Quantification of amino acid transport through interstitial fluid: assessment of four-compartment modeling for muscle protein kinetics

Gore DC, Wolfe RR, Chinkes DL. Quantification of amino acid transport through interstitial fluid: assessment of four-compartment modeling for muscle protein kinetics. Am J Physiol Endocrinol Metab 292: E319–E323, 2007. First published September 5, 2006; doi:10.1152/ajpendo.00399.2005.—The purpose of this study was to assess a novel technique for quantifying in vivo muscle protein metabolism and phenylalanine transport in septic patients and normal volunteers and thereby assess the influence of sepsis on muscle protein kinetics. In patients resuscitated from sepsis, blood flow and edema may influence the extent of muscle loss. Six adult patients septic from pneumonia underwent a study protocol consisting of infusion of isotopic phenylalanine, indocyanine green dye, and sodium bromide; biopsies of skeletal muscle; and sampling from the femoral artery, vein, and interstitial fluid. Study results demonstrate a substantial net catabolism of muscle, an accelerated flux of phenylalanine, and an increased leg blood flow for septic patients compared with normal volunteers. For septic patients and normal volunteers, the rate of phenylalanine transport through the interstitium was rate limiting for the movement of phenylalanine between vasculature and muscle. Measurements demonstrate a concentration gradient of phenylalanine favoring the net efflux of amino acids from the leg in the septic patients. Despite whole body edema, the extracellular fluid volume within muscle of septic patients was similar to normal. These findings demonstrate that the extent of muscle loss in critically ill patients results from the net increase in the rate of muscle protein breakdown, which subsequently drives amino acids through the interstitial compartment down their concentration gradient. Therefore, any effective therapy to correct illness-induced muscle catabolism should be directed at altering the rates of breakdown and synthesis of muscle protein and are not likely related to tissue edema.

catabolism; critical illness; stable isotopes; edema; microdialysis

Regardless of the extent of nutritional support, critically ill patients pervasively lose muscle mass (12). With prolonged illness, this loss of muscle adversely affects immune function, wound healing, and eventual overall survival (15). In an effort to combat this net catabolism of muscle, our group and others have investigated a variety of nutritional regimens as well as various anabolic agents (17). For example, such agents as insulin, growth hormone, testosterone, and others have been shown to augment the rate of muscle protein synthesis and thereby reduce and even negate the net loss of muscle in severely injured and critically ill patients (4–6). Stable isotope tracer methodology has for several years been the primary means for assessing in vivo the efficacy of these various regimens on muscle protein kinetics (1). By combining serial arterial and venous blood sampling, muscle biopsies, and a measure of leg blood flow, quantification of not only the rates of muscle protein synthesis and breakdown but also the rates of amino acid transport into and out of muscle can be determined. Unfortunately, this three-pool compartment model (artery, vein, muscle) fails to assess the influence of the interstitial fluid space on muscle protein kinetics. Given the fact that patients resuscitated from severe injury and sepsis are exorbitantly edematous, it is quite plausible that the changes in the interstitial compartment associated with critical illness may have a demonstrative impact on the movement of amino acids into and out of muscle. The purpose of this study was to assess a novel technique for quantifying not only the kinetics of muscle protein synthesis and breakdown but also the rates of amino acid transport into and out of the interstitial space in critically ill patients compared with healthy normal subjects. It is hoped that, by employing this four-compartment modeling methodology, a comprehensive assessment of injury-induced muscle catabolism, specifically the influence of the interstitial space and extremity blood flow, can be determined.

MATERIALS AND METHODS

Study subjects. The study protocol was completed in eight subjects. All study subjects were septic, with a minimal sepsis severity score of 14. Furthermore, all subjects had pneumonia as an infectious source of their sepsis, as evidenced by lobar consolidation on chest radiographs and bacterial organisms identified on culture of either bronchoscopic lavage or undirected aspiration via the endotracheal tube. At the time of study, all subjects were receiving enteral nutrition delivered continuously via a nasojejunal tube. All subjects had good hemodynamics and adequate urine output. No subject had overt renal (serum creatine ≥1.5 mg/dl) or liver dysfunction (bilirubin ≥4.0 mg/dl), were hy- poxic ($P_{aO_2}/FIO_2$ < 150), or had lactic acidosis (pH ≤7.30, lactate > 5 mmol/l). Patients received fentanyl for analgesia and midazolam for sedation. No subject was chemically paralyzed prior to or during study.

Of the eight patients who completed the metabolic assessment, two subjects were subsequently excluded because the isotopic enrichment of fluid sampled from the interstitial space was exceedingly low. After study completion, blood clots were evident on the microdialysis catheters on both of these subjects, thus strongly suggesting catheter occlusion as a probable explanation for the very low isotopic enrichment of the effluent. The characteristics of the remaining six subjects are presented in Table 1. These subjects ranged in age from 31 to 61 yr and in weight from 56 to 96 kg. Four of these six subjects required mechanical ventilatory support at the time of study. As predisposing factors for the sepsis pneumonia, three patients had suffered recent burn injuries and three subjects had suffered extensive traumatic injuries. Two study subjects subsequently died: one following an acute myocardial infarction 2 days following study; the other patient succumbed to progression of sepsis to multiorgan failure and died 29 days following study.

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were obtained from the vastus lateralis muscle by means of a Berg-
access was standardized for the leg muscle mass as subsequently
central venous access allowed quantification of whole body extracel-
photometric analysis of blood drawn sequentially from the
ml/kg body wt into the femoral artery for 30 min (10). Subsequent
length of the calf, thigh, and foot. Extracellular fluid volume was
assessed by integrating several circumference measurements with the
Leg blood flow measurements were standardized for leg volume as
simultaneously from the femoral vein and from the central vein access (7).
indocyanine green dye (1 mg/min per 20 min) into the femoral artery
to quantify the recovery rate of this amino acid from the
rate of 5
microdialysis probes were perfused with Ringer’s lactate solution at a
[2H5]phenylalanine (2
l/min. A radioactive tracer of phenylalanine was added to
the perfusate to quantify the recovery rate of this amino acid from the
of 3,000 Da) into the vastus lateralis muscle, as described previously
and vein as well as insertion of the microdialysis probes (mol mass
placement of vascular access catheters into an adjacent femoral artery
protocol is diagramed in Fig. 1 and was performed in each patient’s
The University of Texas Medical Branch,
approval by the Institutional
Study protocol. This study was approved by the Institutional
Review Board of The University of Texas Medical Branch,
Galveston, TX, and consent was obtained from family. The study
protocol is diagramed in Fig. 1 and was performed in each patient’s
intensive care unit room. Local anesthesia (1% lidocaine) facilitated
placement of vascular access catheters into an adjacent femoral artery
and vein as well as insertion of the microdialysis probes (mol mass
3,000 Da) into the vastus lateralis muscle, as described previously (8).
After baseline blood sampling, a primed continuous infusion of
[13C]phenylalanine (2 μmol/kg prime; 0.07 μmol·kg⁻¹·min⁻¹) was
given via central venous access and continued for the 5 h of study. The
microdialysis probes were perfused with Ringer’s lactate solution at a
rate of 5 μl/min. A radioactive tracer of phenylalanine was added to
the perfusate to quantify the recovery rate of this amino acid from the
interstitial fluid (8). Leg blood flow was determined by infusion of
indocyanine green dye (1 mg/min per 20 min) into the femoral artery
with subsequent spectrophotometric analysis of blood drawn simul-
taneously from the femoral vein and from the central vein access (7).
Leg blood flow measurements were standardized for leg volume as
assessed by integrating several circumference measurements with the
length of the calf, thigh, and foot. Extracellular fluid volume was
determined by infusion of 3% NaBr solution at a dose rate of 0.75
ml/kg body wt into the femoral artery for 30 min (10). Subsequent
spectrophotometric analysis of blood drawn sequentially from the
central venous access allowed quantification of whole body extracel-
lar fluid volume. Blood drawn sequentially from the femoral venous
access was standardized for the leg muscle mass as subsequently
quantified by dual-energy X-ray absorptiometry (DEXA; Hologic-
QDR-4500A, Waltham, MA). With local anesthesia, muscle biopsies
were obtained from the vastus lateralis muscle by means of a Berg-
strom needle. Muscle biopsies were performed at the second and fifth
hours of study. Dialysate fluid from the microdialysis probes were
collected for subsequent analysis from the fourth to fifth hours of
study. After 4.5, 4.75, and 5 h of isotopic infusion, blood was drawn
simultaneously from the femoral artery and vein, thus completing the
baseline metabolic measurements. Following completion of the study
protocol, each patient underwent DEXA, thereby quantifying muscle
mass within the studied leg.
Sample analysis. Isotopic enrichment of phenylalanine and the
blood and dialysate concentration of unlabeled phenylalanine were
determined by gas chromatography-mass spectrometry (GC-MS),
using an internal standard method (16). Blood and dialysate were
collected in ice-cold tubes containing 2 ml of 15% sulfosalicylic acid
and 20 μl of internal standard ([13C]phenylalanine). Samples were
vortexed and centrifuged. The supernatant was then frozen at −80°C
until processing. After tert-butylimidethylsilyl derivatization, plasma
samples were then analyzed with GC-MS (model 5989; Hewlett-
Packard, Palo Alto, CA) using electron impact ionization.
Muscle biopsies were analyzed for both intracellular and protein-
bound amino acid concentration and isotopic enrichment by use of an
internal standard method of analysis by GC-MS (3). To ~20 mg of
muscle were added 800 μl of 14% perchloric acid and 2 μl of internal
standard. Samples were homogenized and centrifuged, and the super-
natant was collected. This procedure was repeated twice more and the
pooled supernatant processed identically to the blood samples as
described above using tert-butylimidethylsilyl derivatization with
analysis by GC-MS. This method determined the free intracellular
concentration and isotopic enrichment for phenylalanine. For deter-
mination of protein-bound concentration and enrichment, the remain-
ing muscle pellet was washed repeatedly with saline and absolute
ethanol, dried, and then hydrolyzed with 6 N HCl. The protein
hydrolysate was then passed over a cation exchange column (Dowex
AG; Bio-Rad Laboratories, Richmond, CA), dried, esterified, heated,
and subsequently analyzed by GC-MS using chemical impact ioniza-
tion as previously described (16).
Calculations. The net balance of phenylalanine across the leg was
calculated as follows and expressed per 100 ml leg volume:
\[ \text{AVNB} = (C_4 - C_3) \times \text{LBF} \]
where AVNB is net balance of phenylalanine across the leg
(nmol·min⁻¹·100 ml leg volume⁻¹); Ca, Cv is concentration of
phenylalanine from artery and vein, respectively (nmol/ml); and LBF
is leg blood flow (ml·min⁻¹·100 ml leg volume⁻¹). Thus a negative
net balance reflects the net release of phenylalanine from the limb.
Isotopic steady state in the free amino acid pools of blood, inter-
stitium, and muscle is required for the four-compartment modeling

<table>
<thead>
<tr>
<th>Table 1. Patient characteristics</th>
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<tbody>
<tr>
<td>Normal Volunteers (n = 6)</td>
</tr>
<tr>
<td>Age, yr</td>
</tr>
<tr>
<td>Body weight, kg</td>
</tr>
<tr>
<td>Sepsis severity score (all ≥14)</td>
</tr>
<tr>
<td>APACHE II score (all ≥15)</td>
</tr>
<tr>
<td>Mean BP, mmHg</td>
</tr>
<tr>
<td>PaO2/FiO2</td>
</tr>
<tr>
<td>Serum creatinine, mg/dl</td>
</tr>
<tr>
<td>Serum bilirubin, mg/dl</td>
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Values are means ± SD. BP, blood pressure.

Study Protocol Flow Diagram

![Study Protocol Flow Diagram](http://ajpendo.physiology.org/)

Fig. 1. Study protocol flow diagram. ICG, indocyanine green.
calculations. Isotope phenylalanine was infused for 300 min to achieve isotopic equilibrium, which was evidenced by the steady enrichment in the serial blood sampling during the final 30 min of the study protocol. The model is shown schematically in Fig. 2 and provides quantification of amino acid transport into and out of muscle as well as the rates of muscle protein synthesis and breakdown (9). Values are calculated as follows and all are expressed as nanomoles per minute per 100 milliliters of leg volume:

\[ F_{in} = C_a \times LBF \]
\[ F_{out} = C_a \times LBF \]
\[ F_{va} = F_{out} - F_{vi} \]
\[ F_{vi} = \left( \frac{(E_i - E_v) + (E_v - E_i)}{E_i - E_v} \right) \times C_v \times LBF \]
\[ F_{ma} = F_{in} - F_{va} \]
\[ F_{mi} = F_{ma} + \left( \frac{(E_m - E_i) + (E_i - E_m)}{E_m - E_i} \right) \times C_i \times LBF \]
\[ F_{vi} = F_{mo} + AVNB \]
\[ R_m = F_{ma} + F_{mo} \]

where \( F_{in} \) is flow of amino acid into leg, \( F_{out} \) is flow of amino acid out of leg, \( F_{ma} \) is flow of amino acid from artery into muscle, \( F_{vi} \) is flow of amino acid from interstitial space to vein, \( F_{ma} \) is flow of amino acid from artery to interstitial space, \( F_{vi} \) is flow of amino acid from muscle to interstitial space, \( F_{mo} \) is flow of amino acid from muscle to interstitium, \( F_{mi} \) is flow of amino acid from interstitial space to vein, \( F_{vi} \) is flow of amino acid from artery to interstitial space, \( F_{mi} \) is flow of amino acid from artery to vein, \( F_{mo} \) is release of amino acid from bound protein to free concentration in muscle, \( R_m \) is disappearance of amino acid from the intracellular pool into protein, \( R_m \) is total rate of intracellular appearance of phenylalanine; \( E_m, E_i, E_v, \) and \( E_m \) are isotopic enrichment of phenylalanine in arterial plasma, venous plasma, interstitial fluid, and muscle sample, respectively [molar percent excess (MPE)].

Because phenylalanine is an essential amino acid in which production from the leg can come only from breakdown of muscle protein, \( F_{mo} \) for phenylalanine is a direct reflection of the rate of muscle protein breakdown. Likewise, the disappearance of phenylalanine \( (F_{mo}) \) reflects the rate of muscle protein synthesis.

To index the efficiency of transmembrane transport accounting for changes in leg blood flow and alterations in amino acid availability, the rates of amino acid transport were normalized by the rate of amino acid delivery into a given compartment and expressed as follows:

- efficiency \( F_{mi}(\%) = F_{mi}/F_{in} \)
- efficiency \( F_{mi}(\%) = F_{mi}/(F_{ma} + F_{mi}) \)
- efficiency \( F_{mi}(\%) = F_{mi}/(F_{mi} + F_{mo}) \)
- efficiency \( F_{mi}(\%) = F_{mi}/(F_{ma} + F_{mo}) \)

The concentrations of phenylalanine in femoral arterial and venous blood samples and in muscle \( (C_m) \) were measured directly. The phenylalanine concentration in the interstitium \( (C_i) \) was calculated as follows:

\[ C_i = (C_v - C_m)[1 - (F_{mi}/F_{mo})]/(F_{mi}/F_{mo}) \]

Statistical analysis. A Student’s t-test was used to assess statistical significance at \( P \leq 0.05 \).

RESULTS

Measurements in critically ill septic patients are compared with values for normal volunteers that have been reported previously and obtained using identical methodology (9). Characteristics of these septic patients and normal volunteers are noted in Table 1. Leg blood flow and the net balance of phenylalanine across the leg are noted in Table 2. The negative net balance of \(-92 \, \text{nmol} \cdot \text{min}^{-1} \cdot 100 \, \text{ml leg}^{-1} \) is indicative of substantial net muscle catabolism. Calculated values for the rate of phenylalanine transport into the leg and the net flow of phenylalanine out of the leg are shown also in Table 2. These values are significantly higher than those evident for normal volunteers, thus indicating the greater flux of amino acids into and out of muscle for these septic patients. Furthermore, the fractional synthetic rate of muscle protein as shown in Table 2 is substantially greater than reported values for normals (11), another indication of a greater flux of muscle protein in these septic, hypermetabolic patients. Calculated values for muscle protein breakdown \( (F_{mo}) \) and muscle protein synthesis \( (F_{om}) \) are reported in Table 2. These values are substantially greater than those reported for normal volunteers, further indexing the greater net catabolism and faster turnover of phenylalanine within muscle protein for septic patients.

The rates of phenylalanine transport between compartments are shown in Fig. 3. The rate of transport of phenylalanine from artery to muscle in septic patients is similar to that evident in normal volunteers. Furthermore, the rate of flow of phenylalanine from artery into the interstitial compartment and from the interstitial compartment into muscle is also similar between critically ill patients and normal volunteers. In contrast, the flow of phenylalanine from muscle to interstitium and from
interstitium to vein was nearly 20 and 50% higher, respectively, for critically ill patients compared with healthy normal subjects, thereby demonstrating an accelerated transport of amino acid out of muscle and into the venous circulation. The shunt of phenylalanine directly from artery to vein for critically ill patients was more than double that evident for normal volunteers.

The efficiency of transport between tissue compartments is noted in Table 3. Values for septic patients were similar to those of normal volunteers for the rate of transport of phenylalanine from interstitium to muscle, from muscle to interstitium, and from interstitium to vein. The efficiency of transport of phenylalanine from the artery to interstitium was >35% less in the septic patients compared with the normal volunteers. As shown in Table 4, concentrations of unbound phenylalanine were highest in muscle, less for interstitium and artery, and lowest in the venous sample. Compared with normal volunteers, absolute concentrations were higher in septic patients for each compartment except those from the femoral vein.

Extracellular fluid volume was quantified by NaBr dilution and is shown in Table 5. For the critically ill subjects, whole body extracellular water measured 24 liters. When indexed by body weight, these septic patients evidenced an extracellular fluid volume of 30% of their total body mass. For the leg of septic patients, extracellular water measured 1.6 liters. When indexed by the lean body mass of the leg as quantified by DEXA, septic patients evidenced an extracellular fluid volume within leg muscle of 18%.

**Table 3. Efficiencies of phenylalanine transport between compartments**

<table>
<thead>
<tr>
<th>Compartment</th>
<th>Normal Volunteers</th>
<th>Critically Ill Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Artery → interstitium ((F_{im}), %)</td>
<td>56 ± 7</td>
<td>34 ± 4*</td>
</tr>
<tr>
<td>Interstitium → muscle ((F_{mi}), %)</td>
<td>68 ± 3</td>
<td>60 ± 6</td>
</tr>
<tr>
<td>Muscle → interstitium ((F_{mi}), %)</td>
<td>82 ± 8</td>
<td>73 ± 8</td>
</tr>
<tr>
<td>Interstitium → vein ((F_{im}), %)</td>
<td>31 ± 4</td>
<td>40 ± 5</td>
</tr>
</tbody>
</table>

Values are means ± SD. See Calculations for definitions. *P < 0.05.

**DISCUSSION**

Previous application of this four-compartment study methodology to normal healthy volunteers demonstrated that interstitial fluid represents a distinct compartment in the movement of amino acids into and out of muscle, with the flow across the interstitium much slower than the rate of amino acid transient into muscle (9). This finding supported the concept and prior in vitro evidence of an active transport of amino acids into muscle (14). In contrast, the transient of amino acids across the interstitium appears largely dependent on diffusion and thereby rate limiting for the movement of amino acids from the vasculature and into and out of muscle. These results have a significant implication to critically ill and severely injured patients in which alterations in extremity blood flow and exaggerated increases in peripheral edema would be anticipated to have a substantial influence on muscle protein kinetics and possibly play a key role in the extent of muscle catabolism.

In the present study involving modestly septic patients with pneumonia, the absolute rates of phenylalanine transport from artery to interstitium and from interstitium to vein were substantially less than rates of transport into and out of muscle. Thus, in critically ill patients, as in normal healthy subjects, the interstitial compartment functionally limits the rate of amino acid movement between vasculature and muscle. Therefore, while transport mechanisms along the muscle cell membrane actively uptake amino acids into muscle, the delivery of these amino acids to the membrane surface are limited by diffusion through the interstitial fluid space.

On the basis of results from the study of normal volunteers, we theorized that illness-related edema would substantially reduce the rates of amino acid transport through an expanded interstitial fluid compartment. Using the Fick dilution principle, we used NaBr to measure the interstitial fluid compartment in these modestly septic patients. This technique quantified total body extracellular fluid volume as nearly 30% of the body mass, a value 50% greater than the 20% extracellular fluid volume considered normal for a 70-kg man (18). This is consistent with the clinical impression of substantial whole body edema in these patients. However, when the NaBr dilu-

**Table 4. Phenylalanine concentrations within each compartment**

<table>
<thead>
<tr>
<th>Compartment</th>
<th>Normal Volunteers</th>
<th>Septic Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>(C_a)</td>
<td>66 ± 1</td>
<td>78 ± 4*</td>
</tr>
<tr>
<td>(C_i)</td>
<td>71 ± 2</td>
<td>96 ± 5</td>
</tr>
<tr>
<td>(C_m)</td>
<td>77 ± 2</td>
<td>131 ± 14*</td>
</tr>
<tr>
<td>(C_m)</td>
<td>90 ± 4</td>
<td>146 ± 14*</td>
</tr>
</tbody>
</table>

Values are means ± SD in nmol/ml. *P < 0.05.
tion values were indexed by the lean body mass of the leg, the interstitial fluid compartment for muscle measured only 18%. These measurements suggest that, in contrast to the expanded extracellular fluid volume for the entire body, actual edema within the muscle of severely ill patients is minimal. One possibility to explain this discrepancy between extensive whole body edema with yet only a modest increase in the interstitial fluid volume within muscle may be related to the limits of expansion confined by the fascia that surrounds extremity muscles. Thus, despite the septic inflammatory stimulus for edema formation, the interstitial fluid compartment of muscle in these critically ill patients remains similar to the assumed value for the normal healthy subjects. This similarity in interstitial fluid volume may be a significant factor in the observed similarity in transport rates of phenylalanine through the interstitium for both septic patients and healthy volunteers despite the substantial differences in the flow of phenylalanine into and out of the muscle and the rates of muscle protein synthesis and breakdown. Unfortunately, the similarity in rates of amino acid transport through the interstitium between critically ill patients and normal subjects, combined with their similarity in the extracellular fluid volume for muscle, negates our ability to assess any association between extracellular fluid volume and amino acid transport through the interstitial compartment.

Prior kinetic studies have suggested that circulatory changes in subjects following resistance exercise (2) or in patients following a severe injury (13) results in an increased delivery of amino acids into the leg, which then impacts the exchange of amino acids between blood and muscle. By use of four-compartment modeling methodology, this study demonstrated that, despite an increase in leg blood flow and a nearly 40% increase in the delivery of phenylalanine into the leg, there was no overt change in the rate at which this amino acid transverses the interstitium, thereby disproving the contention that blood flow has a major impact on the amino acid transport into and out of muscle. This observation supports further the notion that amino acid transport through the interstitial fluid compartment is rate limiting for the movement of amino acids into and out of muscle. Furthermore, when the rates of phenylalanine transport into and out of each compartment are indexed as a coefficient of transport, this study demonstrates that the efficiency of amino acid transport across the muscle cell membrane remains similar between critically ill patients and normal volunteers. In contrast, the efficiency of transport from the interstitial fluid to the vein is increased slightly with critical illness. Conversely, the transport efficiency from artery to interstitial fluid is decreased in critically ill patients. Measures of the concentration of phenylalanine within each of the compartments demonstrate that the concentration gradient is increased in septic patients, favoring outward transport of amino acids from muscle, a finding which may explain the alterations in transport efficiency between artery through interstitial fluid and interstitial fluid to vein. Undoubtedly, a major factor affecting these changes in concentration within each phenylalanine compartment is the accelerated rate of protein breakdown in excess of the rate of muscle protein synthesis. Results suggest that the increased availability of phenylalanine and other amino acids from the accelerated rate of protein breakdown drives the amino acids out of the muscle and through the interstitium down their concentration gradient. Therefore, it appears that the efflux of amino acids from muscle into the vasculature is primarily related to alterations in the rate of net muscle protein catabolism and is not likely related to any alterations in tissue edema or muscle blood flow. In the context of these results, any effective therapy designed to correct or negate illness-induced muscle catabolism should be focused at altering the rates of protein breakdown and synthesis in muscle. Conversely, any attempt to negate tissue edema or normalize extremity blood flow is not likely to be of any benefit in countering muscle loss. Furthermore, given the passive nature of amino acid diffusion through the interstitium, measures from the interstitial, fourth compartment do not add or alter findings as calculated from a traditional three-compartment study. In view of the greater complexity and invasive nature, this fourth-compartment methodology appears unwarranted.

GRANTS

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REFERENCES