

PAPER

Monitoring adipose tissue blood flow in man: a comparison between the ^{133}Xe washout method and microdialysis

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INTRODUCTION: Adipose tissue blood flow (ATBF) increases after meal intake and a failure to regulate ATBF in the postprandial period seems to be a feature of insulin resistance and obesity. ATBF can be measured quantitatively by the ^{133}Xe washout technique, but the microdialysis ethanol escape method has also been employed to detect relative changes in ATBF.

METHODS: We compared ^{133}Xe washout and the recovery of exogenous ethanol and endogenous urea by microdialysis in abdominal subcutaneous adipose tissue, after physiological stimulation of ATBF by ingestion of oral glucose (75 g) in eight healthy people (age 23–52 y, body mass index (BMI) 19.4–29.6 kg/m²).

RESULTS: The ATBF response was heterogeneous. In subjects responding vigorously to the stimulus as measured by ^{133}Xe washout, the microdialysis ethanol escape was increased (indicating an increase in ATBF). An increased recovery of urea was observed, also indicating an increase in ATBF. The recovery of both small molecules was delayed compared with increased blood flow and failed to return to baseline in response to a rapid decline in ATBF.

CONCLUSION: We conclude that the ^{133}Xe washout technique is more responsive to physiological change in ATBF than ethanol escape or urea recovery by microdialysis.

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Introduction

Adipose tissue blood flow (ATBF) increases after meal intake. Human *in vivo* studies have suggested that ATBF may be involved in regulating biological processes such as triacylglycerol clearance. By increasing the rate of supply of substrate (triacylglycerols) to lipoprotein lipase, the rate of hydrolysis is increased.¹ Experiments in dogs indicate that an increase in ATBF may facilitate the removal of non esterified fatty acids (NEFA) produced during lipolysis² and in accordance with that finding, an increase in ATBF brought about by physical exercise was attenuated when lipolysis was blocked by nicotinic acid. This suggests that NEFAs may regulate ATBF.³ Conversely, a vasoconstrictor effect has

been reported for NEFAs.⁴ Another role for modulation of ATBF could be to regulate the transport of endocrine molecules from the tissue.⁵

A failure to regulate ATBF in the postprandial period seems to be a feature of insulin resistance and obesity.⁶ Quantification of ATBF may therefore be of interest to understand mechanisms of dyslipidaemia and insulin resistance. ATBF can be measured quantitatively by the ^{133}Xe washout technique.^{7,8} Alternatively, the microdialysis ethanol escape method has been employed to detect relative changes in ATBF after local pharmacological delivery or external manipulation such as heating of the subcutaneous tissue.^{9,10} In addition, increased ethanol escape has been reported after glucose ingestion¹¹ and after institution of hyperinsulinaemic normoglycaemia.¹² In this study, we compared ^{133}Xe washout and the recovery of exogenous ethanol and endogenous urea by microdialysis in abdominal subcutaneous adipose tissue after physiological stimulation of ATBF by ingestion of oral glucose. Urea was used as an endogenous marker with the assumption that there is no production in

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adipose tissue and that the background level was in steady state. Hypothetically, the microdialysis probe would drain the tissue of urea and any change in urea recovery would depend on an altered delivery to the tissue, presumably by an altered blood flow. Some of the results have been published previously in abstract form.¹³

Subjects and methods

Adipose tissue ethanol microdialysis

Studies were conducted on eight healthy subjects (six male, two female), median age 42 (23–52) y, with mean BMI 24.0 (19.4–29.6) kg/m² at rest, following an overnight fast. Subjects were asked to refrain from strenuous exercise, smoking or alcohol for 24 h beforehand. A CMA 60 microdialysis catheter (Biotech Instruments Limited, Kimpton, UK) was perfused overnight with sterile Ringer's solution. In the morning of the study, the catheter was inserted into the subcutaneous adipose tissue in the para-umbilical area. The probe was then perfused with Ringer's solution containing 30 mmol/l ethanol at 3 µl/min and allowed to equilibrate for 0.5 h. The effluent dialysate was collected every 10 min during the experiment. In order to minimise evaporation, the dialysate was collected into 500 µl microcentrifuge tubes and immediately placed on ice and subsequently kept at 4°C until analysed. The collected volume was determined by weighing and was diluted with an equal volume of phosphate buffered saline. Ethanol was measured, within 24 h of sampling, in the diluted dialysate using an enzymatic method (Sigma, Poole, UK), adapted for use on an IL Monarch centrifugal analyser (Instrumentation Laboratory, Warrington, UK). The intra-assay coefficient of variation was 3.8% for 30 duplicate (diluted) samples with a mean ethanol concentration of 4.9 mmol/l (1.9–10.1). Results were expressed as the ratio of the concentration of ethanol in the effluent dialysate to that in the perfusate (the outflow: inflow (O/I) ratio).

Adipose tissue ¹³³Xe washout

A dose of 2 MBq of ¹³³Xe in 0.9% saline was injected into the contralateral side, at the same depth as the microdialysis probe, 30 min prior to recording the tissue radioactivity. ATBF_{Xe} was monitored by collecting continuous 20 s readings from a γ-counter probe placed over the exact site of injection and taped firmly in place.⁸ Blood flow was calculated from a semilog plot of disappearance of counts vs time in 10 min intervals. ATBF was then calculated according to the equation ATBF = slope of semilog plot × partition coefficient × 100.^{7,8}

Blood sampling

A cannula was inserted retrogradely into a distal forearm vein and kept patent by a continuous slow infusion of saline (NaCl 9 g/l). The lower part of the forearm was heated to

provide arterialised blood samples. Samples were taken at 10 min intervals throughout the study into heparinised syringes. At time zero, 75 g glucose, dissolved in 200 ml water and flavoured with fresh lemon, was ingested. Plasma glucose was measured the same day on samples stored at 4°C using an enzymatic method.¹⁴ Plasma insulin was measured by radioimmunoassay (Pharmacia and Upjohn, Milton Keynes, UK).

Endogenous urea recovery by microdialysis

In six of the above studies, dialysate urea concentrations were measured in addition to ethanol. Dialysate urea concentrations were measured using a kit method (66-UV; Sigma, Poole, UK) which was adapted for use on the IL Monarch centrifugal analyser. The method was linear to 10 mmol/l and used 3 µl sample. The intra-assay coefficient of variation was 3.2% for 30 duplicate samples with a mean urea concentration of 1.15 (0.46–1.49) mmol/l.

The Central Oxford Research Ethics Committee approved the study and all subjects gave informed consent.

Statistics

Changes in concentrations with time were assessed by repeated-measures analysis of variance (RM-ANOVA) using time and treatment as within-subjects factors. Calculations were done with SPSS for Windows Release 7.1 (SPSS Inc., Chicago, IL, USA).

Peak values for ATBF_{Xe} were calculated as the mean of three points; the maximum value and the two highest adjacent points between 0 and 100 min. Absolute change in ATBF_{Xe} were calculated as peak minus mean baseline. Peak and trough values for urea concentrations and ethanol O/I ratios respectively, were taken between 0 and 150 min. Relative changes for urea and ethanol were calculated as peak or trough values/mean baseline.

Results

ATBF

Mean ATBF_{Xe} increased in response to the oral glucose load. The mean time to peak was 57.5 min (25–85). ATBF_{Xe} peak values were attained between 35 and 55 min after ingestion of glucose and this coincided with the peak plasma glucose concentration of (9.3 mmol/l at 50 min) and of plasma insulin (39.0 mU/ml at 40 min). There was significant variation in ATBF between subjects ($P < 0.005$) with a range of peak values from 2.4 to 23.0 ml/min per 100 g tissue. In fact some people showed a very limited response, if any, whereas others showed a several-fold increased ATBF from baseline (range of 0–4.1).

Microdialysis

Overall, the mean dialysate weight was 28.2 mg (s.e. 0.5). The mean ranged from 26.2 to 30.1 mg for each person.

Ethanol escape

The mean ethanol O/I ratio at baseline was 0.49 (s.e. 0.05). There was no statistically significant change in ethanol O/I ratio with time as determined by RM ANOVA. However, the change in ethanol O/I ratio and change in $ATBF_{Xe}$ were correlated ($r_s = -0.79$, $P < 0.05$), consistent with increased removal of ethanol by increased blood flow. Due to the heterogeneous $ATBF$ response the subjects were divided into two groups, according to the magnitude of the change in $ATBF_{Xe}$ (Figure 1). This created two groups with distinctly different blood flow responses; one group had a mean peak $ATBF_{Xe}$ that was two-fold higher than baseline (Figure 1, panel A), whereas the four subjects with the lowest change in $ATBF_{Xe}$ showed no obvious mean response to the ingestion of glucose (Figure 1, panel B). A decrease of ethanol O/I ratio, was, however only observed in the four subjects with the greater response (Figure 1, panel A). There was no subsequent trend towards return to baseline despite the fact that $ATBF_{Xe}$ rapidly declined after attaining its peak. This suggests that the decrease in ethanol ratio was delayed in comparison with the increase in $ATBF_{Xe}$, and that it was either not sufficiently sensitive, or sufficiently rapid, to respond to the subsequent decrease in $ATBF_{Xe}$.

Urea

There was a two-fold increase in $ATBF_{Xe}$ in the six subjects studied (Figure 2), and the dialysate urea concentration tended to increase from 30 to 80 min after the mean peak $ATBF_{Xe}$. However, a reversal to baseline was not observed

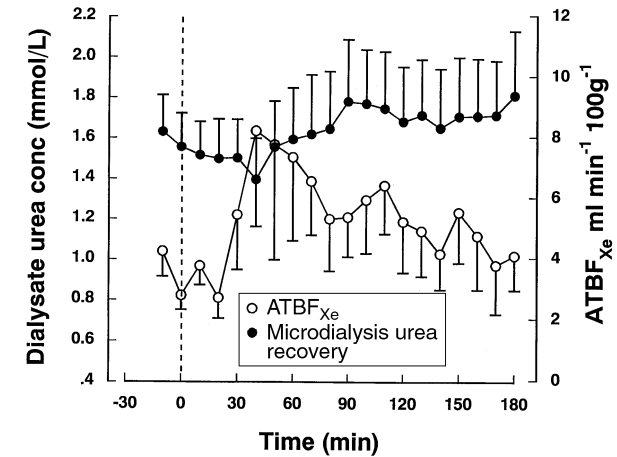


Figure 2 $ATBF_{Xe}$ and microdialysis urea recovery in six subjects before and after the ingestion of glucose. Values shown are mean and s.e.m.

with decreasing $ATBF_{Xe}$. The change in urea concentration was correlated with the change in ethanol O/I ratio ($r_s = -0.886$, $P < 0.05$), but not with change in $ATBF_{Xe}$. These results are consistent with the hypothesis; an increased blood flow would lead to increased delivery of urea to the microdialysis area. The result is also consistent with the ethanol escape experiment in the sense that there was no return to baseline indicating the same kind of delayed response or insufficient sensitivity as with ethanol microdialysis.

Five additional microdialysis studies were performed before and after the ingestion of high carbohydrate test meals in order to assess the performance of alternative small molecules. Sorbitol and sucrose were selected because

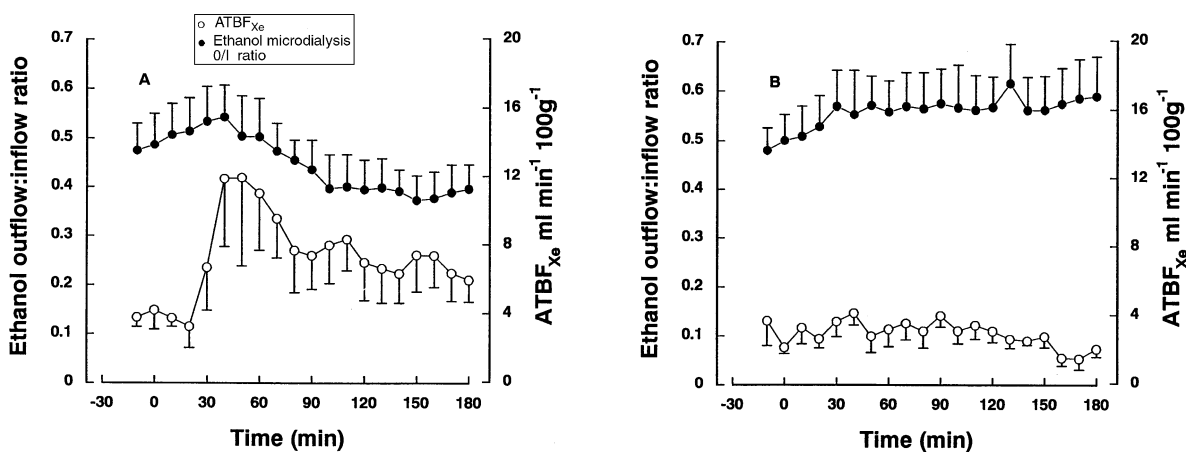


Figure 1 $ATBF_{Xe}$ and ethanol microdialysis O/I ratio measured in eight subjects before and after the ingestion of glucose. Subjects were divided into two groups according to change in $ATBF_{Xe}$ (see text for details) and mean values are shown without error bars for clarity. (A) High responders ($n=4$); (B) low responders ($n=4$). Values shown are mean and s.e.m.

they are not likely to be taken up intracellularly and are therefore more likely to reflect the distribution volume of the extracellular/interstitial space. However, these experiments did not show anything different from the studies using ethanol microdialysis (data not shown).

Discussion

We compared the performance of two methods to estimate physiological changes in ATBF; the ^{133}Xe washout technique and microdialysis of small molecules such as ethanol and urea. The ethanol O/I ratio showed a delayed response (approximately 40 min) to the rapid increase in ATBF_{Xe} elicited by the ingestion of glucose. In addition, late in the experiment, when ATBF_{Xe} had shown a sharp decline, the ethanol O/I ratio showed no tendency to return to baseline.

Urea microdialysis recovery is based on the principle that microdialysis creates a condition of local depletion of endogenous urea, with the assumption that urea is not produced in the tissue¹⁵ or, if produced,¹⁶ it is produced at a constant rate. We hypothesised that this local loss might be more rapidly replenished by increased blood flow. To the best of our knowledge quantification of endogenous urea by microdialysis in adipose tissue has not been employed for this purpose before, but the same principle has been advocated for endogenous glucose in estimating muscle blood flow by microdialysis.¹⁷ The urea results paralleled those of ethanol, which suggest that recovery of small molecules by microdialysis is generally delayed compared with changes in blood flow. The reason for the delayed and different response of microdialysis compared with the xenon washout technique in quantifying changes in ATBF should be considered. One obvious difference is the magnitude or strength of the stimulus needed comparing physiological and pharmacological regulation of ATBF. Enocksson and colleagues reported the effect of microdialysis delivery of a beta-2-adrenoreceptor agonist (terbutaline) and simultaneous quantification of ethanol escape.¹⁸ They used two doses, 10^{-6} and 10^{-5} M of terbutaline, and the ethanol escape was increased at the higher concentration, whereas a lipolytic response was observed at both concentrations. A similar experimental approach with a corresponding effect has been reported by Millet and colleagues¹⁹ at concentrations ranging from 10^{-7} to 10^{-5} M. These concentrations can be compared with those achieved by systemic delivery of epinephrine and simultaneous quantification of ATBF by xenon washout. Samra *et al*¹ reported adipose tissue concentrations of 10^{-9} or 10^{-10} M after a steady-state infusion of epinephrine of 25 ng/kg/min. This infusion provoked a strong lipolytic response and an increase in blood flow from 3 to 17 ml/100 g/min, which is close to a maximal physiological response. Although terbutaline and epinephrine are not absolutely equipotent as beta-2-agonists, there is not likely to be a 100–10000-fold difference in activity. Accordingly, when beta-2-adrenergic agonists have been delivered to adipose tissue by microdialysis it has been done at pharma-

logical levels to enable detection of a change in blood flow by the microdialysis ethanol escape technique.

The time taken to reach equilibrium has been shown to be longer with low flow rates in muscle,²⁰ and this is likely to apply to adipose tissue as well. This may provide an additional explanation for the lack of coherence between and microdialysis based methods and the ^{133}Xe washout method for the quantification of ATBF.

The microdialysis system was perfused overnight and a 30 min equilibration period was allowed before sampling; this period was chosen in accordance with the literature.^{18,19} However, a steady-state ethanol outflow:inflow ratio was not reached until 30 min (Figure 1B) into the experiment. We do not believe that this affects the interpretation of our results, since the subgroup who responded to the stimulus with an increase in ATBF_{Xe} showed the expected decline in outflow:inflow ratio. We have performed additional experiments with a 60 min equilibration period which show an absolutely stable baseline with ethanol outflow:inflow ratios of 0.566 ± 0.051 , 0.569 ± 0.058 and 0.567 ± 0.052 at time points 0, 10 and 20 min. Future studies should allow a 60 min equilibration period to allow for the initial trauma due to insertion of the probe.

Our findings correspond well with those from another tissue bed, skeletal muscle, in which it has recently been concluded that rapid changes in blood flow are poorly reflected by changes in microdialysis ethanol escape.^{21,22}

Although the ethanol escape method has been shown to be comparable to the ^{133}Xe washout technique after external warming,⁹ we conclude that the ^{133}Xe washout technique seems to be more discriminative to monitor physiological changes in ATBF. However, the microdialysis ethanol escape method may still be a useful tool to detect changes in response to local, pharmacological stimuli.

Acknowledgements

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