Metabolism of skin and muscle protein is regulated differently in response to nutrition

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Zhang, Xiao-jun, David L. Chinkes, David Doyle, J. R., and Robert R. Wolfe. Metabolism of skin and muscle protein is regulated differently in response to nutrition. Am. J. Physiol. 274 (Endocrinol. Metab. 37): E484–E492, 1998.—We have measured skin and muscle protein kinetics and amino acid (AA) transport in anesthetized rabbits during 1) 64-h fast, 2) AA infusion, 3) AA plus fat emulsion infusion, and 4) AA plus hyperinsulinemia. L-[ring-13C6]phenylalanine was infused as the tracer, and the ear and hindlimb were used as arteriovenous units to reflect skin and muscle protein kinetics, respectively. Skin protein net balance was not different from zero in all groups, indicating a maintenance of protein mass. In contrast, the muscle net balance differed over a range from −1.6 ± 0.6 after fasting to 0.2 ± 0.2 μmol·100 g−1·h−1 during hyperinsulinemia. In the skin, 59–66% of intracellular free phenylalanine came from proteolysis, and phenylalanine availability from proteolysis was positively correlated to the protein synthesis rate. In conclusion, normal skin maintains its constant protein mass by efficient reutilization of AAs from proteolysis. In contrast to muscle, skin protein is relatively insensitive to control by nutritional and hormonal factors. Because of the metabolic differences, when limb models are used for muscle protein metabolism, the potential contribution by limb skin should be considered.

stable isotopes; mass spectrometry; arteriovenous balance; rabbits

NORMAL SKIN maintains its integrity under a variety of circumstances. For example, during prolonged starvation the majority of muscle mass can be depleted, yet the integrity of the skin barrier is basically preserved. On the other hand, athletes can build up their muscle mass with appropriate training and diet, without risk of overgrown skin. Protein kinetics in the skin have received little attention, and it is uncertain how the skin maintains its relatively constant protein mass under different nutritional and hormonal conditions.

To assess skin protein kinetics, it is necessary to simultaneously quantify skin protein synthesis and breakdown rates. We have previously described a tracer model to accomplish this goal in any tissue in which we can obtain arteriovenous (A-V) difference across the tissue and a tissue biopsy (5). Quantitation also requires a distinct size of tissue sampled to determine the absolute rate of protein synthesis, breakdown, and transmembrane transport of amino acids (AAs) between blood and the tissue intracellular pool (5). We recently applied this tracer model to the rabbit ear for the study of skin protein kinetics (29). The adult rabbit ear satisfies the criteria necessary to quantify skin protein kinetics because the ear exclusively represents skin protein metabolism and has measurable mass, and A-V blood sampling across the ear is possible. In our original study, skin protein kinetics were measured in the postabsorptive state (29). The present study was performed to extend our original observation to various nutritional conditions such as brief starvation, hyperaminoacidemia (with or without lipid infusion), and hyperinsulinemia. These perturbations were selected because of their well-documented anabolic or catabolic effects at the whole body level.

In addition to quantifying the response of skin protein metabolism to a variety of factors, we have simultaneously used the same balance method to quantify the response of the leg. This approach has enabled us to compare and contrast the control mechanisms in the two tissues. Furthermore, this approach enabled us to estimate the influence of limb skin metabolism on the limb A-V balance data. The limb balance technique has been widely used to represent muscle protein metabolism, and the metabolic contribution by the limb skin has not previously been quantified.

METHODS

Animals. Male New Zealand White rabbits (Myrtle's Rabbitry, Thompson Station, TN) weighing ~4.7 kg were used for this study. The rabbits were housed in individual cages and consumed Lab Rabbit Chow HF 5326 (Purina Mills, St. Louis, MO) for weight maintenance. This study was approved by the Animal Care and Use Committee of the University of Texas Medical Branch at Galveston.

Isotopes. L-[ring-13C6]phenylalanine (99% enriched) and L-[ring-H3]phenylalanine (98% enriched) were purchased from Cambridge Isotope Laboratories (Woburn, MA).

Experimental design. There were four groups. In group 1 (fasting group, n = 10), food was deprived for 64 h but rabbits had free access to water. No nutritional or hormonal supply was given during the isotope infusion. Five rabbits of this group were originally designed to match a group of rabbits that consumed very little food after thermal injury. For this reason, during the 64-h fasting period the rabbits were given an intramuscular injection of ketamine (35 mg/kg) and xylazine (5 mg/kg) at the 16th hour for sham injury. A single dose of penicillin G benzathine suspension (Bicillin, 50,000 U/kg; Wyeth Laboratories, Philadelphia, PA) was injected intramuscularly under anesthesia. When the animals awoke, buprenorphine hydrochloride (Buprenex, 0.03 mg/kg; Reckitt & Colman Pharmaceuticals, Richmond, VA) was injected intramuscularly before the isotope infusion study. The other five rabbits in this group were fasted for 64 h without other treatment. Because the prior anesthesia and penicillin had no discernible effects (see RESULTS), the results of all the fasted animals were grouped together.

The other three groups were studied after overnight food deprivation (16 h) with free access to water; during the isotope infusion study these animals were given a balanced mixture of AAs (10% Trasaval, Baxter Healthcare,
Deerfield, IL) at 1.2 ml·kg⁻¹·h⁻¹ (prime: 0.8 ml/kg) with or without other nutritional or hormonal treatment. In group 2 (AA group, n = 5) only the AAs were infused. In group 3 (AA-lipid group, n = 5), the AA infusion was coupled with the infusion of a lipid emulsion (10% Intralipid, Kabi Pharmacia, Clayton, NC) at 3.3 ml·kg⁻¹·h⁻¹ (prime: 0.6 ml/kg). In group 4 (AA-insulin group, n = 5), insulin (Humulin R, Eli Lilly, Indianapolis, IN) was infused at 2.25 ml·kg⁻¹·min⁻¹ after a priming dose calculated to raise the plasma concentration of insulin to 1,000 pmol/l. The insulin infusion solution was prepared in physiological saline containing 0.25% albumin. Arterial blood glucose concentration was measured every 10 min and damped at 6.1 mmol/l by infusion of 25% dextrose solution. The selection of insulin dose was based on our pilot studies in which the dose of insulin was titrated to maintain euglycemia at a constant glucose infusion rate of ~10 mg·kg⁻¹·min⁻¹.

The isotope infusion study was performed under general anesthesia of ketamine and xylazine (29). After the initial anesthesia was induced, polyethylene catheters (PE-90; Becton-Dickinson, Parsippany, Nj) were inserted in the right femoral artery and vein through an incision on the groin. The arterial line was used for arterial blood collection and monitoring of heart rate and mean arterial blood pressure; the venous line was used for infusion. A tracheal tube was placed via tracheostomy. The central ear artery of the left ear was isolated and a flow probe (model 1 RB, Transonic Systems, Ithaca, NY) was placed (29). The flow probe was connected to a small animal blood flowmeter (model T106, Transonic Systems) for measurement of flow rate.

The experimental protocol is illustrated in Fig. 1. Before the start of the infusion of the tracer and nutrients, a blood sample was taken from the arterial line and a muscle specimen was taken from the groin incision for background measurements. The infusion of L-[ring-¹³C]phenylalanine (0.15 µmol·kg⁻¹·min⁻¹; prime: 6 µmol/kg) was started after completion of the surgical procedures in the fasting group; 1 h after start of the infusion of the nutrients in the AA and AA-lipid groups; and 1.5 h after the start of the infusion of insulin-glucose and Travasol in the AA-insulin group. This procedure ensured that before the tracer infusion started a physiological steady state had been achieved, including stable concentrations of blood AAs, fatty acids, glycerol, and glucose.

During the 120-150 min of tracer infusion, four simultaneous arterial and ear-venous blood samples (0.4 ml each) were taken at an interval of 10 min (29). The auricular blood flow rate was recorded at each blood sampling. A skin specimen was then taken from the ventral side of the ear. After completion of the determination of ear balance, under additional 1% lidocaine local anesthesia, the femoral artery was isolated through an incision on the left groin, and the flow probe was placed on the artery for measurement of blood flow rate in the limb. The femoral vein was dissected to facilitate blood collection by puncturing the vessel. The tracer infusion was not interrupted, and the procedure was completed by 170 min. Four simultaneous arterial and femoral-venous blood samples (0.4 ml each) were drawn every 10 min between 190 and 220 min of tracer infusion. The blood flow rate was recorded at each blood sampling, and a muscle specimen was taken from the biceps femoris between 230 and 240 min, which was required by the limb A-V model. These muscle samples were also used for determination of muscle protein fractional synthesis rate (FSR) in the fasting and AA-lipid groups. In the AA and AA-insulin groups, isotope infusion was extended for an additional 60 min, when another muscle sample was taken from the biceps femoris for determination of FSR. At the end of the study, an arterial blood sample was taken to determine plasma insulin concentration.

The blood samples were kept in an ice-water bath until the end of the infusion. The tissue samples were immediately frozen in liquid nitrogen and stored at −70°C for later analysis. The ear was cut off at the skin fold between the base and auricle to measure the ear weight. The tissue composition of the ear and hindlimb (cut at the level of inguinal ligament) was determined by dissection. The skin and cartilage were visually separated from the ear tissue, and the skin, bone, and muscle were isolated in the limb tissue.

The heart rate, mean arterial blood pressure, and rectal temperature were maintained stable by adjusting the infusion rates of anesthetics and physiological saline and heating lamps. These vital signs were recorded every 30 min. The ear skin surface temperature was maintained at 37°C by means of a heating lamp.

Sample analysis. After the collection of samples, an internal standard solution, which contained 29.7 µmol/l of L-[ring-²H₅]phenylalanine, was added to the blood (0.2 ml), and the samples were deproteinized by sulfosalicylic acid (29). The supernatant was processed to make the N-acetyl-n-propyl ester (NAP) derivatives of the AAs (27). Plasma insulin concentration was determined by the microparticle enzyme immunoassay technique (1). Blood glucose concentration was measured on a glucose-lactate analyzer (mode 2300; Yellow Springs Instruments, Yellow Springs, OH). Blood hemoglobin (Hb) concentration was measured on an automated hematologist analyzer (Coulter JT3, Hialeah, FL).

Tissue specimens, either skin or muscle, of ~60 mg were homogenized in 5% perchloric acid three times at 4°C. Phenylalanine was purified from the pooled supernatant by high-performance liquid chromatography and derivatized for the NAP derivatives (27, 29). The muscle precipitate was washed and dried at 80°C as previously described (28). The dry protein pellets were hydrolyzed in 6 N constant boiling HCl. A cation-exchange column (Dowex AG 50W-X8, Bio-Rad)
Laboratories, Richmond, CA) was used to isolate AAs. The samples were prepared for the N-heptafluorobutyryl-n-propyl ester (HFBPPr) derivatives of AA and used to determine \(^{\text{L}}\)-[ring\(^{-13}\text{C}_6\)]phenylalanine enrichment in the protein-bound pool (19).

The isotopic enrichment in the blood and purified phenylalanine from the tissue supernatant were measured on a Hewlett-Packard 5989 gas chromatograph-mass spectrometer (GC-MS) (Hewlett-Packard, Palo Alto, CA) with chemical ionization. Ions were selectively monitored at mass-to-charge ratios of 250, 251, 255, and 256 for phenylalanine (27). \(^{\text{L}}\)-[ring\(^{-13}\text{C}_6\)]phenylalanine enrichment in the protein hydrolysate was determined on a GC-MS (MD 800, Fisons Instruments, Beverly, MA) (19). Isotopic enrichment was expressed as tracer-tracee ratio after correction for the contribution of the abundance of isotopomers of lower weight to the apparent enrichment of isotopomers with larger weight and also a skew correction factor to calculate \(^{\text{L}}\)-[ring\(^{-13}\text{C}_6\)]phenylalanine enrichment (22).

Calculations. The rate of appearance (\(R_a\)) of whole body phenylalanine was calculated by the equation \(R_a = \left(\frac{F}{E_p}\right) - F_{\text{Phe}}\), where \(F\) is the infusion rate of \(^{\text{L}}\)-[ring\(^{-13}\text{C}_6\)]phenylalanine, \(E_p\) is the phenylalanine enrichment (tracer:tracee ratio) in the arterial blood at isotopic plateau, and \(F_{\text{Phe}}\) is the infusion rate of unlabeled phenylalanine in the Travasol solution. The muscle protein FSR was calculated from the equation of the direct incorporation method (16).

Protein kinetics and phenylalanine transport in the skin and muscle were calculated using a three-compartment model (5). The three pools are arterial pool (pool A), venous pool (pool V), and tissue intracellular free pool (pool T) (Fig. 2). Further definitions necessary for the description of phenylalanine kinetics are \(F_{\text{in}}\), the rate of phenylalanine entering the A-V unit via arterial flow (i.e., inflow); \(F_{\text{T,A}}\), the rate of delivery from pool A to pool T (i.e., inward transport); \(F_{\text{V,A}}\), the rate of delivery directly from pool A to pool V (A-V shunting); \(F_{\text{V,T}}\), the rate of delivery from pool T to pool V (i.e., outward transport); \(F_{\text{T,O}}\), the rate of phenylalanine leaving the A-V unit; \(F_{0,T}\), the rate of disappearance (\(R_d\)) from pool T; \(F_{\text{T,O}}\), the rate of phenylalanine from endogenous source. Because phenylalanine is neither synthesized nor degraded in peripheral tissues, endogenous \(R_d\) represents protein breakdown and \(R_d\) represents protein synthesis. Total \(R_d\) into pool T is the sum of inward transport and proteolysis. By measuring phenylalanine enrichment and concentration in the three pools and the blood flow rate, we can calculate the rates of protein kinetics and phenylalanine transmembrane transport (5, 29).

The unique aspect of this model, as opposed to previous models based on A-V balance techniques, is that inclusion of tissue data enables the calculation of the absolute rates of synthesis and breakdown, including the rate at which AAs released from breakdown are directly reincorporated into protein (i.e., intracellular recycling) without ever appearing in the blood. Other A-V models (e.g., Ref. 2) measure only arterial and venous pools (referred to as 2-pool model hereafter) and do not include the rate of intracellular reutilization. We have discussed the limitation of the two-pool model previously (11). Furthermore, our three-compartment model enables calculation of transport rates between blood and the intracellular compartment (5).

Statistics. Data are expressed as means ± SD. Differences among the four groups were evaluated using a one-way analysis of variance. The Student-Newman-Keuls test (14) was used for post hoc multiple comparison testing among the groups. Linear correlation was used to test the relationship between two parameters. A P value < 0.05 was considered statistically significant.

RESULTS

Table 1 lists the general characteristics of the rabbits. The body weight of the rabbits in the four groups was not significantly different. All the rabbits maintained a stable physiological state during the isotope infusion as shown by the relatively constant rectal temperature, heart rate, and mean arterial blood pressure. As described in METHODS, five rabbits in the fasting group received anesthetics, penicillin, and butenorphone during the fasting period to match an injury group (reported elsewhere). Because these rabbits had the same physiological and metabolic responses as the other five rabbits without prior anesthesia and penicillin, the data from these two subgroups were combined.

To determine the tissue composition, we dissected 10 ears and 10 hindlimbs. The weight of the 10 ears was 18.8 ± 1.8 g, which was composed of 78% skin (14.7 ± 1.7 g). The 10 limbs were from seven rabbits weighing 4.5 ± 0.4 kg. On average, one hindlimb contained 215 ± 20 g muscle, 49 ± 5 g skin, and 71 ± 2 g bone. From the above dissection data, the weight of the ear skin was taken to be ear weight × 78% and the weight of limb muscle was taken to be 4.8% × body weight. Because the skin and muscle weights in the ear and limb were known, the measured blood flow rates in the ear artery and femoral artery were normalized to the amount of the skin and muscle in the ear and limb, respectively (see Table 1).

By the end of the tracer infusion, plasma insulin concentrations were 12 ± 9, 58 ± 38, and 74 ± 45 pmol/l in the fasting, AA, and AA-lipid groups, respectively; the value in the fasting group was significantly (P < 0.05 or P < 0.01) lower than in the AA and AA-lipid groups. In the AA-insulin group, insulin concentration...
was raised to 1,055 ± 36 pmol/l by insulin infusion. Blood glucose concentration immediately before anesthesia was 3.8 ± 0.3 mmol/l (drawn from the ear vein of 6 overnight-fasted rabbits). During the 120–240 min of tracer infusion, arterial blood glucose concentrations were 8.7 ± 2.6, 10.2 ± 2.1, and 10.4 ± 1.0 mmol/l in the fasting, AA, and AA-lipid groups, respectively (P > 0.05 among the 3 groups). In the AA-insulin group, it took ~90 min to reach a stable euglycemia; during the 120–240 min of isotope infusion, arterial blood glucose concentration was successfully clamped at 6.1 ± 0.3 mmol/l, which required exogenous glucose infusion at 10.1 ± 0.1 mg·kg⁻¹·min⁻¹.

During the 120–240 min of tracer infusion, isotopic plateaus were maintained in the arterial blood in all rabbits (Fig. 3). The concentrations of phenylalanine in the arterial blood during the 120–150 min of ear balance and 180–210 min of leg balance were almost identical (81.9 ± 18.6 vs. 81.8 ± 17.6 nmol/ml, n = 25).

In the AA, AA-lipid, and AA-insulin groups, the infusion of 10% Travasol delivered unlabeled phenylalanine at 0.6788 µmol·kg⁻¹·min⁻¹. The Rₐ values of endogenous phenylalanine in the whole body were 1.09 ± 0.12, 1.00 ± 0.18, 0.82 ± 0.11, and 0.82 ± 0.15 µmol·kg⁻¹·min⁻¹ in the fasting, AA, AA-lipid, and AA-insulin groups, respectively; the values in the AA-lipid and AA-insulin groups were significantly (P < 0.05 or P < 0.01) lower than in the fasting and AA groups.

Table 2 presents phenylalanine enrichment and concentration in the arterial and venous blood and tissue intracellular free AA pool. The concentration of Hb in the ear-venous blood was higher than that in the arterial blood by 1.8% (133 ± 6 vs. 131 ± 6 g/l, P < 0.05 by paired t-test, n = 12), suggesting a decrease in water content in the venous blood. Therefore, the measured AA concentrations in the ear-venous blood were multiplied by 98.2% to account for the water loss from the skin surface of the ear.

Skin and muscle protein kinetics calculated from the three-compartment model are presented in Table 3. The highest rates of skin protein synthesis and breakdown were observed in the AA group, which was significantly (P < 0.01) higher than those in the other

Table 1. Physiological responses

<table>
<thead>
<tr>
<th>Group</th>
<th>Body Wt, kg</th>
<th>Rectal, T, °C</th>
<th>Heart Rate, beats/min</th>
<th>MAP, mmHg</th>
<th>Ear Wt, g</th>
<th>Blood Flow, ml·100 g⁻¹·min⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting (n=10)</td>
<td>4.7 ± 0.3</td>
<td>38.7 ± 0.5</td>
<td>175 ± 34</td>
<td>72 ± 7</td>
<td>20.1 ± 2.0</td>
<td>5.9 ± 1.5, 3.4 ± 0.6</td>
</tr>
<tr>
<td>AA (n=5)</td>
<td>4.9 ± 0.3</td>
<td>39.7 ± 0.8</td>
<td>188 ± 8</td>
<td>71 ± 9</td>
<td>20.6 ± 1.3</td>
<td>7.7 ± 2.7, 3.7 ± 0.7</td>
</tr>
<tr>
<td>AA-lipid (n=5)</td>
<td>4.7 ± 0.2</td>
<td>38.8 ± 0.4</td>
<td>185 ± 23</td>
<td>79 ± 5</td>
<td>18.3 ± 0.7</td>
<td>7.6 ± 1.7, 4.0 ± 0.6</td>
</tr>
<tr>
<td>AA-insulin (n=5)</td>
<td>4.6 ± 0.1</td>
<td>38.6 ± 0.6</td>
<td>192 ± 16</td>
<td>73 ± 8</td>
<td>18.9 ± 0.9</td>
<td>6.9 ± 1.4, 3.8 ± 0.4</td>
</tr>
</tbody>
</table>

Data are means ± SD; n = no. of rabbits. Values of rectal temperature (T), heart rate, mean arterial blood pressure (MAP), ear blood flow rate, and limb blood flow rate are all averages during the 120–240 min of isotope infusion. AA, amino acid.
Table 2. Phenylalanine concentration and enrichment data

<table>
<thead>
<tr>
<th>Group</th>
<th>Ear</th>
<th>Leg</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>$E_A$</td>
<td>$E_V$</td>
</tr>
<tr>
<td>Fasting (n = 10)</td>
<td>0.1443 ± 0.0162</td>
<td>0.1249 ± 0.0185</td>
</tr>
<tr>
<td>AA (n = 5)</td>
<td>0.0832 ± 0.0128</td>
<td>0.0751 ± 0.0126</td>
</tr>
<tr>
<td>AA-lipid (n = 5)</td>
<td>0.0948 ± 0.0100</td>
<td>0.0879 ± 0.0102</td>
</tr>
<tr>
<td>AA-insulin (n = 5)</td>
<td>0.1005 ± 0.0082</td>
<td>0.0923 ± 0.0105</td>
</tr>
</tbody>
</table>

Data are means ± SD; n = no. of rabbits. $E_A$, $E_V$, and $E_T$ are free phenylalanine enrichments (tracer-to-tracee ratio) in arterial blood, venous blood, and tissue intracellular pool, respectively. $C_A$ and $C_V$ are concentrations of phenylalanine ($\mu$mol/l) in arterial and venous blood, respectively, which were measured by internal standard method.

The phenylalanine transport data are presented in Table 4. The rate of AA entering the ear ($F_{in}$, see Fig. 2) was lowest in the fasting group ($P < 0.01$ vs. AA and AA-lipid groups, $P > 0.05$ vs. AA-insulin group). Because of the smaller A-V shunting ($F_{in}$) in the fasting group ($P < 0.01$ vs. AA and AA-lipid groups, $P < 0.05$ vs. AA-insulin group), the rate of inward transport ($F_{TA}$) in the fasting group was significantly ($P < 0.05$) lower than only the AA group ($P < 0.01$) and not significantly different from those in the AA-lipid and AA-insulin groups. Similarly, whereas the rate of EA entering the limb ($F_{in}$) was lower in the fasting group ($P < 0.01$ vs. AA, AA-lipid, and AA-insulin groups) because of significantly ($P < 0.01$) smaller A-V shunting ($F_{V/A}$) in the fasting group than in the other groups, the rate of inward transport ($F_{TA}$) was significantly lower than only the AA group and not different from the AA-lipid and AA-insulin groups.

When phenylalanine transport data were compared between skin and muscle, the %shunting ($F_{V/A}/F_{in}$) in the skin (84 ± 6%, n = 25) was significantly ($P < 10^{-11}$) greater than in the muscle (57 ± 12%, n = 25). The ratio of inward transport to total R, an indicator of fractional contribution of inward transport to total phenylalanine availability in the tissue intracellular free amino acid pool, was significantly ($P < 10^{-12}$) greater in the muscle (61 ± 9%, n = 25) than in the skin (36 ± 8%, n = 25). The ratio of protein synthesis to total intracellular appearance of AAs, an indicator of the efficiency of protein synthesis, was significantly ($P < 10^{-13}$) greater in the skin (64 ± 8%, n = 25) than in the muscle (32 ± 8%, n = 25).

When the values of protein synthesis and breakdown were pooled from all the groups, significant ($P < 0.01$) linear correlations were found in both skin (Fig. 4A) and muscle (Fig. 4B).

The phenylalanine enrichments in the muscle intracellular free and protein-bound pool are presented in Table 5. The muscle protein FSRs were not significantly different ($P > 0.05$) among groups, which is consistent with the synthesis data from the limb A-V model (see Table 3).

**DISCUSSION**

The present study demonstrated that, in contrast to muscle, skin maintains its protein mass under a variety of conditions. Thus skin protein mass is insensitive to control by nutritional and hormonal factors, which are generally important in other tissues.

We have previously reported the maintenance of skin protein balance in the postabsorptive state using either the ear skin model (29) or a parallel tissue model (8). In the present study, the observation of a maintenance of protein mass in the absence of nutritional intake was extended to 64 h. In contrast, the muscle mass was in strikingly negative balance. This result is in agreement with the data reported by Cherek et al. (12). By determining total skin nitrogen content they demonstrated the maintenance of skin protein mass in rats fasting up to 5 days. Our data indicate that the efficient reutilization of AAs from protein breakdown was the primary mech-
Table 4. Phenylalanine transport in ear skin and limb muscle

<table>
<thead>
<tr>
<th>Group</th>
<th>Ear</th>
<th>Limb</th>
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<tbody>
<tr>
<td></td>
<td>Pheny lalanine, µmol·100 g tissue⁻¹·h⁻¹</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Inflow via artery</td>
<td>Inward transport</td>
</tr>
<tr>
<td>Fasting (n = 10)</td>
<td>23.3 ± 7.0</td>
<td>4.8 ± 1.9</td>
</tr>
<tr>
<td>AA (n = 5)</td>
<td>49.2 ± 17.9*</td>
<td>8.0 ± 3.4*</td>
</tr>
<tr>
<td>AA-lipid (n = 5)</td>
<td>41.4 ± 6.8*</td>
<td>5.0 ± 1.2</td>
</tr>
<tr>
<td>AA-insulin (n = 5)</td>
<td>35.2 ± 9.4</td>
<td>4.3 ± 1.1</td>
</tr>
</tbody>
</table>

Data are means ± SD; n = no. of rabbits. Inflow via artery (F in), rate of delivery into system via artery; inward transport (F T,A), rate of delivery from artery directly to vein; outward transport (F V,T), rate of delivery from tissue free pool to vein; total Ra, total rate of appearance into tissue free pool (i.e., sum of F T,A plus protein breakdown); PS, protein synthesis; %shunting, A-V shunting/inflow via artery ratio. *P < 0.01, †P < 0.05 vs. fasting group; ‡P < 0.01 vs. AA group.

Fig. 4. A: correlation (P < 0.01) between protein synthesis rate and breakdown rate in skin; correlation is expressed by synthesis rate = 1.0528 × (breakdown rate) + 0.4495. B: correlation (P < 0.01) between protein synthesis rate and breakdown rate in muscle; correlation is expressed by synthesis rate = 0.5822 × (breakdown rate) + 1.0525. Unit of protein synthesis and breakdown in skin and muscle is µmol phenylalanine·100g tissue⁻¹·h⁻¹.

The amino acid responsible for the maintenance of protein mass. Not only was balance maintained in skin when nutrient intake was stopped, but the infusion of AA solution with or without lipid or glucose-insulin did not cause a significant improvement in the skin phenylalanine net balance (see Table 3).

Despite the absence of an effect on net balance, the AA infusion stimulated skin protein synthesis and breakdown (see Table 3). Cherel et al. (12) also reported that, despite unchanged skin protein mass in rats fasted for up to 5 days, skin protein FSR decreased from 9.3 to 5.8%/day over this time span. We further demonstrated that the same metabolic feature remained effective in the AA-lipid and AA-insulin groups, in which either fat emulsion or hyperinsulinemia inhibited both skin protein synthesis and breakdown rates (in comparison with AA group) without affecting net balance (see Table 3). These changes could be explained by a dominant role of protein breakdown in determining the rate of protein synthesis in the skin. Because the skin depends on its own proteolysis for the majority of AA availability in the intracellular free AA pool (see Table 4), an inhibition of breakdown would be expected to slow synthesis by decreasing the intracellular availability of AAs. This notion is supported by the almost perfect correlation between the rates of breakdown and synthesis in the skin (Fig 4A). The inward transport of AA from blood played a lesser role in the intracellular AA availability, as only 34–41% of the intracellular AA pool in skin came from blood (see Table 4). Furthermore, the rate of inward transport was counteracted by the equal rate of outward transport (see Table 4), resulting in no net change in AA availability.

In comparison with the skin, the limb protein metabolism was more responsive to nutritional and hormonal treatment (see Table 3). These results support the notion that skeletal muscle is a mobilizable source of AAs in the body. In the AA-lipid and AA-insulin groups the net limb balance was significantly improved because of the inhibition of breakdown rate. The inhibitory effect of lipid and insulin on protein breakdown is...
in agreement with previous reports (13, 18, 24, 26) and the whole body phenylalanine Ra in this study. Whereas the correlation between the rates of muscle protein synthesis and breakdown was significant (Fig. 4B), the r value was less than for skin (0.75 vs. 0.99, respectively). This reflects the fact that muscle relied to a smaller extent on protein breakdown as a source of precursors than did skin.

Some data from the limb model are not consistent with the results we previously obtained in normal subjects. Thus the infusion of AA solution did not improve net balance in the limb and hyperinsulinemia did not stimulate muscle protein synthesis in the rabbits, as it did in human subjects (4, 7). These discrepancies could have been due to the surgery in these rabbits, which apparently induced catabolic stress and modified vascular and metabolic responses. The catabolic stress was reflected by hyperglycemia during the isotope infusion period, even when no glucose was infused. We have previously found that nutrients, either alone or with short-term hyperinsulinemia, are less effective in eliminating net protein catabolism under surgical or burn stress (15, 23). In the current study, infusion of insulin and AA did not stimulate either the inward transport or the muscle synthesis. In the AA group, although the rate of inward transport was greater than in the fasting group, because of the concomitant increase in the rate of outward transport, neither the total phenylalanine Ra in the muscle intracellular pool nor the synthesis rate was significantly increased (see Tables 3 and 4). These results support the notion that the inward AA transport and/or protein synthesis did not respond normally to anabolic stimuli (9, 23). Also the limb blood flow was not increased by either AAs or insulin in the current experiment. In normal humans, we have found an increase in blood flow due to insulin infusion (7) and a significant correlation between leg blood flow and inward AA transport in a variety of circumstances, such as during local insulin infusion (7) and after exercise recovery (10). Therefore, it is likely that the vascular and metabolic responses in these rabbits were modified so that the synthesis rate of muscle protein was not normally responsive to nutrient or hormone treatments. The possible role of stress in modifying the normal responses of muscle does not reduce the significance of the difference between the responses of skin and muscle to nutritional treatments. Rather, it underscores the ability of skin protein mass to be maintained in what is likely to be a catabolic circumstance at the whole body level.

The metabolic differences between skin and muscle were also reflected by the %shunting (see Table 4). The large %shunting (84%) in the skin (in comparison to 57% in muscle) indicated that only a small portion (16%) of AAs in blood were exchanged with the AAs in the intracellular compartment. This may reflect physical shunting of blood in the skin due to its role in thermoregulation, as well as the efficiency of reutilization of intracellular AAs in skin decreasing the demand for blood-borne AAs.

In this study, we used tissue free phenylalanine enrichment as an approximation of labeling of the aminoacyl-tRNA. In muscle tissue, the aminoacyl-tRNA enrichment measured either in pigs (3) or in humans (25) is not different from the muscle free fluid enrichment, which is mainly the intracellular AA enrichment. In the case of skin, no data of aminoacyl-tRNA enrichment have been published. The available data are derived from the use of skin free AA enrichment as the precursor enrichment (8, 12, 20, 21, 29). The skin free fluid is a mixture of intracellular and interstitial fluid. Because the interstitial pool is located between blood vessels and the intracellular pool, the enrichment of interstitial AA should be lower than blood enrichment but higher than the intracellular enrichment. Thus the inclusion of interstitial fluid in the skin samples could only overestimate the true precursor enrichment. Thus the values of skin protein synthesis, breakdown, and intracellular recycling presented in this paper should be considered as minimal estimates of the true values. In any case, conclusions regarding net protein synthesis or breakdown are independent of this issue. Therefore, our conclusions about the differences between muscle and skin have a sound basis. Even though we used a two-pool model based entirely on blood values (2), the responses of skin and muscle protein synthesis, breakdown, and net balance are similar to those calculated from the three-pool model, although the rates of synthesis and breakdown from the two-pool model are much lower.

<table>
<thead>
<tr>
<th>Group</th>
<th>Intracellular Free Enrichment</th>
<th>Protein-Bound Enrichment</th>
<th>Infusion Hours</th>
<th>FSR, %/h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting (n = 10)</td>
<td>0.0798 ± 0.0079</td>
<td>0.000252 ± 0.000055</td>
<td>4.03 ± 0.44</td>
<td>0.0791 ± 0.0162</td>
</tr>
<tr>
<td>AA (n = 5)</td>
<td>0.0551 ± 0.0066</td>
<td>0.000255 ± 0.000053</td>
<td>5.25 ± 0.10</td>
<td>0.0886 ± 0.0180</td>
</tr>
<tr>
<td>AA-lipid (n = 5)</td>
<td>0.0644 ± 0.0100</td>
<td>0.000227 ± 0.000046</td>
<td>4.00 ± 0.49</td>
<td>0.0896 ± 0.0176</td>
</tr>
<tr>
<td>AA-insulin (n = 5)</td>
<td>0.0681 ± 0.0073</td>
<td>0.000309 ± 0.000044</td>
<td>4.79 ± 0.21</td>
<td>0.0948 ± 0.0112</td>
</tr>
</tbody>
</table>

Data are means ± SD; n = no. of rabbits. Enrichment is expressed as tracer-to-tracee ratio. Muscle intracellular free enrichment is used as precursor enrichment. FSR, fractional synthesis rate.
strate that the rat hemicorpus contains 39% by weight of nonmuscle tissue. Because skin and bone have faster protein synthesis rates than muscle, Lobley et al. (17) and Preedy and colleagues (20, 21) state that the metabolic contribution by the nonmuscle tissue could be a considerable portion of the measured protein kinetics in the hemicorpus. Our dissection data showed that the rabbit hindlimb contains 36% by weight of nonmuscle tissue (skin and bone). In particular, the weight of skin was equal to 23% of muscle weight, and the protein synthesis rate in the ear skin was 1.8- to 3.5-fold that in the limb muscle (see Table 3). The real contribution of limb skin to limb A-V data should be smaller than that estimated from the protein mass and synthetic rate because the skin had a large intracellular AA cycle. Using the data in Table 2 and blood flow rates in Table 1, we can calculate that the rates of skin tracer uptake were 0.44 ± 0.15, 0.43 ± 0.09, 0.27 ± 0.08, and 0.27 ± 0.06 µmol·100 g⁻¹·h⁻¹ in the fasting, AA, AA-lipid, and AA-insulin groups, respectively. If the limb skin had the same tracer uptake as the ear skin and 100% of limb skin was drained by the femoral vein, the limb skin accounted for 35 ± 16, 42 ± 11, 25 ± 7, and 26 ± 10% of the total limb tracer uptake. Statistical analysis shows that the rates of skin tracer uptake were significantly (P < 0.05) higher in the fasting than in the AA-lipid and AA-insulin groups, indicating a variable contribution of skin to the limb balance. Consequently, attention should be paid to the nonmuscle tissue when limb models are used to reflect muscle protein metabolism, especially when the skin and muscle have opposite responses. In this study, this source of error would not affect the validity of the comparison between skin and muscle because the inclusion of skin protein kinetics in the limb balance data could only reduce the metabolic differences between skin and muscle. Even if we simultaneously calculate protein kinetics in the limb muscle and limb skin using the parallel tissue model (8), we will reach the same conclusion regarding the different regulation of protein metabolism in these two tissues (data not presented here).

In summary, normal skin maintains a relatively constant protein mass under both catabolic and anabolic conditions. This is because the majority of AAs for synthesis come from protein breakdown and the inward AA transport from blood is counteracted by the outward transport. The skin has a large A-V functional shunting and a large intracellular AA cycle, both of which ensure protein homeostasis and insensitivity to the changes of nutrients and hormones in blood circulation. Whereas the protein mass in the skin is well maintained, the absolute rate of turnover can change in response to nutritional and hormonal conditions. Because the skin may have substantial and variable contribution to the limb balance data, when limb models are used to reflect muscle protein kinetics, potential contribution from the limb skin should be considered.

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