

The 6th International Tutorial Conference of the Research Group on Biochemistry of Exercise, a collaboration between the Nutrition Toxicology and Environmental Research Institute Maastricht and the Nutrition Society was held in Maastricht, The Netherlands on 18–21 February 1999

## Symposium on ‘Metabolic aspects of human nutrition at rest and during physical stress: recent methodological and technical developments’ Session 3: Microdialysis in metabolic and nutrition research

### Microdialysis: use in human exercise studies

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Microdialysis has been used for 25 years to study brain function *in vivo*. Recently, it has been developed for investigations on peripheral tissues. A microdialysis catheter is an artificial blood vessel system which can be placed in the extracellular space of various tissues such as adipose tissue and skeletal muscle in order to examine these tissues *in situ*. Molecules are collected from the tissue by the device and their true interstitial concentration can be estimated. Metabolically-active molecules can be delivered to the interstitial space through the microdialysis probe and their action on the tissue can be investigated locally without producing generalized effects. It is also possible to study local tissue blood flow with microdialysis by adding a flow marker (usually ethanol) to the microdialysis solvent. The microdialysis technique is particularly useful for studies of small and water-soluble molecules. A number of important observations on the *in vivo* regulation of lipolysis, carbohydrate metabolism and blood flow in human skeletal muscle and adipose tissue have been made recently using microdialysis.

#### Microdialysis: Skeletal muscle: Adipose tissue: Blood flow: Exercise

Microdialysis was developed 25 years ago to study brain function *in vivo* (Ungerstedt, 1991). It was not until recently that microdialysis was used to study the function of peripheral tissues (Arner & Bolinder, 1991; Arner & Bülow, 1993; Lafontan & Arner, 1996). The present review will discuss microdialysis, its advantages and disadvantages, focusing on the usefulness for exercise studies in human subjects. For this reason, only studies on adipose tissue and muscle will be considered, since they are the two major peripheral tissues that are involved in energy turnover of exercising subjects, and they can be studied by microdialysis. For brevity, review articles rather than original references will be cited whenever possible.

#### Principles of microdialysis

A microdialysis device is a type of artificial blood vessel system. It is made up of semi-permeable tissue-compatible dialysis tubing which is connected to an inlet and an outlet cannula. Two systems are commonly used (Arner & Bülow, 1993). One system (usually home-made) couples in series the inlet cannula to one end of the dialysis tubing and the outlet cannula to the other end of the tubing. In the other system, which is commercially available, the inlet and outlet cannulas are connected in a double-lumen fashion, and the microdialysis tubing is secured to the tip of the double-lumen catheter. The inlet of the microdialysis catheter is connected to a precision pump and is perfused with a neutral

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dialysis solvent (usually saline (9 g NaCl/l) or Ringer solution) at a low perfusion speed (usually 0.1–10 µl/min) to allow equilibrium dialysis to take place through the microdialysis membrane, which is situated in the interstitial space of the tissue. The outgoing dialysate is collected for analysis and reflects the composition of the interstitial space surrounding the microdialysis probe.

### Performance of the microdialysate system

Microdialysis can be used in two different ways: to collect molecules from the interstitial space; to deliver molecules to this space. It is also possible to simultaneously collect molecules from, and deliver molecules to, the tissue by microdialysis. Furthermore, several different molecules can be determined in the same fraction of the microdialysate.

Molecules are collected from the microdialysate in order to study their concentration in the interstitial fluid, because the dialysate concentration is directly proportional to the interstitial concentration. A number of factors influence the recovery of a particular substance from the interstitial space to the microdialysate, as summarized in Table 1 (Arner & Bolinder, 1991; Arner & Bülow, 1993). The use of a long length dialysis tubing and a slow perfusion rate increases recovery and vice versa. Small and water-soluble molecules pass easily through the microdialysis membrane, whereas large and/or lipophilic molecules do not pass in and out of the membrane, or only to a small extent. Small molecules that bind to carrier proteins have difficulty in passing through the membrane. Usually membranes with a cut-off point of about 1000 Da in molecular mass are used. Water-soluble molecules smaller than 100 Da easily traverse such membranes. It is possible to study larger molecules such as proteins using membranes with a high cut-off point (usually approximately 100 000 Da). However, there are major and unsolved problems with the utilization of such large membranes. As these membranes have a large pore size, microfiltration rather than microdialysis will take place, draining the water space of the investigated tissue so that the composition of the interstitial space will be artificially altered.

Local tissue factors such as tissue resistance are of great practical importance for recovery. These local factors only influence *in vivo* tissue recovery, not *in vitro* recovery, and tissue factors often influence the *in vivo* recovery of different molecules in a unique way. For example, there may be differences in *in vivo* recoveries of glucose, lactate and glycerol both within and between tissues, due to the separate influence of local tissue factors. Thus, it is not useful to 'calibrate' microdialysis probes *in vitro* by, for example, perfusing them in a solvent containing the molecule of interest and determining recovery as dialysate

concentration:external solvent concentration of the molecule.

In kinetic experiments, where the main aim is to follow changes in the concentration of tissue molecules over time, it is often not necessary to consider recovery problems. It might even be appropriate in such situations to use short lengths of membrane (5–10 mm in human subjects) and a fast perfusion rate (3–5 µl/min or more) when rapid events are being investigated, so that the microdialysis solvent can be sampled at frequent time intervals.

A number of different approaches can be applied to the measurement of the true interstitial concentration of a molecule using microdialysis (Table 2). The oldest approach is the mass transfer method (Jacobson *et al.* 1985) in which the perfusion speed is gradually decreased and the changes in the dialysate concentration are measured. Using appropriate formulas the concentration at zero perfusion speed is estimated, which is the actual tissue concentration. However, the accuracy of these formulas is low. The next method to be introduced was equilibrium dialysis (Lönnroth *et al.* 1989). The tissue is microdialysed with solvents containing different concentrations of the molecule of interest, and the relationship between the ingoing and outgoing concentration of the molecule is determined by linear regression analysis. The concentration at which the ingoing concentration equals the outgoing concentration is estimated, and this concentration equals the tissue concentration. Recovery can also be determined *in vivo* using isotopic methods (Jansson *et al.* 1994b). A labelled form of the substance of interest (e.g. [<sup>14</sup>C]glucose) is added to the ingoing dialysis solvent, the ratio outgoing isotope: ingoing isotope is determined and used to correct the measured outgoing concentration of the molecule to the actual tissue concentration of the molecule. However, this technique underestimates true *in vivo* recovery because the local blood flow around the probe does not remove all the isotope. The latter problem can be minimized by adding a vasodilating substance to the ingoing dialysate together with the isotope (Hagström-Toft *et al.* 1997). Finally, the true tissue concentration can be determined directly by performing microdialysis with a long length of membrane (30 mm or more in human subjects) at a low perfusion rate (0.3 µl/min or less; Bolinder *et al.* 1992). Under such conditions recovery is approximately 100 % (Hagström-Toft *et al.* 1997), and the concentration in the outgoing microdialysate equals approximately the actual tissue concentration. However, a problem with the direct method is that water might be drained from the probe to the surrounding extracellular space at a very low perfusion rate (0.1 µl/min or less). However, this problem can be overcome by adding a neutral osmotic agent (i.e. dextran) to the ingoing dialysis solvent (Rosdahl *et al.* 1997).

**Table 1.** Some important factors influencing recovery in microdialysis experiments

Perfusion rate
Length of microdialysis tubing
Molecular cut-off point of the dialysis membrane
Physical and chemical properties of the investigated molecule
Local tissue resistance

**Table 2.** Some ways of determining the true tissue concentration of a molecule in microdialysis experiments

Mass transfer method
Equilibrium dialysis
Isotopic recovery
Direct method

### Analysis of the microdialysate

The total volume of the microdialysate fraction to be sampled is often small and, when recovery is incomplete, the concentrations of the particular compounds to be analysed may be low. In addition, the microdialysate may have to be divided into several subfractions for the determination of many different substances. Thus it is often necessary to use ultrasensitive analytical methods for quantitative analysis (Arner & Bülow, 1993). The most commonly used methods are based on luminescence or HPLC. Recently, commercial autoanalysers have become available which can measure several metabolites in a small microdialysis fraction using methods based on near-dry spectrophotometry.

### Use of microdialysis for blood flow measurements

In kinetic experiments using microdialysis it is necessary to consider the influence of blood flow on the composition of the microdialysate. The tissue concentration at any given time is the net sum of local (production–metabolism) + (input–output) by blood flow. It has been shown experimentally (*in vitro*) that movement of the fluid outside the probe influences the transport of molecules through the microdialysis membrane (Hickner *et al.* 1992). This phenomenon also occurs *in vivo*. Changes in blood flow in the tissue surrounding the probe can have profound effects on the concentrations of substances in the interstitial space that are independent of production or breakdown (Hickner *et al.* 1991; Galitzky *et al.* 1993). One means of taking blood flow into account in kinetic experiments is to ‘clamp’ it by adding a vasodilating agent to the microdialysis solvent (Galitzky *et al.* 1993). Another way is to use microdialysis to study blood flow.

A commonly-used method, the so-called ethanol technique, has been developed to investigate blood flow using microdialysis (Hickner *et al.* 1991). Ethanol is added to the microdialysis solvent and the value for ethanol out : ethanol in is determined. Ethanol *per se* has no effect on tissue blood flow. This technique can be used in skeletal muscle and adipose tissue (Hickner *et al.* 1991; Galitzky *et al.* 1993). It should be noted that the ethanol method only measures relative changes in blood flow; it does not indicate the true rate of blood flow. Nevertheless, it has proved valuable to use the ethanol method in order to investigate blood flow regulation in microdialysis experiments, and also to determine which changes in the composition of the microdialysate are secondary to tissue flow alterations (Arner & Bülow, 1993; Lafontan & Arner, 1996). Although it is not quantitative, the ethanol method is as sensitive as the ‘gold standard’ Xe-washout technique for determining changes in blood flow in adipose tissue (Felländer *et al.* 1996).

### Use of microdialysis in pharmacological experiments

The most unique feature of microdialysis is that it can be used for local manipulation of a tissue *in vivo*. It is possible to expose the tissue to high concentrations of an active compound and at the same time measure the local tissue response without producing the generalized effects

associated with the compound (Arner & Bülow, 1993; Lafontan & Arner, 1996). By adding vasoactive and/or metabolically active compounds to the microdialysis solvent it has been possible to obtain detailed information on the *in vivo* regulation of lipolysis and blood flow in human subcutaneous adipose tissue (Lafontan & Arner, 1996). Recently, such experiments have also proved useful in studies of the regulation of metabolism and blood flow in skeletal muscle (Hickner *et al.* 1994; Enocksson *et al.* 1998).

It is also possible to alter the local hormonal composition using microdialysis and measure the effect *in situ*. For example, muscle and adipose tissue concentrations of catecholamines can be increased by adding an inhibitor of catecholamine metabolism to the microdialysis solvent and subsequently measuring the effect on local metabolism *in situ* (Maggs *et al.* 1995).

### Use of microdialysis to quantify substrate mobilization

Microdialysis can be combined with quantitative blood-flow measurements and arterial determinations, and substrate flux estimated using Fick’s principle (Arner & Bülow, 1993). Using such methods the mobilization rates of glycerol and lactate from subcutaneous adipose tissue have been determined after an overnight fast and following an oral glucose load (Jansson *et al.* 1992, 1994a).

It is possible to combine microdialysis of adipose tissue with venous cannulation of adipose tissue in quantitative studies (Arner & Bülow, 1993). Microdialysis mainly reflects events in fat cells, whereas the adipose vein mirrors extracellular plus intracellular (i.e. fat cell) events. By combining the two techniques it has been possible to evaluate the contribution of intracellular lipolysis in fat cells and extracellular hydrolysis of circulating triacylglycerols to adipose tissue production of glycerol (Summers *et al.* 1998).

### Advantages and disadvantages of microdialysis

The advantages and disadvantages of microdialysis are summarized in Table 3. Microdialysis is an easy-to-learn technique that causes minimal discomfort to the subject. In fact, it can be used to monitor adipose metabolism in infants (Hildingsson *et al.* 1996). It can be utilized for long-term (i.e. several days) monitoring of adipose tissue in freely-moving subjects (Bolinder *et al.* 1993). A unique advantage is that it allows local manipulation and subsequent analysis of the manipulated tissue without producing generalized effects. This feature has proved very useful in pharmacological studies (Lafontan & Arner, 1996). It can also be used to simultaneously study different tissues (e.g. fat and muscle) or different parts of the same tissue (e.g. femoral and abdominal subcutaneous adipose tissue) or different muscle groups.

However, there are drawbacks with microdialysis. It is relatively expensive because it necessitates the use of precision pumps, ultrasensitive analytical tools and sterilization procedures (in human studies). Furthermore, the home-made probes are tedious to construct, and the commercial probes are expensive. Microdialysis cannot

**Table 3.** Advantages and disadvantages of microdialysis

## Advantages

Simple  
 Minimal discomfort  
 Long-term measurements  
 Allows localized pharmacological experiments  
 Allows studies on several tissues and several regions of the same tissues  
 Many different molecules can be investigated at the same time.

## Disadvantages

Rather expensive  
 Calibration problems  
 Difficulties in studying large molecules  
 Hydrophobic compounds cannot be studied  
 Difficulties in studying molecules with strong protein-binding properties

readily be used to study hydrophobic compounds or compounds that bind strongly to carrier proteins. The technique is also not suitable for studying larger proteins, although attempts have been made to measure insulin in adipose tissue using membranes that have a high-molecular-mass cut-off point (Jansson *et al.* 1993). The membranes with a large pore size cause ultrafiltration which may alter the composition of the intracellular space that is being examined. As a 'rule-of-the-thumb', molecules with a size that is larger than one-tenth the dialysis membrane molecular-mass cut-off point cannot be determined with precision in microdialysis experiments. At present it is not possible to use membranes with a larger cut-off point than 100 000 Da. Another problem with microdialysis is calibration when a high perfusion speed is used. Most calibration techniques are indirect and often time-consuming, and some techniques (i.e. the equilibrium dialysis technique) may artificially influence the tissue in subsequent experiments because the methods necessitate exposure of the tissue to high concentrations of the molecule(s) of interest. The direct technique necessitates a very slow perfusion rate which is a drawback in kinetic experiments which involve rapid events and, thereby, frequent sampling.

A consideration of the pros and cons suggests that microdialysis is an attractive approach for the study of adipose tissue and skeletal muscle function *in vivo*. It is particularly useful for investigating small and water-soluble molecules. It can be a valuable method for investigating the function of muscle and fat tissues in exercise.

**Acknowledgements**

This study was supported by Medicus Bromma Ltd.

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