Differences in Estimates of Forearm Protein Synthesis Between Leucine and Phenylalanine Tracers Following Unbalanced Amino Acid Infusion

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We compared the leucine (Leu) and phenylalanine (Phe) tracer-determined response of forearm protein synthesis (PS) before and after stimulation of protein anabolism by intravenous infusion of Leu-enriched, Phe-deficient amino acids and insulin (increased to ≈100 μU/mL) with the euglycemic clamp. Six healthy subjects received primed-constant infusions of L-[ring-$^2H_5$]-Phe and L-[1-^{14}C]-Leu, and steady-state forearm Phe and Leu kinetics were determined. Following the combined infusion, the arterial Leu concentration increased approximately 70% ($P < .001$), whereas Phe decreased about 15% ($P < .01$). Forearm PS and net balance (NB) increased ($P < .05$ or less *v* basal) using both amino acid tracers. However, the relative increments observed with the Leu tracer were more than 75% larger ($P < .05$ or less) than those observed with the Phe tracer, even when the data were corrected for the standard relative abundance of these two amino acids in forearm protein(s). Thus, the calculated changes of forearm PS and NB in response to an unbalanced amino acid infusion with hyperinsulinemia were affected by the plasma level of leucine and phenylalanine, whose tracers were used to estimate forearm protein turnover. Since these two essential amino acids share the same transport system, a competition at this level cannot be excluded.

MUSCLE PROTEIN SYNTHESIS (PS) in humans is usually estimated from the infusion of tracer(s) of essential amino acids by measuring either the tracer incorporation into muscle protein(s) through biopsy[1-8] and/or the exchange across a limb (forearm and/or leg) of the labeled and unlabeled amino acid(s).[2,9-21] A common assumption is that muscle PS can be calculated from the nonoxidative disappearance of any essential amino acid, after correction for the relative abundance of that amino acid in mixed muscle proteins. The same assumptions and calculations can be made regarding the appearance of essential amino acids from forearm proteolysis.

Whether this assumption is valid under all circumstances has not been thoroughly investigated. For instance, the stimulatory effect of hyperaminoacidemia on muscle PS has been commonly determined using tracer(s) of essential amino acid(s) (usually leucine or phenylalanine), whose concentrations were also acutely increased by the exogenous amino acid infusion.[1,7,9,10,12,16,17,20] However, under these conditions, the measured increase in the rate of amino acid disposal into muscle protein(s) could simply be the result of a mass-effect due to the increased concentrations, also involving the tracer. On the other hand, if the theory is valid, the same results, from both a qualitative and a quantitative standpoint, should be simultaneously observed with a tracer of another essential amino acid whose concentration is not increased by the exogenous amino acid infusion.

This general assumption has been recently challenged at the whole-body level.[22] When the concentration of the essential amino acid leucine was acutely elevated by a branched-chain amino acid (BCAA)-enriched, aromatic amino acid (AAA)-deficient amino acid solution, whole-body PS estimated from the leucine tracer technique was also increased. In contrast, a significantly smaller increase was observed when body PS was simultaneously measured using tracers of phenylalanine and tyrosine, whose concentrations were not concurrently increased by the exogenous amino acid infusion.[22] These discrepancies were observed despite a fixed anabolic stimulation induced by concurrent hyperinsulinemia. This observation may be relevant to the fact that many published estimates of PS in humans, in both physiological and pathological conditions, could themselves have been affected by the prevailing plasma amino acid concentration(s) attained during the study, and by the corresponding tracer amino acid(s) used. Whether these discrepancies, described at the whole-body level thus far,[22] can be detected also at the forearm level is not known. Therefore, the aim of this study was to determine the response of forearm (ie, muscle) PS in humans following the simultaneous infusion of tracers of two essential amino acids, leucine and phenylalanine, whose concentrations were changed differently by the exogenous unbalanced amino acid mixture. We used the previously reported[22] BCAA-enriched, AAA-deficient amino acid solution that increased the concentration of leucine and decreased that of phenylalanine. Insulin was also infused with the amino acids to provide a fixed, independent stimulation of muscle protein anabolism.

MATERIALS AND METHODS

Isotopes

1-[^{14}C]-leucine (^{14}C-Leu, ≈55 mCi/mmol), more than 95% pure by high-performance liquid chromatography (HPLC) analysis, and ^{14}C-sodium bicarbonate (≈100 mCi/mmol) were purchased from Amersham (Buckinghamshire, UK). L-[ring-$^2H_5$]-phenylalanine (D_5-Phe, ≈99 atom percent excess) was purchased from Tracer Technologies (Somerville, MA). All tracers were dissolved in 0.9% saline, filtered, and proven to be sterile and pyrogen-free before use.

Experimental Design

Six healthy male subjects (age, 25 to 32 years; body mass index, 22.5 ± 1.2 kg/m^2) were studied after an overnight fast. All subjects were informed about the aims of the study and provided written informed consent. In the absence of an Ethics Committee, the study was approved by the appropriate local authorities (ie, the Dean of the Medical Faculty and the Director of the Department), and it was

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performed according to the recommendations of the local Radiation Safety Officer. Whole-body radiation exposure due to ^{14}C-leucine and ^{14}C-bicarbonate was estimated to be about 100 mrad.[23] All subjects were adapted to a weight-maintaining diet containing about 50% (as percent of total calories) carbohydrate, 30% fat, and 20% protein for at least 1 month prior to the study.

At 7:30 AM, a forearm vein was cannulated with an 18-gauge polyethylene catheter for isotope, glucose, insulin, and amino acid infusions. The brachial artery and a deep forearm vein of the opposite arm were also cannulated for arterial and venous forearm blood sampling. At −180 minutes, after collection of basal blood samples, primed (≈60 times the continuous infusion rate per minute) continuous infusions of ^{14}C-Leu (5,668 ± 482 dpm × kg^{-1} × min^{-1}) and D_5-Phe (0.05 ± 0.001 μmol × kg^{-1} × min^{-1}) were initiated. Venous samples were taken every 30 minutes for 150 minutes to assess the achievement of steady-state isotope and substrate concentrations (data not reported). A steady state was defined as the absence of a slope significantly different from 0 and of changes in the concentration, specific activity, and enrichment greater than 5%. Thereafter, between −30 and 0 minutes, four samples of arterial and deep-venous blood were collected at 10-minute intervals for baseline measurements. During sample collection, blood flow to the hand was occluded with a pediatric sphymomanometer placed around the wrist and inflated above systolic pressure. Each arterial sample was collected starting a few seconds after completion of the corresponding venous sample to avoid acute forearm hypoperfusion. At 0 minutes, an amino acid infusion was started in combination with the euglycemic-hyperinsulinemic clamp for 3 hours. Insulin was infused at a rate of 0.05 U × m^2 body surface^{-1} · min^{-1}. A BCAA-enriched, AAA-deficient solution (L-Amino Acidi Selettivi; Boehringer Mannheim Italia, Milan, Italy) was infused at a constant rate via a calibrated pump. Its composition, expressed as grams per liter, was as follows: alanine 84.3, arginine 34.5, phenylalanine 6.1, glycine 120, isoleucine 68.7, histidine 15.5, leucine 83.4, lysine 41.6, methionine 6.7, proline 69.6, serine 47.6, threonine 37.8, tryptophan 3.7, and valine 71.8. The rate of leucine and phenylalanine exogenous infusion was 1.95 ± 0.13 and 0.13 ± .006 μmol × kg^{-1} × min^{-1}, respectively. Tyrosine and glutamine were not present in this solution. The infused mixture, often used in the parenteral nutrition of patients with liver disease,[24] provided approximately 1 mmol amino acids · min^{-1}. Euglycemia was maintained by a variable-rate glucose infusion using a 20% (wt/vol) dextrose solution. Arterial and deep-venous blood samples were again collected at 120, 150, 160, 170, and 180 minutes. A steady state for all measured parameters was again attained at least by 120 minutes after the start of the combined infusion (data not shown).

Analytical Methods

The plasma concentration and specific activity (SA) of leucine and α-ketoisocaproate (KIC),[25] as well as the plasma phenylalanine concentration,[26] were determined by HPLC. Plasma concentrations of other amino acids were determined as indicated previously.[22] Phenylalanine enrichment was determined by gas chromatography–mass spectrometry as the *tert*-butyl-dimethyl-silyl derivative[27] and expressed as the mole percent enrichment (MPE), ie, the [tracer]/[tracee + tracer] ratio. The arterial and deep-venous blood concentration of ^{14}C-bicarbonate was measured as described previously.[18-20] Plasma flow was determined with a dye-dilution method using indocyanine green (Vert d'Indocyanine; SERB, Paris, France).[18] In four subjects, postinfusion whole-blood and plasma arterial and venous samples were simultaneously obtained for measurement of phenylalanine, leucine, and KIC concentrations, phenylalanine MPE, leucine and KIC SA, and ^{14}C-bicarbonate concentration. Whole-blood analyses were performed as previously described.[18,20] Forearm mass (=volume) was measured by anthropometry by considering the forearm (without the hand) as a truncated cone. Plasma glucose, insulin, and glucagon concentrations were measured as described elsewhere.[22]

Calculations

All kinetic data were calculated under steady-state conditions for the concentration, SA, and MPE, effectively achieved during the last 30 minutes of both the basal and infusion periods (data not shown). Forearm phenylalanine rates of appearance (Ra) and disappearance (Rd) were calculated using the forearm model described by Gelfand and Barrett.[13] Since phenylalanine is not catabolized by muscle, its Rd across the forearm reflects its disposal into PS. Forearm leucine kinetics were calculated using a leucine model similar to that of phenylalanine[13] but also including data for forearm leucine oxidation,

$$\text{Leu Ox} = \frac{*[\text{Bic}_{\text{ven}}] - *[\text{Bic}_{\text{art}}]}{\text{KIC SA}_{\text{ven}}} \times \text{F}, \qquad \text{Eq 1}$$

where $*[\text{Bic}_{\text{ven}}]$ and $*[\text{Bic}_{\text{art}}]$ are ^{14}C-bicarbonate blood concentrations (in dpm per milliter) in artery and vein, respectively, KIC SA_{ven} is venous KIC SA (in dpm per nanomole), and F is forearm plasma flow (in milliliters per minute per 100 mL forearm volume). The rate of leucine disposal into forearm PS (Leu → PS) was then calculated by subtracting Leu oxidation from the Leu Rd:

$$\text{Leu} \rightarrow \text{PS} = \text{Leu Rd} - \text{Leu Ox}. \qquad \text{Eq 2}$$

Net leucine balance into/out of protein was calculated by subtracting the Leu Ra from Leu → PS.

The leucine kinetic data were recalculated also by including KIC (both unlabeled and labeled) in the overall estimation of leucine-carbon exchange across the forearm as previously described,[13] and by using the average between arterial Leu and KIC SA. Data extrapolation from leucine and phenylalanine to estimated protein kinetics was performed using standard assumptions, ie, 9% and 4.2% (on a molar basis) of all amino acid residues in average forearm proteins are represented by leucine and phenylalanine, respectively.[28-30]

Statistical Analysis

All data are expressed as the mean ± SEM. The one-tailed Student *t* test for paired data was used to compare basal versus infusion periods. The Wilcoxon test was used when data distribution was not normal. These paired tests were used also in the comparisons of two sets of data within the same subjects (ie, changes in forearm PS and net balance [NB] using the phenylalanine *v* leucine arteriovenous model). A *P* value less than .05 was considered statistically significant.

RESULTS

Hormone and Metabolite Concentrations

Plasma glucose was maintained at near-baseline values (≈85 mg/dL) throughout the amino acid and insulin infusion. Insulin was increased ($P < .001$) from 11 ± 4 to 100 ± 7 μU/mL, while glucagon did not change (from 93 ± 20 to 96 ± 23 pg/mL). Leucine concentrations increased about 70% ($P < .001$), KIC did not change, and phenylalanine decreased ($P < .01$) by approximately 15% (Table 1). Phenylalanine and leucine forearm arteriovenous concentration differences changed to a positive value following the combined infusion ($P < .05$ or less *v* baseline), indicating net uptake, at variance with KIC, which showed net release (Table 1). The arterial concentration of other measured amino acids increased to a variable extent ($P < .01$ or less *v* baseline; isoleucine, from 48 ± 4 to 163 ± 11 μmol/L; valine, from 208 ± 22 to 431 ± 30; alanine, from 256 ± 23 to 462 ± 22; serine, from 110 ± 8 to 163 ± 5; glycine, from 230 ± 23 to 525±35; and lysine, from 251 ± 12 to 380 ± 26). The tryptophan concentration did not change (from 52 ± 4 to

Table 1. Steady-State Plasma Leu, KIC, and Phe (nmol/mL), ^{14}C-Leu and ^{14}C-KIC Isotope Concentration (dpm/mL) and SA (dpm/nmol), ^{15}N-Leu and D_5-Phe Isotope Concentration (nmol/mL) and MPE, and Blood ^{14}C-Bicarbonate Concentration (dpm/mL) in the Basal State and Following Infusion of Amino Acids and Insulin With the Euglycemic Clamp

Parameter	Basal		Amino Acids + Insulin	
	Artery	Vein	Artery	Vein
Leu	151 ± 6	151 ± 5	254 ± 8*	227 ± 10*†
KIC	43 ± 2	43 ± 2	44 ± 3	46 ± 4†
Phe	59 ± 1	61 ± 1†	51 ± 1*	47 ± 1*
^{14}C-Leu	458 ± 49	389 ± 48†	439 ± 42*	364 ± 39*†
^{14}C-KIC	101 ± 13	94 ± 12†	61 ± 8*	65 ± 8*†
D_5-Phe	4.1 ± 0.2	3.7 ± 0.2†	3.7 ± 0.2*	3.2 ± 0.2*†
^{14}C-Leu SA	3.0 ± 0.3	2.6 ± 0.3†	1.7 ± 0.2*	1.6 ± 0.2*†
^{14}C-KIC SA	2.3 ± 0.2	2.2 ± 0.2†	1.4 ± 0.2*	1.4 ± 0.2*
D_5-Phe MPE	6.9 ± 0.4	6.1 ± 0.4†	7.4 ± 0.5	6.8 ± 0.5*†
^{14}C-Bicarbonate	151 ± 32	164 ± 34†	273 ± 42*	280 ± 43*†

*$P < .05$ or less, infusion *v* basal.
†$P < .05$ or less, vein *v* artery.

58 ± 4). Tyrosine, which was not contained in the infused solution, decreased ($P < .001$) approximately 60%, from 59 ± 6 to 21 ± 2 μmol/L. Glutamine was not determined. Forearm flow increased ($P = .055$) from 26 ± 10 to 33 ± 2 mL × min^{-1} × 100 mL^{-1} forearm volume.

Forearm Phenylalanine Versus Leucine Kinetics

In the fasting state, the forearm Ra of both phenylalanine and leucine exceeded their disposal into PS, indicating a net negative forearm amino acid (ie, protein) balance (Table 2). Following the amino acid and insulin infusion, the phenylalanine Rd increased approximately 70% ($P < .05$), whereas the decrease in Ra (−35%) was not significant. Consequently, phenylalanine NB switched from a negative to a positive value ($P < .01$) (table 2). Leucine disposal into PS (Leu → PS) increased near threefold ($P < .01$), whereas the leucine Ra from degradation decreased slightly although unsignificantly. As a result, leucine NB balance into/out of protein switched from the negative fasting value to a positive value ($P < .01$) (Table 2). Forearm leucine oxidation did not substantially change (13 ± 2 in the basal period *v* 14 ± 3 nmol × min^{-1} × 100 mL^{-1} of forearm in the infusion period), because it increased in three subjects whereas it decreased in the three others. Leucine oxidation was also essentially the same when it was determined using the complete arteriovenous plasma samples in four subjects (basal plasma oxidation, 11 ± 4 nmol × min^{-1} × 100 mL^{-1} of forearm; plasma oxidation following the infusion, 12 ± 7 nmol × min^{-1} × 100 mL^{-1} of forearm).

Forearm PS, degradation and NB, expressed as micrograms of protein per minute × 100 mL of forearm, ie, calculated from the two amino acid tracers after correction for their known average molar abundance in proteins,[28-30] are also reported in Table 2. In the basal state, these rates were not significantly different between the phenylalanine and the leucine data. However, following the anabolic infusion, both PS ($P < .03$) and protein NB ($P < .01$) were significantly greater when calculated using the leucine-derived than the phenylalanine-derived data. Furthermore, the leucine-derived increments versus basal of both PS (+118 ± 29 μg × min^{-1} × 100 mL^{-1} of forearm) and NB (+127 ± 24 μg × min^{-1} × 100 mL^{-1} of forearm), were both significantly greater ($P < .035$ and $P < .05$, respectively) than those calculated from the phenylalanine tracer (+40 ± 16 and +69 ± 25 μg × min^{-1} × 100 mL^{-1} of forearm, respectively) (Fig 1). The change in forearm protein degradation was similar when calculated with the leucine (−10 ± 2 μg × min^{-1} × 100 mL^{-1}) versus the phenylalanine (−29 ± 25) tracer. When KIC was included into the calculations, a significant difference with the phenylalanine-derived data persisted regarding the increment in leucine disposal into PS (+111 ± 30 μg × min^{-1} × 100 mL^{-1}, $P < .04$), whereas the changes of protein breakdown (−4 ± 22) and of NB (+115 ± 24) were not significantly different.

In the four subjects in whom analyses in whole-blood were also performed, the postinfusion phenylalanine-derived estimates of PS, degradation and NB were 158 ± 39, 107 ± 29, and +52 ± 30 μg protein × min^{-1} × 100 mL^{-1} of forearm. Conversely, the corresponding leucine-derived estimates were 400 ± 119, 168 ± 62, and 232 ± 62 μg of protein × min^{-1} × 100 mL^{-1} of forearm, respectively, all significantly lower ($P < .05$ or less) than those calculated with phenylalanine.

DISCUSSION

Our study shows that the relative increase of both forearm PS and NB extrapolated from the leucine tracer was greater than

Table 2. Steady-State Phe and Leu Forearm Ra and Rd Into PS (Rd → PS) and NB Into/Out of Protein, in the Basal State and Following Infusion of Amino Acids and Insulin With the Euglycemic Clamp

Parameter	Basal			Amino Acids + Insulin		
Amino acid data (nmol × min^{-1} × 100 mL^{-1})	Ra	Rd → PS	NB	Ra	Rd → PS	NB
Phe	22 ± 6	14 ± 3†	−8 ± 1	14 ± 3	24 ± 4*†	+10 ± 4*
Leu	61 ± 12	48 ± 8†	−14 ± 6	55 ± 15*‡	128 ± 26*†‡	+74 ± 12*‡
Protein data (μg × min^{-1} × 100 mL^{-1})	PD	PS	NB	PD	PS	NB
Phe-derived	85 ± 21	55 ± 11†	−30 ± 13	56 ± 12	95 ± 17*†	+39 ± 17*
Leu-derived	89 ± 18	69 ± 11†	−20 ± 8	80 ± 22*	187 ± 38*†‡	+107 ± 17*‡

NOTE. These kinetic data were calculated using an arteriovenous approach[13] and arterial amino acid MPE or SA, with the exception of leucine oxidation, calculated using venous KIC SA.
Abbreviation: PD, protein degradation; PS, protein synthesis.
*$P < .05$ or less, infusion *v* basal.
†$P < .05$ or less, PS *v* PD.
‡$P < .05$ or less, Leu *v* Phe.

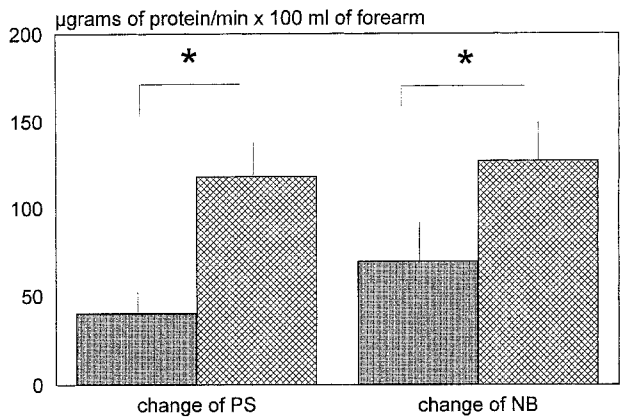

Fig 1. Changes in forearm PS and NB (μg × min^{-1} × 100 mL^{-1} forearm) calculated using either the Phe (■) or Leu (▨) noncompartmental arteriovenous model before and after the unbalanced amino acid and insulin infusion. Data were converted from nmol amino acid to μg protein by assuming that Leu and Phe account, on a molar basis, for 9% and 4.2%, respectively, of amino acid residues in forearm proteins. *$P < .05$ or less, Leu *v* Phe.

that estimated from the phenylalanine tracer (Fig 1 and Table 2) under conditions of increased leucine but modestly decreased phenylalanine following infusion of a BCAA-enriched, AAA-deficient amino acid solution. These differences persisted when the data were expressed as micrograms of protein per minute per 100 mL^{-1} forearm volume, to correct for the average relative abundance of leucine and phenylalanine in the amino acid residues of proteins (we assumed 9% and 4.2% on a molar basis for the two amino acids, respectively).[28-30] Indeed, without such a correction, the phenylalanine kinetic data, if expressed as nanomoles of the amino acid per minute per 100 mL^{-1}, would have been obviously lower than the data for leucine, just because of its lower abundance in proteins and lower turnover rate. Theoretically, the differences in forearm protein kinetics observed between the two amino acids also could be due to intrinsic differences in the metabolism of the two tracers, and not to the experimental conditions. However, such a possibility seems unlikely because (1) amino acid kinetics are proportional to their content in muscle proteins[28]; (2) in postabsorptive conditions, there were no significant differences between these two tracers on estimates of forearm protein turnover (Table 2 and prior studies[2,9,10,16,17,19,20]; (3) in the rat model, leucine-calculated PS was shown to be lower, not greater, than PS estimated with phenylalanine[31]; and (4) the higher leucine- versus phenylalanine-derived PS rates persisted when the data were expressed as the relative change versus baseline.

The reason(s) for the discrepancy in the results obtained with leucine versus phenylalanine tracers is not immediately evident. Selected changes in arterial amino acid levels may modify isotope equilibration between the plasma and the cell, their intracellular disposal, and the resulting kinetic measurements, ie, through a "flooding" effect.[3] Also, tissue amino acyl tRNA charging, as well as peptide-chain elongation, might be selectively modified, eventually resulting in different estimates for the rate of PS and NB. Finally, the BCAAs may have competitively inhibited the transmembrane transport of the AAAs, including phenylalanine,[32] leading to an underestimation of the phenylalanine-derived estimates of forearm PS.

The effects of unbalanced changes in the concentration of two or more essential amino acids, used also as markers of forearm protein turnover, was never directly investigated before. Previous studies from different laboratories were inconclusive[9,10,14-16] because this issue was not directly addressed or forearm leucine oxidation was not measured, therefore preventing the accurate measurement of both PS and NB. Louard et al[15] measured forearm protein turnover using leucine and phenylalanine tracers simultaneously before and after infusion of BCAAs alone. They found (1) increased concentrations of the BCAAs but decreased concentrations of other amino acids, including phenylalanine, and (2) an increased forearm leucine Rd but a decreased forearm phenylalanine Rd, the latter finding indicating decreased PS. By comparing the results of Heslin et al[14] with those of Neumann et al[16] performed under similar experimental conditions, forearm PS determined with the phenylalanine tracer (ie, phenylalanine Rd) was increased, paralleling the increase of phenylalanine concentration.[16] In contrast, the leucine Rd did not increase, paralleling the unchanged arterial plasma leucine concentration.[14,16] However, since forearm leucine oxidation was not measured,[14,16] PS could not be calculated. Nevertheless, despite these experimental differences and some limitations, the results of previous studies agree with the direct demonstration of the present report.

Our group and others[9,10,14,16] also infused insulin with the amino acids to maximize forearm protein anabolism by means of an independent and direct hormonal stimulus, which should be effective whatever the tracer or model used. Furthermore, insulin was shown to affect leucine and phenylalanine turnover to the same extent.[10,12,33] However, it would be interesting to confirm these effects also after infusion of the unbalanced amino acid solution without insulin. As a matter of fact, an increase in amino acid concentrations appears to represent the major protein-anabolic stimulus both at the whole-body level and at the forearm level.[1,7,9,10,12,17,22,34,35] Indeed, a correlation between amino acid delivery and net forearm uptake has been demonstrated.[36]

Interestingly, when KIC was included into the calculations, significant differences between the phenylalanine and leucine tracers persisted as regards forearm PS, whereas the 50% greater NB with the leucine tracer was no longer significant (see Results). This finding thus underlines the contribution by the leucine-deamination product (KIC) to overall forearm leucine-carbon exchange.

A muscle biopsy could not be performed in this study, and therefore, no direct estimate of intracellular amino acid concentration, enrichment, or specific activity, nor tissue amino acyl tRNA labeling could be obtained. Clearly, the lack of these primary measurements may restrict our conclusions. However, muscle biopsy in the human forearm is virtually unfeasible because of the limited muscle mass, whereas it is possible in the leg.[2,37] Therefore, our conclusions may need to be confirmed also using the muscle biopsy technique.

We infused a commercially available amino acid solution rich in some essential amino acids (ie, the BCAAs) but deficient in others (the AAAs).[24] These deficiencies were partly sought, and partly represent an intrinsic limitation of all amino acid

solutions in use when this study was performed (ie, glutamine and tyrosine were absent because of stability or solubility problems).[38,39] We acknowledge these limitations of our study, as well as many other previous studies.[7,9,10,12,14-17,22,33-36] Nevertheless, both whole-body[9,10,17,22,33-35] and muscle[1,7,9,10,12,17,37] PS were apparently stimulated by the hyperaminoacidemia attained through any of the solutions used. Therefore, it is likely that the intracellular amino acid pool(s) supplied the amino acids which were deficient in the infused solution, to cope with the stimulation of PS by other amino acids and/or by hyperinsulinemia. This hypothesis may also explain why forearm proteolysis was not inhibited following the amino acid (and insulin) infusion, at variance with some[9,10,12] but not all[7,36] previous reports. On the other hand, a deficiency of a limited number of amino acids (mostly nonessential) should have restrained both the leucine-derived and phenylalanine-derived PS rates to the same extent.

In conclusion, this study shows that the estimates of forearm PS and NB, following unbalanced hyperaminoacidemia with hyperinsulinemia, are dependent on the systemic (and probably also intracellular) concentration attained by a given amino acid, in this study, leucine and phenylalanine, whose tracers are simultaneously infused to measure forearm protein turnover. These findings indicate the need for a standardized approach in the assessment of the response of forearm (ie, muscle) protein turnover to anabolic stimuli in vivo. Also, a competition between two amino acids sharing the same transport system should be considered.

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