Aust. J. Biol. Sci., 1981, **34**, 419–25

Measurement of Organ Blood Flow using Tritiated Water. I. Hind Limb Muscle Blood Flow in Conscious Ewes

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Abstract

Blood flow rates to a hind limb preparation, consisting predominantly of muscle, were obtained with tritiated water (TOH) diffusion and radioactive microsphere techniques in four conscious ewes (a total of seven comparisons).

During the continuous infusion of TOH, equilibrium between muscle and its venous drainage was attained by 40–60 min after the start of the infusion. Total blood flow to the hind limb measured using TOH was corrected for arteriovenous anastomosis flow to provide a measure of capillary blood flow through the hind limb. The capillary blood flow rates so obtained using TOH were highly correlated with those measured directly in muscle with microspheres (ml min^{-1}, $r = 0{\cdot}934$; ml 100 g^{-1} min^{-1}, $r = 0{\cdot}886$, $P < 0{\cdot}01$, $n = 7$) and the corresponding mean flow values obtained with the two techniques (respectively, $104{\cdot}6 \pm 15{\cdot}1$ *v.* $106{\cdot}5 \pm 14{\cdot}9$ ml min^{-1} and $7{\cdot}2 \pm 0{\cdot}8$ *v.* $7{\cdot}3 \pm 0{\cdot}8$ ml 100 g^{-1} min^{-1}) were not significantly different. There were substantial differences in the capillary blood flow rates for the individual muscles of the hind limb.

Introduction

The hind limb of the sheep is a convenient model for studies of muscle nutrient metabolism *in vivo*. The arterial and venous blood samples required for such studies and for estimates of muscle blood flow with soluble-indicator techniques can readily be obtained from the femoral artery and deep femoral vein by cannulation which requires minimal surgical interference to the animal.

Soluble indicators, for which the distribution of transit times is similar to that of substrates used in and produced by the organ, seem most suitable for blood-flow measurements in studies of organ nutrient metabolism (Zierler 1961). While Domanski *et al.* (1974) and Lindsay *et al.* (1977*a*, 1977*b*) used tritiated water (TOH) as such an indicator to measure muscle blood flow in sheep, they did not report any validation of the technique. On the other hand, Faichney and Hales (1974) compared a TOH diffusion method, similar to that used by Setchell and Waites (1964), to measure blood flow in the testis, with microsphere and inert gas clearance methods to measure muscle blood flow in wether sheep but were unable to obtain equilibrium between arterial and venous blood and tissue water TOH concentration within their 20-min sampling period. Consequently they did not obtain agreement between TOH and microsphere estimates of blood flow in muscles of the upper hind limb and concluded that TOH was not a suitable indicator with which to measure blood flow to skeletal muscle in sheep. However, Sapirstein and Ogden (1956) showed that

failure of marker concentration to reach equilibrium between arterial and venous blood and all the relevant tissues leads to overestimation of measured blood flow in systems in which equilibrium of marker permits accurate measurement of blood flow.

Preliminary observations using a prolonged infusion of TOH in sheep indicated that equilibrium between TOH concentration in the femoral venous and arterial blood water could be obtained between 30 and 60 min (V. H. Oddy, unpublished data). Accordingly, in the present study, TOH was infused for 60 min while arterial and venous blood was sampled continuously during the whole of that period. The resultant flow values obtained were compared with those determined using the microsphere method in the same animals.

Materials and Methods

Animals and Surgical Preparations

The nine mature Merino ewes used in this study were about 112 days pregnant and their live weights ranged from 40 to 50 kg. Five of the ewes were used to determine TOH concentration in tissue and blood at various times after starting an infusion and the other four were used for blood flow measurement. The ewes were accustomed to the experimental environment for 2–4 weeks before the experiment. Each animal was fed 800 g chaffed lucerne hay per day and had free access to water.

With the ewes under Fluothane anaesthesia, polyethylene catheters (1·0 mm int. diam., 1·5 ext. diam., Dural Plastics and Engineering, Dural, N.S.W.), filled with heparin saline were inserted into (*a*) the left ventricle via the left carotid artery, (*b*) the abdominal aorta via the right saphenous artery, (*c*) the right jugular vein and (*d*) the left and right deep femoral veins, via the respective recurrent tarsal veins, with the tip of these catheters lying 3–5 cm anterior to the head of the femur.

Following this procedure, the ewes were kept in metabolism cages and the catheters were flushed daily with heparin saline (500 i.u./ml) until the experiment at least 5 days later. During the experiments, the ambient room temperature ranged from about 18–25°C.

Microsphere Technique

The microsphere technique as described by Hales (1974) was used to obtain measurements of hind limb muscle capillary blood flow. The microspheres (New England Nuclear Co., Boston, Mass.) were 15 $\pm$ 3·0 (mean $\pm$ s.d.) μm in diameter and were labelled with either ^{113}Sn, ^{46}Sc or ^{141}Ce. With the ewe conscious and standing, a dose of microspheres (approximately 15 million) was injected into the left ventricle while blood was being withdrawn from the abdominal aorta at approximately 25 ml min^{-1} (to provide the 'reference organ' for determination of the cardiac output) and from the left and right femoral vein catheters at approximately 15 ml min^{-1} during the time of injection of microspheres and for 45 s after, to determine the percentage of the total flow passing through hind limb arteriovenous anastomoses (AVA's) (Archie *et al.* 1973).

Tritiated Water Technique

Within 10 min of administration of the microspheres, a solution of TOH in saline was infused into the jugular vein at 1 ml min^{-1} for 60 min, using a modification of the method described by Pappenheimer and Setchell (1972). 5 ml of a solution of TOH (1·85 MBq ml^{-1}) was placed in a 20-ml vial and continuously mixed by using a magnetic stirrer. 1 ml TOH (1·85 MBq) was infused during the first minute and the remainder was delivered at an exponentially decreasing concentration by adding a dilute solution of TOH (18·5 or 37 kBq ml^{-1}) in saline at a rate of 1 ml min^{-1} to the vial for the remainder of the infusion period. The intent of this procedure was to produce an 'approximate square wave' of TOH concentration in the arterial blood until equilibrium between blood and muscle water was achieved. Throughout the infusion, a peristaltic pump was used to withdraw blood at a rate of 1·02 ml min^{-1} from each of the two deep femoral vein catheters and the left ventricular catheter. The blood withdrawn through each catheter was heparinized by infusing heparin (500 i.u. ml^{-1}) at a rate of 0·04 ml min^{-1} through a side tube which entered the lumen of the catheter 25 cm from the tip, and the samples of blood were dispensed into separate tubes on an A40 refrigerated fraction collector (Hook and Tucker) at intervals of 3 min.

The concentration of TOH in whole blood was determined using the method of Pappenheimer and Setchell (1972) and the radioactivity was counted on a liquid scintillation counter (model PW4510, Philips, Holland). Measurements of blood flow in the left and right leg were obtained simultaneously with each method in two of the ewes; in the other two ewes blood flow was measured in only one leg because of blockages in the femoral vein catheter. In one of these ewes, No. 74, a second set of blood flow measurements was obtained with the two methods, the first and second doses of microspheres being distinguished by different nuclides.

Tissue Sampling and Processing

On completion of each experiment, the animals were killed with an overdose of sodium pentobarbitone in saturated potassium chloride and the various muscles of each hind limb were dissected. For the determination of microsphere radioactivity, and capillary blood flow, all the leg muscles listed in Table 3 were dissected and on identification sealed in plastic bags and weighed. Each muscle was then finely minced and triplicate subsamples (3–4 g) of each placed in tubes for measurement of the microsphere radioactivity content in an autogamma spectrometer (model 5320, Packard Instruments, La Grange, Illinois).

In order to examine the concentration of TOH in muscle relative to that in blood during an infusion of TOH, triplicate subsamples of B. biceps femoris, M. vastus lateralis and M. semintendinosus were taken within 5 min of death from ewes killed at either 20 or 40 min of the start of TOH infusion. The samples (2–3 g) were cut out as quickly as possible with scissors, and placed in Thunberg tubes to vacuum distill water in which tritium activity was determined by liquid scintillation counting. The dry matter content of samples of these same muscles was also measured after drying at 105°C for 18–24 h.

Calculation of Data

Microsphere method

Capillary blood flow, $\dot{Q}$ (ml 100 g^{-1} min^{-1}), was calculated by multiplying the fraction of the microsphere dose in the tissues by the cardiac output. An assessment of the percentage of the total hind limb blood flow, f, that passed through AVA's was obtained using the formula (Archie *et al.* 1973):

$$f = [(I_{vr}/\dot{Q}_{vr})/(I_{ar}/\dot{Q}_{ar})] \times 100, \quad (1)$$

where I_{ar} and I_{vr} are the counts per minute of radioactivity in, respectively, the arterial and venous reference samples, and $\dot{Q}_{ar}$ and $\dot{Q}_{vr}$ are the withdrawal rates (ml min^{-1}) of these samples.

TOH method

Total blood flow F (ml g^{-1} min^{-1}) through the hind limb was calculated using the Fick equation as described by Kety and Schmidt (1945):

$$F = \left\{ \left[C_v(\text{eq.}).S \right] \Big/ \left[\int_0^{t(\text{eq})} (C_a - C_v)dt \right] \right\} \times 100, \quad (2)$$

where C_a and C_v are the concentration of TOH in arterial and venous whole blood during the approach to equilibrium; C_v(eq) is the concentration of TOH per millilitre of venous blood at equilibrium; S (partition coefficient) is the ratio of the TOH concentration per gram of tissue to venous concentration per gram at equilibrium. It was assumed that the concentration of TOH in tissue water and in arterial and venous blood water was the same at equilibrium, but because the proportions of water in muscle tissue and venous blood differ, a correction was applied to obtain units for S.

From preliminary experiments (Oddy, unpublished data), the mean ($\pm$ s.e.m.) values for the fraction of water in muscle and in femoral venous blood were calculated to be, respectively, $0{\cdot}742 \pm 0{\cdot}004$ and $0{\cdot}834 \pm 0{\cdot}002$ ($n = 15$). Using these values, and the correction factor 1·04, to account for the dilution of blood with heparin, the corrected partition coefficient S required for the calculation of blood flow in equation (2) was 0·855. Capillary blood flow was calculated by

subtracting the amount of blood passing through AVA's from the total blood flow value. Although this derived value of capillary flow includes a capillary component from other tissues of the hind limb such as the skin and bone, the contribution of this component to the total capillary limb flow in the type of preparation and thermal environment used here is not likely to exceed about 5% (J. R. S. Hales, personal communication).

Table 1. Concentration ratio of tritiated water[A] in the muscles of M. vastus lateralis, M. biceps femoris and M. semitendinosus at 20 and 40 min after the start of a TOH infusion

Values given are means and ranges for three 20-min and two 40-min observations. Values for percentage water content of muscles represent means of five observations

Muscle	Mean water content (%) ± s.d.	Time after start of infusion (min)	Tritiated water concn ratio Mean	Range
M. vastus lateralis	75·09 ± 0·85	20	102·4	91–112
		40	97·7	96–100
M. biceps femoris	73·61 ± 1·52	20	86·7	46–95
		40	99·5	99–100
M. semitendinosus	73·81 ± 1·46	20	66·7	60–68
		40	95·1	90–100

[A] (TOH per millilitre of muscle water ÷ TOH per millilitre of venous blood water) × 100.

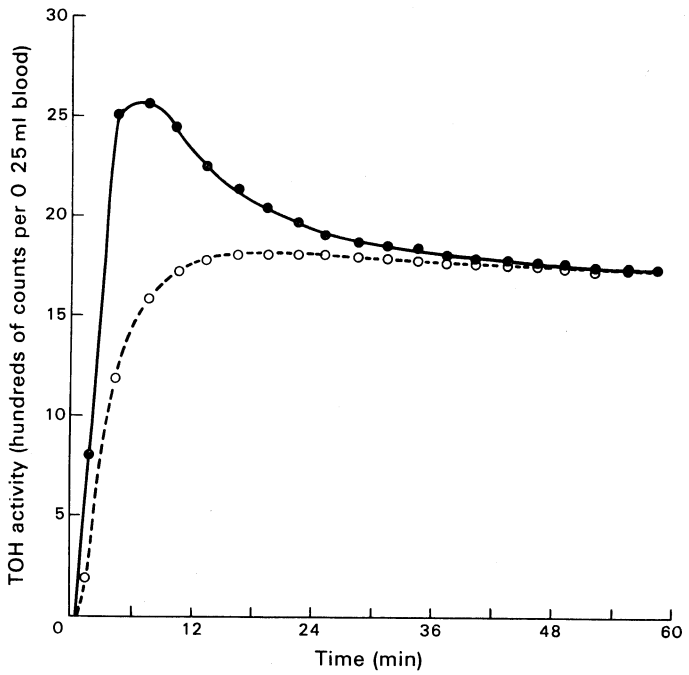

Fig. 1. Time course of TOH activity in ventricular arterial (●) and deep femoral venous blood (○) in one ewe. Infusion of TOH continued for the whole of the blood sampling period (60 min).

Results

TOH Concentration in Blood and Muscle of the Hind Limb

The relative concentration of TOH, expressed as (TOH per millilitre of tissue water ÷ TOH per millilitre of water in femoral venous blood) × 100, at 20 and 40 min

after the start of infusion for M. biceps femoris, M. vastus lateralis and M. semitendinosus are shown in Table 1. Muscles with relatively high blood flow such as M. vastus lateralis were in equilibrium by 20 min after start of TOH infusion whilst muscles through which blood flows more slowly (M. biceps femoris and M. semitendinosus) took 40 min or more to equilibrate (see Table 1).

Table 2. Blood flow to the muscles of the hind limb measured by the TOH and microsphere technique in four conscious ewes

Sheep No.	Total muscle wt (g)[A]	Microsphere technique			TOH technique		
		AVA flow (%)	Capillary flow (ml min^{-1})	(ml $100\,g^{-1}$ min^{-1})	Total flow (ml $100\,g^{-1}$ min^{-1})	Capillary flow (ml min^{-1})	(ml $100\,g^{-1}$ min^{-1})
44	(a) 1649·4	13·0	115·2	7·0	8·8	127·0	7·7
	(b) 1624·1	9·9	184·8	11·4	12·4	180·8	11·1
28	(a) 1317·7	37·7	108·6	8·2	13·6	111·3	8·5
13	(a) 1388·8	46·9	95·3	6·9	10·2	74·9	5·4
	(b) 1320·7	47·0	58·5	4·4	9·3	64·8	4·9
74	(a) 1387·0	3·2	81·8	5·9	7·0	94·3	6·8
	(a) 1387·0	29·9	101·5	7·3	8·2	79·4	5·7
Mean	1439·2	26·8	106·5	7·3	9·9	104·6	7·2
± s.e.m.	±52·4	±6·9	±14·9	±0·8	±0·9	±15·1	±0·8

[A] Values for right and left leg are indicated by (a) and (b) respectively.

Table 3. Mean capillary blood flow and mean weight for the various muscles of the hind limbs in four pregnant ewes

Muscle	Mean weight (g) ± s.e.m. (n = 8)[B]	Mean capillary blood flow ± s.e.m. (ml $100\,g^{-1}$ min^{-1}) (n = 10)[B]	(ml min^{-1}) (n = 10)[B]
M. semitendinosus	102·3 ± 6·9	3·1 ± 0·4	3·3 ± 0·6
M. biceps femoris	273·9 ± 8·1	4·9 ± 0·4	13·6 ± 1·5
M. vastus lateralis	267·4 ± 11·2	11·2 ± 1·1	29·1 ± 2·2
M. adductor, M. semimembranosus	350·5 ± 9·2	4·8 ± 0·4	17·2 ± 1·6
M. gastrocnemius	42·0 ± 2·8	5·5 ± 0·4	2·5 ± 0·3
M. medial vastus, M. rectus femoris	89·1 ± 11·4	12·4 ± 1·6	10·3 ± 0·9
M. gracilis	55·6 ± 2·8	3·3 ± 0·4	1·8 ± 0·2
M. soleus	44·8 ± 3·2	8·6 ± 1·0	3·6 ± 0·4
M. pectineus	39·4 ± 4·4	25·2 ± 1·6	10·9 ± 1·0
Various[A]	126·1 ± 6·2	5·3 ± 0·5	6·5 ± 0·7
M. extensor digiti IV proprinus	40·8 ± 6·5	7·0 ± 0·7	3·2 ± 0·4

[A] Mm. tibialis cranialis, tibialis caudalis, extensor digitorum longus, fibularis longus, flexor hallucis longus, peroneus tertius.

[B] n is based on values obtained from each leg in four ewes (8) plus two further values obtained in one of the ewes that received a second dose of microspheres.

The concentration of TOH in arterial (ventricular) and venous (femoral) blood, plotted as a function of time, is shown for one ewe in Fig. 1. The time taken for all

muscles to reach equilibrium varied with the overall rate of hind limb blood flow and was between 40 and 60 min from the start of the infusion.

Estimates of Blood Flow Obtained using Microspheres and TOH

Blood flow values obtained with each method, the percentage AVA flow and the total weights of the muscles of the hind limb are shown in Table 2. The mean capillary blood flows obtained with microspheres (and the mean weights) for the various muscles of the hind limb are shown in Table 3.

The capillary blood flow values calculated from the total flow obtained with the TOH method were highly correlated with the estimates obtained by the microsphere technique (ml min^{-1}, $r = 0{\cdot}934$; ml $100\,g^{-1}min^{-1}$, $r = 0{\cdot}886$, $P < 0{\cdot}01$, $n = 7$). The corresponding mean flow values provided by the two methods were, $104{\cdot}5 \pm 15{\cdot}1$ and $106{\cdot}5 \pm 14{\cdot}9$ ml min^{-1} ($7{\cdot}2 \pm 0{\cdot}8$ and $7{\cdot}3 \pm 0{\cdot}8$ ml $100\,g^{-1}\,min^{-1}$) respectively, and did not differ significantly. The proportion of the total blood flow (as assessed by microspheres) that passed through AVA's in the hind limb ranged from 3 to 47% (Table 2).

Discussion

The TOH method as used in this study provides a measure of total blood flow to the whole of the hind limb tissues drained by the deep femoral vein and would include bone and skin as well as muscle. As the skin of the hind limb of sheep contains the majority if not all of the AVA's in the leg (Hales 1974) and the flow through these AVA's can be a significant proportion of the total leg blood flow (Table 2 and Hales *et al.* 1978), it was necessary to correct the TOH values by subtracting AVA flow to allow a valid comparison with the estimates of capillary blood flow provided by the microsphere method.

There was good agreement between the derived estimates (TOH) and measured estimates (microspheres) of muscle capillary blood flow in the hind leg even though there was considerable variation in the level of capillary blood flow between individual muscles. Such heterogeneity of flows within the system being measured does not invalidate the use of the TOH method provided that equilibrium between the TOH concentration in all the muscles and the venous outflow is reached (Sapirstein and Ogden 1956). As the microsphere method can be considered to provide an accurate measure of blood flow because it relies simply upon mechanical trapping of particles within the vasculature, it appears from the present findings that the TOH diffusion method described can be used for measuring blood flow in a preparation consisting largely of skeletal muscle in conscious sheep.

Nevertheless, some differences between individual pairs of flow values obtained with the two methods could be expected. Estimates of flow obtained with microspheres relate to flow during the short time interval of lodgement of the microspheres (approximately 30 s; Hales and Cliff 1977) while those provided by the TOH method represent mean values over a period of 60 min. Further, sheep femoral arterial flow when monitored with electromagnetic flow probes frequently exhibited increases of short duration (5 s to 1 or 2 min) that reached two to three times base level (J. R. S. Hales, personal communication).

Our findings are not in accord with the conclusions reached by Faichney and Hales (1974), that the TOH diffusion method was not suitable for measuring blood flow in

skeletal muscle of sheep. This is largely due to the methodology adopted in each case. We infused TOH, and sampled, for up to 60 min, where Faichney and Hales (1974) infused TOH for no longer than 7 min and sampled blood for only 20 min, which as can be seen in Fig. 1 is insufficient time to achieve equilibrium. However, in the experiments in which they measured tissue TOH concentration the corrected flow values obtained were similar to those reported here ($9 \cdot 9$–$10 \cdot 5$ ml $100\ g^{-1}\ min^{-1}$). Moreover, they compared the resultant TOH values with those obtained using microspheres for the muscles of the upper hind limb rather than with all the muscles of the leg, and as can be seen in Tables 2 and 3 many of these muscles (for example, M. biceps femoris, M. gastrocnemius, M. semimembranosus, and M. semitendinosus) have capillary blood flows considerably lower than the weighted mean capillary blood flow to all muscles of the leg.

Our results indicate that the TOH method used is suitable for measuring total blood flow in the hind leg, and because capillary flow arises predominantly from muscle, is therefore a useful tool in studies of nutrient flux across the leg muscle of sheep.

Acknowledgment

The authors wish to thank Mr S. A. Lane for his skilled technical assistance.

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Manuscript received 10 November 1980, accepted 21 April 1981