~2.5 cm, and then having it exit through the skin. The needle was withdrawn, the microdialysis probe was threaded through the catheter, and the catheter was then removed. Microdialysis probes were taped in place, with the inlet tube connected to a syringe placed in a Harvard infusion pump (Harvard Apparatus, South Natick, MA). Subjects were assisted to a semireclining position in a comfortable chair, where they remained for the entire experiment.

Microdialysis probes were perfused at 2.0 $\mu\text{l}/\text{min}$ with Ringer solution containing 2.5 mM glucose but no glycerol for 60 min. No samples were collected in this first 60 min after probe insertion, as has been suggested previously (4, 9), to allow for equilibration of the microdialysis system and to allow the initial trauma of probe insertion to subside. A baseline dialysate sample was collected over the subsequent 30 min. Glycerol was then included in the perfusion medium at concentrations of 100, 300, 500, 700, or 900 μM in random order for 1 h per concentration. One microdialysis sample was collected over the final 30 min at each perfused glycerol concentration.

The 100 and 300 μM glycerol perfusates were prepared with labeled glycerol (^{13}C glycerol) to obtain data for the IR method, whereas all other glycerol solutions contained unlabeled glycerol. The concentrations of labeled glycerol were selected to span the range of previously reported interstitial glycerol concentrations (2, 11, 12).

When the IR method was conducted over a period of 5 h in control experiments ($n = 5$), recovery as calculated from the IR method did not change, with values of 62.2 ± 4.6 , 65.5 ± 6.4 , 66.5 ± 4.8 , 69.4 ± 5.7 , and 64.4 ± 5.2 over the 1st through 5th h, respectively [$P =$ not significant (NS)]. The IR method was therefore shown to be stable over the length of the present experiments.

In Vitro Studies

In vitro studies were conducted to determine whether the NNF and IR methods yielded similar estimates of the glycerol concentration in a solution bathing the microdialysis probe under carefully controlled laboratory conditions. A secondary goal of these experiments was to characterize in vitro relative recovery across the probe membrane. For these studies, the microdialysis probes were positioned in beakers containing solutions of known glycerol concentrations at 20°C. The recovery of glycerol over the microdialysis probe membrane was determined by three methods: 1) direct measurement, determined from the measured glycerol concentrations in the dialysate and beaker solutions; 2) the NNF method; and 3) the IR method.

Direct recovery. Microdialysis probes ($n = 3$) were placed in a beaker containing Ringer solution and 300 μM glycerol and perfused at 2.0 $\mu\text{l}/\text{min}$ with a Ringer solution containing no glycerol. A 30-min equilibration period was allowed before a 30-min sample collection. Glycerol concentrations in the beaker and dialysate solutions were determined, and recovery was calculated using the equation

$$\text{Relative recovery} = [\text{glycerol}]_{\text{dialysate}} / [\text{glycerol}]_{\text{beaker}} \times 100\%$$

NNF method. The NNF method was conducted as previously described by Lönnroth et. al. (15). Microdialysis probes ($n = 3$) were placed in a beaker containing Ringer solution and 300 μM glycerol and perfused at 2.0 $\mu\text{l}/\text{min}$ with a Ringer solution containing 0, 100, 300, 500, 700, or 900 μM glycerol. A 30-min equilibration period was allowed before a 30-min sample collection period for each perfusate concentration.

IR method. Microdialysis probes (3) were placed in a beaker containing Ringer solution and ~300 μM glycerol and perfused at 2.0 $\mu\text{l}/\text{min}$ with a Ringer solution containing 300 μM [^{13}C]glycerol. The ratio of labeled to unlabeled glycerol in the perfusate and the dialysate was determined by gas chromatography-mass spectrometry (GC-MS) as described in *Sample storage and analyses*.

Two microdialysis probes were also placed in a beaker containing Ringer solution and 300 μM glycerol. Probes were perfused at 1, 2, and 4 $\mu\text{l}/\text{min}$ with a Ringer solution containing either 300 μM [^{13}C]glycerol or [$^2\text{H}_5$]glycerol. The [$^2\text{H}_5$]glycerol was used only in initial in vitro studies before the acquisition of [^{13}C]glycerol. The IR method was performed at multiple flow rates in vitro to determine the validity of this method over a range of flow rates typically used in vivo. Dialysate samples were collected for 30 min after a 30-min equilibration period at each perfusate flow rate. Microdialysis probe recovery was determined in these two probes using the IR method and was compared with the recovery determined in two separate probes from measurement of unlabeled glycerol concentration in the dialysate and the beaker, i.e., relative recovery = $[\text{glycerol}]_{\text{dialysate}} / [\text{glycerol}]_{\text{beaker}} \times 100\%$.

Sample storage and analyses. Perfusate and dialysate samples were collected in 150- μl polypropylene vials (CMA/Microdialysis, Stockholm, Sweden). The concentrations of total glycerol in the perfusates and dialysates were determined using an enzymatic-fluorometric method as previously described (22).

Measurement of the ratio of unlabeled to labeled glycerol was performed using a modification of the negative ion chemical ionization (NCI) GC-MS method described by Gilker et. al. (8). A sample of dialysate (17 μl) was pipeted into a small glass vial and evaporated to dryness under nitrogen. Samples were reconstituted with 150 μl of a 3:1 mixture of heptafluorobutyric acid (HFB) and ethyl acetate to form the tris(hydroxymethyl)aminomethane-heptafluorobutyl ester

derivatives, placed in a heating block at 70°C for 10 min, and evaporated under nitrogen. Samples were then reconstituted with 100 µl of ethyl acetate before analysis.

The NCI spectrum of the HFB derivative was obtained using a model 5988A Hewlett-Packard GC-MS instrument. For gas chromatography, a DB-17 column was used (30 M, 0.25 mm ID, 0.25-µm film thickness; J and W Scientific, Folsom, CA) with a helium flow rate of 0.5 ml/min, a column temperature of 100°C for 1 min, increasing 45°C/min to 280°C, and a split ratio of 20:1. The NCI mass spectrometer conditions were: source temperature, 120°C; injector port and detector temperature, 250°C; emission, 300 µA; and ion source pressure, 0.5–0.6 Torr. Methane was used as the Cl⁻ reactant gas. The [M]⁻ ions corresponding to nominal mass 680 for unlabeled glycerol, mass 681 for [¹³C]glycerol, and mass 685 for [1,1,2,3,3-²H₅]glycerol were monitored. The ion ratios of 681/680 or 685/680 in the perfusates and dialysates, after correction for background [¹³C]- or [²H₅]glycerol in the samples, were then used to calculate recovery of glycerol over the microdialysis probe membrane as described below.

Calculations

NNF method. Relative recovery of the microdialysis probes was determined for the NNF method from the slope of the regression of the difference between the dialysate and perfusate glycerol concentrations vs. perfusate glycerol concentration (15). Interstitial (or beaker) glycerol concentrations were predicted from the regression line as the concentration at which there was no net flux of glycerol across the membrane (i.e., [glycerol]_{dialysate} - [glycerol]_{perfusate} = 0) as described by Lönnroth et al. (15).

IR method. Relative recovery for the IR method was calculated as

$$1 - ([^{13}\text{C}]\text{glycerol}_{\text{dialysate}} / [^{13}\text{C}]\text{glycerol}_{\text{perfusate}})$$

or

$$1 - ([^2\text{H}_5]\text{glycerol}_{\text{dialysate}} / [^2\text{H}_5]\text{glycerol}_{\text{perfusate}})$$

where the concentration of [¹³C]- or [²H₅]glycerol in the dialysate was calculated from the ratio of labeled to unlabeled glycerol in the dialysate determined by GC-MS and the total concentration of glycerol in the dialysate determined using an enzymatic-fluorometric method (22). The concentration of [¹³C]- or [²H₅]glycerol in the perfusate was calculated from the ratio of labeled to unlabeled glycerol in the perfusate determined by GC-MS, and the total concentration of glycerol in the perfusate was determined using an enzymatic-fluorometric method. It was assumed that the net flux of labeled glycerol out over the microdialysis membrane is equal to the net flux of unlabeled glycerol in over the microdialysis membrane over a given sampling period (14).

Interstitial (or beaker) concentrations were calculated from IR data as the unlabeled dialysate glycerol concentration divided by the relative recovery ([glycerol]_{dialysate}/relative recovery) (21).

Glycerol release. Glycerol release was calculated from the following equation, as described by Jansson et al. (11)

$$\text{Glycerol release} = (V - A) \cdot Q \cdot (I - \text{hematocrit})$$

where V is the calculated venous plasma glycerol concentration (µmol/ml) near the microdialysis probe, I is the average interstitial glycerol concentration (µmol/ml) as determined by NNF and IR, A is the arterial concentration (assumed to be 0.06 µmol/ml) (1, 11), and Q is the blood flow [assumed to be

2.5 and 3.0 ml · min⁻¹ · 100 g⁻¹ in the femoral and abdominal adipose tissue, respectively (7)].

The venous plasma glycerol concentration was estimated as follows

$$V = (I - A) \cdot (1 - e^{-PS/Q}) + A$$

where PS is the permeability surface area product for glycerol [~5 ml · 100 g⁻¹ · min⁻¹ (11)].

Statistics

Differences between methods (NNF vs. IR) of determining microdialysis probe recovery and interstitial glycerol concentrations and differences between abdominal and femoral sites with respect to microdialysis probe recovery and interstitial concentration were evaluated using two-tailed paired Student's *t*-tests. All data are expressed as means ± SE. The level of significance was set at *P* < 0.05.

RESULTS

In Vivo

IR method. The dialysate glycerol concentrations were 112.4 ± 11.0 and 193.3 ± 21.0 µM in abdominal probes perfused with 100 and 300 µM glycerol, respectively. Corresponding values in femoral probes were 171.8 ± 17.3 and 249 ± 32.4 µM. The ratios of labeled to unlabeled glycerol in the dialysate, after correction for background (natural abundance) [¹³C]glycerol, were 0.488 ± 0.12 and 1.28 ± 0.22 in abdominal probes perfused with 100 and 300 µM glycerol, respectively. These ratios were 0.494 ± 0.215 and 0.923 ± 0.423 in femoral probes perfused at 100 and 300 µM, respectively.

Relative recoveries, as calculated from the IR method, were 68.4 ± 7.5 and 64.8 ± 5.3% when abdominal probes were perfused with 100 and 300 µM glycerol, respectively (*P* = NS). Corresponding values in femoral probes were 60.0 ± 11.6 and 62.3 ± 12.5% (*P* = NS).

Interstitial glycerol concentrations, as calculated using the IR method, were 117.3 ± 15.2 and 141.4 ± 26.3 µM around abdominal probes when perfused with 100 and 300 µM glycerol, respectively (*P* = NS). Corresponding values for femoral probes were 240.2 ± 45.5 and 242 ± 41.8 µM (*P* = NS).

Because there were no intraprobe differences in the relative recoveries or the interstitial glycerol concentrations calculated from data obtained while the probes were perfused with 100 or 300 µM labeled glycerol, the data obtained within a probe using labeled 100 and 300 µM glycerol perfusates were pooled. Data from the two abdominal probes were also pooled. The interprobe coefficients of variation (CVs) for probe recovery in the abdominal site were 15.7 ± 6.1 and 10.0 ± 2.8% for the IR method (at 100 and 300 µM glycerol in the perfusate, respectively). The interprobe CVs of interstitial glycerol concentration in the abdominal site were 18.4 ± 9.3 and 19.7 ± 3.3% using the IR method when probes were perfused with 100 and 300 µM labeled glycerol, respectively.

NNF method. Correlation coefficients for the regression lines drawn to calculate microdialysis probe recovery

ery and interstitial glycerol concentration using the NNF method were $r = 0.967 \pm 0.008$ (range 0.89 to 0.998). The interprobe CV of probe recovery using the NNF method in the abdominal site was $17.1 \pm 7.8\%$. The interprobe CV of interstitial glycerol concentration in the abdominal site was $42.4 \pm 21.7\%$ using the NNF method. This high CV was due to one very high interprobe CV of 127%; the mean CV without this value was $21.4 \pm 6.1\%$.

Microdialysis probe recovery. Microdialysis probe membrane relative recoveries were 57.4 ± 3.6 and $61.2 \pm 10.1\%$ in femoral adipose tissue and 55.2 ± 6.0 and $66.6 \pm 4.2\%$ in abdominal adipose tissue using NNF and IR, respectively (Fig. 1; both $P = \text{NS}$).

Calculated interstitial glycerol concentration. Interstitial glycerol concentrations in femoral adipose tissue were 236.4 ± 42.7 and $241.1 \pm 39.6 \mu\text{M}$, calculated using NNF and IR methods, respectively (Fig. 2). Interstitial glycerol concentrations in abdominal adipose tissue were 151.4 ± 29.7 and $129.4 \pm 18.7 \mu\text{M}$, calculated using the NNF and IR methods, respectively (Fig. 2). Across both methods, the mean glycerol concentrations were 238.7 ± 40.8 and $140.7 \pm 24.0 \mu\text{M}$ in the femoral and abdominal sites, respectively ($P < 0.05$, $n = 5$).

There was a strong correlation within a given probe between the interstitial glycerol concentrations calculated using the IR method and the interstitial glycerol concentrations calculated from NNF data (Fig. 3; $r = 0.87$, $P < 0.001$, $n = 15$).

Glycerol release. The estimated rates of glycerol release, using previously reported (7) values of adipose tissue blood flow (thigh, $2.5 \text{ ml} \cdot \text{min}^{-1} \cdot 100 \text{ g}^{-1}$; abdomen, $3.0 \text{ ml} \cdot \text{min}^{-1} \cdot 100 \text{ g}^{-1}$), were $0.239 \pm 0.055 \mu\text{mol} \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1}$ in the femoral subcutaneous adipose tissue and $0.121 \pm 0.036 \mu\text{mol} \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1}$ in abdominal subcutaneous adipose ($P < 0.05$).

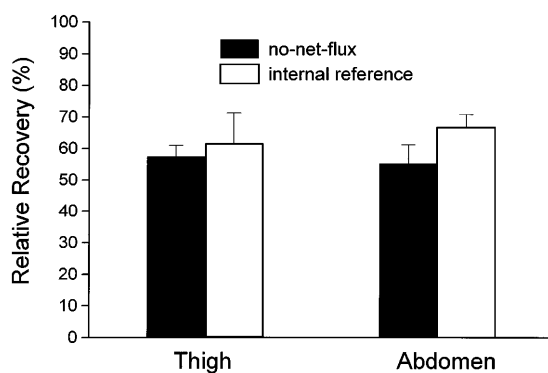


Fig. 1. Microdialysis probe relative recovery. Microdialysis probes were placed in abdominal and femoral adipose tissue in lean premenopausal women ($n = 5$) and perfused at $2.0 \mu\text{l}/\text{min}$ with a Ringer solution containing 2.5 mM glucose and glycerol at concentrations of $100, 300, 500, 700,$ or $900 \mu\text{M}$ in random order for $1 \text{ h}/\text{concentration}$. One microdialysis sample was collected over the final 30 min at each perfused glycerol concentration. The 100 and $300 \mu\text{M}$ glycerol concentrations were labeled with ^{13}C . Microdialysis probe recoveries were calculated according to no-net-flux (NNF) and internal reference (IR) methods. Data are presented as means \pm SE. Values are not significantly different.

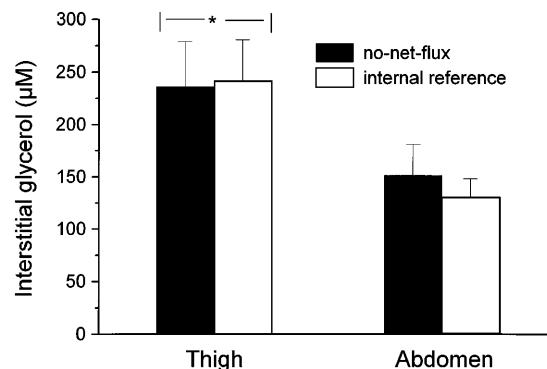


Fig. 2. Estimated interstitial glycerol concentration. Microdialysis probes were placed in abdominal and femoral adipose tissue in lean premenopausal women ($n = 5$) and perfused at $2.0 \mu\text{l}/\text{min}$ with a Ringer solution containing 2.5 mM glucose and glycerol at concentrations of $100, 300, 500, 700,$ or $900 \mu\text{M}$ in random order for $1 \text{ h}/\text{concentration}$. One microdialysis sample was collected over the final 30 min at each perfused glycerol concentration. The 100 and $300 \mu\text{M}$ glycerol concentrations were labeled with ^{13}C . Interstitial glycerol concentrations were calculated according to NNF and IR methods. Data are presented as means \pm SE. *Significantly different from abdomen; $P < 0.05$.

In Vitro

Microdialysis probe recoveries determined in vitro using the IR method were 84.4 ± 2.0 , 68.2 ± 9.0 , and $57.6 \pm 2.0\%$ at perfusion flow rates of $1, 2,$ and $4 \mu\text{l}/\text{min}$, respectively. These values were similar to corresponding values calculated from the measured dialysate and beaker glycerol concentrations: 86.3 ± 4.4 , 70.3 ± 0.6 , and $59.9 \pm 0.2\%$ at $1, 2,$ and $4 \mu\text{l}/\text{min}$, respectively ($n = 2$). In a separate set of in vitro experiments performed at a perfusion rate of $2 \mu\text{l}/\text{min}$, microdialysis probe recovery was similar using IR and NNF (recovery: 69.6 ± 3.6 and $67.5 \pm 4.5\%$, respectively) to the recovery calculated using direct measurement of dialy-

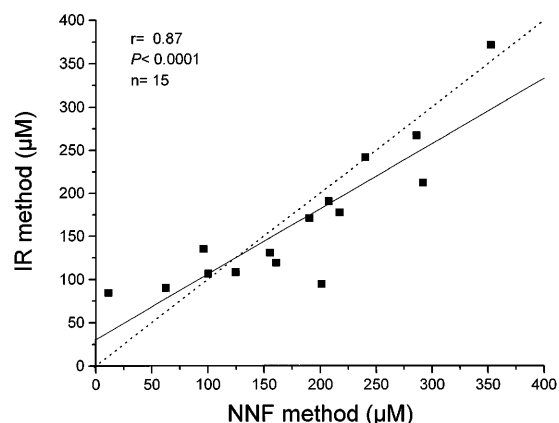


Fig. 3. Estimated interstitial glycerol concentration. Microdialysis probes were placed in abdominal and femoral adipose tissue in lean premenopausal women and perfused at $2.0 \mu\text{l}/\text{min}$ with a Ringer solution containing 2.5 mM glucose and glycerol at concentrations of $100, 300, 500, 700,$ or $900 \mu\text{M}$ in random order for $1 \text{ h}/\text{concentration}$. One microdialysis sample was collected over final 30 min at each perfused glycerol concentration. The 100 and $300 \mu\text{M}$ glycerol concentrations were labeled with ^{13}C . Interstitial glycerol concentrations around each probe were calculated according to NNF and IR methods.

sate and beaker glycerol concentrations (recovery: 65.3%).

DISCUSSION

The significant physiological finding of this study was that interstitial glycerol concentration was higher in femoral subcutaneous adipose tissue than in abdominal subcutaneous adipose tissue in resting, nonobese females. With the use of previously reported rates of blood flow in the femoral and abdominal adipose tissue of lean subjects (7), the rate of glycerol release was estimated to be twofold higher in femoral than in abdominal subcutaneous adipose tissue. Although these calculated rates of glycerol release are only approximations, the markedly higher interstitial glycerol concentrations and rates of release in the femoral adipose tissue suggest that lipolytic rate is higher in femoral than abdominal adipose tissue in nonobese women. This may be explained by differences in fat cell size, since femoral adipocytes have been reported to be larger than abdominal adipocytes in lean women (19, 20). There is evidence from both *in vitro* and *in vivo* investigations that rates of lipolysis are directly related to fat cell size (3, 6, 10, 11). Rebuffe-Scrive et al. (19, 20) reported a greater lipolytic rate per cell *in vitro* in femoral than abdominal adipocytes from lean premenopausal women. The difference in lipolytic rate was due to cell size, since no difference was evident when data were adjusted for cell size. In an *in vivo* study using microdialysis, Jansson et al. (11) reported higher interstitial glycerol concentrations in obese than lean men, a difference that was also ascribed to an increased lipolytic rate in larger fat cells. Although interstitial glycerol concentration could also be affected by lipoprotein lipase activity, this is not likely the case in the present study since the subjects were in the postabsorptive state after an overnight fast, a time when lipolysis, rather than triglyceride storage, would predominate.

Arner et al. (1) reported no difference in dialysate glycerol concentrations from microdialysis probes placed in the abdominal and gluteal subcutaneous adipose tissue in six lean premenopausal women. They also performed the NNF method of determining interstitial concentration in two men and two women in that study, finding very similar interstitial glycerol concentrations in the abdominal and gluteal regions. Although the findings of Arner et al. do not support the large difference between femoral and abdominal interstitial glycerol concentrations found in the current study, possible reasons for these conflicting data are potential differences in interstitial concentration or blood flow between the gluteal and femoral regions, as well as differences in subject characteristics.

Under most experimental conditions, the dialysate glycerol concentration is not a direct measure of interstitial glycerol concentration due to incomplete recovery of glycerol over the microdialysis membrane. Microdialysis probe recovery and interstitial concentration must be derived from calibration procedures employed during microdialysis. The NNF method of *in vivo*

calibration is well established as an accurate determinant of interstitial concentrations under steady-state conditions (11, 13, 15). However, this method requires 5–6 h to conduct and is therefore impractical to use on the same day as most experimental studies. The extrapolation to zero method also requires considerable time to conduct. Whereas interstitial concentration can be more directly obtained when a very low perfusion flow rate through the microdialysis probe is used to attain nearly 100% recovery (likely $<0.5 \mu\text{l}/\text{min}$ with a 3-cm dialysis membrane) (5), the sample volume attained per unit time is very small compared with commonly used perfusion flow rates. The IR method may therefore be the most useful of the calibration methods, because this method allows for sample-to-sample determination of interstitial concentrations and can be performed over a wide range of perfusion flow rates. With the use of radioactive isotopes, the IR method was shown to yield values for microdialysis probe recovery and interstitial concentrations comparable to those obtained using the NNF method under steady-state conditions (13, 16). The addition of a stable isotope-labeled glycerol to the perfusate in the present study yielded *in vitro* recoveries similar to the actual measured *in vitro* recovery and yielded recoveries and interstitial concentrations *in vivo* similar to those obtained using the NNF method (Figs. 1 and 2). Furthermore, there was a very strong correlation between the interstitial concentration calculated using IR and NNF methods (Fig. 3). The stable isotope-labeled IR method may therefore be the method of choice when interstitial concentrations are required in a short amount of time.

The interstitial glycerol concentrations determined in abdominal subcutaneous adipose tissue in this study are similar to those obtained in abdominal subcutaneous adipose tissue in young men by Jansson et al. (11, 12) and Barbe et al. (2). The abdominal interstitial glycerol concentration was reported by Jansson et al. to be similar to (lean men) or greater than (obese men) that of femoral interstitial glycerol concentration in subcutaneous adipose tissue of men, which is in contrast to the finding of a higher interstitial concentration in the femoral than abdominal adipose tissue in women in the present study. These *in vivo* data collectively indicate that there are gender-related, site-specific differences in lipolytic rate. The data of Jansson et al. (11, 12), Barbe et al. (2), and Arner et al. (1), demonstrating interstitial glycerol concentrations in subcutaneous adipose tissue of between 100 and 250 μM , have been verified in the current study using two microdialysis methods of determining interstitial glycerol concentration. These data seem to outweigh the findings of Maggs et al. (17), who recently reported interstitial glycerol concentrations in the range of 2.8 mM using the NNF method.

The present experiments were conducted in resting humans in the absence of pharmacological perturbations of lipolysis. Further validation of the IR method is necessary to determine the usefulness of the method under conditions of physiological or pharmacological

perturbations. It should also be noted that the concentrations of labeled glycerol used in the perfusates were selected to span the range of previously reported interstitial glycerol concentrations (1, 2, 11, 12), with no differences observed in the data obtained using the IR method when the probes were perfused with 100 or 300 μM labeled glycerol.

In summary, we found that the interstitial glycerol concentration, as determined with microdialysis using the NNF and IR methods, was higher in femoral than in abdominal adipose tissue of nonobese, premenopausal women. Calculated rates of glycerol release were also higher in the femoral than abdominal adipose tissue. Importantly, our findings from both in vivo and in vitro experiments demonstrate that stable isotope-labeled glycerol can be used as an internal reference under resting conditions to estimate interstitial glycerol concentrations.

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