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Amino acid flooding doses for measuring rates of protein synthesis*

Review Article

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Summary. The development, advantages and disadvantages of using the amino acid flooding dose technique to determine *in vivo* rates of protein synthesis are examined in this review. A discussion of the use of this procedure in animals greater than 5 kg is included. The flooding dose procedure reduces the disparity between isotope enrichment in different amino acid precursor pools, which should theoretically improve the precision and accuracy of protein synthesis measurements. However, the possibility must be considered that the large doses of amino acids injected or infused in conjunction with this technique may influence protein turnover due to attendant metabolic effects. Therefore, a judicious choice of an amino acid and an evaluation of the experimental parameters involved in this procedure are required to optimize the accuracy of results obtained.

Keywords: Amino acids – Flooding dose – Protein synthesis

Introduction

Many researchers in the past two decades have sought to measure rates of protein synthesis in various tissues and to elucidate how those rates are influenced by factors such as nutrition (Reeds and Fuller, 1983), age (Attaix et al., 1988a), environment (Scott et al., 1992), physiological state (Baracos et al., 1991) and hormones (Early et al., 1990; Preedy and Garlick, 1986). To that end, methods have been developed to determine rates of protein turnover, and especially of protein synthesis. Continuous infusion (Garlick et al., 1973; Waterlow, Garlick and Millward, 1978) or flooding dose (Garlick et al., 1980) of a

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tracer amino acid are two of the most widely used techniques for determining rates of protein synthesis in tissues. In both, a tracer amino acid (labelled with either a radioactive or stable isotope) is used to monitor the incorporation of precursor (amino acid) into product (individual or heterogeneous mixtures of intracellular proteins). Primarily for safety reasons, the use of amino acids labelled with stable isotopes such as ^{15}N and ^{13}C has been gaining popularity (Millward et al., 1982; Rennie et al., 1982; Yang et al., 1984; Ford et al., 1985; Krishnamurti and Schaefer, 1987).

Since its development for work with laboratory animals (McNurlan et al., 1979; Garlick et al., 1980), the flooding dose technique has been adapted for use in a variety of species, including small domestic animals (Attaix et al., 1986) and man (Garlick et al., 1989). However, indiscriminate use of the method without thoroughly considering its ramifications may be imprudent. Therefore, this review will focus on the assumptions and limitations involved in the use of the flooding dose technique, particularly with large animals.

Methodology

The flux through the free amino acid pool includes inputs of amino acids from digestion and proteolysis and outputs to protein synthesis and cellular oxidation. There is a dynamic turnover of the protein-bound amino acid pool as well as the free pool (Fig. 1). Output of amino acids to protein synthesis is usually estimated from the rate of incorporation of a tracer amino acid into cellular protein.

The flooding dose technique was developed to avoid the necessity of approximating the specific radioactivity (SRA; or % enrichment in the case of stable isotopes) of the labelled amino acid in the true precursor pool for protein synthesis, the aminoacyl-tRNA. This is usually estimated from the SRA of the label in the extracellular (plasma) or the intracellular free amino acid pools. With the continuous infusion method, dilution of label with unlabelled amino acids from proteolysis usually results in a lower SRA in the intracellular pool compared with plasma. Controversy therefore exists as to whether the aminoacyl-tRNA SRA is closer to that of the extracellular or intracellular pool (Airhart et al., 1974; Khairallah and Mortimore, 1976; Martin et al., 1977; McKee et al., 1978; Rannels et al., 1982). This disparity between the SRA of different precursor pools can be seen in results from experiments with large animals, summarized

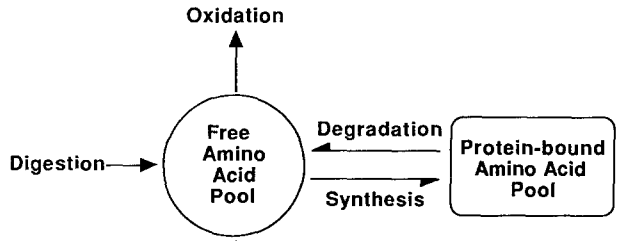

Fig. 1. Turnover of free and bound amino acids

in Table 1. The SRA of label in the intracellular pool is shown as a percentage of the extracellular pool SRA.

With the flooding dose technique, a large amount of unlabelled amino acid is injected along with the tracer in order to rapidly equilibrate all free amino acid pools. Theoretically, this results in a convergence of the tracer amino acid SRA in the extracellular and intracellular free amino acid pools. The actual extent of flooding in various tissues following administration of an amino acid flooding dose to subjects weighing over 5 kg is shown in Table 2. If the flooding dose is sufficient, the SRA of tracer in the precursor pool will not decline appreciably over the short period in which incorporation of label into protein is measured (Fig. 2). This simplifies estimation of the fractional synthesis rate (FSR) of protein in tissue, which is calculated using a two-pool model, according to the following equation (McNurlan et al., 1979):

$$k_s = S_B/(S_A \cdot t)$$

where k_S is the FSR of protein (%/day), S_B is the SRA of the product (protein-bound amino acid pool), S_A is the average SRA of the precursor (free amino acid or aminoacyl-tRNA pool) between times t_0 and t, and t is the actual time of incorporation in days.

Following the flooding dose injection, groups of animals are sacrificed at specific times post-injection to determine the SRA of the tracer in the precursor and product pools. Over the experimental period, the SRA of the protein-bound amino acid will rise in a linear fashion (Attaix et al., 1986). The slope is dependent

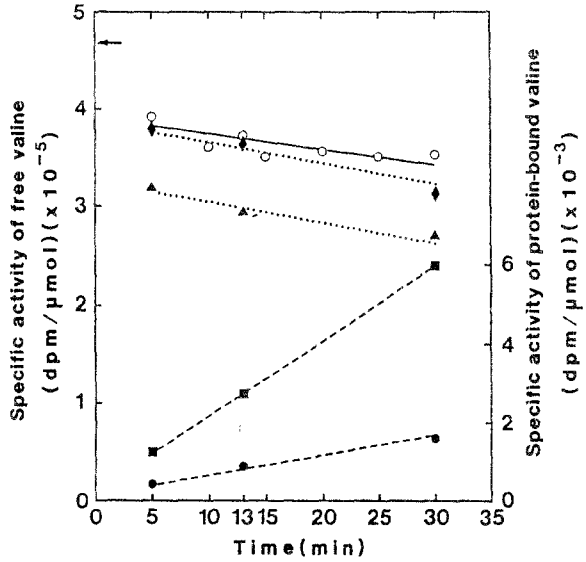

Fig. 2. Time courses for free and protein-bound valine specific radioactivities in tissues of one-week-old lambs after a flooding dose injection of 12.8 mmol of [^{3}H] valine/5 kg body wt. The arrow indicates the injected specific radioactivity. Free valine specific radioactivity was measured in the plasma (o), the tensor fasciae latae muscle (♦) and the jejunum (▲). Protein-bound valine specific radioactivity is shown for the tensor fasciae latae muscle (●) and the jejunum (■). Each point is the mean value for 3 to 9 lambs. (From Attaix et al., 1986)

on the FSR of protein in the tissue. For example, intestinal tissue, which has a rapid turnover rate, will have a steeper slope than muscle, which has a slow turnover rate.

Under ideal circumstances, it should be possible to extrapolate the SRA of tracer in the extracellular and intracellular free amino acid pools back to that of the injection, and to extrapolate the SRA of tracer in the protein-bound amino acid pool back to zero. However, this is usually not the case in actual experiments, possibly due to a lag in the equilibration of the precursor pools. Recent experiments with rabbit cardiac muscle have shown that following a flooding dose of leucine, there may be a delay in the equilibration of leucyl-tRNA, but not intracellular free leucine, with the plasma free leucine pool (Robinson and Samarel, 1990). This delay in equilibration brings into question a fundamental assumption of the flooding dose technique, which is that immediate equilibration occurs between all free amino acid pools and the aminoacyl-tRNA pool, thus removing doubt about the SRA of the precursor pool. Few experiments compare the SRA of the putative precursor pool(s) to that of the aminoacyl-tRNA, mainly due to technical difficulties. Specifically, the tissue concentration of aminoacyl-tRNA is low (Airhart et al., 1974; Samarel, 1991) and its half-life is only a few seconds (Airhart et al., 1974; Martin et al., 1977), requiring rapid freezing and/or homogenization of tissues.

In experiments where the SRA of an aminoacyl-tRNA was determined, it was usually found to be intermediate to those of the extracellular and intracellular free amino acid pools following a single injection (Airhart et al., 1974; Khairallah et al., 1977; Martin et al., 1977; Ferrara et al., 1977; Lundholm et al., 1991), perfusion (Khairallah and Mortimore, 1976; McKee et al., 1978; Watkins and Rannels, 1980) or a primed continuous infusion of tracer (Watt et al., 1991). However, Everett et al. (1981) showed that the SRA of all three pools were equal in rat heart tissue following a 1 hour continuous infusion of leucine and Parmacek et al. (1986) demonstrated that the SRA of rabbit heart tRNA equalled that of plasma following a 2 hour continuous infusion of leucine. Possibly, cardiac tissue equilibrates more rapidly *in vivo* than other tissues.

Various interpretations of the disparity between the SRA of the aminoacyl-tRNA and the intracellular and extracellular free amino acid pools have been expounded. Khairallah and Mortimore (1976) suggested that the intracellular pool is compartmentalized, and proposed a model for the compartmentation of intracellular valine in perfused rat liver comprising three pools. The first pool, identified as a small precursor pool, exchanges with both the extracellular pool and a second large intracellular pool. A third pool, derived from proteolysis, feeds into the second pool but does not mix with it. This model accounts for their observation that the rise in the intracellular valine SRA is slower than that of the aminoacyl-tRNA.

Alternatively, tRNA may be charged from both the extracellular and intracellular free amino acid pools. Based on results from rat liver protein synthesis *in vivo*, Airhart et al. (1974) advanced a model for tRNA charging from a labile valine pool within the plasma membrane. Both the extracellular and intracellular pools contribute to the membrane pool, resulting in a valyl-tRNA with a SRA intermediate to the two.

A third model, proposed by Khairallah et al. (1977), incorporates features of both mentioned above. Instead of tRNA charging from a small intracellular pool near transportation sites, they postulated that the precursor pool was located in the plasma membrane. Consequently, only amino acids en route through the membrane, regardless of direction of flow, could be activated by aminoacyl-tRNA synthetases. The membrane pool could exchange with the extracellular pool and a large intracellular pool. This model also permits estimation of the protein degradation rate, by assuming that amino acids released by degradation feed directly into the large intracellular pool.

Another possibility is that tRNA itself may be compartmentalized between the cytosol and organelles such as the mitochondria and nuclei (Martin et al., 1977; Samarel, 1991). There may be a pool of tRNA charged from the extracellular pool and another charged from one or more intracellular pools (Airhart et al., 1974).

Several authors have theorized that whether tRNA was charged from a compartmentalized intracellular pool or from both the intracellular and extracellular pools, differences in SRA between them could be overcome by expanding the free amino acid pool with a large dose of unlabelled amino acid. Experimental results from perfusions of liver (Mortimore et al., 1972), heart (McKee et al., 1978), and lung (Watkins and Rannels, 1980) which showed that raising the perfusate amino acid concentration resulted in an equilibration of the extracellular, intracellular, and tRNA SRA support this hypothesis.

Selection of an amino acid

Metabolic effects of amino acids

The criteria for choosing an amino acid tracer for protein turnover studies have been reviewed by Waterlow, Garlick and Millward (1978). Briefly, these criteria include the requirements that (1) the tracer amino acid is essential; (2) the metabolism of the amino acid is relatively uncomplicated without reversible biochemical reactions that would confound the measurement of isotope enrichment; (3) neither the amino acid tracee itself nor its metabolic by-products should affect protein turnover; and (4) the tracer should be simple to analyze, have a small pool size, be inexpensive and readily available. We suggest that an additional requirement should be included when the flooding dose technique is used. Namely, that pharmacological levels of an amino acid tracee should not produce any metabolic effects that could alter protein synthesis or degradation. Evidence presented in the previous section suggests that either an injection (seconds to minutes) or an infusion (hours) of an amino acid flooding dose is successful in reducing the disparity in tracer SRA or enrichment between different precursor pools. Undoubtedly, this has been an important factor in the extensive adoption of the flooding dose technique to measure the FSR of protein.

Four amino acids have been widely used with the flooding dose technique, including leucine (Schaefer et al., 1986; Okita et al., 1988; Dorup and Clausen, 1989; Early et al., 1989; Garlick et al., 1989; Irvine et al., 1990), valine (Attaix et al., 1986; Attaix et al., 1988a, 1988b; Sayegh and Lajtha, 1989) and tyrosine

(Morgan et al., 1989; Funabiki et al., 1990). Phenylalanine appears to be by far the most popular choice, especially in small animals (Holt et al., 1990; MacLennan and Edwards, 1990; Muramatsu et al., 1990; Preedy and Peters, 1989, 1990; Preedy et al., 1990a, 1990b). Phenylalanine was chosen for many of these experiments due to its essential biochemical requirement in mammals, its reasonably uncomplicated, non-reversible metabolism, its comparatively low expense, and its ease of analytical quantification.

Elevating concentrations of various amino acids in the body fluid compartment, including those mentioned above, can jeopardize metabolic homeostasis. For example, with inborn errors of metabolism such as phenylketonuria and maple sugar disease, plasma concentrations of phenylalanine and leucine, respectively, are raised by degrees of magnitude (Rodwell et al., 1985). Other amino acids are reported to regulate metabolic events such as protein metabolism, including glutamine (MacLennan et al., 1988; Hammarqvist et al., 1989; Millward, 1990; Vinnars et al., 1990; Wu and Thompson, 1990) and tryptophane (Thibault and Roberge, 1987; Thibault and Roberge, 1988; Lin et al., 1988).

The use of branched-chain amino acids (BCAA), especially leucine, for estimating protein synthesis is controversial because they can have substantial effects on protein turnover (Young et al., 1989; Mitch and Clark, 1984; Morgan and Chua, 1984; Harper et al., 1984). Reported effects include altering muscle tissue sensitivity to insulin (Garlick and Grant, 1988), improving nitrogen balance (Hasselgren et al., 1988; Okita et al., 1988), increasing the FSR of protein in tissue, and reducing protein degradation (Smith, 1985; Trenkle, 1986; Garlick et al., 1988; Smith et al., 1992). On the other hand, several studies have failed to demonstrate a stimulation of protein synthesis by BCAA (McNurlan et al., 1982, 1991; Gibson et al., 1985; Morrison et al., 1988; Hammarqvist et al., 1988). Inconsistent metabolic effects have been reported for leucine, including changes in plasma concentrations of amino acids (Schaefer et al., 1986; Papet et al., 1988a, 1989), but not in concentrations of metabolites such as glucose, glucagon, and cortisol (Attaix et al., 1987; Papet et al., 1988b, 1989) nor in baseline physiological parameters (Schaefer et al., 1986). Leucine is also reported to either increase (McNurlan et al., 1982; Lobley et al., 1990a) or not affect (Attaix et al., 1987; Papet et al., 1989) insulin levels. Differences in nutritional state and tissue responsiveness to amino acid concentrations likely account for some of these discrepancies (Schaefer et al., 1986). In addition, species differences in tissue sensitivity to BCAA may account for some of these apparent contradictions (Early et al., 1989).

Misgivings about the possible effects of a leucine flooding dose on protein turnover likely led to its replacement by phenylalanine (McNurlan et al., 1979; Garlick et al., 1980). The use of phenylalanine in the flooding dose technique has been widely adopted for several species, including large animals such as sheep (Southorn et al., 1991). However, a phenylalanine flooding dose can have metabolic effects during or shortly following an injection. In rats, these effects included reduced plasma sodium and calcium concentrations and elevated creatine kinase concentrations by 10 minutes post-injection (Preedy et al., 1990a). In sheep, elevated plasma insulin levels were observed immediately following a flooding injection of phenylalanine (Lobley et al., 1990a; Southorn et al., 1991).

It could be argued that these effects may either directly or indirectly alter protein turnover.

Valine was chosen by Attaix et al. (1986, 1987) for use of the flooding dose technique in lambs due to its lack of attendant metabolic effects. Lobley et al. (1990a) have also suggested valine as the preferable amino acid for flooding doses in sheep since it does not affect insulin secretion. However, recent work in our lab with sheep showed that a flooding infusion of valine (4 g/hour for 8 hours) eventually brought about changes in plasma aminograms (Schaefer et al., 1991). Plasma concentrations of threonine, phenylalanine, tryptophane and lysine were reduced while those of histidine and isoleucine were elevated.

Even though amino acid flooding injections and infusions have been shown to have metabolic effects, it is possible that, given the short time over which they

Table 1. A comparison of tissue intracellular and extracellalar (plasma) presursor pool specific activity using the continuous infusion method in animals having a body mass greater than 5 kg

Method	Reference	Species and mass	Isotope	Tissue	Isotope enrichment[1]
Continuous infusion	Lobley et al. (1980)	cattle 236–628 kg	^{3}H tyr ^{3}H leu (8 h)	muscle liver GIT	89% 35% 49%
Continuous infusion	Early et al. (1990)	cattle 273 kg	^{3}H phe (4 h)	muscle liver rumen heart	24% 23% 11% 54%
Continuous infusion	Scott (1991)	calves ~65 kg	^{3}H phe (8 h)	muscle liver rumen heart	69–77% 54–60% 54–56% 69–82%
Continuous infusion	Davis et al. (1981)	lambs 16 kg	^{3}H leu (7 h)	muscle liver rumen heart	28–73% 15–39% 14–18% 36–61%
Continuous infusion	Schaefer et al. (1986)	sheep 58 kg	^{3}H leu (8 h)	muscle liver rumen heart	70% 27% 50% 80%
Continuous infusion	Baracos et al. (1991)	goats 51–53 kg	^{3}H phe (8 h)	muscle liver rumen heart	62% 40% 59% 62%
Primed continuous infusion	Smith et al. (1992)	man 69 kg	^{13}C val (6 h)	muscle	~75%

[1] Data either reported in or calculated from the above studies is expressed as the intracellular free amino acid specific activity as a percentage of the extracellular (plasma) free amino acid activity for control animals.

are administered (from minutes to hours), protein synthesis will not be affected. However, it is also conceivable that amino acid flooding, if continued for sufficient time and at a sufficient concentration, would have some metabolic effects including alterations in protein turnover.

Choice of an amino acid

In view of the foregoing discussion, the optimal choice of an amino acid tracer and tracee for use in a flooding dose procedure is not immediately evident. Metabolic effects notwithstanding, the ability of the amino acid to reduce the disparity in precursor pool enrichment should be a primary consideration, and should be validated with a comparison of the enrichment in the intracellular and extracellular pools to that of the tRNA pool. Obled et al. (1989) compared the rates of whole body protein synthesis in rats obtained with flooding doses and continuous infusions of five amino acids. When threonine and lysine were used, the flooding dose technique was found to yield estimates of whole body rates of protein synthesis similar to those obtained with the continuous infusion method. However, when leucine was used, values obtained with the flooding dose technique were intermediate to the minimum and maximum estimates calculated from the SRA of the extracellular and intracellular free amino acid pools, respectively, following a continuous infusion. They hypothesized that the relationship between the SRA of the tRNA pool and the intracellular and extracellular free amino acid pools will vary depending on the amino acid used, mainly because of the size of the intracellular pool and the heterogeneity of intracellular protein.

Use of the flooding dose technique in mammals larger than 5 kg

Attaix and co-workers (Attaix et al., 1986, 1988a, 1988b; Attaix and Arnal, 1987) have used the flooding dose technique to determine the FSR of protein in tissues from lambs weighing up to 21 kg. However, certain factors may preclude its use in larger subjects. A serious concern is the volume of injectate administered. For small laboratory animals, most researchers follow the guidelines set out by McNurlan et al. (1979) and Garlick et al. (1980) regarding the amino acid dose, i.e., about 10 μCi and 100 μmol/100 g body weight for leucine and 50 μCi and 150 μmol/100 g body weight for phenylalanine in a volume of 1 ml. If these doses are extrapolated to a 70 kg subject, 7 mCi and 70 mmol of leucine in a volume of 700 ml or 35 mCi and 105 mmol of phenylalanine in a volume of 700 ml would be required in an injection.

Studies with subjects weighing more than 20 kg have generally used doses and volumes less than those extrapolated above. For studies with humans, Garlick et al. (1989) used a flooding dose of 26.7 mmol (3.5 g) of [1-^{13}C]leucine/70 kg in a volume of 175 ml and injected it over 4 minutes. Using a similar technique to study protein synthesis in humans with cancer, Heys et al. (1991) used a flooding dose of 24.4 mmol (3.2 g) of [1-^{13}C]leucine/70 kg in a volume of 200 ml, which was injected over 10 minutes. For lambs averaging about 21 kg, Attaix et al. (1988a; 1989) used a dose equivalent to 62 mmol and

Table 2. A comparison of tissue intracellular and extracellular (plasma) presursor pool specific activity using a flooding dose method in animals having a body mass greater than 5 kg

Method	Reference	Species and mass	Isotope	Tissue	Isotope enrichment[1]
Flooding dose	Attaix et al. (1986)	lamb 5 kg	^{3}H val 12.8 mmol/5 kg (0.4 min)	muscle liver jejunum	>89% >81% >77%
Flooding dose	Schaefer et al. (1986)	sheep 58 kg	^{3}H leu 22.8 mmol/h (8 h)	muscle liver rumen heart	93% 95% 80% 100%
Flooding dose	Attaix et al. (1989)	lambs 21 kg	^{3}H val 12.8 mmol/5 kg$^{0.75}$ (2.8 min)	pancreas	>67%[2]
Flooding dose	Lobley et al. (1990b)	lambs 45 kg	^{13}C leu 0.85 mmol/kg (10 min)	muscle	100%
Flooding dose	Southorn et al. (1991)	lambs 33 kg	^{3}H phe 1.5 mmol/kg (12 min)	liver small intestine	75% 75%
Flooding dose	Garlick et al. (1989)	man 67–88 kg	^{13}C leu 0.38 mmol/kg (4 min)	muscle	>90%
Flooding dose	Smith et al. (1992)	man 69 kg	^{13}C leu 0.38 mmol/kg (10 min)	muscle	~78%

[1] Data either reported in or calculated from the above studies is expressed as the intracellular free amino acid specific activity as a percentage of the extracellular (plasma) free amino acid activity for control animals.

[2] Tissue homogenate specific activity is expressed as a percentage of the injected specific activity. This value is the lowest percentage observed in sixteen tissues, including the viscera (gastrointestinal tract, liver), skin, skeletal muscles and the viscera-free carcass (D. Attaix, personal communication).

13 mCi of [^{3}H]valine/70 kg in a volume of 84 ml, which took about 2.8 minutes to inject. For slightly heavier lambs (33 kg), Southorn et al. (1991) used a dose comparable to 105 mmol and 2.3 mCi of [^{3}H]phenylalanine/70 kg in a volume of 300 ml (B. G. Southorn, personal communication), and injected it over 12 minutes. With these sizable volumes, it is impossible to instantaneously inject the tracer. This results in uncertainty about the definition of zero time and, consequently, calculation of a positive intercept for the protein-bound tracer SRA. Attaix et al. (1986) addressed this problem by subtracting the protein-bound SRA at zero time from each experimental time point. To avoid many of these complications associated with injecting large volumes of tracee, Schaefer et al. (1986) elected to use an amino acid flooding infusion rather than a flooding injection in mature sheep (57 kg). An infusion of 22.8 mmol of leucine/hour

was optimal with respect to reducing the disparity between intracellular and extracellular (plasma) precursor pool amino acid SRA in liver, skeletal muscle and heart, but not rumen, tissues.

The solubility of the amino acid chosen also plays a role in determining the injection volume. For example, an amino acid with a low solubility will require a larger injection volume, which will in turn prolong the injection. Valine, which has a solubility of 83.4 g/l at 0°C, is about four times more soluble than leucine and phenylalanine.

A further consideration with use of the flooding dose technique in large subjects is the protracted sampling time. With large domestic animals in particular, it is difficult to humanely sacrifice them and sample tissues quickly. Any delay in the time between slaughter and freezing the tissues can result in an erroneous calculation of the FSR of protein. Accurate calculation of protein synthesis depends on maintenance of a plateau SRA in the precursor pool. If tissue sampling takes too long and the precursor SRA drops, the FSR of protein will be overestimated. Baracos et al. (1991) addressed this problem following a continuous infusion of phenylalanine in goats by rapidly removing whole limbs from anesthetized animals and immediately freezing them in liquid nitrogen.

In addition to the theoretical reasons discussed above, use of the flooding dose technique in large animals may be precluded by the practical aspect of cost. The amount of label that is required to adequately flood all tissue pools may be prohibitively expensive. In addition, except for studies with humans, subjects are usually sacrificed at short intervals. This is extremely costly for experiments with large domestic animals because the commercial value of the carcass cannot be recovered.

Conclusions

The amino acid flooding dose technique for measuring protein synthesis reduces the disparity among isotope enrichment in the precursor pools. Theoretically, this should allow a more precise and accurate estimation of tissue protein synthesis rates and thus be a valuable tool to biological sciences. However, controversy continues to surround the use of this technique. First is the debate whether or not the large dose of unlabelled tracee amino acid employed in this procedure can directly or indirectly affect protein turnover (see McNurlan et al., 1991 vs. Smith et al., 1992). Second is the question whether there is even evidence to confirm that the tracer isotope enrichment in the intracellular and extracellular free amino acid pools actually equals that of tRNA (see Watt et al., 1991). Finally, no consensus exists regarding the choice of an optimal amino acid tracer-tracee. Currently, empirical evidence suggests that with smaller mammals (< 5 kg) an intraperitoneal injection of phenylalanine (40 μCi and 150 μmol/100 g body weight) is the most accepted flooding dose method for estimating rates of protein synthesis. Certainly, intraperitoneal injections overcome the need to restrain or immobilize an animal, which is advantageous since restraint in itself is reputed to alter protein synthetic rates (Preedy and Garlick, 1984). Alternatively, with larger animals (> 5 kg), one logical conclusion from the foregoing discussion would be that a valine infusion in the order of 600–700 μmol/hour/kg,

given over minutes or hours with sufficient label consistent with the sensitivity of available analytical techniques, would be a reasonable experimental guideline.

In terms of methodological approaches to measuring protein synthesis, there have been few developments in recent years that have been as interesting to follow as the literature relating to the use of amino acid flooding doses. It must be kept in mind, however, that the use of the flooding dose technique in general, and its use with large animals in particular, is still a comparatively novel technique, despite over ten years of application. Further validation of this procedure will depend on research aimed specifically at assessing the disparity among precursor pool isotope enrichment compared to that of the tRNA benchmark.

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References

Airhart J, Vidrich A, Khairallah EA (1974) Compartmentation of free amino acids for protein synthesis in rat liver. Biochem J 140: 539–548

Attaix D, Arnal M (1987) Protein synthesis and growth in the gastrointestinal tract of the young preruminant lamb. Br J Nutr 58: 159–169

Attaix D, Manghebati A, Grizard J, Arnal M (1986) Assessment of *in vivo* protein synthesis in lamb tissues with [^{3}H]valine flooding doses. Biochim Biophys Acta 882: 389–397

Attaix D, Papet I, Grizard J, Arnal M (1987) Absence d'effets de l'injection de surcharges de L-valine et L-leucine sur les teneurs plasmatiques en insuline et en glucagon de l'agneau préruminant. Reprod Nutr Develop 27: 297–298

Attaix D, Aurousseau E, Bayle G, Rosolowska-Huszcz D, Arnal M (1988a) Respective influences of age and weaning on skeletal and visceral muscle protein synthesis in the lamb. Biochem J 256: 791–795

Attaix D, Aurousseau E, Manghebati A, Arnal M (1988b) Contribution of liver, skin and skeletal muscle to whole-body protein synthesis in the young lamb. Br J Nutr 60: 77–84

Attaix D, Aurousseau E, Bayle G, Rosolowska-Huszcz D, Manghebati A, Arnal M (1989) Influences of age and weaning on *in vivo* pancreatic protein synthesis in the lamb. J Nutr 119: 463–470

Baracos VE, Brun-Bellut J, Marie M (1991) Tissue protein synthesis in lactating and dry goats. Br J Nutr 66: 451–465

Davis SR, Barry TN, Hughson GA (1981) Protein synthesis in tissues of growing lambs. Br J Nutr 46: 409–419

Dorup I, Clausen T (1989) Effects of potassium deficiency on growth and protein synthesis in skeletal muscle and the heart of rats. Br J Nutr 62: 269–284

Early RJ, Thompson JR, Christopherson RJ (1989) Net blood exchange of branched-chain amino and α-keto acid across the portal-drained viscera and hindlimb of cattle during infusions of leucine and insulin. Can J Anim Sci 69: 131–140

Early RJ, McBride BW, Ball RO (1990) Growth and metabolism in somatotropin-treated steers: III. Protein synthesis and tissue energy expenditures. J Anim Sci 68: 4153–4166

Everett AW, Prior G, Zak R (1981) Equilibration of leucine between the plasma compartment and leucyl-tRNA in the heart, and turnover of cardiac myosin heavy chain. Biochem J 194: 365–368

Ferrara M, Arnal M, Fauconneau G (1977) Mesure de la synthèse protéique *in vivo* à l'aide du ^{14}C lysyl-tRNA^{lys} dans le foie d'agneau en croissance. Ann Biol anim Bioch Biophys 17: 1061–1068

Ford GC, Cheng KN, Halliday D (1985) Analysis of (1-^{13}C)leucine and (^{13}C)KIC in plasma by capillary gas chromatography/mass spectrometry in protein turnover studies. Biomed Mass Spec 12: 432–436

Funabiki R, Yagasaki K, Hara H, Nyumura N, Takeda A (1990) Measurement of the rate of whole body protein synthesis by intraperitoneal injection of a large dose of alanyltyrosine with [^{14}C]tyrosine. Agr Biol Chem Tokyo 54: 113–119

Garlick PJ, Grant I (1988) Amino acid infusion increases the sensitivity of muscle protein synthesis *in vivo* to insulin: effect of branched chain amino acids. Biochem J 254: 579–584

Garlick PJ, Millward DJ, James WPT (1973) The diurnal response of muscle and liver protein synthesis *in vivo* in meal fed rats. Biochem J 136: 935–945

Garlick PJ, McNurlan MA, Preedy VR (1980) A rapid and convenient technique for measuring the rate of protein synthesis in tissues by injection of [^{3}H]phenylalanine. Biochem J 192: 719–723

Garlick PJ, Burns HJG, Palmer R (1988) In: Burns HJG (ed) Baillière's clinical gastroenterology, vol. 2, no. 4. Baillière-Tindall, London, pp 915–940

Garlick PJ, Wernerman J, McNurlan MA, Essen P, Lobley GE, Milne E, Calder GA, Vinnars E (1989) Measurement of the rate of protein synthesis in muscle of postabsorptive young men by injection of a "flooding dose" of [1-^{13}C]leucine. Clin Sci 77: 329–336

Gibson JNA, Morrison JL, Rennie MJ (1985) Elevation of blood branched chain amino acid concentration has no effect on protein synthesis or breakdown in human leg tissues. Eur J Clin Invest 15: A9

Hammarqvist F, Wernerman J, von der Decken A, Vinnars E (1988) The effects of branched chain amino acids upon post-operative muscle protein synthesis and nitrogen balance. Clin Nutr 7: 171–175

Hammarqvist F, Wernerman J, Rutsom A, von der Decken A, Vinnars E (1989) Addition of glutamine to total parenteral nutrition after elective abdominal surgery spares free glutamine in muscle, counteracts the fall in muscle protein synthesis and improves nitrogen balance. Ann Surg 209: 455–461

Harper AE, Miller RH, Block KP (1984) Branched-chain amino acid metabolism. Ann Rev Nutr 4: 409–454

Hasselgren PO, LaFrance R, Pedersen P, James JH, Fischer JE (1988) Infusion of a branched-chain amino acid-enriched solution and α-ketoisocaproic acid in septic rats: effects on nitrogen balance and skeletal muscle protein turnover. J Parent Enter Nutr 12: 244–249

Heys SD, Park KGM, McNurlan MA, Calder AG, Buchan V, Blessing K, Eremin O, Garlick PJ (1991) Measurement of tumour protein synthesis *in vivo* in human colorectal and breast cancer and its variability in separate biopsies from the same tumour. Clin Sci 80: 587–593

Holt TL, Ward LC, Thomas BJ, Davey JF, Shepherd RW (1990) The effect of an anabolic steroid, methenolone enanthate, on growth, body composition and skelelal muscle protein synthesis in the growing rat. Nutr Res 10: 535–545

Irvine JW, Ottaway JH, Saunderson CL (1990) Turnover of sarcoplasmic proteins in the breast muscle of rapidly growing chicks. Int J Biochem 22: 187–192

Khairallah EA, Mortimore GE (1976) Assessment of protein turnover in perfused rat liver: Evidence for amino acid compartmentation from differential labeling of free and tRNA-bound valine. J Biol Chem 251: 1375–1384

Khairallah EA, Airhart J, Bruno MK, Puchalsky D, Khairallah L (1977) Implications of amino acid compartmentation for the determination of rates of protein catabolism in livers in meal fed rats. Acta Biol Med Germ 36: 1735–1745

Krishnamurti CR, Schaefer AL (1987) Measurement of plasma leucine flux and protein synthesis in pregnant ewes using gas chromatography and mass spectrometry. Nutr Rep Intl 35: 683–692

Lin FD, Smith TK, Bayley HS (1988) A role for tryptophan in regulation of protein synthesis in porcine muscle. J Nutr 118: 445–449

Lobley GE, Milne V, Lovie JM, Reeds PJ, Pennie K (1980) Whole body and tissue protein synthesis in cattle. Br J Nutr 43: 491–502

Lobley GE, Connell A, Buchan V, Milne E, Calder AG, Skene PA (1990a) Use of a large dose, stable isotope procedure to measure muscle protein synthesis in sheep. Proc Nutr Soc 49: 134A

Lobley GE, Connell A, Milne E, Buchan V, Calder AG, Anderson SE, Vint H (1990b) Muscle protein synthesis in response to testosterone administration in wether lambs. Br J Nutr 64: 691–704

Lundholm K, Ternell M, Zachrisson H, Moldawer L, Lindstrom L (1991) Measurement of hepatic protein synthesis in unrestrained mice-evaluation of the "flooding technique". Acta Physiol Scand 141: 207–219

MacLennan PA, Smith K, Weryk B, Watt PW, Rennie MJ (1988) Inhibition of protein breakdown by glutamine in perfused rat skeletal muscle. FEBS Lett 237: 133–136

MacLennan PA, Edwards RHT (1990) Protein turnover is elevated in muscle of mdx mice *in vivo*. Biochem J 268: 795–797

Martin AF, Rabinowitz M, Blough R, Prior G, Zak R (1977) Measurements of half-life of rat cardiac myosin heavy chain with leucyl-tRNA used as precursor pool. J Biol Chem 252: 3422–3429

McKee EE, Cheung JY, Rannels DE, Morgan HE (1978) Measurement of the rate of protein synthesis and compartmentation of heart phenylalanine. J Biol Chem 253: 1030–1040

McNurlan MA, Tomkins AM, Garlick PJ (1979) The effect of starvation on the rate of protein synthesis in rat liver and small intestine. Biochem J 178: 373–379

McNurlan MA, Fern EB, Garlick PJ (1982) Failure of leucine to stimulate protein synthesis *in vivo*. Biochem J 204: 831–838

McNurlan MA, Essen P, Heys SD, Buchan V, Garlick PJ, Wernerman J (1991) Measurement of protein synthesis in human skeletal muscle: further investigation of the flooding technique. Clin Sci 81: 557–564

Millward DJ (1990) Muscle glutamine and the regulation of muscle protein balance in endotoxemia. Clin Nutr 9: 34

Millward DJ, Davies CTM, Halliday D, Wolman SL, Matthews DE, Rennie MJ (1982) Effect of exercise on protein metabolism in humans as explored with stable isotopes. Fed Proc 41: 2686–2691

Mitch WE, Clark AS (1984) Specificity of the effects of leucine and its metabolites on protein degradation in skeletal muscle. Biochem J 222: 579–586

Morgan HE, Chua BHL (1984) In: Adibi SA, Fekl W, Langenbeck U, Schauder P (eds) Branched chain amino and keto acids in health and disease. Karger, Basel, pp 15–21

Morgan JB, Jones SJ, Calkins CR (1989) Muscle protein turnover and tenderness in broiler chickens fed cimaterol. J Anim Sci 67: 2646–2654

Morrison WL, Gibson JNA, Rennie MJ (1988) Skeletal muscle and whole-body protein turnover in cardiac cachexia: influence of branched chain amino acid administration. Eur J Clin Invest 18: 648–654

Mortimore GE, Woodside KH, Henry JE (1972) Compartmentation of free valine and its relation to protein turnover in perfused rat liver. J Biol Chem 247: 2776–2784

Muramatsu T, Takai D, Kita K, Okumura JI (1990) Studies on the measurement of whole-body protein degradation *in vivo* in the chicken. Int J Biochem 22: 153–158

Obled C, Barre F, Millward DJ, Arnal M (1989) Whole body protein synthesis: studies with different amino acids in the rat. Am J Physiol 257: E639–E646

Okita M, Watanabe A, Tsuji T (1988) Effect of branched chain amino acid on ^{15}N-incorporation into liver and skeletal muscle proteins following ^{15}N-ammonium chloride administration to carbon tetrachloride-intoxicated rats. J Nutr Sci Vitaminol 34: 85–96

Papet I, Breuille D, Glomot F, Arnal M (1988a) Nutritional and metabolic effects of dietary leucine excess in preruminant lamb. J Nutr 118: 450–455

Papet I, Gardy-Godillot M, Dalle M, Grizard J, Arnal M (1988b) Absence d'effets d'un exces alimentaire en leucine sur les cinetiques postprandiales du glucagon et du cortisol plasmatiques chez l'agneau préruminant. Reprod Nutr Develop 28: 161–162

Papet I, Grizard J, Gardy-Godillot M, Dalle M, Arnal M (1989) Lack of an effect of a high leucine test meal on plasma insulin in preruminant lambs. Nutr Res 9: 941–945

Parmacek MS, Magid NM, Lesch M, Decker RS, Samarel AM (1986) Cardiac protein synthesis and degradation during thyroxine-induced left ventricular hypertrophy. Am J Physiol 251: C727–C736

Preedy VR, Garlick PJ (1984) Reduced rates of muscle protein synthesis during tail-vein infusion of rats. Biochem Soc Trans 12: 700–701

Preedy VR, Garlick PJ (1986) The response of muscle protein synthesis to nutrient intake in post-absorptive rats: The role of insulin and amino acids. Biosci Rep 6: 177–183

Preedy VR, Peters TJ (1989) Protein synthesis of muscle fractions from the small intestine in alcohol-fed rats. Gut 31: 305–310

Preedy VR, Peters TJ (1990) The acute and chronic effects of ethanol on cardiac muscle protein synthesis in the rat *in vivo*. Alcohol 7: 97–102

Preedy VR, Hammond B, Bottiglieri TG, Marway JS, Peters TJ (1990a) Measurement of protein synthesis by the phenylalanine flooding dose technique: Effect of phenylalanine and anaesthesia on plasma electrolyte, enzyme and metabolite levels. J Pharm Pharmacol 42: 851–856

Preedy VR, Marway JS, Salisbury JR, Peters TJ (1990b) Protein synthesis in bone and skin of rat are inhibited by ethanol: implications for whole body metabolism. Alcoholism 14: 165–168

Rannels DE, Wartell SA, Watkins CA (1982) The measurement of protein synthesis in biological systems. Life Sci 30: 1679–1690

Reeds PJ, Fuller MF (1983) Nutrient intake and protein turnover. Proc Nutr Soc 42: 463–471

Rennie MJ, Edwards RHT, Halliday D, Matthews DE, Wolman SL, Millward DJ (1982) Muscle protein synthesis measured by stable isotope techniques in man: the effects of feeding and fasting. Clin Sci 63: 519–523

Robinson ME, Samarel AM (1990) Regional differences in *in vivo* myocardial protein synthesis in the neonatal rabbit heart. J Mol Cell Cardiol 22: 607–618

Rodwell VW (1985) In: Martin DW, Mayes PA, Rodwell VW, Granner DK (eds) Harper's review of biochemistry, 20th edn. Lange Medical Publications, Los Altos CA, pp 293–318

Samarel AM (1991) *In vivo* measurements of protein turnover during muscle growth and atrophy. FASEB J 5: 2020–2028

Sayegh JF, Lajtha A (1989) *In vivo* rates of protein synthesis in brain, muscle, and liver of five vertebrate species. Neurochem Res 14: 1165–1168

Schaefer AL, Davis SR, Hughson GA (1986) Estimation of tissue protein synthesis in sheep during sustained elevation of plasma leucine concentration by intravenous infusion. Br J Nutr 56: 281–288

Schaefer AL, Scott SL, Lepage P (1991) The effect of a valine flooding dose on plasma and intracellular fluid aminograms in sheep. Can J Anim Sci 71: 1279 (Abstr.)

Scott SL (1991) Effect of a cold environment on protein synthesis and endocrine kinetics in growing cattle. Ph.D. Thesis, University of Alberta, Canada

Scott SL, Christopherson RJ, Thompson JR, Baracos VE (1992) The effect of a cold environment on protein and energy metabolism in calves. Br J Nutr (in press)

Smith K, Barua JM, Watt PW, Scrimgeour CM, Rennie MJ (1992) Flooding with L-[1-^{13}C]leucine stimulates human muscle protein incorporation of continuously infused L-[1-^{13}C]valine. Am J Physiol 262: E372–E376

Smith TK (1985) Effect of leucine-rich dietary protein on *in vitro* protein synthesis in porcine muscle. Proc Soc Exp Biol Med 180: 538–543

Southorn BG, Kelly JM, McBride BW (1991) Protein synthesis measurements in sheep using a "flooding" dose technique. Can J Anim Sci 71: 1279 (Abstr.)

Thibault L, Roberge AG (1987) Dietary protein-induced changes in serum dopamine β-hydroxylase activity, glucose, insulin, cortisol and pyridoxyl-5′-phospate levels in normal and immobilized cats. J Clin Biochem Nutr 3: 55–64

Thibault L, Roberge AG (1988) Dietary protein and carbohydrate effects on blood parameters related to stress in cats. Physiol Behav 42: 1–5

Trenkle A (1986) In: Dutt RH (ed) Proceedings of the symposium on food animal research, Lexington, Kentucky 1986. Intersociety Research Committee, Lexington, pp 80–91

Vinnars E, Hammarqvist F, von der Decken A, Wernerman J (1990) Role of glutamine and its analogs in posttraumatic muscle protein and amino acid metabolism. J Parent Enter Nutr 14 [Suppl]: 125S–129S

Waterlow JC, Garlick PJ, Millward DJ (1978) Protein turnover in mammalian tissues and in the whole body. North-Holland Publishing Company, Amsterdam

Watkins CA, Rannels DE (1980) Measurement of protein synthesis in rat lungs perfused *in situ*. Biochem J 188: 269–278

Watt PW, Lindsay Y, Scrimgeour CM, Chien PAF, Gibson JNA, Taylor DJ, Rennie MJ (1991) Isolation of aminoacyl-tRNA and its labeling with stable-isotope tracers: use in studies of human tissue protein synthesis. Proc Natl Acad Sci 88: 5892–5896

Wu G, Thompson JR (1990) The effect of glutamine on protein turnover in chick skeletal muscle *in vitro*. Biochem J 265: 593–598

Yang RD, Matthews DE, Bier DM, Lo C, Young VR (1984) Alanine kinetics in humans: influence of different isotopic tracers. Am J Physiol 247: E634-E638

Young LH, Gelfand RA, King JE, Revkin JH, Barrett EJ (1989) In: Kinney JM, Borum PR (eds) Perspectives in clinical nutrition. Urban and Schwarzenberg, Baltimore, pp 261–273

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