Importance of sites of tracer administration and blood sampling in relation to leucine metabolism

Practical considerations

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For the same infusion site of L-[1-¹⁴C]leucine, sampling downstream of arterial blood underestimates leucine turnover, whereas sampling of venous blood overestimates turnover. Further, the lungs release a small but consistent amount of leucine into the blood. Unlabelled leucine also is produced by the portal-drained viscera, and some is removed immediately by the liver. These sources of leucine should thus be considered in turnover calculations.

L-[1-¹⁴C]Leucine has become a popular tracer for the study of whole-body and tissue protein turnover (Waterlow *et al.*, 1978). However, in spite of the work of Katz and co-workers (Katz *et al.*, 1981), little attention has been given to the influence of its precise site of administration and sampling. Katz (1982) infused tracer into the aorta and sampled blood from the vena cava, and then infused tracer into a vein and sampled from the aorta. These were termed the A-V and V-A modes respectively, and thus infusion of tracer and blood sampling were on either side of the body tissues. Katz (1982) then concluded that the specific radioactivity of mixed venous blood is more indicative of tissue specific radioactivity than is that of arterial blood, especially for metabolites with a rapid turnover time, such as lactate and alanine. Indeed, this difference in specific radioactivity of venous and arterial blood often has been used to determine the contribution of individual tissues to whole-body metabolism (Bergman *et al.*, 1974; Bergman & Heitmann, 1978).

Leucine is an essential amino acid, and hence the largest pools of leucine are stored as protein. Therefore the assumptions made about its compartmentation may differ from those for lactate and alanine, which have been studied in detail (Katz, 1982). Since we are interested in leucine metabolism (Bergman & Pell, 1982), we decided to study the influence of sites of tracer administration and blood sampling on leucine metabolism and turnover. In addition, the effect on turnover calculations caused by leucine release from the portal-drained viscera and its subsequent hepatic metabolism was in-

vestigated. We have concluded that additional considerations must be combined with those of Katz (1982).

Experimental

Animals

Four non-pregnant, non-lactating, cross-bred ewes, weighing 50–65 kg, were housed in individual pens under constant lighting and at a temperature of 19°C. They were fed on a maintenance diet of 29 g of a pelleted grain and hay mixture (Agway, Syracuse, NY, U.S.A.)/h (approx. 700 g/day) and hence rumen fermentation and nutrient absorption were in a steady state. Water and a salt lick were always available.

Experimental procedures

At least 1 week before an experiment, catheters were inserted into a femoral artery so that the tip lay in the caudal aorta, the portal and hepatic veins and a branch of the mesenteric vein (Katz & Bergman, 1969a). The remaining experimental procedures were divided into two parts performed approx. 1 week apart.

For the first part, an angiography catheter (Edwards Laboratories, Santa Ana, CA, U.S.A.) was inserted into a jugular vein such that the catheter tip lay in the pulmonary artery. This catheter had an inflatable balloon at its tip so that blood flow would guide the tip through the heart and into the pulmonary artery. A polyvinyl catheter was inserted into the other jugular vein such that its tip

lay in the right ventricle. Confirmation of the two sites of the catheter tips was made by blood-pressure recordings. The following day, L-[1-¹⁴C]leucine (50 μ Ci/ μ mol; Amersham, Arlington Heights, IL, U.S.A.) was infused into the pulmonary artery at a concentration of 0.5 μ Ci/ml; 50 ml was given as a priming dose and 46.3 ml/h thereafter. The specific

infused [1-¹⁴C]leucine also is diluted by leucine absorbed by the portal-drained viscera, but the liver removes some of this dietary leucine before mixing with tracer. Therefore turnover must be corrected for that proportion of unlabelled leucine which is absorbed from the gut and immediately removed the liver (Wolff & Bergman, 1972).

Corrected turnover = turnover + (portal production $\times$ fractional uptake of [1-¹⁴C]leucine by liver)

Portal production = portal utilization + net production

radioactivity of blood [1-¹⁴C]leucine reached a plateau after about 2 h. Five paired sets of blood samples were simultaneously withdrawn from the aorta and the right ventricle at 10 min intervals, starting 2.5 h after the priming dose of tracer. In this manner, blood was sampled at sites both before and after the point of tracer administration. Samples were placed immediately in tubes submerged in ice, and deproteinization was performed after collection of each individual pair of blood samples. Both the pulmonary-arterial and right-ventricular catheters were removed after collection of the fifth pair of samples.

The general experimental protocol described above was used for the second part of the procedures, with the following exceptions. A polyvinyl catheter was inserted into only one jugular vein such that its tip was in the vena cava. [1-¹⁴C]Leucine was infused into this catheter and four sets of blood samples were withdrawn from the femoral-arterial, portal- and hepatic-vein catheters at 30 min intervals. Blood flow to the portal-drained viscera and liver was determined by a dilution method (Katz & Bergman, 1969b) using *p*-aminohippuric acid.

Chemical analyses

Whole blood for leucine analysis was deproteinized with an equal volume of 10% (w/v) sulphosalicylic acid. Analysis was performed with a JEOL 6AL amino acid analyser by ion-exchange chromatography (Joo *et al.*, 1976). The analyser was equipped with a stream-splitter so that one half of the column effluent was mixed with ninhydrin for determination of amino acid concentration and the remaining half was collected in fractions for the determination of ¹⁴C content.

Whole-blood *p*-aminohippuric acid concentrations were determined colorimetrically as described by Katz & Bergman (1969b).

Calculations

Leucine turnover was calculated as follows:

$$\text{Turnover } (\mu\text{mol/min}) = \frac{\text{infusion rate of [1-}^{14}\text{C]leucine } (\mu\text{Ci/min})}{\text{whole-blood sp. radioactivity of [1-}^{14}\text{C]leucine } (\mu\text{Ci}/\mu\text{mol})}$$

$$\text{or } \left(\frac{S_a - S_p}{S_a} \right) \cdot F_p \cdot C_a + \text{net production}$$

where S_a and S_p are the ¹⁴C radioactivities (nCi/l) in arterial and portal blood respectively, F_p is the portal blood flow (l/min) and C_a is the concentration of leucine in arterial blood (μ mol/l).

All statistical comparisons were made by a paired *t* test.

Results and discussion

In this study, L-[1-¹⁴C]leucine was infused into the pulmonary artery and blood was sampled from two sites, the first being the aorta. This is equivalent to infusing tracer into a vein and sampling from an artery, and hence may be termed the V-A mode, after Katz (1982). In this mode blood is sampled between the site of tracer infusion and metabolism by the tissues, and it is a common method of determining turnover. The second sampling site was the right ventricle. Here, the tissues were between the site of tracer infusion and blood sampling; this may be called the A-V mode. The right ventricle is a better sampling site for this mode than the vena cava used by Katz (1982), as there is a risk that whole-body mixed venous blood will not be obtained from just the vena cava.

Table 1 shows the mean leucine concentrations, specific radioactivities and turnover rates obtained. Also, Fig. 1 illustrates the specific radioactivities of leucine in blood during a typical experiment. The specific radioactivities were significantly lower in right-ventricular blood than in arterial blood, resulting in significantly higher leucine turnover values in the A-V mode than in the V-A mode by about 24%. This is a substantial difference and represents the two extreme values that may be obtained, i.e. sampling before or after metabolism by body tissues. The immediate problem is quantitative; which value is correct? It is obvious that the sites of both infusion and sampling must be considered.

Table 1. *Leucine concentrations, specific radioactivities and turnover*

L-[1-¹⁴C]Leucine was infused into the pulmonary artery at approx. 23 μCi/h and blood was sampled from the aorta and right ventricle. Five observations were made per sheep. *Significantly different from aorta, *P* < 0.01; †significantly different from V-A, *P* < 0.01.

Sheep no.	Leucine concn. (μmol/l)		Leucine specific radioactivity (nCi/μmol)		Leucine turnover (μmol/min)	
	Aorta	Right ventricle	Aorta	Right ventricle	V-A mode	A-V mode
1	101.4 ± 1.6	99.5 ± 1.1	3.85 ± 0.16	3.29 ± 0.11	99.7	116.7
2	83.7 ± 4.9	81.0 ± 2.8	3.00 ± 0.14	2.44 ± 0.05	127.9	157.1
3	92.7 ± 1.9	90.8 ± 0.6	3.39 ± 0.05	2.81 ± 0.028	113.1	136.8
4	99.6 ± 2.1	98.6 ± 1.8	3.03 ± 0.08	2.29 ± 0.02	126.7	167.5
Mean ± S.E.M.	94.4 ± 4.0	92.5 ± 4.3*	3.32 ± 0.20	2.71 ± 0.22*	116.9 ± 6.6	144.5 ± 11.3†

Fig. 1. *Whole-blood L-[1-¹⁴C]leucine specific radioactivities during an experiment*
Specific radioactivities (nCi/μmol) are for arterial (O) and right-ventricular (●) blood from sheep no. 3. Means ± S.E.M. are shown.

The infusion site should provide a uniform specific radioactivity of tracer to all tissues. Katz (1982) suggests infusing into the left heart chamber, thus by-passing the pulmonary circulation. The arterio-venous differences for leucine (Table 1) illustrate that this is still not entirely correct, at least for leucine, as blood sampled from the aorta had a 2% higher leucine concentration than did right-ventricular blood. Clearly, the lungs were releasing leucine derived from protein degradation into the blood and therefore should be considered as being metabolically active, as are other body tissues. Hence infusion of tracer ideally should be before the pulmonary circulation.

The theory underlying all steady-state turnover studies is that the specific radioactivity of tracer at the sampling site is equivalent to that at its site of metabolism. It is assumed that tissues are in equilibrium with blood and that rapid mixing of

tracer occurs in the whole body. Thus it is attractive to consider venous blood as typical of tissue specific radioactivity, because it represents the result of metabolism. However, even though all tissues receive arterial blood with a constant specific radioactivity of tracer, the actual specific radioactivity of tracer supplied to all cells may not be constant and could decrease across the capillary bed, owing to release of unlabelled metabolite. Therefore the specific radioactivity of tracer supplied to the entire tissue is less than that of the arterial supply. By a similar argument, the specific radioactivity of tracer in the venous drainage will be less than that for the entire tissue. As a result, the sampling of blood from the arterial supply will tend to underestimate turnover, and sampling blood from the venous drainage will overestimate turnover. This could explain, for the most part, the discrepancies in turnover listed in Table 1.

Unlabelled metabolite also is produced by the portal-drained viscera, and some of this immediately is removed by the liver before mixing with infused tracer, resulting in a further source of error for turnover estimation. Table 2 shows the effects of this hepatic removal for both fed and 3-day-starved sheep. For leucine, there are only two unlabelled sources that may enter the portal blood: dietary leucine and leucine resulting from protein catabolism. In our study, this led to an underestimation of turnover of 5.6% in fed and 3.7% in starved sheep. The error is likely to be even greater for other metabolites which may be synthesized *de novo* by the portal-drained viscera and therefore should be considered whenever possible.

In summary, infusion of tracer for the determination of whole-body turnover should ideally be at a site where all tissues, including the lungs, receive the same specific radioactivity of tracer. Blood sampling between the site of infusion and the tissues will result in an underestimation of turnover and sampling after the tissue will result in an overestimation of turnover. The by-passing of the arterial supply carried by the portal blood first entering the

Table 2. *Venoarterial differences and production of unlabelled leucine by the portal viscera and its effect on leucine turnover*

L-[1-¹⁴C]Leucine was infused into the jugular vein of fed and 3-day-starved sheep at approx. 23 μ Ci/h, and blood was sampled from the aorta, portal vein and hepatic vein. Four observations were made per sheep. Corrected turnover significantly different from apparent turnover: * $P < 0.001$; † $P < 0.02$.

	Sheep no.	Portal vein — artery (μ mol/l)	Portal production (μ mol/min)	Hepatic fractional uptake	Turnover (μ mol/min)	
					Apparent	Corrected
Fed sheep	2	6.09 $\pm$ 2.0	41.9	0.162	107.7	114.5
	3	7.06 $\pm$ 1.26	50.6	0.133	108.2	114.9
	5	4.29 $\pm$ 1.70	46.1	0.127	106.7	112.6
	6	10.10 $\pm$ 3.11	45.7	0.142	106.5	113.0
	Mean $\pm$ S.E.M.	6.89 $\pm$ 1.22	46.1 $\pm$ 1.8	0.141 $\pm$ 0.008	107.3 $\pm$ 0.4	113.7* $\pm$ 0.6
Starved sheep	2	-4.62 $\pm$ 1.94	20.8	0.100	80.0	82.3
	3	-3.20 $\pm$ 1.17	24.3	0.120	75.6	80.9
	5	-1.53 $\pm$ 1.57	28.9	0.206	94.1	97.0
	6	-3.52 $\pm$ 2.34	23.0	0.099	92.0	94.8
	Mean $\pm$ S.E.M.	-3.22 $\pm$ 0.64	24.2 $\pm$ 1.71	0.131 $\pm$ 0.025	85.4 $\pm$ 4.5	88.7† $\pm$ 4.2

liver also should be taken into account, or else turnover will be further underestimated.

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