

An isotopic method for measurement of muscle protein synthesis and degradation *in vivo*

Eugene J. BARRETT,* James H. REVKIN, Lawrence H. YOUNG, Barry L. ZARET, Ralph JACOB and Robert A. GELFAND

Department of Medicine, Sections of Endocrinology and Cardiology, Yale University School of Medicine, P.O. Box 3333, 333 Cedar Street, New Haven, CT 06510, U.S.A.

In eight anaesthetized post-absorptive dogs we measured the concentration and specific radioactivity of phenylalanine and leucine in arterial and femoral-venous plasma, together with hindlimb flow during a continuous infusion of L-[ring-2,6-³H]phenylalanine and L-[1-¹⁴C]leucine. The femoral-venous plasma concentration was greater than arterial for both phenylalanine and leucine ($P < 0.05$ for each). Despite net amino acid release there was a significant removal of both labelled phenylalanine and labelled leucine. Consequently, a significant dilution of specific radioactivity was observed between artery and vein for both radio-tracers. The uptake of leucine from the arterial circulation by the hindlimb exceeded by 2.6-fold that of phenylalanine; the measured molar ratio of leucine to phenylalanine in hindlimb muscle protein averaged 2.4 ± 0.1 . Since phenylalanine is neither synthesized nor degraded by muscle tissue, the measured removal of tracer and the dilution of tracer specific radioactivity across the hindlimb can be used to estimate rates of phenylalanine incorporation into, and release from, tissue protein. The estimated rate of protein synthesis by hindlimb averaged 644 ± 250 nmol of phenylalanine/min. This was exceeded by the rate of tissue protein degradation (987 ± 285 nmol of phenylalanine/min). The present results demonstrate that the dilution of the specific radioactivity of labelled phenylalanine can be readily measured across dog hindlimb. This measurement, coupled with an estimate of tissue blood flow, can provide a readily measured, non-destructive, method for estimation of protein turnover in specific muscle beds *in vivo*. Measurements can be made repeatedly over time in a single experiment, allowing the study of factors which regulate protein turnover. The method developed here in dogs can be readily extended to clinical studies.

INTRODUCTION

Skeletal muscle, owing to its bulk (approx. 27 kg in the 70 kg man) and high protein content (12–16 g% wet wt.), is the major store of body protein. During periods of fasting or injury, net release of amino acids derived from skeletal-muscle protein provides the liver and kidneys with substrate for gluconeogenesis (Cahill *et al.*, 1972). This muscle protein is subsequently repleted during feeding (Millward *et al.*, 1983). For any amino acid whose intracellular concentration is remaining constant, the net release or uptake by muscle is the difference between its rates of production and utilization. For phenylalanine, which is neither synthesized nor degraded in muscle tissue, at steady state the net balance across muscle tissue is the difference between the rates of synthesis and degradation of protein. In preparations of isolated skeletal (Jefferson *et al.*, 1977) and cardiac (Morgan *et al.*, 1971) muscle, radiolabelled phenylalanine has been used to estimate the rates of synthesis and degradation of total tissue protein and to examine the effects of insulin and other hormones on muscle protein turnover *in vitro* (see Morgan *et al.*, 1979, for review).

In vivo, measurements of muscle protein turnover have utilized either pulse injection (Garlick *et al.*, 1980) or continuous infusions (Pain & Garlick, 1974) of labelled amino acids, combined with measurement of the tracer

content of tissue protein from biopsy or necropsy specimens. These latter techniques, owing to the large amount of tracer and large size of tissue sample required, are not applicable to clinical studies. In man, no generally accepted method is available to assess directly rates of protein synthesis and degradation in skeletal muscle. Inferences regarding effects of insulin, amino acid availability and other factors on muscle protein turnover in man have been based on net balance measurements across muscle (Abumrad *et al.*, 1982; Pozefsky *et al.*, 1969) or on whole-body radioisotope turnover measurements (Waterlow *et al.*, 1978).

Several authors have suggested that measurement of the exchange kinetics of leucine across the forearm (Cheng *et al.*, 1985) or hindlimb (Oddy & Lindsay, 1986) could provide an index of muscle protein synthesis and degradation. Since leucine is metabolized in muscle both by incorporation into protein and via transamination and oxidation, quantification of radiolabelled leucine exchange across the forearm necessitates the use of two tracers and measurement of the arterial and venous specific radioactivities (or enrichment) not only of leucine, but of 4-methyl-2-oxopentanoic acid and bicarbonate as well.

In the current work we have taken advantage of the single metabolic fate of phenylalanine in muscle to develop a simple method for simultaneously estimating

* To whom reprint requests should be addressed, in the Endocrinology Section.

the rates of skeletal-muscle protein synthesis and breakdown, based on the combined measurement of the dilution of L-[ring-2,6-³H]phenylalanine and the net phenylalanine balance across the dog hindlimb *in vivo*. These measurements do not require tissue samples and can therefore be considered for use in clinical studies. Furthermore, measurements can be made repetitively during a single experiment, thus allowing assessment of the effect of experimental interventions.

METHODS

Eight adult mongrel dogs of either sex weighing between 18 and 26 kg were anaesthetized with sodium pentobarbital and ventilated on room air. Catheters (18 gauge) were placed in the left femoral artery and vein and in the carotid artery. For measurement of hindlimb flow, Indocyanine Green dye was continuously infused in the left femoral artery (150 µg/h) and the dilution of the dye was determined in femoral-venous plasma. A continuous infusion of L-[ring-2,6-³H]phenylalanine (200 µCi/h) and a primed (10 µCi) continuous (0.20 µCi/min) infusion of L-[1-¹⁴C]leucine were given in a peripheral vein. The leucine and dye infusions were begun 2.5 h before, and the phenylalanine infusion was begun 1 h before, and continued throughout, the blood sampling period. Simultaneous blood samples were obtained from the carotid artery and left femoral vein at 10 min intervals over 30 min.

From each blood sample, portions of whole blood and plasma were diluted 1:1 with 6% (w/v) sulphosalicylic acid, and samples of the supernatant were taken for amino acid analysis on a Dionex D-500 amino acid analyser. A second portion of supernatant (1 ml) was passed over a Dowex 50W ion-exchange column, which retains the amino acids, but not their oxo acid derivatives. The amino acids were eluted with 1 M-NaOH, collected in scintillation vials and counted for radioactivity in a Packard Minaxi liquid-scintillation system by using a dual radioisotope-counting mode and an external standard. In control experiments, recovery of labelled leucine and phenylalanine from the Dowex column was greater than 95%. At the end of a 3 h infusion of labelled phenylalanine in the dog, more than 95% of the ³H in plasma amino acids is present in phenylalanine and less than 1% is in tyrosine.

The rate of protein degradation across the hindlimb was estimated from the dilution of phenylalanine specific radioactivity between arterial and femoral-venous plasma according to the following formula:

$$D = [\text{Phe}]_{\text{art}} \times \{[\text{SA}_{\text{art}}/\text{SA}_{\text{vein}}] - 1\} \times F \quad (1)$$

where D is the rate of phenylalanine release from protein (nmol/min), SA_{art} and SA_{vein} denote the specific radioactivity of phenylalanine in artery and vein respectively, $[\text{Phe}]_{\text{art}}$ is the arterial plasma phenylalanine concentration and F is the hindlimb plasma flow. This is analogous to the equation used by Rannels and colleagues to estimate the rate of protein degradation in the recirculating perfusate of rat hindlimb (Jefferson *et al.*, 1977) and heart (Rannels *et al.*, 1975).

The relationship given by eqn. (1) can be derived straightforwardly, and begins with finding an expression for the specific radioactivity of phenylalanine in venous plasma. Venous phenylalanine specific radioactivity

(SA_{vein}) will be determined by the relative rates of entry of labelled and unlabelled phenylalanine into the vein. All phenylalanine in venous plasma originated from either arterial inflow or tissue protein degradation. The contribution of arterial inflow to venous phenylalanine must equal $[\text{Phe}]_{\text{art}} \times F \times (1 - \text{ER})$, where ER is the extraction ratio for labelled phenylalanine, i.e.

$$\text{ER} = \{[\text{Phe(d.p.m.)}]_{\text{art}} - [\text{Phe(d.p.m.)}]_{\text{vein}}\} / [\text{Phe(d.p.m.)}]_{\text{art}} \quad (2)$$

Similarly, the contribution of protein-derived phenylalanine to venous phenylalanine will equal $D \times (1 - \text{ER})$ (i.e. phenylalanine derived from protein breakdown is assumed to be treated identically with that entering muscle from arterial plasma). Therefore the flux of phenylalanine into venous blood is given by the expression:

$$\begin{aligned} \text{Total Phe entry into vein} &= [\text{Phe}]_{\text{art}} \times F \times (1 - \text{ER}) + (D) \times (1 - \text{ER}) \\ \text{or} &= ([\text{Phe}]_{\text{art}} \times F + D) \times (1 - \text{ER}) \end{aligned} \quad (3)$$

The flux of radioactivity into the vein is given by the difference between radioactivity (d.p.m.) entering muscle from the artery minus that retained within muscle, i.e.:

$$\text{Flux of radioactivity into the vein} = (\text{d.p.m.})_{\text{art}} \times F \times (1 - \text{ER}) \quad (4)$$

The specific radioactivity of phenylalanine in venous blood is simply the flux of radioactivity into the vein divided by the flux of phenylalanine into the vein [i.e. eqn. (4)/eqn. (3)]:

$$\text{SA}_{\text{vein}} = (\text{d.p.m.})_{\text{art}} \times F / ([\text{Phe}]_{\text{art}} \times F + D) \quad (5)$$

This can be solved for the rate of degradation, D , by simple algebraic rearrangement to give eqn. (1). We explicitly note that eqn. (1) derived in this manner is equivalent to assuming that the specific radioactivity of phenylalanine in the pool utilized by muscle for protein synthesis is measured by venous phenylalanine specific radioactivity. The rate of protein synthesis by the tissue was estimated as the difference between net phenylalanine balance across the hindlimb {net balance = $([\text{Phe}]_{\text{art}} - [\text{Phe}]_{\text{vein}}) \times F$ } and the rate of protein degradation obtained in eqn. (1), or:

$$\text{Synthesis} = \text{balance} + \text{degradation} \quad (6)$$

The rates of protein synthesis and degradation obtained with these calculations are expressed as nmol/min of phenylalanine incorporated into or released by hindlimb tissue protein. Values reported are the means \pm S.E.M.

RESULTS

In these post-absorptive dogs there was a net release of most amino acids across the hindlimb, as shown in Fig. 1. Release of alanine and glutamine predominated over that of other amino acids. The hindlimb plasma flow in these animals averaged 33 ± 3.5 ml/min. The net release of amino acids that cannot be synthesized by muscle (e.g. phenylalanine and tyrosine) indicates that protein degradation exceeded synthesis in hindlimb tissues. The arterial and venous concentration and specific radioactivity of phenylalanine and leucine did not change during the 30 min sampling period, indicating that both tracers were at a steady state in plasma (results not shown). Despite the net release of both phenylalanine

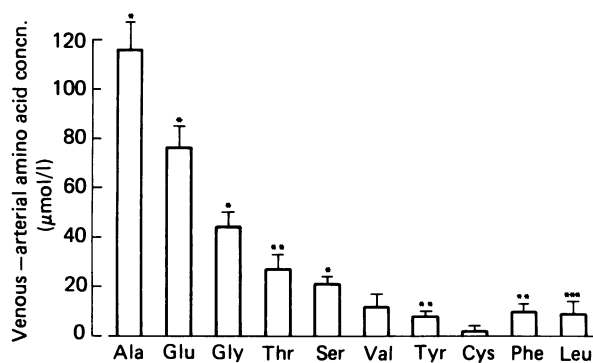


Fig. 1. Difference in concentration between arterial and femoral venous plasma for ten selected amino acids

Significant concentration differences are indicated by:
* $P < 0.001$, ** $P < 0.01$, *** $P < 0.05$.

and leucine by hindlimb, there was a highly significant extraction of both [^3H]phenylalanine and [^{14}C]leucine from plasma (Table 1). The combination of tracer extraction and addition of unlabelled phenylalanine and leucine to venous blood resulted in dilution of their specific radioactivities in femoral-venous plasma (Table 1).

The concentration and specific radioactivity of phenylalanine and leucine were also measured in whole-blood samples obtained from the arterial and femoral-venous catheters. There was a significant net release of both leucine and phenylalanine ($P < 0.05$ for each), and a highly significant extraction of both [^{14}C]leucine and [^3H]phenylalanine ($P < 0.001$ for each), with the consequent dilution of specific radioactivity of each tracer in venous blood (Table 1).

The rate of uptake of plasma phenylalanine into hindlimb tissue is obtained from the product: plasma L-[ring-2,6- ^3H]phenylalanine extraction ratio \times plasma [phenylalanine] $_{\text{art}} \times$ plasma flow. This is a measure of the incorporation of arterial-plasma phenylalanine in newly synthesized hindlimb protein (Table 2). The flux of

leucine into hindlimb tissue, calculated in an identical manner, exceeded that for phenylalanine. This was seen regardless of whether plasma (leucine 2.6-fold greater than phenylalanine) or whole-blood samples (leucine 3.2-fold greater) were used. This would be expected from the molar ratio of leucine to phenylalanine in hindlimb muscle protein. In biopsy samples of dog hindlimb muscle this ratio of leucine/phenylalanine was 2.4 ± 0.1 ($n = 3$) in protein purified as described by Morgan *et al.* (1971). The tissue uptake of leucine in excess of this presumably reflects one of the alternative metabolic fates of leucine, or a greater leucine/phenylalanine ratio in a subset of proteins that have a higher turnover rate.

When the exchange of unlabelled and ^3H -labelled phenylalanine across hindlimb was measured in whole blood rather than plasma samples, the estimated rates of hindlimb phenylalanine uptake and net hindlimb phenylalanine balance were similar to those obtained with plasma samples (Table 2). This is in keeping with previous reports that red-cell phenylalanine does not significantly participate in muscle phenylalanine exchange (Jefferson *et al.*, 1977). In contrast, the hindlimb uptake of leucine and net hindlimb leucine balance were each significantly greater when whole blood was analysed (Table 2). This also agrees with results of previous studies indicating a role for red-cell leucine in tissue leucine exchange (Felig *et al.*, 1973).

As indicated above, the hindlimb uptake of phenylalanine from plasma could be used as one estimate of the rate of protein synthesis in this tissue. The rate of protein degradation would be predicted by the difference between the net balance and the rate of synthesis. However, the rates of both synthesis and degradation of protein determined in this manner must be considered as minimal estimates, since the calculations do not allow for any reutilization of phenylalanine derived locally from protein degradation in muscle. If we assume that phenylalanine derived from proteolysis and that delivered from arterial plasma are used without preference for protein synthesis, then the rate of protein degradation (estimated by eqn. 1) averaged 987 ± 285 nmol of phenylalanine/min and that of protein synthesis (ob-

Table 1. Hindlimb phenylalanine and leucine exchange

The arterial and femoral-venous concentrations and the radioactivities, the extraction ratios (eqn. 2) and the specific radioactivities of phenylalanine and leucine are given. The P values refer to the comparison between arterial and venous samples.

| | | Plasma | | Whole blood | |
|--------------------------------------|--------------|------------------|----------------|------------------|----------------|
| | | Phenylalanine | Leucine | Phenylalanine | Leucine |
| Amino acid ($\mu\text{mol/l}$) | Artery | 51 ± 5 | 119 ± 7 | 51 ± 5 | 128 ± 10 |
| | Femoral vein | 61 ± 7 | 128 ± 10 | 56 ± 6 | 137 ± 10 |
| | P | < 0.002 | < 0.05 | < 0.05 | < 0.05 |
| Radioactivity (d.p.m./ml) | Artery | 13360 ± 1270 | 1680 ± 200 | 10581 ± 1055 | 1425 ± 183 |
| | Femoral vein | 10270 ± 900 | 1270 ± 150 | 8534 ± 922 | 1092 ± 158 |
| | P | < 0.01 | < 0.02 | < 0.001 | < 0.001 |
| Extraction ratio (%) | | 23 ± 5 | 24 ± 4 | 19 ± 3 | 23 ± 3 |
| Specific radioactivity (d.p.m./nmol) | Artery | 281 ± 36 | 14.3 ± 1.9 | 221 ± 33 | 11.7 ± 1.9 |
| | Femoral vein | 174 ± 20 | 10.0 ± 1.3 | 164 ± 26 | 8.4 ± 1.5 |
| | P | < 0.002 | < 0.01 | < 0.001 | < 0.001 |

Table 2. Uptake, release and net balance of phenylalanine and leucine in plasma and whole blood

For calculations, see the text. Results are expressed as nmol/min per hindlimb.

| | Muscle uptake | Muscle release | Net balance |
|---------------|---------------|----------------|-------------|
| Plasma | | | |
| Phenylalanine | 364 ± 116 | 698 ± 149 | -335 ± 78 |
| Leucine | 952 ± 240 | 1230 ± 254 | -291 ± 105 |
| Whole blood | | | |
| Phenylalanine | 515 ± 125 | 760 ± 142 | -246 ± 101 |
| Leucine | 1669 ± 263 | 2124 ± 282 | -455 ± 178 |

tained from eqn. 6) 644 ± 250 nmol/min. The phenylalanine content of protein purified from dog hindlimb muscle averaged 294 ± 14 nmol/mg of protein ($n = 3$). Therefore the rate of hindlimb protein degradation averaged 3.4 mg/min and that of synthesis 2.2 mg/min.

DISCUSSION

The results of the current study demonstrate that the extraction of labelled phenylalanine and dilution of phenylalanine specific radioactivity across hindlimb muscle are readily measured in the intact animal. To the extent that hindlimb phenylalanine metabolism reflects that of skeletal muscle, these parameters of radioisotope flux can be used to estimate the rates of synthesis and breakdown of muscle protein. By using the current method, the flux of arterial phenylalanine into protein synthesis can be precisely quantified (see Table 2). However, the total flux of phenylalanine into protein also includes some contribution of phenylalanine supplied locally by proteolysis. It is evident from the dilution of phenylalanine specific radioactivity between arterial and venous plasma (Table 1) that phenylalanine taken up by muscle tissue near the venule end of a capillary may have originated from either proteolysis or arterial plasma. Precise estimation of the magnitude of re-incorporation of proteolysis-derived phenylalanine back into protein is problematic and would require determining the specific radioactivity of the precursor pool for muscle cell protein synthesis. This precursor pool specific radioactivity would be accurately reflected by measurement of the specific radioactivity of phenylalanine in phenylalanyl-tRNA (Stirewalt *et al.*, 1985). However, owing to the small amount of phenylalanine present in phenylalanyl-tRNA (approx. 0.1% of cellular phenylalanine content), the measurement of aminoacyl-tRNA specific radioactivity *in vivo* has been possible only in small rodents receiving large doses of tracer. Available information indicates that *in vivo* the specific radioactivity of heart muscle leucyl-tRNA is in rapid equilibrium with that of plasma during either a pulse (Martin *et al.*, 1977) or a continuous infusion (Everett *et al.*, 1981) of labelled leucine. Since leucine and phenylalanine enter muscle at similar rates (Morgan *et al.*, 1971), we for the present assume that phenylalanine in venous plasma is in isotopic equilibrium with phenylalanyl-tRNA. In accord with this, in the isolated heart perfused at physiological concentrations of phenylalanine, the specific radioactivity

of heart muscle phenylalanyl-tRNA differs from that of the perfusate by only 10–20% after only 3–5 min of perfusion (McKee *et al.*, 1978). We are keenly aware that to date there are no results available to indicate whether or not skeletal-muscle phenylalanyl-tRNA or leucyl-tRNA achieves isotopic equilibrium with radiolabelled tracer in venous plasma. It follows that the absolute rates of protein synthesis and degradation estimated by using eqns. (1) and (6) will reliably indicate total protein phenylalanine turnover only if such isotopic equilibrium prevails. Any proteolysis-derived phenylalanine which is reincorporated back into protein without equilibrating with labelled phenylalanine is not detected, and turnover is underestimated.

It should be recognized that tracer methods such as the L-[1-¹⁴C]leucine turnover technique, which are widely used *in vivo* to estimate leucine flux and, when corrected for leucine oxidation, whole-body protein synthesis and breakdown, similarly assume rapid equilibration of leucine derived from tissue proteolysis with the labelled leucine pool. In the absence of rapid equilibration, the [¹⁴C]leucine turnover method will underestimate both the leucine flux and the rate of protein synthesis. The observation that arterial leucine specific radioactivity is greater than that of either tissue or venous-plasma leucine (Table 2) indicates that underestimation of leucine flux will predictably occur. This difficulty is not necessarily resolved by use of the specific radioactivity of plasma 4-methyl-2-oxopentanoic acid, for, although the latter more accurately reflects the tissue leucine specific radioactivity (Schwenk *et al.*, 1985), this may not be equivalent to that of the precursor pool for protein synthesis. Thus, in perfused heart muscle the specific radioactivity of both phenylalanyl- and leucyl-tRNA is higher than that of cytosolic phenylalanine and leucine when these amino acids are present at physiological concentrations (Martin *et al.*, 1977; McKee *et al.*, 1978; Morgan *et al.*, 1979). It is of particular interest that, in heart muscle *in vivo*, the specific radioactivity of plasma leucine and leucyl-tRNA equilibrate rapidly (within 2 min) and both significantly exceed that of leucine in heart cytosol (Martin *et al.*, 1977).

It is not currently known whether, *in vivo*, labelled phenylalanine, like leucine, rapidly equilibrates between the plasma and heart muscle tRNA pool. Available evidence does suggest that, similarly to leucine, phenylalanine incorporation into muscle protein begins immediately upon addition of tracer to muscle perfusate and increases linearly with time. This contrasts with other amino acids (e.g. glycine and lysine), which display a linear rate of tracer incorporation into protein only after a lag period, presumably required for labelling of some cellular amino acid pool. This variable behaviour for different amino acids has been described in muscle (Jefferson *et al.*, 1977; Morgan *et al.*, 1971) and other cell types (Khairallah & Mortimore, 1976; Robertson & Wheatley, 1979).

As noted above, in isolated perfused heart at physiological phenylalanine concentrations the phenylalanyl-tRNA specific radioactivity is 10–20% less than that of the perfusate, but each was greater than that of cytosolic phenylalanine (McKee *et al.*, 1978). Raising the perfusate phenylalanine concentration to values 5–8-fold greater than in normal plasma results in a uniform specific radioactivity of the perfusate, tRNA and cytosolic pools. The latter method has been used

extensively in studies of isolated perfused heart and perfused hemicorpus, and more recently has been adapted for studies in rats *in vivo* (Garlick *et al.*, 1980). However, this manipulation cannot be used with the current method *in vivo*, since it would so greatly increase the flux of phenylalanine into the hindlimb from arterial plasma that it would be very difficult to detect the dilution of phenylalanine specific radioactivity that occurs across hindlimb muscle.

Since in muscle L-[ring-2,6-³H]phenylalanine has a single metabolic fate, i.e. incorporation into protein (Williams *et al.*, 1981), we have emphasized its use to measure muscle protein turnover. However, it is of interest that the pattern of exchange of blood leucine across hindlimb paralleled that of phenylalanine. As shown in Table 2 the uptake of blood leucine by hindlimb was 3.2-fold greater than that of blood phenylalanine and the release of leucine from muscle was 2.8-fold greater. Each value slightly exceeds the relative ratio of leucine to phenylalanine in hindlimb muscle protein (2.4 ± 0.2). Since leucine can be transaminated and oxidized by muscle as well as incorporated into protein, uptake of leucine in excess of its incorporation into protein would be expected. We further note that, if the arterial concentration and specific radioactivities of blood leucine given in Table 1 are substituted in eqn. (1), the rate of leucine released from hindlimb protein averages 2540 nmol/min, or 2.6-fold the rate of phenylalanine release from protein, approximately that predicted from the average amino acid composition of muscle protein. Although these latter calculations serve to illustrate the similarity of the measurements of muscle protein turnover by using leucine and phenylalanine in the basal state, the single metabolic fate of phenylalanine in muscle allows direct estimation of the protein-synthesis rate from eqn. (6), whereas if leucine is used some estimation of the rates of both transamination and oxidation is required.

This is well illustrated by the studies by Cheng *et al.* (1985) and Oddy & Lindsay (1986), directed at modelling the exchange of leucine tracer across a limb. In each of these studies the authors recognized the need to quantify separately the flux of leucine into the 4-methyl-2-oxopentanoic acid pool and the total rate of 4-methyl-2-oxopentanoic acid oxidation in muscle in order to estimate rates of protein synthesis and degradation. One limitation common to both studies was the exclusive use of plasma samples to estimate tracer exchange of leucine by the limb. As we have indicated in the Results section, it appears clear that in the dog the red cell participates in the inter-organ exchange of leucine, and whole-blood measurements will more correctly reflect the overall radioisotope exchange occurring between the muscle and the circulating leucine pool. Preliminary studies also indicate this to be the case in man (R. A. Gelfand, unpublished work). Use of plasma samples for measurement of either the net balance or tracer-exchange kinetics of leucine across the limb leads to a systematic underestimation of the rates of leucine flux. For phenylalanine in the current study no such discrepancy was apparent. In the absence of specific information regarding the participation of red cells and plasma in the exchange of any amino acid across a tissue bed, whole-blood measurements would be preferred.

Estimates of the fractional rate of protein synthesis in human skeletal muscle have been obtained from

measurement of the incorporation of either lysine (Halliday & McKeran, 1975) or leucine (Rennie *et al.*, 1982) labelled with stable isotopes into muscle protein obtained in biopsy samples. Fractional synthesis rates of 1.5 ± 0.3 and $2.4 \pm 1.0\%$ /day were reported for myofibrillar and total muscle protein respectively. In the post-absorptive growing rat the fractional protein-synthesis rate averaged $10.4 \pm 0.6\%$ /day. In the latter study a bolus infusion of unlabelled phenylalanine and [³H]phenylalanine was used in an effort to achieve a uniform labelling of the muscle tRNA specific radioactivity (Garlick *et al.*, 1983). In the current study, if one assumes that hindlimb muscle receives the majority of hindlimb blood flow, and blood flow to dog hindlimb muscle averages 3 ± 1 ml/min per 100 g of tissue (Liang *et al.*, 1982), then the half-life of hindlimb muscle protein can be obtained from the measured rate of hindlimb protein breakdown (3.4 mg/min; see the Results section), and averaged $1.7 \pm 0.5\%$ /day.

In summary, as discussed extensively in several reviews (Morgan *et al.*, 1979; Rannels *et al.*, 1982), the most precise quantification of the rate of muscle protein synthesis can be achieved only by using invasive methods which allow direct measurement of the specific radioactivity of either the aminoacyl-tRNA pool or even the peptidyl-tRNA. The requirement for use of tissue sampling and large amounts of radioisotope precludes application of these methods to clinical investigation. As a result, there is currently no generally accepted method for measuring protein turnover *in vivo* in muscle tissue. The phenylalanine tracer-exchange method described here takes advantage of several attractive properties of L-[ring-2,6-³H]phenylalanine. The method does require both arterial and selective venous catheterization, yet these techniques are already in wide use in the clinical investigation of other aspects of both skeletal and cardiac muscle metabolism. This method may allow more detailed investigation of the humoral, substrate and other factors which regulate protein turnover in specific tissues under a variety of physiological and pathological conditions.

This work was supported by grants from the American Heart Association (AHA 84-1264) and from the U.S. Public Health Service AM00888 (E.J.B.) and AM34241 (R.A.G.)

REFERENCES

- Abumrad, N., Rabin, D., Wise, K. & Lacy, W. (1982) *Metab. Clin. Exp.* **31**, 463-470
- Cahill, G. F., Aoki, T. T. & Marliss, E. B. (1972) *Handb. Physiol., Sect. 7: Endocrinol.* **1**, 463-577
- Cheng, K. N., Dworzak, F., Ford, G. C., Rennie, M. J. & Halliday, D. (1985) *Eur. J. Clin. Invest.* **15**, 349-354
- Everett, A., Prior, G. & Zak, R. (1981) *Biochem. J.* **194**, 365-368
- Felig, P., Wahren, J. & Raf, L. (1973) *Proc. Natl. Acad. Sci. U.S.A.* **70**, 1775-1779
- Garlick, P. J., McNurlan, M. A. & Preedy, V. R. (1980) *Biochem. J.* **192**, 719-723
- Garlick, P. J., Fern, M. & Preedy, V. R. (1983) *Biochem. J.* **210**, 669-676
- Halliday, D. & McKeran, P. O. (1975) *Clin. Sci. Mol. Med.* **49**, 581-590
- Jefferson, L., Li, J. & Rannels, D. E. (1977) *J. Biol. Chem.* **252**, 1476-1483
- Khairallah, E. A. & Mortimore, G. E. (1976) *J. Biol. Chem.* **251**, 1375-1384

- Liang, C., Doherty, J., Failace, R., Mackawa, K., Arnold, S., Gavras, H. & Hood, W. (1982) *J. Clin. Invest.* **69**, 1321–1336
- Martin, A. M., Rabinowitz, M., Blough, R., Prior, G. & Zak, R. (1977) *J. Biol. Chem.* **252**, 3422–3429
- McKee, E. E., Cheung, J. Y., Rannels, D. E. & Morgan, H. (1978) *J. Biol. Chem.* **253**, 1030–1040
- Millward, D. J., Odedra, B. & Bates, P. C. (1983) *Biochem. J.* **216**, 583–587
- Morgan, H. Earl, D., Broadus, A., Wolpert, E., Giger, K. & Jefferson, L. S. (1971) *J. Biol. Chem.* **246**, 2152–2162
- Morgan, H., E., Rannels, D. E. & McKee, E. E. (1979) *Handb. Physiol., Sect. 2: Cardiovasc. Syst.* **1**, 845–871
- Oddy, V. H. & Lindsay, D. B. (1986) *Biochem. J.* **233**, 417–425
- Pain, V. M. & Garlick, P. J. (1974) *J. Biol. Chem.* **249**, 4510–4514
- Pozefsky, T., Felig, P., Tobin, J., Soeldner, J. & Cahill, G. (1969) *J. Clin. Invest.* **48**, 2273–2282
- Rannels, D. E., Kao, R. & Morgan, H. E. (1975) *J. Biol. Chem.* **250**, 1694–1701
- Rannels, D. E., Wortell, S. A. & Watkins, C. A. (1982) *Life Sci.* **30**, 1679–1690
- Rennie, M. J., Edwards, R. H. T., Halliday, D., Matthews, D. E., Wolman, S. L. & Millward, D. J. (1982) *Clin. Sci.* **63**, 519–523
- Robertson, J. & Wheatley, D. (1979) *Biochem. J.* **178**, 699–709
- Schwenk, W. F., Beaufre, B. & Haymond, M. W. (1985) *Am. J. Physiol.* **249**, E646–E650
- Stirewalt, W. S., Low, R. B. & Slaiby, J. M. (1985) *Biochem. J.* **227**, 355–362
- Waterlow, J. C., Garlick, P. J. & Millward, D. J. (1978) *Protein Turnover in Mammalian Tissues and in the Whole Body*, Elsevier/North-Holland Publishing Co., New York
- Williams, I., Sugden, P. & Morgan, H. (1981) *Am. J. Physiol.* **240**, E677–E681

Received 16 October 1986/3 March 1987; accepted 23 March 1987