In vivo muscle amino acid transport involves two distinct processes

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Miller, Sharon, David Chinkes, David A. MacLean, Dennis Gore, and Robert R. Wolfe. In vivo muscle amino acid transport involves two distinct processes. Am J Physiol Endocrinol Metab 287:E136–E141, 2004; 10.1152/ajpendo.00092.2004.—We have tested the hypothesis that transit through the interstitial fluid, rather than across cell membranes, is rate limiting for amino acid uptake from blood into muscle in human subjects. To quantify muscle transmembrane transport of naturally occurring amino acids, we developed a novel 4-pool model that distinguishes between the interstitial and intracellular fluid compartments. Transport kinetics of phenylalanine, leucine, lysine, and alanine were quantified using tracers labeled with stable isotopes. The results indicate that interstitial fluid is a functional compartment insofar as amino acid kinetics are concerned. In the case of leucine and alanine, transit between blood and interstitial fluid was potentially rate limiting for muscle amino acid uptake and release in the postabsorptive state. For example, in the case of leucine, the rate of transport between blood and interstitial fluid compared with the corresponding rate between interstitial fluid and muscle was 247 ± 36 vs. 610 ± 95 nmol·min⁻¹·100 ml leg⁻¹, respectively (P < 0.05). Our results are consistent with the process of diffusion governing transit from blood to interstitial fluid without selectivity, and of specific amino acid transport systems with varying degrees of efficiency governing transit from interstitial fluid to muscle. These results imply that changes in factors that affect the transit of amino acids from blood through interstitial fluid, such as muscle blood flow or edema, could play a major role in controlling the rate of muscle amino acid uptake.

stable isotopes; interstitial fluid; microdialysis

THE PROCESS OF TRANSPORT of amino acids into and out of muscle cells is governed by a variety of amino acid transporters. Because a variety of intracellular processes, including the rate of protein synthesis, are directly affected by the intracellular availability of amino acids, these transporters play an important physiological role. Accordingly, the characteristics of muscle amino acid transport have been well documented in in vitro systems (e.g., Ref. 14). However, there is little information about the in vivo regulation of muscle amino acid transmembrane transport. We have previously described an in vivo three-pool model that enables quantification of the rate of transit of amino acids between blood and muscle tissue (2). Although this model has provided the first in vivo data on the rates of muscle uptake and release of naturally occurring amino acids in various physiological circumstances, the model is inadequate to provide a complete picture of the transport processes. This shortcoming exists because the model combines the two separate processes of transit through the interstitial fluid and transport across the muscle cell membrane.

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METHODS

Study design. Five healthy volunteers [4 males, 1 female; age 27 ± 8 yr, weight 84 ± 6 kg, body mass index (kg/m²) 23 ± 1] were recruited to participate in the study approved by the Institutional Review Board at the University of Texas Medical Branch (UTMB). All subjects provided written, informed consent and were determined to be healthy and free of metabolic disease at a preliminary health screening. Subjects reported to the General Clinical Research Center at UTMB the evening before the scheduled study and were fasted overnight. The following morning, peripheral venous catheters were placed for primed, continuous infusions of stable isotope tracers. Tracer infusion rates (IR) and priming doses (PD) were as follows: l-[^3]H]phenylalanine: IR = 0.10 μmol·kg⁻¹·min⁻¹, PD = 2 μmol/kg; l-[1-13C]leucine: IR = 0.12 μmol·kg⁻¹·min⁻¹, PD = 4.8 μmol/kg; l-[^1-2H]alanine: IR = 0.08 μmol·kg⁻¹·min⁻¹, PD = 7.2 μmol/kg; l-[1-13C]lysine: IR = 0.35 μmol·kg⁻¹·min⁻¹, PD = 35 μmol/kg. Femoral arterial and venous samples and tissue biopsies, the new four-pool model utilizes values from samples obtained from interstitial fluid by use of microdialysis. Use of multiple naturally occurring amino acids labeled with stable isotopes enabled us to investigate different transport systems simultaneously.

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3,000 Da) were inserted into the vastus lateralis muscle of the leg, as described previously (11). After insertion, the probes were attached to a perfusion pump (CMA model 102) and perfused at a rate of 5.0 μl/min with 5% Ringer solution. Small amounts of glucose and lactate (3.0 mM and 0.5 mM, respectively) were also included in the perfusate to reduce the potential of interstitial drainage of substrates. Recovery rates were determined with radiolabeled tracers of amino acids. Sampling started after isotopic steady state was achieved and following a 30-min equilibration period after probe placement.

**Infusion protocol.** Isotopic steady state in the free amino acid pools in blood, interstitial fluid, and muscle is required to apply the model. Tracers were infused for 260 min to achieve steady state. Dialysate sample collection began 60 min after placement of probes to allow for equilibration between perfusate and the interstitial environment, and samples were collected continuously over 45-min time intervals, yielding a sample volume of ~225 μl per probe (3 probes placed in each leg). The first muscle biopsy was taken at ~215 min to measure the isotopic enrichment (i.e., tracer/tracer ratio) of free amino acid tracers in the muscle. Biopsies of 30–50 mg were taken from the lateral portion of the right vastus lateralis muscle, ~20 cm above the knee, using a 5-mm Bergstrom biopsy needle (Umeå, Sweden). Blood and visible fat and connective tissue were quickly removed from the specimen, and the tissue was immediately frozen in liquid nitrogen and stored at −80°C for later analysis. To measure leg blood flow, peripheral and femoral venous blood samples were obtained 10 min after start of a continuous infusion of indocyanine green dye into the femoral artery. Femoral arterial and venous blood was taken at ~10-min intervals before and after the muscle biopsy. To allow sampling from the femoral artery, the dye infusion was stopped for <10 s and then quickly resumed. A second muscle biopsy was taken at ~245 min to correspond to the last 45 min of dialysate collection, and blood samples were taken at 235 and 255 min. The free intracellular and interstitial steady-state amino acid enrichments were used to calculate the parameters of the four-pool arteriovenous model (see Calculations). Sample analyses. Enrichments of blood and interstitial and intracellular free amino acids were determined on the tert-butyldimethylsilyl derivatives with a gas chromatograph-mass spectrometer (model 5973, Hewlett-Packard, Palo Alto, CA), and selected ion monitoring (15). An appropriate internal standard of amino acids labeled differently from the infused amino acids was used to quantify concentration of each amino acid, as described previously (2). Dialysate samples were processed in the same manner as blood supernatant and muscle homogenate, as described in Ref. 6, except that there was no need to precipitate protein from dialysate samples, and they were eluted through a lesser volume of acid-washed Dowex ion exchange resin to maximize recovery. Isotopic enrichment data are expressed as tracer-to-tracer (t/T) ratio, with appropriate corrections for the contribution of overlapping isotopomers (15). Muscle enrichments were adjusted to account for the small amount of interstitial fluid present in the extracted intracellular free amino acid sample, as we will describe. Calculations. The model shown schematically in Fig. 1 was used to calculate transport rates of phenylalanine, leucine, lysine, and alanine. The same model enables calculation of muscle protein synthesis and muscle protein breakdown. Calculations associated with the model are outlined below.

Some parameters of the four-pool model (e.g., protein synthesis and breakdown) are the same as we have previously described for the three-pool model in which the interstitial and free intracellular pools are not distinguished. The 3-pool model is presented in full in Ref. 2.

**Transport of amino acids between blood and interstitial fluid.** The t/T ratio enrichment of amino acids in the vein (E_V) depends on the percentage of amino acids in the vein that come from interstitial fluid (p_VIA), the t/T ratio in the artery (E_A), and the t/T ratio in interstitial fluid (E_I):

\[
E_V = p_{VIA} \times E_I + (1 - p_{VIA}) \times E_A.
\]

If the above equation is solved for p_VIA, then

\[
p_{VIA} = (E_A - E_V)/(E_A - E_I).
\]

The rate at which amino acids are transported from interstitial fluid to the vein (F_VA) is equal to the percentage of amino acids in the vein that come from interstitial fluid times the rate at which amino acids leave the vein (F_out), i.e.,

\[
F_{VA} = p_{VIA} \times F_{out} = [(E_A - E_V)/(E_A - E_I)] \times C_V \times BF.
\]

The rate at which amino acids go from the artery to the vein without being transported into interstitial fluid (F_VA) is equal to the rate that amino acids leave the vein minus the rate that amino acids are transported from interstitial fluid to the vein, i.e.,

\[
F_{IA} = F_{IA} - F_{VA}.
\]

**Transport of amino acids between interstitial fluid and muscle.** The t/T ratio of amino acids in interstitial fluid will depend on the percentage of amino acids in interstitial fluid that come from the artery (p_IA), the t/T ratio in the artery, and the t/T ratio in muscle:

\[
E_I = p_{IA} \times E_A + (1 - p_{IA}) \times E_M.
\]

If the above equation is solved for p_IA, then

\[
p_{IA} = (E_I - E_M)/(E_A - E_M).
\]

The percentage of amino acids in interstitial fluid that come from the artery is also equal by definition to the rate that amino acids are transported from the artery to interstitial fluid divided by the rate that amino acids are transported from the artery and muscle to interstitial fluid, i.e.,

\[
p_{IA} = (F_{IA} + F_{IM})/F_{IA}.
\]

If we combine the two above equations, we get

\[
F_{IM} = f_{IA} \times (1 - p_{IA})/p_{IA} = F_{IA} \times (E_A - E_I)/(E_A - E_M).
\]
The rate at which amino acids are transported from interstitial fluid to muscle is equal to the rate that amino acids are transported from muscle to interstitial fluid plus the net balance across muscle, i.e.,

$$F_{MI} = F_{IM} + (F_{in} - F_{out})$$

**Correction of intercellular concentrations and enrichments.** Approximately 15% of the water volume of samples obtained from muscle biopsies comes from the interstitial space rather than the intracellular space. Thus account must be taken of this portion to obtain the intracellular amino acid concentration and t/T ratio. The intracellular concentration is corrected by the following formula:

$$C_M = \frac{(C_{meas} - p_V \times C_t)}{C_t(1 - p_V)}$$

where $C_M$ is the true intracellular amino acid concentration, $C_{meas}$ is the measured amino acid concentration in the muscle biopsy, $C_t$ is the interstitial amino acid concentration, and $p_V$ is the proportion of the water volume in the muscle biopsy that is in the interstitial space.

To correct the intracellular amino acid t/T ratio, we first calculate the percentage of amino acids in the muscle biopsy that are in interstitial fluid (denoted $p_A$) via the formula

$$p_A = \frac{[C_t \times p_V]}{[C_t \times p_V + C_M \times (1 - p_V)]}$$

The correct muscle t/T ratio is therefore calculated using the formula

$$E_M = \frac{(E_{meas} - p_A \times E_t)}{(1 - p_A)}$$

where $E_M$ is the true intracellular amino acid t/T ratio, $E_{meas}$ is the measured amino acid t/T ratio in the muscle biopsy, and $E_t$ is the t/T ratio of the interstitial space.

**Statistical analysis.** Student’s t-test and ANOVA followed by Tukey’s test for multiple comparisons were used as appropriate. Statistical significance was set at the 0.05 level.

**RESULTS**

An isotopic equilibrium was achieved, meaning that t/T ratios were similar throughout the sampling period. Therefore, values were averaged for each amino acid. t/T ratios were consistently highest in the artery, followed by the vein, interstitial fluid, and muscle (Table 1). The ratios between intracellular and arterial t/T ratios corresponded well with previously published values (2). Phe (0.52 ± 0.06) showed the highest ratio, followed by Leu (0.49 ± 0.04), whereas Lys (0.29 ± 0.02) and Ala (0.22 ± 0.02) were significantly lower ($P < 0.01$ vs. Phe, $P < 0.05$ vs. Leu). Calculation of the ratio between interstitial fluid and muscle showed a significantly greater ($P < 0.05$) correspondence between these two pools than between blood and muscle. Muscle-to-interstitial fluid ratios were 0.82 ± 0.08, 0.76 ± 0.05, 0.65 ± 0.04, and 0.66 ± 0.08 for Phe, Leu, Lys, and Ala, respectively. The corresponding ratios between interstitial fluid and blood were 0.65 ± 0.06, 0.64 ± 0.04, 0.65 ± 0.10, and 0.33 ± 0.03 for Phe, Leu, Lys, and Ala. Concentrations are shown in Table 2. In contrast to the situation with isotopic t/T ratios, concentrations in the interstitial fluid and blood were generally much closer together than those between interstitial fluid and intracellular fluid. Model-derived transport rates indicated a dependency on the particular amino acid (Table 3). In the cases of Phe, Leu, and Ala, exchange between blood and interstitial fluid were slower than those between interstitial and intracellular fluid. In contrast, the rates were comparable between compartments for Lys. The rates of irreversible loss ($F_{OM}$) were dependent on the amino acid tracer. In the cases of Phe and Lys, the $F_{OM}$ corresponded to rates of muscle protein synthesis, because neither has any other fate in muscle, but the absolute values differ because of the proportional contribution of each amino acid to muscle protein. Similarly, the different rates of synthesis or breakdown ($F_{MO}$) reflect the rate of breakdown in Leu, Phe, and Lys, because none of these is synthesized in muscle, but the values again differ because of the composition of muscle protein. $F_{MO}$ for Ala includes both breakdown and de novo synthesis.

It is reasonable to assess the rates of exchange between compartments in the context of the prevailing availability of amino acids. Thus $F_{IA}$ (transport from artery to interstitial fluid) can be evaluated as a function of $F_{IA}$ (arterial concentration × flow), and $F_{MI}$ can be evaluated as a function of appearance of amino acids in the interstitial fluid, i.e., $E_{IA} + F_{IM}$. When normalized for amino acid availability, the transit rates can be considered to express “transport efficiency.” Thus a higher transport efficiency ratio reflects a relatively greater ability to transport a given amount of amino acids. The transport efficiency of all amino acids tested was comparable from arterial blood to interstitial fluid, whereas the efficiency was generally greater between interstitial and intracellular fluid than from arterial blood to interstitial for all cases but Lys (Table 4). In the case of Lys, transport efficiency into the intracellular compartment was numerically (but not significantly) lower than transport from arterial blood to interstitial fluid (Table 4). An alternative way in which to quantify the relative efficiency of transit across a membrane is to calculate the permeability-surface area product (ps) (13). The ps describing the movement from blood to interstitial fluid was similar for all amino acids and ranged from 2.5 ± 0.7 to 3.2 ± 0.6 ml·min⁻¹·100 ml⁻¹, whereas the rate of movement from interstitial fluid to muscle, expressed as clearance, ranged from 1.0 ± 0.5 (Lys) to 6.6 ± 1.4 (Ala) ml·min⁻¹·100 ml⁻¹, in a manner consistent with the values of efficiency for transport shown in Table 4.

The parameter $F_{MA}$ in our three-pool model represents transport from artery to tissue, ignoring the distinction between interstitial and intracellular compartments. Comparison of transport calculated by the three-pool and the four-pool models is shown in Fig. 2. The $F_{MA}$ determined by the three-pool model reflects the slower of the two transport rates calculated by the four-pool model, which in three of the four cases was

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**Table 1. Tracer/tracee ratios of compartments of 4-pool model**

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Interstitial Fluid</th>
<th>Muscle Free Pool</th>
<th>Femoral Artery</th>
<th>Femoral Vein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenylalanine</td>
<td>0.0863±0.0064</td>
<td>0.0712±0.0087</td>
<td>0.1379±0.0083</td>
<td>0.1087±0.0072</td>
</tr>
<tr>
<td>Leucine</td>
<td>0.0530±0.0027</td>
<td>0.0404±0.0026</td>
<td>0.0836±0.0042</td>
<td>0.0655±0.0033</td>
</tr>
<tr>
<td>Lysine</td>
<td>0.0252±0.0036</td>
<td>0.0070±0.0008</td>
<td>0.0423±0.0066</td>
<td>0.0349±0.0046</td>
</tr>
<tr>
<td>Alanine</td>
<td>0.0221±0.0027</td>
<td>0.0167±0.0020</td>
<td>0.0641±0.0044</td>
<td>0.0414±0.0006</td>
</tr>
</tbody>
</table>

Values (ratios) are means ± SE.
FIA (Phe, Leu, and Ala). Only in the case of Lys did the FMA more closely correspond to FMI (interstitial to intracellular).

DISCUSSION

We have previously described a method for measuring amino acid kinetics across the leg that quantifies muscle protein synthesis and breakdown, the rate at which amino acids enter muscle from the artery, the rate at which amino acids leave muscle for the vein, and the rate at which amino acids enter the vein from the artery without entering muscle (i.e., “shunt”). With the additional measurement of amino acid enrichment in interstitial fluid, we can quantify transit through the interstitial fluid as a separate compartment from the intracellular compartment. Our results indicate that this is an important distinction, because interstitial fluid is a functional compartment insofar as amino acid kinetics are concerned.

The importance of the interstitial fluid as a kinetic compartment was predicted from our previous application of compartmental modeling to the study of glucose kinetics. It was shown that interstitial fluid (as reflected by thoracic duct sampling) represented a distinct compartment from blood when a compartmental model was constructed from the decay in enrichment after a bolus of labeled glucose (6). Because the transfer of amino acids from blood to interstitial fluid presumably occurs by a diffusion mechanism similar to that for glucose, delay in transit of amino acids from blood to the intramuscular fluid in muscle was also expected. Furthermore, the existence of at least one pool between blood and the intracellular compartment can be predicted from the complexity of the mathematical model required to describe the uptake of methylenaminoisobutyric acid (an amino acid analog) across the forearm, although those authors (4) did not identify the interstitial fluid as a separate compartment. No previous study has utilized the direct measurement of interstitial fluid isotopic enrichment to quantify muscle amino acid uptake, and from prior indirect evidence, the importance of distinguishing the interstitial pool could not have been readily appreciated. The interstitial fluid constitutes only ~15% of the muscle tissue fluid (9). Furthermore, the concentrations of amino acids in the intracellular fluid, although variable, are generally well in excess of those in interstitial fluid. Thus a muscle “tissue” sample of free amino acids largely reflects those amino acids in the intracellular pool. Nonetheless, it is clear from Fig. 2 that, for several amino acids, transit through the interstitial fluid is the rate-limiting step for uptake into the cell in the postabsorptive state. When this is the case, a transport value based on intracellular t/T ratio alone (FMA in the 3-pool model) in fact predominantly reflects the rate of exchange between blood and interstitial fluid, rather than uptake into muscle cells from interstitial fluid. Expressed differently, a pooled expression of transmembrane transport (i.e., FMA) reflects the rate-limiting step in transport, which may be either transit through the interstitial fluid or transport into the muscle cell from the interstitial fluid, depending on the amino acid. Because FMA is similar to FIA for Phe, Ala, and Leu, transit through the interstitial fluid must be the rate-limiting step for these amino acids. Thus FMA cannot increase without an increase in FIA for those amino acids.

It is important to note that the functional importance of the interstitial fluid in terms of transport does not invalidate our previous studies that did not include measurements from interstitial fluid. This is true primarily because we are only using steady-state equations, so any delays in transport of amino acids between blood and the intracellular space do not affect the calculations. The difference between the four-pool and three-pool models is similar to the difference between the three-pool model and the traditional two-pool model, which uses only blood measurements (see Ref. 7). The two-pool model gives the rate at which amino acids are irreversibly taken up from blood, which is a function of both transport and

Table 2. Concentrations of compartments of 4-pool model

<table>
<thead>
<tr>
<th>Compartment</th>
<th>Interstitial Fluid</th>
<th>Muscle Free Pool</th>
<th>Femoral Artery</th>
<th>Femoral Vein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenylalanine</td>
<td>77±2</td>
<td>90±4</td>
<td>66±1</td>
<td>71±2</td>
</tr>
<tr>
<td>Leucine</td>
<td>123±12</td>
<td>184±22</td>
<td>121±6</td>
<td>122±9</td>
</tr>
<tr>
<td>Lysine</td>
<td>247±33</td>
<td>654±71</td>
<td>214±23</td>
<td>223±27</td>
</tr>
<tr>
<td>Alanine</td>
<td>308±60</td>
<td>1692±80</td>
<td>232±35</td>
<td>256±40</td>
</tr>
</tbody>
</table>

Values are means ± SE expressed in nmol/ml.

Table 3. Transport rates between compartments, and protein synthesis and breakdown

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>FIA</th>
<th>FIA/FIM</th>
<th>FIA/FMI</th>
<th>FIA/FMA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenylalanine</td>
<td>253±9</td>
<td>420±56</td>
<td>711±62</td>
<td>888±132</td>
</tr>
<tr>
<td>Leucine</td>
<td>271±8</td>
<td>447±46</td>
<td>780±71</td>
<td>965±142</td>
</tr>
<tr>
<td>Lysine</td>
<td>118±8</td>
<td>173±41</td>
<td>413±82</td>
<td>503±153</td>
</tr>
<tr>
<td>Alanine</td>
<td>135±15</td>
<td>247±36</td>
<td>296±89</td>
<td>385±56</td>
</tr>
<tr>
<td>FIM</td>
<td>338±143</td>
<td>610±95*</td>
<td>183±64</td>
<td>1,872±286*</td>
</tr>
<tr>
<td>FMA</td>
<td>358±146</td>
<td>637±103</td>
<td>252±44</td>
<td>1,940±285</td>
</tr>
<tr>
<td>FVl</td>
<td>153±16</td>
<td>274±44</td>
<td>367±96</td>
<td>461±65†</td>
</tr>
<tr>
<td>FVD</td>
<td>100±29</td>
<td>180±34</td>
<td>451±76</td>
<td>1,095±87</td>
</tr>
<tr>
<td>FVM</td>
<td>82±26</td>
<td>153±28</td>
<td>382±92</td>
<td>1,019±82</td>
</tr>
</tbody>
</table>

Values are means ± SE in nmol/min·1·100 ml leg⁻¹. FIA, arterial blood to interstitial fluid; FIM, interstitial to intracellular fluid; FMI, intracellular to interstitial fluid; FV, intracellular fluid to venous blood. *Significantly different from FIA, for that amino acid: P < 0.05 (Leu), P < 0.01 (Ala); †significantly different from FIA, P < 0.05 (Leu), P < 0.01 (Ala).

Table 4. Efficiency of transport

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Phenyllaline</th>
<th>Leucine</th>
<th>Lysine</th>
<th>Alanine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Artery to interstitial</td>
<td>FIA/FIA</td>
<td>56±7</td>
<td>58±6</td>
<td>41±10</td>
</tr>
<tr>
<td>Intestinal to intracellular</td>
<td>[FMA/(FIA + FMA)]</td>
<td>68±3*</td>
<td>69±5*</td>
<td>33±10</td>
</tr>
</tbody>
</table>

Percentage values are means ± SE. *Significantly different from lysine, P < 0.05.
synthesis, so conclusions can only be drawn about the irreversible loss and not the individual pathways of transport and synthesis without adding measurements from the intracellular muscle pool. Similarly, in the three-pool model, the calculation of transport from plasma to muscle is correct, but conclusions about the individual pathways of diffusion into interstitial fluid and transport from interstitial fluid to muscle cannot be drawn without adding measurements of interstitial fluid. The calculation of protein synthesis and breakdown is not affected by adding measurements of interstitial fluid.

The calculation of irreversible loss of Leu is complicated by the fact that Leu exchanges with o-ketoisocaproate and can be lost to oxidation via this route. Account can be taken of the extent of this exchange by using multiple tracers, e.g., t-[1-13C,15N]leucine, since nitrogen labels but not carbon labels will be lost in this cycle. A similar problem exists for some other amino acids, such as Ala. However, it should be noted that, regardless of the tracer used, the transport rates described by our model are correctly calculated even in the presence of exchange reactions within the cell. To calculate the relative rates of transport into interstitial fluid from plasma and intracellular spaces, the model utilizes the interstitial fluid enrichment relative to the corresponding plasma and intracellular enrichments. For example, if the interstitial fluid enrichment is halfway between the plasma and intracellular enrichments, then interstitial fluid must receive amino acids in equal amounts from each of the two pools. If the intracellular enrichment is altered by cycling with other substrates, the interstitial enrichment will also be altered accordingly, so the calculation will remain correct.

Certain assumptions must be made with our model that we know to be imprecise. The most problematic assumptions are that the blood flow to the leg is entirely directed to muscle, and that the sampled muscle is reflective of all the muscle in the leg. These assumptions are common to our three-pool model and have been discussed in detail previously (2). The errors stemming from these assumptions are not likely large in most circumstances. Furthermore, transport rates reflect interstitial and tissue samples taken from the same muscle, and the same assumptions apply to all amino acids. Also, for calculating the intracellular enrichment, we have assumed that the tissue fluid obtained by muscle biopsy is 15% interstitial fluid. Although the value of 15% is established for normal volunteers, that value could be different in certain clinical situations, such as peripheral edema. Therefore, we have also calculated the value for the intracellular t/T ratio by assuming that the percentage of interstitial fluid was either 0 or 30%. The impact of the assumed percentage on the calculated t/T ratio depended on the amino acid tracer, owing to the different intracellular pool sizes and transmembrane concentration gradients for each amino acid. In the case of Ala, so much less Ala is in the interstitial fluid compared with the intracellular fluid that there was no difference in the calculated t/T ratio, regardless of whether the interstitial fluid was assumed to be 0 or 30% of the total. On the other hand, in the case of Phe, the calculated t/T ratios were 0.068 ± 0.008, 0.065 ± 0.009, and 0.061 ± 0.009 for assumed values of 0, 15, and 30%, respectively. The differences for Leu were less extreme (0.042 ± 0.003 for 0 vs. 0.039 ± 0.004 for 30%). Because a reasonable guess of the true percentage can be made on the basis of the clinical condition, the assumption of the contribution of the interstitial fluid to total tissue fluid enrichment should therefore not cause a significant error in the calculation in the model parameters. Therefore, relative rates should be valid. Finally, a potential technical problem with the four-pool model is that a measurable difference in the t/T ratios in all four pools is required. Given the closeness of the enrichments in the interstitial and intracellular fluid compartments, one can easily envision a circumstance in which the values would be too close to reliably distinguish between them. Nonetheless, in the current study, differences in t/T ratios between pools were large enough to measure.

The use of naturally occurring amino acids, as opposed to analogs (e.g., Ref. 4), is an advantage of our approach, because transport mechanisms are specific. Furthermore, we were able to quantify the transport rates of several amino acids simultaneously, because detection of the stable isotope tracers involves isolation of individual amino acids before analysis. Our results therefore provide insight into the nature of a variety of transport systems. Thus movement from the arterial blood to the interstitial fluid (as well as in the opposite direction) was similar for all amino acids tested when account was taken of amino acid availability (i.e., transport efficiency). This supports the notion that this process is governed by diffusion. Further support for this notion derives from the fact that the ps were similar for all amino acids and consistent with values from comparable compounds known to transit from blood to interstitial fluid by diffusion (13). In contrast, the efficiency of transport between the interstitial and intracellular compartments was highly variable and presumably reflected the transport systems involved for each amino acid. This observation held regardless of whether transport efficiency was expressed as shown in Table 4, or if it was expressed as the clearance of interstitial fluid by muscle. The similarity of the efficiency with which Phe and Leu are transported into muscle from interstitial fluid is consistent with the in vitro observation that the two amino acids are transported by the same transport system (system L) (14). Lys, on the other hand, is transported by the y+ system (14), which our data indicate is the least efficient system in terms of maintaining a high rate of exchange between intra- and extracellular compartments. Nonetheless, the intracellular Lys pool is much larger than that of any other essential amino acid, meaning that the transporter is able to maintain a large transmembrane concentration gradient. Ala transport is extremely rapid, reflecting transport via both the ASC and A transport systems, and perhaps also system L (14).

Our transport efficiency data are consistent with in vivo kinetic studies. Several studies have demonstrated a similar behavior of Phe and Leu kinetics with respect to muscle protein metabolism (e.g., Ref. 2). In contrast, acute changes in plasma Lys flux and oxidation are often sluggish compared with those of other essential amino acids. For example, during aerobic exercise, no change was observed in Lys flux or oxidation, even though Leu flux and oxidation changed rapidly (16, 17). Presumably the large intracellular pool of Lys accommodates acute changes in requirements for utilization, whereas the oxidation of other essential amino acids, such as Phe and Leu, is more closely tied to changes in transmembrane transport and thus reflected more directly by acute changes in plasma flux. The extremely efficient transmembrane transport of Ala is essential in enabling the rapid and large changes in Ala flux to occur that are often required because of the role of Ala as the most important means of interorgan transfer of nitrogen from
muscle to liver. For example, in response to even moderate aerobic exercise, Ala release increases severalfold in a matter of minutes (17).

Although it was not the primary purpose of our study, the demonstration that the t/T ratio of interstitial fluid was close to the intracellular t/T ratio has a direct bearing on the measurement of the rates of muscle protein synthesis and breakdown. In the measurement of muscle protein synthesis, whether by the direct incorporation technique or by use of our three- or four-pool model, it is necessary to assume a value for the enrichment of the precursor for protein synthesis. It is generally assumed that the charged tRNA enrichment represents the true precursor enrichment, but this value is difficult to measure accurately and requires a prohibitively large sample size for most human studies. When both tRNA and intracellular free amino acid enrichment have been measured simultaneously, the relationship of those two values has been variable (e.g., Refs. 8 and 10) and has led to controversial approaches such as the flooding dose technique (5) to overcome uncertainty regarding the true precursor enrichment. The amino acids charged in the tRNA must come from either the interstitial fluid or the intracellular pool, and because for amino acids such as Phe and Leu there is little difference in the enrichments in these two pools, the tRNA enrichment must be reasonably reflected by the intracellular free enrichment. This notion is supported by the recent study of Nair and associates (Bauman et al., Ref. 1), who found that during a constant tracer infusion the measured muscle tRNA-leucine enrichment was approximately the same as that of the intracellular free leucine. Furthermore, data in the current paper enabled us to assess the potential error made in our previous studies, in which we assumed that the interstitial fluid enrichment was the same as the venous enrichment to calculate the intracellular free enrichment (2). For both Phe and Leu, there was no significant difference in t/T ratio, regardless of whether the intracellular t/T ratio was calculated by correcting for the contribution of the interstitial fluid enrichment by using either the directly measured value or an assumed value equal to the femoral venous enrichment, or by using no correction at all (i.e., tissue free enrichment).

The practical significance of these calculations is that it is reasonable to use tissue free Leu or Phe enrichment as representative of the true precursor enrichment for muscle protein synthesis.

If our general hypothesis is correct, then circulatory responses would be expected to have a direct impact on muscle amino acid transport. Changes in the extent of perfusion of muscle tissue would be expected to affect the exchange rate between blood and muscle, and tissue edema would also affect muscle amino acid transport. Although there are no studies directly assessing these possibilities insofar as exchange rates between blood and interstitial fluid are concerned, existing data are consistent with expectations. Thus, after resistance exercise, muscle blood flow is increased and amino acid exchange between muscle and blood is also accelerated (3). Similarly, muscle edema is prevalent in severely burned patients, and muscle amino acid transmembrane transport is impaired (12). Thus, whereas specific experiments are needed to directly determine the physiological importance of the blood-interstitial exchange of amino acids in various circumstances, it seems likely that transit through the interstitial fluid is an important process governing the rate at which amino acids are taken up by muscle.

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GRANTS

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REFERENCES