Adipose tissue metabolism in the postprandial period: microdialysis and arteriovenous techniques compared

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SUMMARY

Microdialysis has been used extensively for determination of interstitial concentrations of substances in animal brain and, more recently, in human brain, muscle, and adipose tissue (21). The microdialysis probe is placed in the tissue of interest, and an isotonic fluid is infused through it. Hydrophilic substances diffuse across the microdialysis membrane, and their concentrations in the fluid leaving the probe can be measured. It is then possible to calculate the interstitial concentrations of these substances (18). In the case of adipose tissue, for instance, interstitial glycerol concentrations have been used as an index of lipolysis (1).

Another technique that has been used for studying adipose tissue metabolism is the arteriovenous technique (8). Cannulation of a vein draining adipose tissue and an artery or an arterialized vein at the wrist allows calculation of arteriovenous differences for both hydrophilic and hydrophobic molecules across adipose tissue. It is therefore possible to assess adipose tissue uptake and release of substances and to calculate the rates of action of the enzymes lipoprotein lipase (LPL) and hormone-sensitive lipase (HSL) in vivo under different physiological conditions.

Adipose tissue uptake of fatty acids, triglycerides, NEFA, and glycerol; hormone-sensitive lipase; lipoprotein lipase

METHODS

We studied 10 subjects (5 female) aged 22 to 65 (median 43.5) yr with body mass index 19 to 35 (median 25) kg/m². Subjects were asked to refrain from smoking, alcohol, and “unaccustomed exercise” for 24 h before the study. They were asked to eat a fat-free meal on the evening before the study and then to fast from 8:00 PM, also avoiding caffeinated drinks. The studies were approved by the Central Oxford Research Ethics Committee, and all subjects gave informed consent.

Experimental methods. A 10-cm, 22-gauge Secalon Hydrocath catheter (Ohmeda, Swindon, UK) was introduced over a guide wire into a superficial vein on the anterior abdominal wall and threaded toward the groin so that its tip lay just superior to the inguinal ligament. As described previously (8), this provided access to the venous drainage from the subcutaneous abdominal adipose tissue, uncontaminated by muscle drainage and with a relatively minor contribution from skin. This adipose tissue depot has been shown to be representative of whole body adipose tissue (7).

A cannula was inserted in a retrograde fashion into a vein draining a hand heated in a box at 60°C, to provide arterialized samples. Blood gas analysis was performed every 30 min throughout the study to ensure adequate arterialization (oxygen saturation was always >97%). Both the arterial and the venous cannulas were kept patent by continuous infusion of saline.
of isotonic saline. Simultaneous blood samples were taken from the arterialized vein (for simplicity referred to below as arterial) and the abdominal adipose vein at −30, 0, 30, 60, 90, 120, 180, 240, 300, 360 min. Subjects were fed at 0 min; five subjects were fed 60 g fat and 85 g carbohydrate (in the form of a bowl of cereal and a strawberry-flavored milkshake), and five subjects were fed 40 g fat and 100 g carbohydrate (given as tuna fish on toast with a banana). Data from the two sets of studies have been combined, since the comparison between techniques is made within subjects. Adipose tissue blood flow (ATBF) was measured immediately after each blood sample using the $^{133}$Xe washout method (16), as described by Summers et al. (20).

A microdialysis probe (CMA 60; CMA, Stockholm, Sweden) with a 30-mm-long membrane was inserted under local anesthetic into the same subcutaneous adipose tissue depot. Ringer solution was infused through the probe at 0.3 µl/min, after a 16-min priming period. The effluent was collected at 30-min intervals throughout the study period, starting at −45 min (allowing for dead space time), and the sample time was taken as the midpoint of the collection period. This microdialysis system has a long dialysis membrane perfused at a slow rate, resulting in virtually complete recovery of glycerol (10), and therefore did not require calibration.

Analyses. Arterial and adipose venous blood samples were rapidly deproteinized with 7% (wt/vol) perchloric acid. Whole blood glycerol concentration was measured using enzymatic methods on an IL Monarch centrifugal analyzer (Instrumentation Laboratory, Warrington, Cheshire, UK). In the five subjects who received the fish meal, plasma was also prepared from the samples, and plasma glycerol concentration was measured enzymatically on the same analyzer. In both groups, plasma TAG concentrations were measured enzymatically (with correction for free glycerol; see Ref. 12), and a portion of the arterial samples was heparinized for blood gas analysis and hematocrit estimation. Microdialysate glycerol was measured using an enzymatic fluorometric method, with a CMA 600 microdialysis analyzer (CMA/Microdialysis).

Calculations and statistical analyses. ATBF was calculated as described by Larsen et al. (16). The partition coefficient for $^{133}$Xe was taken as 10 ml/g. Any error in the estimation of the partition coefficient should not affect the results, as two measurements are being compared within the same subject. Plasma flow was calculated as $\text{ATBF} \times (1 - \text{hematocrit})$, and plasma water flow was calculated by multiplying plasma flow by 0.94.

There is a very close relationship between whole blood and plasma glycerol concentrations (6). This was confirmed by comparison of whole blood and plasma glycerol concentrations in 120 samples from the fish meal studies (including the results of 1 subject who did not undergo microdialysis). Whole blood glycerol concentrations (µmol/l) for the five subjects who consumed the milkshake meal were converted to plasma glycerol concentrations (µmol/l) using a regression equation derived from the direct comparison

\[(1.13 \times \text{blood glycerol concentration}) + 7.58\]

Plasma glycerol concentrations were converted to plasma water concentrations by dividing by 0.94.

Calculated venous plasma water glycerol concentrations ($C_{vcalc}$) were calculated from interstitial glycerol concentrations using Fick’s law of diffusion for a thin membrane (13)

\[J = PS(C_i - C_v)\]

where $J$ is substrate flux, $P$ is the membrane permeability of the substrate, $S$ the membrane surface area, and $C_1$ and $C_2$ the concentrations on the two sides of the membrane ($C_1$ being higher than $C_2$). From this equation, a further equation can be derived (19)

\[C_{vcalc} = [(C_i - C_a) \times (1 - e^{-PS/0})] - C_a\]

where $C_i$ is interstitial water concentration, $C_a$ is arterial plasma water concentration, $Q$ is plasma water flow, and PS is the permeability surface area product. PS was taken as 3 ml·100 g$^{-1}$·min$^{-1}$ for glycerol, as similar size molecules are known to have this value (17). It was assumed that the PS product remained constant within the range of blood flows recorded.

To compare the two different methods, the difference between actual venous plasma water glycerol concentration ($C_v$) and $C_{vcalc}$ was plotted against the mean of the two concentrations for each subject at each time point. The limits of agreement were then calculated as $d - 2s$ to $d + 2s$, where $d$ is the mean difference between the two methods and $s$ is the standard deviation of the differences. Repeated measures ANOVA was used to compare concentrations in each subject, analyzing the effects of both site of measurement and time. Where appropriate, a paired t-test was used to compare maximum or minimum values with the basal value (taken as the value at 0 min) or with each other using the Bonferroni correction.

Adipose tissue glycerol release was calculated as the product of whole blood arteriovenous difference and ATBF. $C_{vcalc}$ was converted to a plasma glycerol concentration by multiplying by 0.94 and then to a whole blood concentration using the regression equation above. Glycerol release derived from the action of HSL was calculated using the equation

\[\text{HSL action} = \text{total glycerol release} - \text{TAG extraction}\]

where TAG extraction was the product of whole blood arteriovenous difference and ATBF (9). The glycerol arteriovenous difference due to HSL action was calculated by dividing HSL action by ATBF.

RESULTS

Interstitial, venous, and arterial plasma glycerol concentration. Arterial and adipose venous plasma glycerol concentrations changed with time ($P < 0.001$); high fasting concentrations fell significantly to a minimum at ~60 min ($P < 0.01$ and $P < 0.005$, respectively) before increasing significantly ($P < 0.01$ and $P < 0.05$, respectively) to concentrations at 360 min that were not significantly different from those at 0 min. Dialysate glycerol concentrations (interstitial glycerol) also changed with time ($P < 0.001$), again reaching a minimum at 60 min.

Mean interstitial glycerol followed a similar pattern to mean venous glycerol in subcutaneous adipose tissue during the postprandial period, except that the increase in mean interstitial glycerol concentration after 60 min plateaued at 210 min (Fig. 1). Venous plasma and interstitial glycerol concentrations were not significantly different within individuals. Arterial plasma glycerol concentration was lower than venous and interstitial concentrations within individuals ($P < 0.01$).

$C_{vcalc}$. The agreement between venous plasma water glycerol concentration calculated from interstitial con-
centrations and the actual concentrations was almost complete (Fig. 2). The two measures were not significantly different and followed an identical course, with the calculated concentration continuing to increase during the postprandial period to reach fasting concentrations.

The two measurements in each subject at each time point were correlated \( r_s = 0.7, P < 0.001 \), but the plot of differences between \( C_v \) and \( C_{vcalc} \) against the means of the two (Fig. 3) shows that there were considerable differences in the results obtained from the two methods. The limits of agreement were \(-2174 \) to \( 291 \) µmol/l, with a mean difference between the two methods of \( 58.6 \) µmol/l. The mean difference between measurements for each subject was not correlated with the age, sex, body mass index, or ATBF of the subjects.

Glycerol release. The mean whole blood glycerol release across adipose tissue calculated from the microdialysis and adipose tissue vein data at each time point differed markedly (Fig. 4). Glycerol release calculated from the microdialysis data was significantly lower than that calculated from the adipose vein data by \( \sim 40\% \) \( (P < 0.05) \), but there was no significant difference between glycerol release calculated from the microdialysis data and the HSL-derived glycerol (calculated from the adipose tissue vein data). Similarly, the arteriovenous differences for total glycerol calculated from the two different techniques were different \( (P < 0.05) \), whereas there was no significant difference between the arteriovenous difference for glycerol calculated from the microdialysis data and the glycerol arteriovenous difference due to HSL action (Fig. 5).

**DISCUSSION**

Microdialysis has practical advantages over the arteriovenous technique. It is an easier technique to use in unselected subjects; the cannulation of an adipose tissue vein can be time consuming or unsuccessful, whereas insertion of a microdialysis probe is quick and straightforward. Microdialysis can be used in different...
Adipose tissue depots, whereas the arteriovenous technique can only be used in the anterior subcutaneous adipose tissue depot because of the existence of an anepineurosis below this depot, removing the problem of mixed venous drainage from muscle and adipose tissue. Also, microdialysis allows the local introduction of effectors, such as catecholamines, so that the physiology and pharmacology of adipose tissue lipolysis can be studied more closely (3, 4). Blood flow can be measured simultaneously during microdialysis experiments by using ethanol infusion through the probe (11). In view of the advantages of the microdialysis technique, it would be a useful tool in the investigation of postprandial metabolism if interstitial glycerol concentrations do reflect intravascular LPL action as well as intracellular HSL action.

Postprandial interstitial glycerol concentrations followed a similar pattern to venous plasma glycerol concentrations and were not significantly different from them. The venous plasma water glycerol concentrations calculated from the interstitial glycerol concentrations decreased in the first hour postprandially due to HSL suppression and subsequently increased again to fasting concentrations as LPL activity increased (following a similar pattern to the venous plasma water concentrations obtained by catheterization and not differing significantly from them). However, in spite of these apparent similarities, there were substantial differences for individuals at any given time point between the venous plasma water glycerol concentrations calculated using the two different techniques; the mean difference and the limits of agreement between the two methods indicate that these differences are large enough to be clinically important. The differences between whole blood glycerol concentrations calculated from microdialysis data and those measured in the adipose tissue vein samples were then further amplified by multiplication with plasma water flow during the calculation of glycerol release. The glycerol release calculated from the catheterization technique was consistently 40% higher than that calculated from the interstitial data. This agrees with previous findings of a twofold difference in calculated glycerol release after the ingestion of a glucose load (19).

The calculation of venous plasma water glycerol concentrations from interstitial glycerol concentrations relies on several assumptions, for example, that the PS product is 3 ml·100 g⁻¹·min⁻¹. Other investigators have assumed a PS product as high as 5 ml·100 g⁻¹·min⁻¹ (15). Changing the estimate of the PS product from, for example, 2 to 3 ml·100 g⁻¹·min⁻¹ can alter the term 1 - e⁻PS,Q,1 by up to 40%, depending on the plasma flow (19), but in the current study, altering the PS product from 3 ml·100 g⁻¹·min⁻¹ did not appear to improve the compatibility of the venous plasma water glycerol concentrations calculated from the two techniques. Assumptions made in the calculation of venous plasma water glycerol concentrations from interstitial concentrations may make the results unreliable compared with those calculated from the adipose venous plasma concentrations.

This study suggests that adipose tissue interstitial glycerol concentrations reflect HSL action more closely than LPL action; not only is glycerol release calculated from microdialysis data more closely related to HSL-derived glycerol release than to total adipose tissue glycerol release, but, also, interstitial glycerol concentrations plateau at 210 min, during the period of increased LPL and low HSL activity. This indicates that the glycerol produced by the action of LPL on circulating lipoprotein particles, in this case chylomicrons, remains mostly in the intravascular compartment escaping in the adipose tissue venous plasma, with little passing across the capillary endothelium into the interstitial space. The contribution of glycerol derived from LPL action on the capillary wall (more distant from the microdialysis probe) is therefore less than that derived from HSL action in fat cells (closer to the probe).

Although microdialysis does appear to be a useful tool for the investigation of adipose tissue metabolism in the postprandial period, with regard to the calculation of glycerol release, the catheterization method would seem to be superior due to the assumptions necessary for the calculation of venous concentrations from microdialysis data, at least when the contribution of LPL is considered. It is possible that lipolysis around the microdialysis probe may be affected by local trauma resulting from insertion of the probe or by the local anesthetic used. Also, microdialysis cannot (unlike the arteriovenous technique) be used to measure hydrophobic molecules, and the analytic techniques may be easier for the arteriovenous technique and the results easier to quantitate (depending on the system used). Overall, both techniques have a role to play in the study of postprandial adipose tissue metabolism. Microdialysis probably reflects glycerol released from fat cells, whereas the arteriovenous technique also measures glycerol released from chylomicron-TAG.

Fig. 5. Postprandial adipose tissue glycerol concentrations for 10 subjects. Estimated glycerol arteriovenous difference due to HSL (●) and mean whole blood glycerol arteriovenous difference calculated from microdialysis data (□) and from adipose vein data (▲).
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