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Symposium on 'Metabolic aspects of human nutrition at rest and during physical stress: recent methodological and technical developments'
Session 2: Arterio–venous differences in metabolic and nutrition research

Arterio–venous differences to study macronutrient metabolism: introduction and overview

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The arterio–venous difference technique is now well established in the study of organ and tissue metabolism. This technique requires samples to be obtained of the arterial blood supplying and the venous drainage from a tissue, together with a measurement of the blood flow through the tissue. The technique is most appropriate when the arterial concentration and tissue metabolism of a substance are constant, and when the blood flow is stable. If these criteria are not satisfied, care is needed in the interpretation of the results obtained. It should be recognized that the arterio–venous difference technique only measures the net exchange of a substance with the tissue, and that tracers are needed if unidirectional flux needs to be estimated. The other factors which must be borne in mind when intending to use this technique are the transit times of blood and the substance of interest through a tissue, the volume of distribution of the substance in the tissue, and the possibility that the venous samples obtained are derived from a mixture of different tissues.

Arterio–venous difference: Tissue metabolism: Fick Principle: Arterialization

The use of arterio–venous (A-V) concentration differences to study the metabolism of tissues and organs is based on a principle first proposed by Adolph Fick in 1870. This principle was not Fick's first contribution to the understanding of physiology, as in 1855 he proposed a law of diffusion in fluids, in which the rate of diffusion was determined by the concentration gradient. However, in both cases his contributions were the formulation of a theory, and it took up to 25 years for supporting experimental evidence to be obtained (see Hamilton, 1962; Landis & Pappenheimer, 1963).

The Fick Principle

This principle was initially based on the transport of gas in the blood being used to determine the cardiac output. If the whole-body O_2 consumption, and the arterial and mixed-venous O_2 contents were measured, the rate of blood flow through the lungs (the cardiac output) could be calculated. Thus, in general terms, if the amount of a substance (x) entering or leaving the blood per unit time is measured as the blood passes through a tissue or organ, the concentration of x in the arterial inflow to and venous outflow from the tissue or organ, then the blood flow through the tissue or organ can be calculated from:

$$\text{blood flow} = \text{uptake}/(\text{arterial concentration} - \text{venous concentration}),$$
$$\text{or} = \text{output}/(\text{venous concentration} - \text{arterial concentration}).$$

The converse of this equation can then be used to estimate the rate of metabolism of a known substance by the tissue or organ, i.e. if blood flow through the organ and the arterial

Abbreviation: A-V, arterio–venous.

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and venous concentrations of the substance of interest are measured, the rate of uptake or output of that substance can then be calculated:

uptake = flow × (arterial−venous concentration),
and output= flow × (venous−arterial concentration).

Criteria for applying the Fick Principle

The Fick Principle is now widely used to estimate tissue and organ metabolism, but it is important that when using this approach an investigator understands the criteria applicable to its use. The three fundamental criteria which underpin the use of the Fick Principle are that blood flow should be stable, and arterial concentration and tissue metabolism of the substrate should be constant (Zierler, 1961). Related to these criteria are the needs to accurately and reproducibly measure blood flow and the A-V concentration difference. If non-steady-state conditions exist, the Fick Principle needs to be modified, and investigators should consult Zierler (1961).

One major concern in relation to these criteria is what is meant by constancy of blood flow, as arterial blood flow through most tissues and organs varies due to cardiac cycle and respiratory effects. In physiological stable conditions this concern is not too serious, provided the periodicity describing the variation in flow is short in relation to the mean transit of a substance through the vascular bed concerned (Zierler, 1961). If the example of phasic flow illustrated in Fig. 1 is considered, this factor could be regarded as constant provided the mean transit of a substance through the organ was 10 s, but not if it was 2 s. The time taken for a substance to pass through a tissue (transit time) depends on the vascular path taken and the volume of distribution of the substance. Thus, in tissues that only have a capillary network, the transit time will be longer than that in tissues with A-V anastamoses (A-V shunts) in which some blood passes to the venous side of the circulation without passing through the capillary bed. This phenomenon of blood flowing through A-V anastamoses in some tissues forms the basis of the arterialization technique to obtain blood which reflects arterial concentrations (see p. 873). The other factor which influences the time taken for a substance present in arterial blood to appear in the venous drainage from a tissue is the volume of distribution of that substance in the tissue. Thus, the volumes of distribution of non-esterified fatty acids, glucose and alcohol will be substantially different, affecting the time taken for a change in arterial concentration to be reflected by a change in venous concentration. As non-esterified fatty acids are transported in plasma bound to albumin, the initial distribution will be restricted to the plasma volume. Glucose freely diffuses throughout the extracellular compartment and so will occupy approximately one-third of the tissue fluid, whereas alcohol freely passes through cell membranes and so will distribute throughout the entire fluid volume of the tissue.

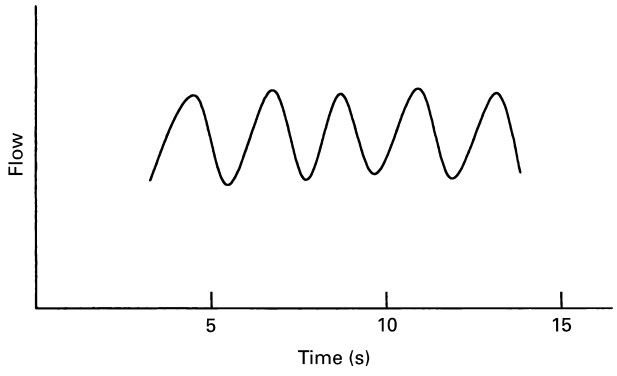

Fig. 1. Illustration of variation in blood flow.

Volume of distribution

The influence of volume of distribution of a substance on its appearance in the venous blood is illustrated by McGuire *et al*. (1976). They showed that the blood transit time through the forearm was 1–2 min (partly dependent on whether the hand circulation was arrested), but that glucose transit through the forearm was approximately 3 min, and in some circumstances could be longer.

The practical importance of the effects of the volume of distribution on using the A-V difference method to assess tissue metabolism relates to the timing of blood sampling. As can be seen from Fig. 2, when the arterial concentration of a substance rises from one steady-state value to another, the change in the venous concentration lags behind, even if there is no change in tissue metabolism. Conversely, when arterial concentration subsequently falls, the venous will remain elevated for some time. These effects are due to some of the initial increase in the arterial concentration being lost from the circulation into the tissue distribution volume of the substance of interest, and it then taking some time for the material distributed throughout the tissue to return to the venous blood at the later stages. This effect illustrates the difficulty in using the Fick Principle to study organ metabolism in the non-steady-state condition, and the importance in having a number of paired samples of arterial and venous blood in order that some assessment can be made of the existence of a steady-state. At least four sets of duplicate values should be obtained in order to obtain a reliable value for A-V difference. In some tracer studies only one set of values is obtained (to save time and cost) and, therefore, many of the recent studies have produced unreliable data.

In some experimental situations it may not be possible to obtain steady-state conditions. Such a situation would be

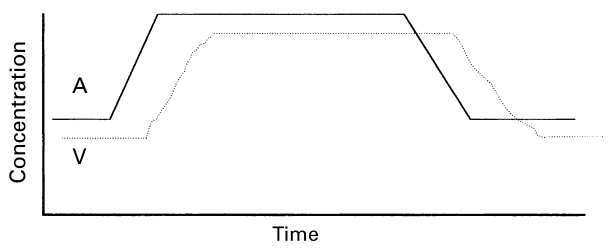

Fig. 2. Illustration of lag between change in arterial (A) and venous (V) concentrations.

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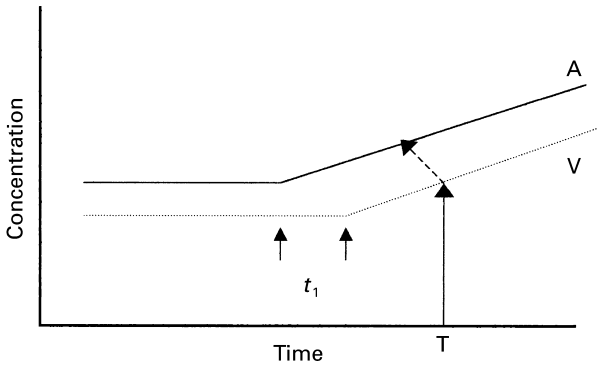

Fig. 3. Illustration of potential errors in simultaneous arterial (A) and venous (V) sampling. ↑, Sample time for V; ⇡, equivalent sample time for A due to lag time between arterial blood entering the organ or tissue and venous blood leaving the organ or tissue.

when the arterial concentration was rising, and it becomes essential that multiple blood samples of arterial and venous blood are obtained, and some estimate is made of the volume of distribution and transit time. These problems are illustrated in Fig. 3, where time interval, t_1, illustrates the effect of volume of distribution and transit time causing a lag in the venous concentration. In addition, a pair of arterial and venous samples taken at time T would overestimate the A-V difference, as the venous blood leaving the organ at T corresponds to arterial blood entering the organ some time earlier, where this time is dependent on the transit time through the vasculature of the organ.

Quantification of the arterio–venous difference technique

The most important principle which must be recognized when the A-V difference technique is used to study organ and tissue metabolism is that it only allows the measurement of the net flux of a substance, i.e. either net uptake or net output. If it is necessary to obtain data on unidirectional flux, then isotopic tracers must also be used.

The net flux is easily calculated from:

$$\text{net flux} = \text{A-V difference} \times \text{flow},$$

but care must be taken to ensure the correct units are used. If flow is measured in ml/min (or ml/100 g tissue per min), then the concentrations should be expressed per ml blood. Furthermore, if blood flow is measured but the concentrations are measured in plasma, then the packed cell volume needs to be included in the equation to reduce blood flow to plasma flow (which is usually approximately 55 % total blood flow).

Other data which can be derived from the A-V difference technique are the net fractional extraction and the clearance. The former is the proportion of the arterial concentration removed by the tissue, and is determined as:

$$\text{fractional extraction} = \frac{\text{A-V concentration difference}}{\text{arterial concentration}}.$$

The clearance is the volume of blood completely cleared of the substance in one pass through the tissue in unit time, given by:

$$\text{clearance} = \frac{\text{flux}}{\text{arterial concentration}}.$$

These additional terms are valuable in helping to identify the mechanism of any change in tissue uptake–output, as to whether it was the result of a change in blood flow (in which case the extraction and clearance would not change), or of transport of substance into the tissue (where both extraction and clearance would be affected). A more detailed discussion of these calculations is provided by Frayn & Macdonald (1997).

Blood sampling

Arterial

Ideally tissue or organ metabolism should be studied by sampling the arterial inflow and venous drainage from that tissue alone. If arterial sampling is to be used, it is reasonable to assume that all systemic arterial (i.e. not the pulmonary) blood has the same composition, and thus even if splanchnic or leg metabolism is being studied it is appropriate to obtain samples from the radial artery at the wrist. In some situations there may be difficulties in obtaining arterial samples due to the lack of trained staff, and many investigations then resort to the arterialized-venous (heated hand) technique. This technique was initially evaluated by McGuire *et al*. (1976), who showed that with good arterialization the glucose concentration in the arterialized sample obtained from the hand vein was very close to the arterial concentration. Subsequently, Liu *et al*. (1992) showed that this arterialization technique remained valid for estimating arterial glucose concentration during hypoglycaemia, and that arterialized-venous concentrations of adrenaline and noradrenaline were within 10 % of the arterial values until more severe degrees of hypoglycaemia occurred (Liu *et al*. 1993). One important methodological aspect of the arterialization technique which must be taken into account is the potential effects on body temperature and skin blood flow. Gallen & Macdonald (1990) showed that the ‘hot-air’ technique for arterializing venous blood in one hand had no effect on body temperature, but did increase blood flow in the contralateral hand. However, when the contralateral hand was rendered ischaemic during the measurement of contralateral forearm blood flow, there was no effect of the ‘hot-air’ method on forearm blood flow. By contrast, both the heated blanket and warm-water techniques for arterializing venous blood cause body temperature to rise and have effects on the contralateral forearm.

It must be recognized that, in some circumstances, the arterialized-venous sampling technique is not an adequate alternative to arterial sampling. The best example of this inadequacy is the study of O_2 consumption by adipose tissue compared with skeletal muscle. In most studies where the comparisons have been made, the O_2 saturation of arterialized-venous blood is within 2 % of the arterial blood (i.e. 95+ % *v*. 97–98 %). When studying muscle O_2 uptake

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this difference is of minor importance, as the muscle tissue extracts approximately 50 % of the O_2 from the arterial blood. However, the O_2 content of the venous blood draining abdominal subcutaneous adipose tissue is only 10 % lower than the arterial value (Frayn *et al*. 1989). Thus, using arterialized-venous samples could underestimate adipose tissue O_2 uptake by 25 %. Thus, care should be taken when using arterialization of venous blood to obtain estimates of the inflow concentrations of substances being studied with the A-V difference technique.

Venous

The venous drainage from the organs of the body can only be sampled by insertion of catheters via a superficial vein. The correct location of these catheters is of course essential if valid samples are to be obtained. In principle, it is easier to obtain venous samples from skeletal muscle, as cannulation of the femoral vein or the antecubital vein is technically much easier. However, one must be aware that the femoral vein does not just receive blood from the muscle of the leg, but also drains adipose tissue and skin. In exercise this factor is not a major problem, but it does pose difficulties at rest. In the forearm, retrograde cannulation of the antecubital vein (Andres *et al*. 1956) allows the tip of the cannula to lie in a vein draining predominantly skeletal muscle. However, even with such a cannulation technique it is essential that blood flow into and out of the hand is prevented before the deep forearm venous samples are obtained. This step is necessary because some of the venous drainage from the hand appears to pass in the deep forearm veins, and failure to occlude this hand venous drainage will lead to errors in the measurement of muscle venous blood (Holling, 1939; Gallen & Macdonald, 1990).

Blood flow

The application of the Fick Principle to estimate tissue metabolism obviously requires reproducible and accurate measurements of blood flow in order that valid data are obtained. A detailed discussion of the various techniques available to measure blood flow is beyond the scope of the present paper; for present purposes it is sufficient to note that Doppler ultrasound, indicator dilution, isotope clearance, laser Doppler flowmetry and plethysmography are the major methods which can be used (see Rådegran, 1999). When an appropriate blood flow technique is used to measure organ blood flow, and a sample can be obtained of mixed venous blood leaving the organ (plus arterial blood), the Fick Principle can be used to produce valid estimates of whole-organ metabolism. However, problems can arise when trying to assess metabolism of one tissue type (usually skeletal muscle) when a measurement is made of blood flow to a mixture of tissues. The best example of this problem is when the forearm is studied in order to obtain information about skeletal muscle. Most of the techniques described earlier measure blood flow to the total forearm, and thus the result obtained is the weighted average of the flows to the individual tissues, based on the proportion of forearm volume occupied by each of the types of tissue, i.e. skin, subcutaneous adipose tissue, skeletal muscle and bone). Thus, if a sample of deep venous blood draining skeletal muscle is obtain from the forearm, assumptions must be made about the relationship between muscle and total forearm blood flows. It is critical that this relationship does not change during a period of measurement, otherwise erroneous conclusions may be drawn.

Examples of the use of the arterio–venous difference technique to assess tissue metabolism

The value of the A-V difference technique in the study of human metabolism has been demonstrated on many occasions. One example of its use to study muscle glucose metabolism at rest comes from our investigation of the insulin resistance of starvation (Mansell & Macdonald, 1990). This study confirmed many previous demonstrations that consuming a low-carbohydrate diet or starving for a few days produces insulin resistance, but also showed that the main feature of the resistance was the inability of insulin to stimulate glucose oxidation in starving subjects. However, using the A-V difference technique to estimate forearm glucose uptake revealed that whilst in the normally-fed state insulin promoted glucose uptake into the forearm, when the subjects were starving this process did not occur. Thus, the insulin resistance of starvation is not just due to a failure of insulin to stimulate glucose oxidation, but is contributed to by a failure of skeletal muscle to take up glucose. The obvious conclusion from this study was that in starving subjects glucose administered is preferentially taken up by the liver for storage as glycogen, but this process has not been confirmed experimentally.

Other examples of the use of the A-V difference technique to study tissue metabolism, which are of nutritional interest, concern the assessment of forearm muscle O_2 consumption responses to food ingestion. Astrup *et al*. (1986, 1989) have used this technique to show that forearm O_2 consumption increases after oral glucose intake. Interestingly the main effect occurs some time after the uptake of glucose by the forearm, and coincides with an increase in plasma adrenaline (a potent stimulus of thermogenesis and O_2 consumption; Astrup *et al*. 1986). A subsequent study using the A-V difference technique to assess forearm O_2 consumption provided further evidence that catecholamines can stimulate resting muscle O_2 uptake (Astrup *et al*. 1989). This latter study showed that β-adrenoceptor antagonism reduced the forearm O_2 uptake response to the ingestion of a mixed meal.

Common problems and sources of error in the arterio–venous difference technique

Analysis of blood samples

As already mentioned, it is critical that if plasma concentrations are measured, this factor is taken into account when calculating the uptake or output of a substance. It is also

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very important that high levels of analytical precision are available. For example, the A-V difference in glucose concentration across subcutaneous adipose tissue is approximately 0·1 mmol/l at rest, and only rises to approximately 0·5 mmol/l after meal ingestion (Frayn *et al*. 1989). Thus, it is often necessary to measure the arterial and venous samples in triplicate to obtain sufficient analytical precision.

Experimental design

A major issue when studying muscle metabolism in the forearm or leg in resting subjects is minimizing confounding effects on limb blood flow. As mentioned earlier, most of the techniques measure total limb blood flow. Thus, effects of room temperature on skin blood flow or vasoactive hormones on skin or muscle blood flow must be minimized, or preferably avoided. An example of the problem of poor experimental design comes from one of our own studies, which only ever appeared in abstract form because of this factor (Gallen *et al*. 1991). This experiment was designed to measure whole-body and forearm muscle O_2 consumption responses to the intravenous infusion of adrenaline. The whole-body responses were readily demonstrated, but there was no effect on estimated forearm O_2 consumption. However, the experiment was conducted at a thermoneutral environmental temperature (30°), which will have produced a higher forearm skin blood flow than would occur at a room temperature of 22°. Unfortunately, adrenaline is a potent vasoconstrictor of skin blood vessels, at the same time as producing vasodilation in skeletal muscle. Thus, during the adrenaline infusion the proportional contribution of skin (reduced) and muscle (increased) to total forearm blood flow will have changed. The overall increase in forearm blood flow will have been much less than at a lower room temperature. As a deep vein was used to sample blood from forearm muscle to calculate the A-V difference for O_2, but total forearm blood flow was used to calculate O_2 consumption, there was no apparent change in forearm muscle O_2 consumption. This finding is at variance with many other studies demonstrating effects of adrenaline to stimulate muscle O_2 consumption at rest, and obviously due to a basic error in the experimental design.

Conclusions

The A-V difference technique, with accurate measurement of blood flow, provides a powerful tool for the assessment of tissue and organ metabolism. However, there are a number of methodological and experimental design issues that must be taken into account when using this technique.

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