

# Branched-Chain Amino Acids: Metabolism, Physiological Function, and Application

## Branched-Chain Amino Acids as Fuels and Anabolic Signals in Human Muscle<sup>1-3</sup>

Michael J. Rennie,<sup>\*4</sup> Julien Bohé,<sup>†</sup> Ken Smith,<sup>\*</sup> Henning Wackerhage,<sup>\*\*</sup> and Paul Greenhaff<sup>\*</sup>

<sup>\*</sup>School of Biomedical Sciences, University of Nottingham, Graduate Entry Medical School, Derby City General Hospital, Derby DE22 3DT, UK; <sup>†</sup>Medical Intensive Care Unit, Lyon-Sud University Hospital, 69465 Pierre-Bénite, France; and <sup>\*\*</sup>Medical Sciences, University of Aberdeen, Aberdeen AB24 3FX, UK

**ABSTRACT** During exercise, there is an increase in amino acid (AA) oxidation accompanied by a depression in whole-body protein synthesis and an increase in protein breakdown. Leucine oxidation increases in proportion to energy expenditure, but the total contribution of BCAA to fuel provision during exercise is minor and insufficient to increase dietary protein requirements. When investigating the effects of AA on the control of muscle protein synthesis (MPS), we showed that increased availability of mixed AAs caused a rise in human MPS to about the same extent as complete meals. Leucine alone (and to some extent other essential, but not nonessential, AAs) can stimulate MPS for a short period, suggesting that leucine acts as a signal as well as a substrate. MPS stimulation by infused AAs shows tachyphylaxis, returning to basal rates after 2 h, possibly explaining why chronically elevated leucine delivery does not elevate MPS clinically. Increased availability of essential amino acids (EAAs) results in dose-related responses of MPS, but, in elderly subjects, there is blunted sensitivity and responsiveness associated with decreased total RNA and mRNA for signaling proteins and signaling activity. Increases of MPS due to EAAs are associated with elevation of signaling activity in the mammalian target of rapamycin (mTOR)/p70 ribosomal subunit S6 kinase eukaryotic initiation factor 4 binding protein 1 pathway, without requiring rises of plasma insulin availability above 10  $\mu$ U/mL. However, at insulin of <5  $\mu$ U/mL, AAs appear to stimulate MPS without increasing mTOR signaling. Further increasing availability of insulin to postprandial values increases signaling activity, but has no further effect on MPS. *J. Nutr.* 136: 264S–268S, 2006.

**KEY WORDS:** • protein • fuel • protein turnover • muscle

In this article, we will review work concerning amino acids (AAs)<sup>5</sup> as fuel during exercise and also the control of lean body mass, particularly muscle mass, by AAs. We will also set the work within the context of results obtained by others, although our interpretation of some of these may differ from those of the

original authors. The questions of most interest are: What are the effects of contractile activity on AA transport and metabolism (including protein turnover); to what extent does exercise influence the efficiency of protein metabolism in the human body; what are the mechanisms by which AAs influence muscle size (and possibly composition); what goes wrong after injury and during disease and aging; and to what extent can exercise and optimal nutrition ameliorate any problems?

### Exercise and amino acid and protein metabolism

The two most powerful environmental influences upon muscle protein metabolism are obviously nutrition and exercise, but most of the information available 25 y ago said little about the effects of these on the components of AAs and protein metabolism (i.e., AA transport, intermediary metabolism in muscle, and protein turnover). Joe Millward persuaded John Waterlow to provide us with some <sup>15</sup>N glycine and he was also persuaded to measure whole-body protein turnover by the <sup>15</sup>N ammonia end-product method in the experiments we designed to be carried out in the basement of the London School of Hygiene and Tropical Medicine courtesy of Mervyn Davies. These were probably the first studies using a stable

<sup>1</sup> Published in a supplement to *The Journal of Nutrition*. Presented at the conference "Symposium on Branched-Chain Amino Acids" held May 23–24, 2005, in Versailles, France. The conference was sponsored by Ajinomoto USA, Inc. The organizing committee for the symposium and guest editors for the supplement were Luc Cynober, Robert A. Harris, Dennis M. Bier, John O. Holloszy, Sidney M. Morris, Jr., and Yoshiharu Shimomura. Guest editor disclosure: L. Cynober, R. A. Harris, D. M. Bier, J. O. Holloszy, S. M. Morris, Y. Shimomura: expenses for travel to BCAA meeting paid by Ajinomoto USA; D. M. Bier: consults for Ajinomoto USA; S. M. Morris: received compensation from Ajinomoto USA for organizing BCAA conference.

<sup>2</sup> Author Disclosure: No relationships to disclose.

<sup>3</sup> Supported by the UK BBSRC, MRC, The Wellcome Trust, and by the US National Institutes of Health. We are grateful to Professor Robert R. Wolfe for providing facilities in his laboratory and for the Shriners Burns Institute of Galveston for financial support for M.J.R. and J.B.

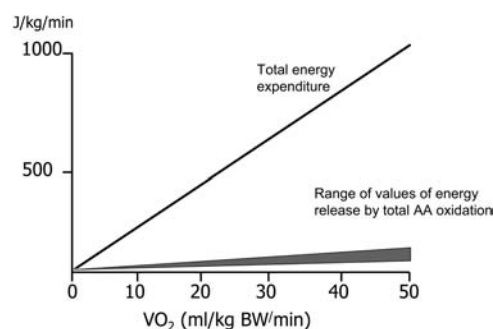
<sup>4</sup> To whom correspondence should be addressed. E-mail: michael.rennie@nottingham.ac.uk.

<sup>5</sup> Abbreviations: AA, amino acids; EAA, essential amino acids; eIF4 BP-1, eukaryotic initiation factor 4 binding protein 1; MPS muscle protein synthesis; mTOR, mammalian target of rapamycin; p70 S6 kinase, p70 ribosomal subunit S6 kinase.

isotope tracer to measure AA and protein metabolism during exercise. Later, we used  $^{13}\text{C}$  leucine to confirm some of the initial results. What was shown quite clearly was that, as a result of moderate, medium-term exercise—nearly 4 h of walking uphill on a treadmill—there were substantial changes in whole-body AA and protein metabolism (1). There was a substantial increase in the flux of  $^{15}\text{N}$  glycine and marked increases in urinary ammonia and urea as a result of exercise. We interpreted the results as suggesting that AA oxidation increased during exercise with whole-body protein synthesis depressed and whole-body protein breakdown possibly elevated. In the postexercise period, the data suggested that AA oxidation fell and the net balance between protein synthesis and breakdown became positive. One of the difficulties of interpretation of our data was that we were measuring protein metabolism by means of the appearance of  $^{15}\text{N}$  in urea and ammonia in urine during exercise. However, renal blood flow was sufficiently decreased that glomerular filtration rate decreased and therefore the whole-body urea pool was elevated; thus there was an apparent temporal disjunction between the changes in metabolism and when we observed them. In particular, the temporal relation between the period of exercise and the rise and return to basal values of AA oxidation was not clear. We therefore carried out experiments in which we used  $^{13}\text{C}$  leucine as a tracer and were gratified to observe that exercise caused a sudden marked increase in the appearance of  $^{13}\text{CO}_2$  in the breath and that the elevated rate of leucine oxidation was apparently confined to the exercise period alone. We then investigated the relation between the intensity of exercise and leucine oxidation and were able to show that there was a linear relation with energy consumption during exercise and the oxidation (2). Furthermore, we showed that the extent of leucine oxidation could be reduced by consumption of glucose during exercise (3–6). In experiments carried out in the perfused rat hindlimb, we found that BCAAs, including leucine, were transported into muscle by a low-affinity, high-capacity system (7), suggesting that increased blood flow to muscle as occurs during exercise would be a major stimulatory factor in increasing leucine oxidation by delivering more leucine to the BCAA transaminase and ketoacid dehydrogenase.

The relevance of AA utilization as a fuel to nutrition is one of considerable controversy. Leibig, who believed that protein contained some mystical vital force imbued by virtue of its biological origin, was convinced that the most obviously vital of biological tissues, namely muscle, would be fueled by protein; however, his students Fick and Wislicenius produced evidence that threw great doubt on this question, almost completely disproving it in their Faulhorn experiment [see (8) for discussion]. Taking into account the extent to which AAs could act as a fuel for muscle and the number of hours a day a physically active person exercises, we would not expect that this would much alter their protein requirements. In our opinion, and we do not believe that any of the results in the succeeding years have done anything to materially change the facts, the accelerated oxidation of leucine and other BCAAs during exercise is insufficient to increase the protein requirements of even very highly active individuals because the contribution of protein to fuel metabolism is relatively small— $<20\%$  of the total energy requirements (Fig. 1).

In fact, recent evidence suggests that training increases the efficiency of protein metabolism so that net balance is improved with exercise, whether moderate, medium-term endurance exercise or resistance training. Also, the amount of protein required to build and maintain muscle can easily be obtained through normal diet sufficient to satisfy the total energy requirements of the individual concerned because most diets



**FIGURE 1** Calculated total amount of energy released during steady-state exercise and contribution of AA oxidation (shaded portion). Values calculated from data in Refs. 3–6.

contain 10%–15% protein. Recent evidence shows that endurance training does not increase leucine oxidation and that protein metabolism becomes more efficient [i.e., increased protein balance achieved at a lower rate of oxidation (9)]. Sadly, this kind of scientific argument has had almost no influence upon the mystical adherence of athletes and there are still a substantial number of scientists who maintain that physical exercise increases protein requirements, reinforced by the commercial pressure of internet advertising to sell worthless protein supplements.

#### *The anabolic effects of amino acids per se on human muscle protein turnover*

As we first showed 25 y ago (10), feeding small aliquots of an enteral feed at half-hour intervals approximately doubled muscle protein synthesis (MPS) measured as the incorporation of  $^{13}\text{C}$  leucine into human muscle sampled by a needle biopsy. Subsequently (11), we found that infusing mixed AAs at a moderate rate sufficient to increase plasma AA concentrations to a value similar to that seen after ingestion of a small meal caused an increase in MPS of about the same magnitude as that caused by a complete meal. There was no change in blood glucose concentration and the rise in plasma insulin availability was modest, from 7–10  $\mu\text{U/mL}$ . The MPS stimulation was thus somewhat of a surprise because 1) it was generally assumed that AAs could not have a mass action effect in driving protein synthesis (partly because the  $K_m$  of the aminoacyl tRNA synthetase was sufficiently low that, at all likely concentrations of intramuscular AAs, the enzyme would be saturated) and 2) protein synthesis was widely believed to be insulin dependent. Nevertheless, the results were clear—AAs stimulated human MPS in the absence of any other exogenous substrate and without a large rise in insulin concentration. We also showed that, unlike in rodents, insulin alone would not stimulate MPS unless there was sufficient availability of AAs (12,13) and, although some workers have been unable to obtain an effect of stimulation of MPS by insulin (14), others have been able to demonstrate its anabolic effect using both incorporation and arteriovenous tracer flux measurements (15).

At about this time, there was considerable controversy regarding the relative efficacy and accuracy of the two main approaches to the measurement of MPS that differed in their method of delivery of labeled AAs to muscle. It was our contention that the large flooding-dose method (using  $^{13}\text{C}$  leucine or  $\text{D}_5$ -phenylalanine) (16) produced artifactually high rates of MPS and that this elevation was one of the reasons why it was difficult for workers using this method to demonstrate increases in MPS on feeding (17). To resolve this problem, we carried out a number of studies in which we constantly infused AA tracers

and, after making a measurement of MPS, arranged for a flooding dose of a variety of nonessential and essential amino acids (EAAs) to be superimposed (18). It had not been our intention specifically to investigate the effects of particular AAs, but we found that we could stimulate MPS by about 2-fold using flooding doses of EAAs (like valine, leucine, and phenylalanine) but not nonessential AAs (like serine, alanine, or proline). In fact, this work turned out to be useful in two ways: First, it alerted us to the possibility that indeed there was a substantial signaling role for EAAs, and particularly leucine, in stimulating human MPS (at least within the time frame of our experiment); and second, it paved the way for our later use of the flooding-dose method utilizing labeled proline to measure collagen synthesis in muscle, bone, tendon, and ligament.

Looking back, it seems that these studies inadvertently proved something to which many of us had been resistant, i.e., the idea that provision of a single AA could stimulate MPS in the absence of an abundance of other AAs. Hitherto, there had been a wealth of data provided from animal studies suggesting that BCAAs and leucine, in particular, did stimulate MPS in isolated animal preparations (19,20), but there had been no unequivocal demonstration of the effect in human muscle (until we inadvertently carried out the crucial study). Obviously, if leucine were capable of independently stimulating MPS, substrate would have to be drawn from the intracellular pool and the plasma and, as long as there was a sufficiency of AA delivered (e.g., by protein breakdown in other tissues), synthesis would continue. However, it could be predicted that, under these circumstances, intramuscular AA concentrations should fall and indeed this is what had previously been reported in studies on the effects of leucine infusion on human muscle carried out by Swedish workers (21).

#### Latency, duration, and dose response relation between amino acid availability and MPS

It seemed obvious that, if AAs were having a signaling role, we needed more information about the nature of the effects in muscle and therefore we began, with colleagues in Bob Wolfe's laboratory in Galveston, to investigate how long it took for AAs to have their effect, how big the effect was, how long it lasted, and what the dose response relation was.

The results of the first study (22) were somewhat of a surprise. We designed the study to allow us to make measurements of MPS before and during the infusion of mixed AAs over a period of 6 h. There appeared to be a latent period of about one-half hour before any increase in incorporation of AAs could be measured, but then there was a rapid and rather large stimulation of protein synthesis—somewhat larger than we had previously obtained when making measurements over periods of about 4–6 h. We also observed something that in retrospect seems obvious, i.e., that MPS turned off after about 2 h despite the continued availability of AAs, returning to basal postabsorptive values. In fact, it now seems that the behavior of muscle in the presence of exogenous AAs was similar to that predicted by Joe Millward (23) in suggesting that there was an upper limit to the amount of protein that could be contained within the muscle at a given time, determined ultimately by the muscle RNA:protein ratio and the connective tissue extracellular three-dimensional network.

The implications of our findings are interesting in three ways: First, the results suggest that there must be a desensitization of the signaling mechanism that senses and transmits information concerning the availability of AAs; second, it would seem that long-term infusions of parenteral AA-containing solutions are unlikely to maintain an anabolic effect

beyond a couple of hours and that any AA not taken into tissue protein will simply be catabolized to urea. Last, it seems that, in the future, if accurate values of human MPS are to be obtained, then studies need to be confined to periods of <3 h, with 2.5 h seeming to be optimal; values obtained during studies of 4–6 h and longer periods are likely to be wrong.

When we carried out studies in which we made measurements of MPS at different rates of infusion of mixed AAs (24), we obtained evidence of a curvilinear relation between MPS and plasma EAAs, but not nonessential AAs (many of which are, of course, synthesized, using nitrogen transaminated from BCAAs). Revisiting the data we were also able to find a good relation between the increase in MPS and extracellular leucine concentration (Fig. 2).

An interesting feature of the results was that there appeared to be a relation between intracellular AAs and MPS that was counterintuitive; in other words, with low and modest increases in extracellular AAs, intracellular EAAs actually showed a fall as protein synthesis was stimulated, although, with large increases in the rate of infusion of mixed AAs, all intramuscular concentrations of AAs were elevated. We have been able to reproduce the effect during administration of oral EAAs (25) so the evidence for the existence of the phenomenon seems quite firm. This behavior of AAs appeared to suggest that the stimulation of MPS itself was causing a fall in intracellular AA concentrations possibly due to their increased utilization for protein. This is a surprising conclusion because our previous work in animals (7) suggested strongly that rates of AA transport were much faster than any possible rate of MPS; thus the results are puzzling. Nevertheless, we believe our interpretation to be correct because a similar fall in intracellular AAs can be observed in human muscle when leucine alone is infused or when proteins deficient in particular AAs like albumin are fed as meals [see (24) for discussion]. In this circumstance, any stimulation of protein synthesis due to leucine and albumin would be short lived given the nature of albumin's deficiency in isoleucine. The other conclusion from this work is that any nutrient sensing must be due to an *extracellular* sensor because it is difficult to understand how an intracellular sensor could cause a rise in MPS under circumstances in which intramuscular leucine concentration was falling.

We later repeated the studies of the dose response on MPS to exogenous EAAs delivered not by intravenous infusion but orally (25). In these studies, we were again able to show a strong curvilinear relation between availability of plasma AAs and

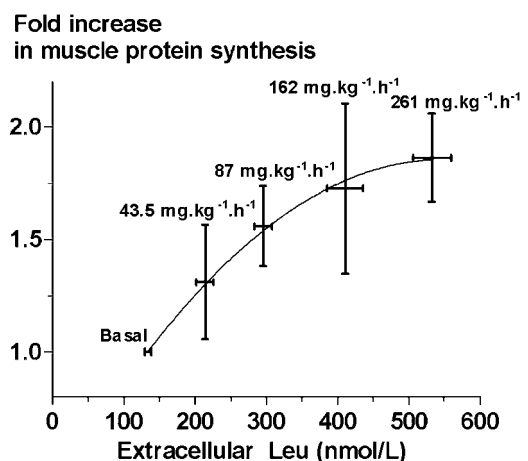


FIGURE 2 Relation between increase in human MPS and plasma leucine concentration during infusion of mixed AAs as described in Ref. 24. Values are means  $\pm$  SEM.



MPS in terms of both absolute and relative increase; the relations were most clearly marked when the rates were related to plasma leucine concentration or the area under the plasma leucine curve for 3 h after administration of the oral dose.

### Nutrient sensing and signaling

In the latter studies, we showed clearly that administration of exogenous AAs stimulated changes in phosphorylation of elements of the mammalian target of rapamycin (mTOR) pathway with increases in phosphorylation of mTOR itself and of the p70 ribosomal subunit S6 kinase (p70 S6 kinase) and eukaryotic initiation factor 4 binding protein 1 (eIF4 BP1) with relatively small doses of EAAs—10 g (25). The Boirie group (26) were also able to obtain evidence of stimulation of this pathway with a combination of hyperinsulinemia and hyperaminoacidemia, but our studies were carried out at concentrations of insulin that were clamped at 10  $\mu$ U/mL, suggesting that, as we had previously hypothesized, large increases in insulin were not required for an anabolic stimulation of MPS by AAs. We also found that, in elderly men studied during an insulin clamp at 10  $\mu$ U/mL, feeding EAAs resulted in a smaller rise in MPS, accompanied by smaller increases in anabolic signaling, but without any deficits in protein turnover in the postabsorptive state.

### The role of insulin in control of muscle protein turnover

The relation between AA availability, insulin availability, and stimulation of muscle anabolism is still far from clear. In the studies alluded to above, we have evidence that AA-induced increases in plasma insulin concentrations to values above 10  $\mu$ U/mL had little effect on MPS, but this does not rule out the permissive effect for insulin at concentrations between 0 and 10  $\mu$ U/mL. In very recent studies, we have attempted to address this question by measuring MPS and breakdown using the arteriovenous tracer flux technique as well as by measuring MPS as the leucine incorporation into muscle. The results show unequivocally that, at insulin concentrations below 5  $\mu$ U/mL, exogenous AAs stimulate MPS (27) but, surprisingly, given our previous results, do not stimulate phosphorylation of anabolic signaling proteins in the mTOR pathway. When insulin was administered to achieve concentrations of about 15, 30, and 100  $\mu$ U/mL, there was no further increase in MPS measured by either of the two methods, but there was a marked depression of muscle protein breakdown that appeared to be maximal at 15  $\mu$ U/mL (27). In addition, there appeared to be a strong dose response relation between the concentration of insulin and phosphorylation of mTOR and p70 S6 kinase (M. J. Rennie, P. L. Greenhaff, and H. Wackerhage, unpublished results). These results strongly suggest that, in the absence of insulin, AAs are able to signal their presence without involvement of p70 S6 kinase or mTOR, but that the effects of insulin and AAs must share a final common pathway because the effects are maximal in the presence of high physiological insulin concentrations. It is possible that AAs signal through other nutrient-sensing and signaling pathways involving eukaryotic initiation factor 2B and glycogen synthase kinase or hitherto unrecognized pathways involving protein kinase B.

### Implications for protein requirements and clinical practice

Muscle has a substantial ability to detoxify BCAAs producing glutamine and alanine, and oxidizing the

branched-chain keto-acids. This metabolic activity is accelerated during muscular exercise largely driven by increased blood flow through muscle under circumstances of normal AA concentrations. There seems to be little evidence that higher rates of physical activity increase protein requirements beyond those achievable by eating a normal diet that satisfies energy requirements. Providing increased exogenous BCAAs is likely to stimulate MPS (and possibly decreased muscle protein breakdown), but the effect is likely to be short lived given the muscle-full phenomenon. It may be that administration of AAs needs to mimic a pattern of meal feeding to allow the set point of muscle fullness to be adjusted to allow the success of anabolic stimuli.

### LITERATURE CITED

1. Rennie MJ, Edwards RH, Krywawych S, Davies CT, Halliday D, Waterlow JC, Millward DJ. Effect of exercise on protein turnover in man. *Clin Sci*. 1981; 61:627–39.
2. Millward DJ, Davies CT, Halliday D, Wolman SL, Matthews D, Rennie M. Effect of exercise on protein metabolism in humans as explored with stable isotopes. *Fed Proc*. 1982;41:2686–91.
3. Davies CTM, Halliday D, Millward DJ, Rennie MJ, Sutton JR. Glucose inhibits CO<sub>2</sub> production from leucine during whole-body exercise in man. *J Physiol (Lond)*. 1982;332:41–42P.
4. Rennie MJ, Halliday D, Davies CTM, Edwards RHT, Krywawych S, Millward DJ, Matthews DE. (1981) Exercise induced increase in leucine oxidation in man and the effect of glucose. In: Walser M, Williamson, JR, editors, *Metabolism and Clinical Implications of Branched Chain Amino and Keto Acids*. New York: Elsevier-North Holland, p. 361–6.
5. Bowtell JL, Leese GP, Smith K, Watt PW, Nevill A, Rooyackers O, Wagenmakers AJM, Rennie MJ. Modulation of whole body protein metabolism, during and after exercise, by variation of dietary protein. *J Appl Physiol*. 1998;85:1744–52.
6. Bowtell JL, Leese GP, Smith K, Watt PW, Nevill A, Rooyackers O, Wagenmakers AJ, Rennie MJ. Effect of oral glucose on leucine turnover in human subjects at rest and during exercise at two levels of dietary protein. *J Physiol*. 2000;525:271–81.
7. Hundal HS, Rennie MJ, Watt PW. Characteristics of acidic, basic and neutral amino acid transport in the perfused rat hindlimb. *J Physiol (Lond)*. 1989; 408:93–114.
8. Rennie MJ. (1996) Influence of exercise on protein and amino acid metabolism. In: Rowell LB, Shepherd JT, editors, *American Physiological Society Handbook of Physiology: Exercise*. pp. 995–1035. Oxford University Press, New York.
9. Gaine PC, Viesselman CT, Pikosky MA, Martin WF, Armstrong LE, Pescatello LS, Rodriguez NR. Aerobic exercise training decreases leucine oxidation at rest in healthy adults. *J Nutr*. 2005;135:1088–92.
10. Rennie MJ, Edwards RH, Halliday D, Matthews DE, Wolman SL, Millward DJ. Muscle protein synthesis measured by stable isotope techniques in man: the effects of feeding and fasting. *Clin Sci (Lond)*. 1982;63:519–23.
11. Bennet WM, Connacher AA, Scrimgeour CM, Smith K, Rennie MJ. Increase in anterior tibialis muscle protein synthesis in healthy man during mixed amino acid infusion: studies of incorporation of [1-<sup>13</sup>C]leucine. *Clin Sci (Lond)*. 1989;76:447–54.
12. Bennet WM, Connacher AA, Smith K, Jung RT, Rennie MJ. Inability to stimulate skeletal muscle or whole body protein synthesis in type 1 (insulin-dependent) diabetic patients by insulin-plus-glucose during amino acid infusion: studies of incorporation and turnover of tracer L-[1-<sup>13</sup>C]leucine. *Diabetologia*. 1990;33:43–51.
13. Bennet WM, Connacher AA, Scrimgeour CM, Jung RT, Rennie MJ. Euglycemic hyperinsulinemia augments amino acid uptake by human leg tissue during hyperaminoacidemia. *Am J Physiol*. 1990;259:E185–94.
14. Gelfand RA, Barrett EJ. Effect of physiologic hyperinsulinemia on skeletal muscle protein synthesis and breakdown in man. *J Clin Invest*. 1987;80:1–6.
15. Biolo G, Fleming RYD, Wolfe RR. Physiologic hyperinsulinemia stimulates protein synthesis and enhances transport of selected amino acids in human skeletal muscle. *J Clin Invest*. 1995;95:811–9.
16. Garlick PJ, Wernerman J, McNurlan MA, Essen P, Lobley GE, Calder GA, Vinnars E. Measurement of the rate of protein synthesis in muscle of postabsorptive young men by injection of a “flooding dose” of [1-<sup>13</sup>C]leucine. *Clin Sci*. 1989;77:329–36.
17. McNurlan MA, Essen P, Milne E, Vinnars E, Garlick PJ, Wernerman J. Temporal response of protein synthesis in human skeletal muscle to feeding. *Br J Nutr*. 1993;69:117–26.
18. Smith K, Reynolds N, Downie S, Patel A, Rennie MJ. Effects of flooding amino acids on incorporation of labeled amino acids into human muscle protein. *Am J Physiol*. 1998;275:E73–8.

19. Buse MG, Reid SS. Leucine: a possible regulator of protein turnover in muscle. *J Clin Invest.* 1975;56:1250–61.
20. Buse MG, Weigand DA. Studies concerning the specificity of the effect of leucine on the turnover of proteins in muscles of control and diabetic rats. *Biochim Biophys Acta.* 1977;475:81–9.
21. Alvestrand A, Hagenfeldt L, Merli M, Oureshi A, Eriksson LS. Influence of leucine infusion on intracellular amino acids in humans. *Eur J Clin Invest.* 1990;20:293–8.
22. Bohé J, Low JF, Wolfe RR, Rennie MJ. Latency and duration of stimulation of human muscle protein synthesis during continuous infusion of amino acids. *J Physiol.* 2001;532:575–9.
23. Millward DJ. A protein-stat mechanism for regulation of growth and maintenance of the lean body mass. *Nutr Res Rev.* 1995;8:93–120.
24. Bohé J, Low A, Wolfe RR, Rennie MJ. Human muscle protein synthesis is modulated by extracellular, not intramuscular amino acid availability: a dose-response study. *J Physiol.* 2003;552:315–24.
25. Cuthbertson D, Smith K, Babraj J, Leese G, Waddell T, Atherton P, Wackerhage H, Taylor PM, Rennie MJ. Anabolic signaling deficits underlie amino acid resistance of wasting, aging muscle. *FASEB J.* 2005;19:422–4.
26. Guillet C, Prod'homme M, Balage M, Gachon P, Giraudet C, Morin L, Grizard J, Boirie Y. Impaired anabolic response of muscle protein synthesis is associated with S6K1 dysregulation in elderly humans. *FASEB J.* 2004;18:1586–7.
27. Greenhaff PL, Peirce N, Simpson E, Hazell M, Babraj J, Waddell T, Smith K, Rennie M. Dose-response relationship during hyperaminoacidaemia between insulin and leg protein turnover in healthy young men studied by tracer amino acid exchange. *J Physiol* 2005;558P.