Model to assess muscle protein turnover: domain of validity using amino acyl-tRNA vs. surrogate measures of precursor pool

Gianna Toffolo,1 Robert Albright,2 Michael Joyner,2 Niki Dietz,2 Claudio Cobelli,1 and K. Sreekumaran Nair2

1Department of Information Engineering, University of Padova, 35131 Padua, Italy; and 2Division of Endocrinology, Mayo Clinic, Rochester, Minnesota 55905

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Address for reprint requests and other correspondence: K. Sreekumaran Nair, Mayo Clinic and Foundation, 5-194 Joseph, 200 First St. SW, Rochester, MN 55905 (E-mail: nair.sree@mayo.edu).

Model to assess muscle protein turnover: domain of validity using amino acyl-tRNA vs. surrogate measures of precursor pool. Am J Physiol Endocrinol Metab 285: E1142–E1149, 2003; 10.1152/ajpendo.00106.2003.—Current models to measure protein turnover across muscle bed are based on many surrogate measures of amino acyl-tRNA. We measured muscle protein turnover based on tracer-to-tracee ratios of the stable isotopes of leucine, phenylalanine, and ketoisocaproate (KIC) in artery and vein and muscle amino acyl-tRNA and muscle tissue fluid (TF) in 26 healthy subjects. A three-compartment model calculation based on arteriovenous and tRNA measurements was first performed and its domain of validity assessed. The results were then compared with those using simpler approaches based on surrogate measures of tRNA such as those of TF and KIC and a one-compartment model based on arteriovenous amino acids. In 96% of cases, the model using tRNA was applicable, but only in a lower percentage of cases were the results using surrogate measures applicable. Protein breakdown, protein synthesis, and shunting of amino acids from artery to vein were consistently underestimated, and fluxes of amino acid from artery to intracellular compartment and from intracellular compartment to vein were overestimated, when surrogate measures were used. The one-compartment model also underestimated protein breakdown and synthesis. Measurements using tissue fluid gave results closer to those based on tRNA. In conclusion, a three-compartment model using arteriovenous samples and amino acyl-tRNA provides measurements of muscle protein turnover of acceptable precision in 96% of cases. The precision was unacceptable in a substantial percentage of cases, and the accuracy of the estimation of protein fluxes was significantly affected when surrogate measures were used.

simultaneous measurement of the synthesis and breakdown of muscle proteins is critical to define the regulation of muscle protein balance. Arteriovenous models using amino acid tracers have been extensively utilized to study the regulation of muscle protein synthesis and breakdown in humans (2, 15, 19, 20). However, these techniques do not measure the immediate pool from which amino acid incorporation to protein (protein synthesis) occurs or the pool in which amino acids appear from protein breakdown. The model that is most extensively used is a one-compartment model, in which samples are taken from the venous side and the arterial side and tracer-to-tracee ratios (TTRs), tracer concentrations, and blood flow are used for calculations (2, 15). The amino acid fluxes calculated using the arterial TTRs provide the correct estimates of the tracer rate of appearance and disappearance in the arterial compartment. This approach underestimates the true values. Conversely, the use of the venous TTR could either under- or overestimate the amino acid fluxes (16).

A three-pool compartment model, in which the muscle tissue fluid compartment was introduced between arterial and venous compartments, was an advance on the one-pool model (4). This approach allows quantification not of only protein synthesis and breakdown but also of the amino acid fluxes between plasma and muscle tissue fluid. In these studies, both in animals (4) and in humans (5), the muscle tissue fluid (a mixture of intracellular and extracellular pools) was sampled. A correction was applied for the mixing of extracellular fluid to estimate the intracellular fluid (4). The correction was based on the assumption that the interstitial TTR coincides with either the arterial TTR (4) or the venous TTR (5) and that extracellular space accounts for 14–16% of free water space. Studies performed in humans demonstrated that muscle tissue fluid enrichment better represents amino acyl-tRNA than other surrogate measures such as plasma α-ketoisocaprate (KIC; when carboxyl-labeled leucine was used as a precursor pool) or plasma amino acids (12). However, these studies were performed in the fasted state, and it is also unclear whether physiological interventions affect electrolyte shift across the cell membrane (8) and may invalidate the assumptions involved in the estimation of intracellular pool from the mixed-muscle tissue fluid pool. Ideally, intracellular fluid should be used to measure the appearance of amino acids from protein breakdown and amino acyl-tRNA as the precursor from which amino acid is incorporated into protein. We do not have the technological means to...
sample directly the intracellular muscle pool and to measure its TTR. In studies in which carboxyl-labeled leucine is used as a tracer, KIC, the deamination product of leucine (appears only from the intracellular compartment), was used as a surrogate measure of intracellular compartment (13, 17). The KIC TTR in muscle tissue fluid does not always match that of tissue fluid leucine TTR, challenging the concept that plasma KIC TTR represents intracellular TTR (12). The model based on a leucine tracer has been proposed to estimate protein kinetics across the forearm or leg compartments as well as across the splanchnic bed (7, 15). The main problem with this approach is that the net balance of leucine across artery and vein represents a net effect of leucine appearance rate from protein breakdown and synthesis, and its disposal comprises both catabolism (transamination and oxidation) and incorporation into protein. The present study was therefore undertaken to use muscle amino acyl-tRNA as the precursor for protein synthesis and also to represent the intracellular compartment into which the amino acids appear from protein breakdown. To the best of our knowledge, amino acyl-tRNA is the obligatory precursor of protein synthesis, and acylation of tRNA occurs in the intracellular pool. It is therefore the best available approach to measure the true precursor of protein synthesis and the pool in which amino acids appear from protein breakdown. We compared the results from this model with those obtained from the one-compartment and three-compartment models, which used surrogate measures of amino acyl-tRNA to measure protein synthesis and breakdown.

METHODS

Materials

The isotopes L-[U-13C]leucine [98 atom percent excess (APE)] and L-[15N]phenylalanine (99 APE) were purchased from Cambridge Isotope Laboratories (Woburn, MA). The chemical isotopic and optical purity were tested before use. Sterile solutions of the isotopes were prepared and confirmed to be bacteria and pyrogen free before use in humans.

Subjects

Twenty-six normal healthy volunteers were studied (age 28.9 ± 2.4 yr; body mass index 23.9 ± 1.1 kg/m²; fasting plasma glucose 92.3 ± 2.1 mg/dl). The study protocol was approved by the Institutional Review Board of Mayo Clinic and Foundation.

Protocol

Subjects were admitted to the General Clinical Research Center at St. Mary’s hospital on the evening before the study. The study was performed after an overnight fast. All subjects were given a standard meal at 6:00 PM and then a 500-calorie snack at 10 PM on the night before the study. That evening, subjects had an intravenous line inserted into a forearm vein to infuse isotopes and to draw baseline blood samples. The forearm intravenous catheter was kept patent with a saline infusion. On the morning of the study, baseline venous samples for isotopic measurements were taken, and a primed continuous infusion of isotopes was started intravenously. The isotopes included 1.0 mg·kg⁻¹·h⁻¹ L-[U-13C]leucine and 0.75 mg·kg⁻¹·h⁻¹ L-[15N]phenylalanine. The priming doses were given at a rate of 1.0 mg/kg L-[U-13C]leucine, 1.0 mg/kg [15N]phenylalanine, and 0.2 mg/kg [13C]sodium bicarbonate.

Between 8:00 and 9:00 AM, catheters were inserted into the femoral vein and artery for infusion as well as collection of samples. The arterial and venous lines were maintained by normal saline infusion. Samples were collected from femoral artery and vein at baseline state and then at 150, 160, 170, and 180 min.

Three hours after the infusion was started, muscle samples were collected by needle biopsy technique after local anesthesia and immediately frozen in liquid nitrogen. Blood flow was measured by indicator dye dilution technique using intra-arterial infusion of indocyanine green (cardiogreen) as previously described (1, 6).

Analysis

Samples were processed for measurement of trace ratios in arterial and venous plasma, muscle tissue fluid, and muscle amino acyl-tRNA, as has been previously described (12). TTRs of leucine and KIC in arterial and venous plasma as well as in tissue fluid were calculated as previously described (10, 11).

Models

Three-compartment model. The three-compartment model of amino acid transport and protein kinetics in skeletal muscle (4) is shown in Fig. 1. 3. F_in and F_out denote the endogenous amino acid (tracee) inflow and outflow of the region, equal to the product of blood flow through the region by tracer concentration in artery and vein. F_in and F_out represent the rates of amino acid transport from artery to tissue and from tissue back to vein, and F_out represents the direct amino acid flow from artery to vein without entering the intracellular space. PS and PB represent the amino acid rate of utilization and production, virtually equal to the rate of protein synthesis and breakdown. By writing mass balance equations for the tracer and the tracee at steady-state conditions, equations for model fluxes have been derived (5). Briefly, PS and PB can be derived from the tracer and tracee
arteriovenous differences across the organ and the TTR of the intracellular precursor of protein synthesis (TTRt)

\[ \frac{f_{in} - f_{out}}{TTR_a} = \Phi \frac{c_a - c_v}{TTR_v} \]  

(1)

where a is arterial, t is tissue fluid, and v is venous.

\[ PB = PS - F_{in} + F_{out} = PS - \Phi(c_a - c_v) \]  

(2)

where \( F_{in} \) and \( F_{out} \) denote the amino acid tracer inflow and outflow of the region, equal to the product of blood flow through the region by tracer concentration in artery and vein.

Intercompartmental fluxes can be derived as

\[ F_{va} = \frac{TTR_a - TTR_v}{TTR_a - TTR_v} F_{out} \]  

(3)

\[ F_{ta} = F_{in} - F_{va} \]  

(4)

\[ F_{vt} = F_{out} - F_{va} \]  

(5)

The three-compartment model structure of Fig. 1 is consistent with experimental data that satisfy the constraint

\[ TTR_a \geq TTR_v \geq TTR_t \]  

(6)

A proof is given in Appendix. All model fluxes assume nonnegative values provided that Eq. 6 holds.

**Traditional approach.** The model underlying the traditional approach is shown in Fig. 1, right. Because this model is used in conjunction with measurements taken from artery and vein only, it does not allow estimation of protein synthesis and breakdown, but only amino acid turnover in plasma, commonly referred to as amino acid rate of appearance (Ra) and rate of disappearance (Rd). Two estimates are possible for Rd and Ra, depending on the assumption that uptake in the region takes place at the TTR either in artery

\[ R_d = \frac{f_{in} - f_{out}}{TTR_a} = \Phi \frac{c_a - c_v}{TTR_v} \]  

(7)

\[ R_a = R_d - F_{in} + F_{out} = R_d - \Phi(c_a - c_v) \]  

(8)

or in vein

\[ R_d = \frac{f_{in} - f_{out}}{TTR_a} = \Phi \frac{c_a - c_v}{TTR_v} \]  

(9)

\[ R_v = R_d - F_{in} + F_{out} = R_d - \Phi(c_a - c_v) \]  

(10)

TTRs and total (tracer + tracee) concentrations (Ctot) in artery (TTRa, Ctot,a) and vein (TTRv, Ctot,v) have been calculated as the average of four values measured in samples collected during the plateau phase at 150, 160, 170, and 180 min after the beginning of the tracer infusions.

From TTRa, TTRv, Ctot,a, and Ctot,v, the tracer (Ca and Cv) and tracee (Ct and Cv) concentrations have been calculated, e.g., for the artery

\[ C_a = C_{tot,a} \frac{1}{1 + TTR_a} \]  

(11)

### Precision of Estimated Fluxes

To quantify the effect of both analytical errors and plateau variability on the precision of estimated fluxes, a Monte Carlo analysis was performed (9). Briefly, 1,000 data sets were generated for each individual by adding to his nominal data set, namely \([\Phi, TTR_a, TTR_v, TTR_t, C_{tot,a}, C_{tot,v}]\), 1,000 different realizations of the errors affecting these data. Errors were assumed Gaussian, zero mean, and independent. For fluxes, a constant coefficient of variation equal to 5% was assumed. For TTRs and concentrations \(C_{tot}\), the standard deviations of the errors were experimentally determined from the four replicates at 150, 160, 170, and 180 min. On the basis of these assumptions, the RANDN Matlab function was used to generate the errors. Model fluxes were calculated from each of the 1,000 data sets. The resulting 1,000 sets of fluxes were averaged and the standard deviations calculated. For all fluxes, the mean value virtually coincided with the value calculated from the nominal data set, whereas the standard deviation provided a measure of the precision in an individual of the estimated fluxes resulting from plateau variability and analytical errors.

### Statistics

All values are stated as means ± SE. Model fluxes are expressed as micromoles per minute. Comparison between groups was performed by the Wilcoxon paired test. A two-tailed probability value <0.05 was considered statistically significant.

### RESULTS

**Concentrations, TTRs, and Three-Compartment Model Fluxes**

Average values of blood flow, phenylalanine, and leucine concentrations (tracer + tracee) in artery and vein are shown in Table 1, together with phenylalanine and leucine TTRs in artery and vein, tRNA, and tissue fluid. The three-compartment model fluxes calculated by using tRNA and different surrogates in 26 subjects are shown in Table 2. In some subjects, some fluxes assumed negative values because the data were not consistent with the model constraint (Eq. 6). More precisely, the arterial TTR was always higher than the venous ones. With reference to the additional constraint, TTRv ≥ TTRa, the relationships are represented graphically in Fig. 2. With phenylalanine, the constraint is satisfied in all but one subject with the tRNA TTR as TTRa, whereas it is violated in three

<table>
<thead>
<tr>
<th>Blood Flow, ml/min</th>
<th>Phe, μmol/l</th>
<th>Leu, μmol/l</th>
<th>TTR-Phe, %</th>
<th>TTR-Leu, %</th>
<th>TTR-KIC, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>V</td>
<td>A</td>
<td>V</td>
<td>A</td>
</tr>
<tr>
<td>Mean</td>
<td>315</td>
<td>46.9</td>
<td>52.3</td>
<td>109.5</td>
<td>113</td>
</tr>
<tr>
<td>SD</td>
<td>22</td>
<td>1.4</td>
<td>1.5</td>
<td>4.6</td>
<td>4.2</td>
</tr>
</tbody>
</table>

TTR, tracer-to-tracee ratio; KIC, α-ketoisocaproate; t-fluid, tissue fluid.
subjects when the tissue fluid value is used as TTR\textsubscript{t}. Similarly, with plasma leucine, the constraint is satisfied in all but one subject with the tRNA TTR as TTR\textsubscript{t}, whereas it is violated in, respectively, six, four, and one subject with the measurements in tissue fluid, arterial KIC, and venous KIC as TTR\textsubscript{t}. Because the three-compartment model is not applicable on the data of these subjects, average values were recalculated excluding them (Table 3). The resulting average TTRs and fluxes are obviously different from the previous one, but all of the qualitative comparisons (tRNA vs. surrogates, three-compartment model vs. traditional approach) remained the same.

**Three-Compartment Model: Use of tRNA TTR vs. Tissue Fluid Surrogate**

The TTRs measured in tissue fluid were significantly higher than the tRNA values, for both phenylalanine and leucine, as indicated in Table 3. This overestimation of the intracellular TTR by the tissue fluid measurement has major consequences on the three-compartment model fluxes PS, PB, and F\textsubscript{va}, which were significantly underestimated compared with those obtained with the tRNA TTR, whereas F\textsubscript{ta} and F\textsubscript{vt} were significantly overestimated (Table 3).

### Table 2. Average values of three-compartment model fluxes in 26 subjects

<table>
<thead>
<tr>
<th></th>
<th>PB</th>
<th>F\textsubscript{va}</th>
<th>F\textsubscript{ta}</th>
<th>F\textsubscript{vt}</th>
<th>PS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phenylalanine</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tRNA</td>
<td>7.53 ± 0.80</td>
<td>4.22 ± 4.64</td>
<td>10.74 ± 4.84</td>
<td>12.38 ± 4.85</td>
<td>5.89 ± 0.73</td>
</tr>
<tr>
<td>Tissue fluid</td>
<td>5.12 ± 0.49*</td>
<td>-3.47 ± 8.67*</td>
<td>18.14 ± 8.92*</td>
<td>19.78 ± 8.92*</td>
<td>3.48 ± 0.40*</td>
</tr>
<tr>
<td><strong>Leucine</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tRNA</td>
<td>15.42 ± 1.60</td>
<td>18.97 ± 2.24</td>
<td>15.77 ± 1.58</td>
<td>16.75 ± 2.83</td>
<td>14.44 ± 1.92</td>
</tr>
<tr>
<td>Tissue fluid</td>
<td>10.73 ± 0.92\¥</td>
<td>9.83 ± 8.83\¥</td>
<td>24.91 ± 8.07\¥</td>
<td>25.89 ± 8.07\¥</td>
<td>9.75 ± 1.09\¥</td>
</tr>
<tr>
<td>KIC\textsubscript{a}</td>
<td>10.59 ± 0.87\¥</td>
<td>8.45 ± 2.01\¥</td>
<td>26.30 ± 2.22\¥</td>
<td>27.28 ± 2.15\¥</td>
<td>9.61 ± 1.02\¥</td>
</tr>
<tr>
<td>KIC\textsubscript{v}</td>
<td>11.47 ± 0.99\¥</td>
<td>12.78 ± 1.92\¥</td>
<td>21.97 ± 1.91\¥†</td>
<td>22.94 ± 1.86\¥†</td>
<td>10.49 ± 1.15\¥†</td>
</tr>
</tbody>
</table>

Values are means ± SE in μmol/min. PB, protein breakdown; PS, protein synthesis; F\textsubscript{va}, flow from from artery (a) to vein (v); F\textsubscript{ta}, flow from artery to tissue; F\textsubscript{vt}, flow from tissue to vein. \*Significantly different value (P < 0.05) compared with tRNA. †Significantly different value (P < 0.05) compared with tissue fluid.

### Three-Compartment Model vs. Traditional Approach

The traditional approach uses the TTR either in artery or in vein to calculate the amino acid Ra and Rd. Arterial and venous TTRs significantly overestimate the intracellular (tRNA) TTR for both phenylalanine and leucine (Fig. 3). With the arterial TTR, the amino acid Ra (Phe 2.13 ± 0.22; Leu 6.72 ± 0.79) significantly underestimated PB; similarly their Rd (Phe 3.76 ± 0.31; Leu 7.70 ± 0.56) significantly underestimated PS (Fig. 3). By use of the venous TTR, a similar picture is obtained for the Ra (Phe 2.81 ± 0.31; Leu 8.67 ± 0.89) and Rd (Phe 4.45 ± 0.40; Leu 9.65 ± 0.75).

### Leucine Three-Compartment Model: Use of Arterial or Venous KIC as Intracellular Leucine Surrogate

The TTR of KIC in artery or vein has often been used as a surrogate for intracellular leucine TTR. Both measurements do not significantly differ from the leucine TTR measured in tissue fluid (Table 3). However, they differ significantly from the leucine tRNA TTR. When KIC TTRs where used in the three-compartment model formulas as TTR\textsubscript{t} to calculate model fluxes, PS and PB were significantly lower than the corresponding values estimated with intracellular (tRNA) TTR but similar to...
those obtained with the tissue fluid measurement. With respect to the intercompartmental fluxes, $F_{va}$, $F_{ta}$, and $F_{vt}$, they are all significantly different from the corresponding values estimated with the tRNA TTR. The use of KIC in artery but not in vein allows one to estimate values of intercompartmental fluxes that are not significantly different from those obtained with tissue fluid TTR.

**Precision of Three-Compartment Model Fluxes**

From the analysis of the four replicates collected at four different time points, the TTR measurements were affected by errors having an approximately constant standard deviation, with values shown in Table 4 for phenylalanine, leucine, and KIC. Conversely, the concentration measurements were affected by errors having a fairly constant coefficient of variation, also shown in Table 4. The average precision of the three-compartment model fluxes due to these errors, calculated by the Monte Carlo method, was acceptable for all parameters: slightly higher for PS (phenylalanine 36%; leucine 25%), lower for PB (phenylalanine 25%, leucine 15%), and for the intercompartmental fluxes $F_{va}$ (phenylalanine 19%, leucine 12%), $F_{ta}$ (phenylalanine 22%, leucine 14%), and $F_{vt}$ (phenylalanine 17%, leucine 11%). The aforesaid figures refer to the case where the tRNA TTR was used. When surrogate measurements were used, the average precision of all fluxes was very similar, with the only exception being that of $F_{va}$. Its average precision increased to 27% with phenylalanine tissue fluid, to 21% with leucine tissue fluid, and to 29 and 22% with arterial and venous KIC.

**DISCUSSION**

In the present study, the rate of PS and PB in human skeletal muscle was measured using the three-compartment model (Fig. 1), assuming amino acyl-tRNA TTR as the obligatory precursor of PS and the measure of intracellular pool into which amino acids appear from PB. For measurement of PS, the obligatory precursor is amino acyl-tRNA based on all available evidence (3, 12, 21). The use of the true precursor of PS is an essential requisite, since the expressions for PS and PB, Eqs. 1 and 2, are derived from the tracer and tracee net balance equations across the region as a function of inward and outward fluxes and of the TTR of the precursor of protein synthesis. The estimation of PS and PB based on the present approach gave results that are significantly higher than those based on either the single-compartment model or the three-pool model.

![Fig. 3. TTR measured in t-RNA, tissue fluid, artery, and vein and PS and PB (μmol/min) derived with the 3-compartment (3comp) model vs. amino acid R∗ and Rs derived with the 1-compartment (1comp) model for phenylalanine (A) and leucine (B). *Significantly different values (P < 0.05).](image-url)
### Table 4. Analytical error affecting TTRs and concentrations and total error, also including variations of plateau values

<table>
<thead>
<tr>
<th>TTR (constant SD)</th>
<th>C (constant CV)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Analytical</td>
</tr>
<tr>
<td>Phe</td>
<td>0.0005</td>
</tr>
<tr>
<td>Leu</td>
<td>0.0002</td>
</tr>
<tr>
<td>KIC</td>
<td>0.0002</td>
</tr>
</tbody>
</table>

C, concentrations; CV, coefficient of variation.

in which tissue fluid is used as a surrogate measure of tRNA.

Previous studies have demonstrated that tissue fluid amino acid enrichment was the best available correlate of muscle amino acyl-tRNA (12). However, the tissue fluid also does not truly represent amino acyl-tRNA enrichment. Theoretically, the pool in which amino acids are acylated to tRNA is the intracellular pool in which amino acids appearing from PB and transported from outside the cell membrane are uniformly mixed. On the basis of these assumptions, we used amino acyl-tRNA enrichment as the precursor for protein synthesis as well as the pool into which unlabeled amino acids appear from protein breakdown. The uniqueness of the present approach is that the $R_d$ of amino acids from the intracellular pool using both leucine and phenylalanine tracers represents PS. When tissue fluid is used as the surrogate measure of amino acyl-tRNA, the measurement of leucine $R_d$ represents both PS and leucine catabolism, because the acylation to tRNA and transamination of leucine take place in the same compartment. In contrast, leucyl-tRNA has only one fate, incorporation into protein, and therefore the $R_d$ of leucine is protein synthesis. Although the assumption to use amino acyl-tRNA as the precursor is unchallenged by any evidence, the use of amino acid-tRNA as an equivalent to the pool into which amino acids from PB appear remains to be fully validated. It remains to be resolved whether acylation of amino acids to tRNA and catabolic processes such as transamination of leucine occur in the same compartment (12). There are no alternative approaches to the use of amino acyl-tRNA TTR as equivalent to intracellular TTR, and this approach is the best available based on all theoretical and practical reasons. This approach is theoretically more logical when phenylalanine is used as a tracer, because phenylalanine, tyrosine, and lysine have no catabolic fates in muscle.

The TTR was highest in the artery followed by the vein and then tRNA in all subjects but one for both phenylalanine and leucine. As shown in APPENDIX, the aforementioned hierarchical relationship between different compartments is a condition that is a prerequisite to applying the three-compartment model. The model in fact provides a simplified description of the system to arrive at linear, constant coefficient equations to be solved for unknown fluxes. In particular, the tissue fluid compartment is treated as a homogeneous, well-mixed pool with a single TTR value, and tracer gradients both along the direction of blood flow and transversely along the inner cellular space. The model also assumes that all amino acid molecules leave the blood at the arterial site and that all molecules reenter the blood at the venous site. An intermediate pool, represented by the extracellular compartment, is not included. As a consequence of these simplifications, the domain of validity of the model is limited to the case where intracellular TTR assumes a value intermediate between those in artery and vein, since only in this case all fluxes assume positive values. This condition is met with tRNA in all but two studies, thus indicating that, only in a small percentage of cases (4%), model assumptions are in evident conflict with the data. More complex models are required to be consistent even with these two studies, e.g., models accounting for a dishomogeneity of the intracellular compartment (15). Unfortunately, they are not identifiable on the basis of the available data. Therefore, the two studies were excluded from our analysis.

The estimate of protein synthesis was equal to 6.07 ± 0.74 when the phenylalanine tracer was used and to 14.67 ± 1.85 with the leucine tracer. PB was equal to 7.71 ± 0.82 with phenylalanine and 15.61 ± 1.65 with leucine. The higher values obtained with leucine as a tracer are consistent with the higher contribution of leucine to the amino acid composition in muscle proteins (14). The three-compartment model also provides estimates of amino acid fluxes in artery, vein, and intracellular space. $F_{va}$ and $F_{va}$ represent the amino acid fluxes from the arterial to the interstitial pool and then to either the intracellular space ($F_{ta}$) or to the venous pool ($F_{va}$), eventually after recirculating in the interstitial pool. $F_{va}$ represents the flux of amino acids that reenter the blood at the venous site from the intracellular space. They are all composite fluxes: $F_{ia}$ is related to the transfer of molecules from blood to interstitial space and then from interstitial space into the cell, $F_{va}$ to the arteriovenous shunt and to the recirculation of amino acids between blood and interstitial space, and $F_{va}$ to the transfer of particles from the intracellular to interstitial space and then to blood. The results using Eqs. 3–5 and the tRNA TTR showed that ~40 and 43%, respectively, of phenylalanine and leucine molecules leaving the arterial pool will reach the intracellular space. This flux represents 44% (phenylalanine) and 49% (leucine) of the rate at which amino acids enter the cell; de novo synthesis accounts for the remaining portion. Of the total amino acid flux leaving the intracellular space, 56% (phenylalanine) and 52% (leucine) reenter the blood at the venous site, and the remaining portion contributes to protein synthesis.

We have also analyzed the effect of measurement error and plateau variability on the precision of the estimated fluxes. Our analysis indicates that the three-compartment model formulas tend to amplify the data variability, as expected. However, the 2–3% precision of concentration data and the 5–12% (phenylalanine) and 2–4% (leucine) precision of TTR data resulted in acceptable precisions of all of the estimated fluxes. The
The present approach represents the most accurate and precise picture of amino acid-protein kinetics currently available, against which simpler approaches using easier-to-measure surrogate measurements of the intracellular TTR and/or a simpler model were tested.

The TTR in tissue fluid has been proposed in the literature as a surrogate for the intracellular value (4). In our 26 subjects, tissue fluid TTR overestimated the intracellular value for both phenylalanine and leucine. In some subjects (three with phenylalanine, six with leucine), the extent of this overestimation was such that the TTR was even higher in tissue fluid than in vein (Fig. 2), thus contradicting the constraint, Eq. 6. Applicability of the three-compartment model is thus more critical with tissue fluid than with tRNA, since model assumptions are in evident contrast with experimental data in a larger percentage (~18 vs. 4%) of cases. As with tRNA, critical data were excluded, because the three-compartmental model predicts unreliable negative values for some fluxes. Even after these subjects were excluded, tissue fluid TTR significantly overestimated the intracellular value. The PS, PB, and Fva obtained by using tissue fluid in the three-compartment model formulas were significantly underestimated on the basis of both phenylalanine and leucine tracers, whereas Fva and Fvt were significantly overestimated (Table 3). As a consequence, the model predicts that a higher portion of amino acid particles will reach the intracellular space (56% for phenylalanine, 64% for leucine). This flux represents the higher component, 60% (phenylalanine) and 67% (leucine) of the rate at which amino acid enter the cell, whereas de novo synthesis accounts for only 40% (phenylalanine) and 33% (leucine). Similarly, the model overestimated the portion of the total amino acid flux leaving the intracellular space that reenters the blood at the venous site, 72.5% (phenylalanine) and 70% (leucine), whereas protein synthesis accounts for only 27.5% (phenylalanine) and 30% (leucine). Thus the use of tissue fluid TTR leads to an underestimation of protein turnover (PS and PB) not only in absolute terms but also in cases of intercompartmental fluxes.

The TTR in either artery or vein was used to calculate the amino acid Rs and R4 based on the one-compartment model. Our data further confirmed that these fluxes underestimate PS and PB (Fig. 3). Importantly, lower values are obtained with both the arterial and the venous TTRs. However, because the venous TTR is closer to the intracellular value than to the arterial one, differences between the three-compartment model fluxes and their counterparts provided by the conventional approach are somewhat lower, albeit still significant, when the venous site is used.

Finally, we have also examined the use of the KIC TTR measured in artery (or vein) as a surrogate measure of intracellular leucine TTR. The three-compartment model was not applicable in four subjects with arterial KIC and in one subject with venous KIC. Results in our normal subjects indicate that the TTR of arterial KIC reflects that of leucine in tissue fluid but not the intracellular one (as represented by leucyl-tRNA). Fluxes estimated with arterial KIC are not significantly different from those estimated with tissue fluid leucine, but they are both significantly different from the reference values estimated with leucyl-tRNA (Fig. 5).

A previous study (12) demonstrated that tissue fluid leucine enrichment and tissue fluid (as well as plasma) KIC enrichment values are different. This difference between leucine tissue fluid isotopic enrichment and KIC enrichment varies in different study conditions. In addition, amino acyl-tRNA enrichment was also different from both tissue fluid and KIC enrichment. These observations are further confirmed by the present study. On the basis of the previous report (12), it was suggested that the pool in which leucine transamination takes place is different from the pool in which amino acid is acylated to tRNA. An alternative explanation is that KIC transport across the cell membrane is different from that of leucine and, therefore, that muscle tissue fluid KIC enrichment reflects neither intracellular leucine nor plasma KIC. This hypothesis was not fully supported when plasma KIC enrichment decreased (during a meal ingestion) but muscle tissue fluid enrichment remained higher (12).

The present approach can potentially be used to measure synthesis rates of specific muscle proteins (amino acid-tRNA to specific proteins). Although factors such as amino acids and insulin may enhance translation of mRNA to protein, there is increasing evidence to show that the effect on transcription is more gene specific (18). As a result, synthesis of specific proteins may be enhanced whereas others may be inhibited by factors such as insulin. Future studies may therefore have to focus more on specific proteins, and such studies will become increasingly important when combined with the measurement of gene transcripts. Similar specificity occurring in muscle protein breakdown remains to be determined. The present technique allows the measurement only of total muscle protein breakdown but not those of individual muscle proteins. Another limitation of the present study is that, to measure the transport of amino acid across the cell membrane, it is ideal to measure the flux of amino acid from interstitial fluid (extracellular fluid) to intracellular compartment. In the present study, we assumed that arterial TTR of leucine and phenylalanine would reach the same levels as those of interstitial fluid during the steady state. Similarly, the transport of amino acid from within the cell to the interstitial fluid and from there to the venous side has also not been measured in the present study.

In summary, the present study provides a technique to measure protein synthesis and protein breakdown in muscle in the most accurate way so far reported, based on the amino acyl-tRNA pool representing the precursor pool of protein synthesis and intracellular pool. It will help to better understand the regulation of protein balance in muscle and flux of amino acids between different compartments. Some of the limitations related to the use of a three-compartment model to interpret the data have been reported. These limitations
became more critical when the three-compartment model was used with surrogate measurements of the intracellular pool. Moreover, the use of surrogate measures of tRNA significantly affected the estimate of protein and amino acid fluxes.

**APPENDIX**

To derive the constraint

$$ TTR_a \geq TTR_v \geq TTR_t $$  \hspace{1cm} (A1)$$

which holds for the three-compartment model of Fig. 1, let us first prove that the TTR in compartment V assumes an intermediate value between those of the two donor compartments A and T. Let us write steady-state tracee and tracer mass balance equations for compartment V

$$ F_{va} + F_{vt} = F_{out} $$  \hspace{1cm} (A2)$$

$$ F_{va}TTR_a + F_{vt}TTR_t = F_{out}TTR_v $$  \hspace{1cm} (A3)$$

By dividing Eq. A2 into Eq. A3, the following equation is obtained

$$ F_{va} = \frac{TTR_a - TTR_v}{TTR_v - TTR_t} $$  \hspace{1cm} (A4)$$

Because the left-hand side of Eq. A4 is the ratio between two positive fluxes, it can assume only positive values. Then the right-hand side is also positive, and this occurs only if $TTR_v$ assumes an intermediate value between $TTR_a$ and $TTR_t$; that is, either

$$ TTR_a \geq TTR_v \geq TTR_t $$  \hspace{1cm} (A5)$$

If a similar reasoning is applied to compartment T, which receives material from compartment A and from PB (PB is a cold flux; i.e., its TTR equals zero), TTR results to be intermediate between zero and $TTR_a$

$$ TTR_a \geq TTR_t \geq 0 $$  \hspace{1cm} (A6)$$

Given that Eqs. A5 and A6 must hold simultaneously, the second disequality of Eq. A5 is rejected, whereas the first one, equivalent to Eq. A1, is proved.

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