Measurement of blood flow and amino acid metabolism in the skin of sheep

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ABSTRACT

This paper reports preliminary studies to determine the normal variation in blood flow, amino acid uptake and protein metabolism in a defined area of skin on 45 kg Romney wethers.

Four sheep were catheterised in branches of the deep circumflex iliac artery and vein to allow measurement of arterio-venous concentration differences and blood flow (using dye dilution techniques) in a 560 cm^2 patch of skin on the lateral abdominal flank.

Blood flow to the infused area ranged between 5 and 30 ml/min, giving an average for the total skin of about 7% of cardiac output. Net uptake of amino acids (tyrosine and phenylalanine) across the area gave a net protein gain by the total skin of 3 to 4 g/d. Uptake of ^{14}C-tyrosine across the skin patch gave an estimate of protein synthesis of 10 g/d in the total skin.

It is intended to develop the preparation to allow comparisons of protein:energy metabolism in both normal and fleece weight selected sheep.

Keywords Skin; blood flow; protein synthesis; sheep.

INTRODUCTION

The rate of whole body production in the growing animal is the result of a series of influences including intake, digestive efficiency and metabolic efficiency. Within the whole body, the productive gain by individual tissues depends on their ability to retain a proportion of the flux of nutrients in the circulation, ie. the combination of nutrient concentration in the blood and the rate of blood flow. Regulation of this tissue gain occurs at many levels. However, the rapidity and sensitivity with which changes in blood supply can alter nutrient supply to individual tissue suggests that changes in blood supply may be one level at which partitioning, such as between skin and muscle, can be controlled.

Until recently, the majority of studies undertaken on metabolism in the skin were based on slaughter (Davis *et al.*, 1981) or *in vitro* procedures (Leng and Stephenson, 1965). This type of work showed that the skin of sheep made a very significant contribution to levels of whole body protein synthesis (of about 20%), .and that the skin also maintained and unusual energy metabolism with a high production of lactate and some utilisation of acetate. However, these studies all gave single time-point measurements about skin metabolism even though it is a very dynamic system with radical shifts in blood flow as a result of the demands of body temperature control. It is well acknowledged that there is an effect of environmental temperature on wool growth (Hopkins and Richards, 1979). While the mechanism for this effect is still unclear, changes in blood flow to the skin are implicated, although apart from the work of Hales (1983) using labelled microspheres there have been few direct measurements of blood flow to the skin of sheep.

Hoey and Hopkins (1983) developed an arterial cannulation system in the tail of sheep, which allowed manipulation of metabolism by either pulse or continuous infusion of hormones, pharmaceuticals or nutrients into a tissue which is predominantly skin. Unfortunately, their preparation does not permit direct measurement of blood supply or metabolic changes associated with their infusion, and so this group sought an alternative approach.

The arterio-venous preparation developed at Biotechnology Division enables simultaneous measurements of blood flow and both uptake and output of metabolites in a defined area of skin over small increments of time under normal and modified conditions of growth.

EXPERIMENTAL

Surgical Preparation

Fine bore polyvinylchloride catheters were implanted into 4 sheep, under fluothane anaesthesia, in the descending lateral branches of the deep circumflex iliac artery (A) and vein (V) as in Fig. 1. The catheter tips were advanced 3 cm proximally to approximate the bifurcations of these vessels in the region of the subiliac lymph gland. This allowed infusion of tracers into, and sampling of blood from the cranial lateral branches supplying the skin and subcutaneous (*cutaneous trunci*) muscle on the lateral abdominal flank from a skin area of approximately 560cm^2. The area supplied by A and

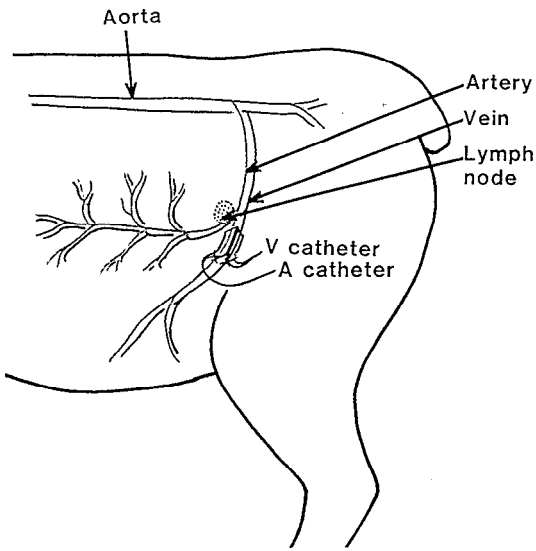

FIG. 1 Positions of catheters placed into descending lateral branches of the deep circumflex iliac artery (A) and vein (V) in relation to the deep circumflex iliac lymph node.

drained by V was defined in each animal before any experimental work was undertaken by use of radio-opaque dye and X-rays, and at slaughter by injection and tracing of coloured latex.

A third catheter was implanted in the saphenous artery (S) and temporary catheters were placed in both jugular veins with the tip of the first (J_1) positioned in the jugular itself, while the tip of the second (J_2) was advanced into the right auricle.

Patency of all catheters was maintained by infusion (60 ml/d), or regular flushing, with heparinised (30 iu/ml) saline.

Measurements

Blood flow to the skin area was measured by continuous infusion of para-amino hippuric acid (PAH) at a known rate into A and measurement of concentration at V against background whole body levels of PAH at S. Cardiac output was obtained by infusion of PAH into J_1 and measurement of the concentration of PAH in J_2 compared with background concentration at S.

Estimates of protein gain (Pg), protein synthesis (Ps) and protein degradation (Pd) in the skin area were obtained from the difference between S and V in concentration and specific radioactivity (SRA) of 1-^{14}C-tyrosine infused into J_1 at 25 μci/h. Whole body protein synthesis was estimated from the irreversible loss rate of the 1-^{14}C-tyrosine corrected for oxidation from appearance of $^{14}CO_2$. Because no oxidation of tyrosine occurs in the skin or muscle, Pg, Ps and Pd may be calculated as follows:

$$Pg = (\text{nmol tyr/ml in S} - \text{nmol tyr/ml in V}) \times \text{Blood flow}$$

$$Ps = \frac{(\text{DPM. ml}^{-1} \text{ in S} - \text{DPM.ml}^{-1} \text{ in V}) \times \text{Blood flow}}{\text{Precursor SRA (S or V)}}$$

$$Pd = Ps - Pg$$

Because of the problems in assessing the SRA of the true percursor pool used in calculating Ps, an independent flood measurement of Ps was made on each animal by infusing into A, 3 mg/ml phenylalanine together with 16 μci/ml (2, 6)-^{3}H-phenylalanine at 0.4 ml/min for 30 min. This was followed by taking biopsy and/or slaughter samples of skin and subcutaneous muscle. Ps was then calculated as:

$$Ps = \frac{\text{DPM/min/g protein}}{\text{DPM/nmol phenylalanine}}$$

(McNurlan *et al.*, 1979)

TABLE 1 Mean measurements of blood flow, together with protein synthesis, gain and degradation in the skin of wethers using an arterio-venous preparation. (S-V) values are the difference between the saphenous artery and the deep circumflex iliac vein. Total skin values are corrected values based on proportional weight of the skin patch of 4%.

Parameter	Skin patch	Site	
		Total skin	Whole body
Weight (kg)	0.18 ± 0.02	4.5	45 ± 4
Blood flow (ml/min)	13.6 ± 3.6	341	5036 ± 248
Protein synthesis (g/d)			
(S-V)	0.4 ± 0.1	9.9	174 ± 6
Flood	2.2 ± 0.3	54	-
Protein gain (g/d)	0.14 ± 0.05	3.56	-
Protein degradation (g/d)			
(S-V)	0.26 ± 0.06	5.34	-
Flood	2.1 ± 0.4	50.4	-

RESULTS AND DISCUSSION

The area of skin drained by the surgical preparation amounted to 560 cm^2 and weighed 180 g; 4% of the total skin weight (Table 1). Contributing to the 180 g total is the lateral cutaneous trunci, and the weight of skin after removal of this muscle layer was 120 g.

Blood flow to the area averaged 13 ml/min (range 5 - 30 ml/min) and the total skin used about 7% of cardiac output. This is very similar to the measurements made by Hales (1983) who used labelled microspheres and obtained a figure of 8% cardiac output. This level of blood flow indicates that the skin gets the same or higher proportions of cardiac output than the same weight of resting muscle (Oddy *et al.*, 1985).

Levels found for whole body protein synthesis are low. This may indicate that although the feed intake of animals had recovered to maintenance or higher, they had still not completely recovered from surgery.

As expected, values of Ps obtained by the S-V difference procedure are lower than those obtained using the phenylalanine flood. The flood procedure eliminates the errors associated with differences in SRA of precursor pools and so the results can be treated with more confidence. Because Pd is calculated by difference from Ps and Pg, the estimate selected for Ps also affects the estimated size of Pd.

Overall the range of values of Ps found in the total skin are lower than the 42 to 123 g/d measured in 20 kg lambs by Davis *et al.* (1981). This is probably a consequence of incomplete recovery from surgery, as already discussed, and also the fact that the present work was carried out on more mature animals. However, the fractional rates of protein synthesis measured in the skin (1-6%/d) are of a similar magnitude to those found for the muscle of sheep (Oddy and Lindsay, 1986).

Some preliminary measurements have also been made on the uptake of oxygen by the skin. Estimates of 1 to 2% of whole body oxygen consumption were obtained. This is significantly less than the utilisation of oxygen by other tissues such as resting muscle (Oddy *et al.*, 1985). There is some evidence (Black and Reis, 1979) that the skin uses a proportion of its glucose anaerobically and these low estimates support this.

The association of skin protein synthesis rates, similar to those found in muscle, with much lower estimates of oxygen utilisation indicates that the interrelationship of protein and energy metabolism in the skin may be quite different to that in other tissues. It seems probable that the metabolism in the skin enables the sheep to conserve whole body amino acid and energy supplies when under temperature or metabolic (exercise) stress without compromising skin protein (wool) production. Further work is being undertaken to investigate these relationships in more detail.

CONCLUSIONS

The preliminary results presented suggest that this surgical arterio-venous preparation will be a valuable tool for studying metabolism in the skin. Further technical development work is needed as maintenance of catheter patency over extended periods is still a problem; partly because of gradually increasing pressure from the lymph node located where the catheter tips are positioned, and because of knee movement when the animal is sitting which causes movement of the catheter occulding the catheter tip. It is intended to use the preparation to investigate the interrelationships of protein and energy-yielding substrates in the skin of normal sheep as well as investigating the metabolic differences in the skin of high wool producing sheep and their responses to metabolic challenges.

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