Microdialysis of skeletal muscle at rest

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Techniques in human skeletal muscle research are by necessity predominantly 'descriptive'. Microdialysis has raised high expectations that it could meet the demand for a method that allows 'mechanistic' investigations to be performed in human skeletal muscle. In the present review, some views are given on how well the initial expectations on the use of the microdialysis technique in skeletal muscle have been fulfilled, and the areas in which additional work is needed in order to validate microdialysis as an important metabolic technique in this tissue. The microdialysis catheter has been equated to an artificial blood vessel, which is introduced into the tissue. By means of this 'vessel' the concentrations of compounds in the interstitial space can be monitored. The concentration of substances in the collected samples is dependent on the rate of perfusate flow. When perfusate flow is slow enough to allow complete equilibration between interstitial and perfusate fluids, the concentration in the perfusate is maximal and identical to the interstitial concentration. Microdialysis data may be influenced by changes in blood flow, especially in instances where the tissue diffusivity limits the recovery *in vivo*, i.e. when recovery *in vitro* is 100 %, whereas the recovery *in vivo* is less than 100 %. Microdialysis data indicate that a significant arterial–interstitial glucose concentration gradient exists in skeletal muscle but not in adipose tissue at rest. While the concentrations of glucose and lactate in the dialysate from skeletal muscle are close to the expected values, the glycerol values obtained for muscle are still puzzling. Ethanol added to the perfusate will be cleared by the tissue at a rate that is determined by the nutritive blood flow (the microdialysis ethanol technique). It is concluded that microdialysis of skeletal muscle has become an important technique for mechanistic studies in human metabolism and nutrition.

Microdialysis: Skeletal muscle: Glucose: Lactate: Blood flow

Techniques in human skeletal muscle research are by necessity predominantly 'descriptive'. Muscle biopsy, nuclear magnetic resonance spectroscopy (MRS) and positron emission tomography (PET) are examples of such techniques. Methods allowing investigations of regulatory processes can only be applied to human tissues in exceptional cases, one example being *in vitro* incubation of skeletal muscle biopsies. Microdialysis has raised high expectations that it could meet the demand for a method that allows 'mechanistic' investigations to be performed in human skeletal muscle.

Microdialysis was developed as a technique for the sampling of neurotransmitters in the brain of small animals, and is now considered a major research tool in brain neurochemistry (Hamberger *et al*. 1991; Hamani *et al*. 1997).

The method also has the potential to become the method of choice for sampling the interstitial fluid of skeletal muscle, both with respect to small molecules such as glucose, as well as to biological macromolecules. In addition, based on the determination of flow-limited clearance of substances from the microdialysis perfusion solution, microdialysis can be used to monitor the nutritive blood flow around the dialysis catheter.

In the present paper, some views will be given on how well the initial expectations on the use of the microdialysis technique in skeletal muscle have been fulfilled, and the areas in which additional work is needed in order to validate microdialysis as an important metabolic technique in this tissue.

Basic principle

The microdialysis catheter is inserted into the tissue using a guide cannula, and is perfused using a precision pump with a physiological buffer solution (perfusate) at rates of the order of μl/min. After entering through inflow tubing, the

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perfusate passes through the membrane section of the microdialysis catheter, where the tubing is replaced by a semipermeable dialysis membrane of varying length. Finally, the perfusate enters outflow tubing and can be collected at the outlet. Water-soluble molecules in the interstitial space will diffuse along a concentration gradient through the microdialysis membrane into the perfusate. Substances which have been added to the perfusate will diffuse in the opposite direction to reach the interstitial space, where effects may be exerted on the cells lining it. In this way the microdialysis catheter has been equated to an artificial blood vessel which is introduced into the tissue. By means of this 'vessel' the concentrations of compounds in the interstitial space can be monitored. By introducing hormones or other metabolically-active compounds into this 'blood vessel' advanced mechanistic studies can be performed using the microdialysis technique. The pore size of the microdialysis membrane determines the size of the molecules that can pass through it. This factor is discussed in more detail by Arner (1999). Different designs of microdialysis catheters are in current use. The type in most widespread use, and which has been used in our laboratory, is a double-lumen microdialysis catheter (Rosdahl *et al*. 1993). Another design is a linear catheter in which the dialysis membrane is secured between the inlet and outlet tubing (Lönnroth *et al*. 1987). The concentration of substances in the collected samples is dependent on the rate of perfusate flow (the lower rate, the higher concentration). When perfusate flow is slow enough to allow complete equilibration between interstitial and perfusate fluids, the concentration in the perfusate is maximal and identical to the interstitial concentration (see pp. 920–921). A general advantage with the microdialysis technique is that large proteins are filtered by the microdialysis membrane and, therefore, the samples may be analysed without further purification.

Influence of changes in blood flow on substance concentrations measured in dialysate

Microdialysis was developed for use in the brain, where the relative variations in blood flow are comparatively small. In skeletal muscle, on the other hand, blood-flow variations even at rest may be substantial. This factor is secondary to the fact that changes in the vascular resistance in skeletal muscle tissue constitute an important buffer against acute changes in blood pressure. The difference between the two tissues probably explains why it was not acknowledged earlier that microdialysis data may be influenced by changes in blood flow. The blood-flow dependency of dialysate concentrations is the result of the microdialysis catheter competing for interstitial compounds with other processes (this factor is also discussed by Arner, 1999). The glucose concentration in the interstitial space may be used as an example. The interstitial concentration is determined by the balance between cellular glucose uptake (removing glucose) and capillary blood circulation (delivering glucose). At steady-state, an increase in capillary blood flow therefore causes the interstitial glucose concentration to increase (and vice versa if capillary blood flow decreases). This balance is determined by vascular physiology and does not suggest a problem with microdialysis. However, this balance is disturbed by the microdialysis catheter, which functions as an 'extra' blood vessel removing an additional amount of glucose from the interstitial space. This factor will, at perfusion flow rates resulting in an incomplete recovery, reduce the concentration of substances in the immediate vicinity of the dialysis membrane (Johnson & Justice, 1983; Jansson *et al*. 1988; Benveniste *et al*. 1989; Hickner *et al*. 1992). It would be logical to assume that this local drainage will make the interstitial glucose concentration in the vicinity of the microdialysis catheter much more sensitive than normal to variations in blood flow. We have shown in several examples that blood flow changes in fact may result in artifactual changes in dialysate concentrations of glucose both in skeletal muscle and adipose tissue (Hickner *et al*. 1992; Rosdahl *et al*. 1993; Rosdahl, 1998). We believe that this problem, due to local drainage of substances in the vicinity of the microdialysis catheter, can be expected in all instances where the tissue diffusivity limits the recovery *in vivo*, i.e. when recovery *in vitro* is 100 %, whereas the recovery *in vivo* is less than 100 % (Rosdahl, 1998). As with glucose, we have found that changes in blood flow have pronounced effects on microdialysis concentrations of several other substances, including glutamate, glutamine and leucine (Rosdahl *et al*. 1998*c*), whereas other substances, e.g. lactate, are less affected (Rosdahl *et al*. 1993, 1998*c*).

Assessment of interstitial concentrations

Microdialysis is often performed under conditions where there is incomplete equilibration between interstitial and dialysate concentrations. Thus, the actual *in vivo* recovery (dialysate divided by interstitial concentration) must be known in order to calculate the correct interstitial concentration. Several methods have been introduced for this purpose, including the techniques of no-net flux, and extrapolation-to-zero-flow, as well as the use of internal standards. These methods are described and discussed by Arner (1999) and Bangsbo (1999). As indicated earlier, in some instances this calculation may be further complicated if local substance drainage occurs around the microdialysis catheter.

The need for calibration of the microdialysis catheters can be obviated if a combination of a long length of microdialysis membrane and a low perfusion flow rate is used to permit complete equilibration between the interstitial and perfusate fluids. This idea was first put forward during several microdialysis investigations in the brain, and has also been used in adipose tissue (Johnson & Justice, 1983; Van Wylen *et al*. 1986; Alexander *et al*. 1988; Bolinder *et al*. 1992).

Loss of microdialysis perfusate

When we attempted to use very low perfusate flow rates with 30 mm catheters in skeletal muscle, it was found that most of the perfusate was lost into the tissue. We found this problem to be more pronounced in skeletal muscle than in adipose tissue. In fact, at 0·2 µl/min almost no sample was obtained from the skeletal muscle catheters, whereas half

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the perfused volume was recovered from the catheters in adipose tissue. With the size of microdialysis catheters used, this low perfusate flow rate was necessary, both in skeletal muscle and adipose tissue, in order to obtain full equilibration for glucose between perfusate and interstitial fluids. Loss of perfusate occurs to some extent also at higher perfusate speeds, but is less pronounced due to the shorter period for equilibration (Hickner *et al*. 1995). The loss of perfusate fluid can be completely ameliorated by the addition of a colloid to the perfusate (Rosdahl *et al*. 1997). We have used Dextran-70 (35–40 g/l; Amersham Pharmacia Biotech, Uppsala, Sweden), but other colloids such as albumin may also be used. What is the major cause of the loss of perfusate fluid? We initially assumed that it was due to the colloid osmotic pressure of the tissue, which acted unopposed by osmotically-active agents in the perfusate. However, tissue colloidal osmotic pressure in skeletal muscle is reported to be much lower (12–13 mmHg) than that of a 35–40 g solution of Dextran-70 (26-33 mmHg). This difference may indicate that the major factor responsible for the loss of perfusate fluid is in fact the colloidal osmotic pressure of plasma, which is of the order of 27 mmHg (Noddeland, 1982; Wiig *et al*. 1988). It is possible, however, that hydrostatic pressure inside the microdialysis catheter could be an additional cause of the fluid shift. Incidentally, the loss of perfusate is markedly increased if the hydrostatic back pressure in the microdialysis catheter is increased by elevating the position of the outflow tubing. Conversely, the perfusate fluid loss is diminished if the outflow tubing is placed as low as possible, and may be completely inhibited if the outflow tubing is of sufficient length. This factor would be of interest if, for some reason, it was desirable to avoid the use of a colloid in the perfusate.

Interstitial concentrations may be measured with microdialysis at a low flow rate

In experiments in which there was a gradual decrease in the perfusate flow rate (1·33, 0·66, 0·33, 0·16 and 0·075 μl/min), the glucose concentration in dialysate samples stabilized at the maximal level at 0·16 μl/min. This finding was obtained in both skeletal muscle and adipose tissue, but dialysate glucose reached the plasma level in adipose tissue only. The glucose concentration in skeletal muscle dialysates remained significantly below that of cubital venous plasma, even if the perfusate flow rate was lowered to 0·075 μl/min. We have suggested that this finding indicates that a marked arterial–interstitial glucose concentration gradient exists in skeletal muscle at rest. This explanation is in agreement with the results of studies of other investigators (Maggs *et al*. 1995; Müller *et al*. 1996), and most studies report that glucose concentrations in plasma equal those in the interstitial space of adipose tissue (Lönnroth *et al*. 1987; Bolinder *et al*. 1989). The result is further supported by the finding that urea, which is not taken up by skeletal muscle, is not lower in skeletal muscle dialysates than in plasma (Rosdahl *et al*. 1998*a*), and is not different at low flow rates in skeletal muscle and adipose tissue dialysates. Changes in the interstitial glucose concentration during insulin stimulation should reflect the insulin sensitivity of the tissue (Rosdahl *et al*. 1998*b*). The possibility of measuring this factor directly by microdialysis at a low flow rate opens interesting areas of research. The interstitial lactate concentration (as measured at a low flow rate) was 2·4 mM, and complete equilibration occurred at a higher perfusion flow than that for glucose (0·3 μl/min). Assuming an intracellular lactate concentration of 1 mM, and an intracellular *v.* interstitial pH difference of 7·0 *v.* 7·4), the expected interstitial lactate concentration was calculated by Rosdahl (1998). The basis of this calculation is that it is only the undissociated lactate molecule (2 mmol/mol total lactate) that equilibrates over the cell membrane to yield equal concentrations on both sides. The lactate ion concentration (998 mmol/mol total lactate) is therefore inversely related to the H^+ concentration (Roos, 1975), i.e. a 2·5-fold difference. The measured interstitial lactate concentration of 2·4 mM is therefore close to the expected level.

While the concentrations of glucose and lactate in the dialysate from skeletal muscle are close to the expected values, the glycerol values obtained in muscle are puzzling. We have repeatedly found very high values during the first hour following catheter insertion, but thereafter glycerol values are lower than in venous blood (Rosdahl *et al*. 1998*a*). However, a similar finding was not obtained in a study by Hagström-Toft *et al*. (1997). Glycerol values which are lower in the interstitial space than in plasma would indicate glycerol uptake by the muscle.

We believe that this observation must be satisfactorily explained before dialysate concentrations of glycerol can be taken to indicate the rate of lipolysis in skeletal muscle.

Stability over time

Results were collected from microdialysis catheters which were inserted in the *m. quadriceps femoris* of healthy subjects and perfused at a constant rate for 8 h (Rosdahl *et al*. 1998*a*). Briefly, the results indicate that glucose, lactate and glycerol levels stabilized 90 min after catheter insertion, whereas urea levels stabilized faster. This initial instability is likely to reflect the trauma of insertion, and may be expected to differ with the catheter type used.

Using microdialysis to assess nutritive blood flow in skeletal muscle: the microdialysis ethanol technique

I have discussed earlier that in some instances blood-flow variations may seriously affect microdialysis results in skeletal muscle. This finding led to the idea that the microdialysis technique may also be used to monitor blood-flow changes around the catheter. We have proposed that ethanol added to the perfusate will be cleared from the tissue area adjacent to the catheter at a rate that is determined by the nutritive blood flow.

We have shown that this is indeed the case, and using a perfused gastrocnemius preparation the nature of the blood-flow-dependent ethanol clearance from the dialysis catheter has been explored (Wallgren *et al*. 1995). The principle of the microdialysis ethanol technique is quite simple. The perfusate is supplemented with ethanol at a concentration low enough not to influence tissue physiology or metabolism; the highest concentration used has been 5 mM. The concentration of ethanol in the dialysate outflow is then

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determined. The values, given as outflow : inflow concentration decrease with increasing blood flow in an exponential manner. As discussed by Arner (1999), the technique has also been evaluated in human adipose tissue and found to correlate well with ^{133}Xe clearance. Recently, the technique was validated further in rat hindquarters (Stallknecht *et al*. 1999) perfused at flow rates of 0–21 ml/100 g per min. In addition, it was found that ethanol and [^{14}C]ethanol gave similar results. As pointed out by Arner (1999), the ethanol method can be made as sensitive as the 'golden standard' Xe-washout technique. However, as shown earlier by Wallgren *et al*. (1995), the sensitivity increases with decreasing perfusate flow rate. If perfusion flow rates are too high the method is unable to detect blood-flow changes (Hickner *et al*. 1995). This factor must be taken into account when attempting to validate the technique. However, Stallknecht *et al*. (1999) and also Rådegran *et al*. (1998) noted that muscle contractions led to a decreased outflow : inflow, independent of flow. This finding indicates that the microdialysis ethanol technique may not be suitable for use in contracting muscle, possibly due to large changes in diffusion conditions secondary to movement of the catheter.

As previously indicated (Wallgren *et al*. 1995) results obtained with the microdialysis ethanol technique may in theory be recalculated to yield quantitative blood-flow results. However, this calculation demands knowledge of the diffusion coefficient of ethanol through the tissue around the catheter, a factor which is difficult to determine with any precision. In practice, therefore, the outflow : inflow values can only be expressed as quantitative blood-flow data after calibration with ^{133}Xe clearance. Based on such a calibration, the diffusion coefficient of ethanol may be calculated and this value could probably be used as long as the experimental conditions remain unchanged.

Our experience of mechanistic studies in skeletal muscle using microdialysis

Insulin-induced hypoglycaemia results in vasodilatation in rat skeletal muscle. Using the microdialysis ethanol technique with the perfusate supplemented with adrenergic agonists and antagonists, it has been possible to show the adrenergic nature of this response. In brief, the results showed that the blood-flow increase was based on a strong β-adrenergic vasodilatatory component, which was counteracted by a vasoconstrictive response mediated by adrenergic α-receptors (Hickner *et al*. 1994).

In a recent study the microdialysis technique was used to examine the hormonal regulation of the release of amino acids from skeletal muscle. The release was monitored indirectly by measurement of interstitial concentrations. To distinguish metabolic from vasoactive influences, the effect of both metabolically-active hormones and pure vasodilators (lacking metabolic effects) were included in the protocol. A significant new finding was that adrenaline perfusion induced an initial short-lasting anabolic effect, and furthermore it could be shown that β-agonists stimulate the glutamine synthetase (*EC* 6.3.1.2) step (Rosdahl *et al*. 1998*c*). This study further illustrated that although isoprenaline is as strong a glycolytic stimulator as adrenaline, it results in a much lower increase in dialysate lactate concentration. This finding is probably due to the fact that isoprenaline is a more potent vasodilator than adrenaline. This result therefore illustrates that concentrations in the interstitial space are governed by metabolic factors as well as circulatory factors.

The last example is a study in human subjects in which the whole-body insulin sensitivity was measured using the euglycaemic–hyperinsulinaemic clamp (Rosdahl *et al*. 1998*b*). Microdialysis was used concomitantly in the gastrocnemius and brachioradialis muscles. The study is an example of how microdialysis can be used to detect changes in insulin-mediated glucose disposal in specific tissues during a glucose clamp.

In conclusion, microdialysis has become an important technique for studies of human metabolism and nutrition. However, although simple to perform, there are several pitfalls, and caution is therefore warranted when interpreting data from microdialysis studies in skeletal muscle.

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