Leucine Supplementation Has an Anabolic Effect on Proteins in Rabbit Skin Wound and Muscle[1,2]

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ABSTRACT We investigated the effect of leucine supplementation on protein metabolism in skin wounds and muscle in anesthetized rabbits. L-[ring-$^{13}C_6$]phenylalanine was infused on d 7 after the ear was scalded, and the scalded ear and uninjured hindlimb were used as arteriovenous units to reflect protein kinetics in skin wounds and muscle. In comparison with a commercially available amino acid solution (10% Travasol), isonitrogenous [1638 μmol/(kg · h)] infusion of the amino acid solution with supplemental leucine to account for 35% of total nitrogen increased the net phenylalanine balance ($P < 0.05$) in the skin wound and muscle from -6.7 ± 6.1 to 0.9 ± 1.4 and from -4.4 ± 2.4 to -1.0 ± 0.4 μmol/(100 g · h), respectively. Infusion of leucine alone did not significantly improve the net phenylalanine balance in either skin wounds [-4.0 ± 4.6 μmol/(100 g · h)] or muscle [-2.7 ± 0.7 μmol/(100 g · h)]. We conclude that leucine supplementation had an anabolic effect on proteins in skin wounds and muscle, provided that adequate additional amino acids were also available. J. Nutr. 134: 3313–3318, 2004.

KEY WORDS: • *stable isotopes* • *gas chromatograph-mass spectrometer* • *fractional synthesis rate* • *arteriovenous balance*

The amino acid (AA)[4] leucine (Leu) has multiple metabolic functions. As an energy source, Leu can provide calories via oxidation (1,2). The amino group released during Leu oxidation can be used to synthesize alanine and glutamine (1,3,4) to meet their increased demand in catabolic states (4). Leu can stimulate pancreatic section of insulin (5), thereby regulating substrate metabolism. More importantly, a series of studies demonstrated beneficial effects of Leu on protein metabolism under diverse conditions including normal subjects (6,7), recovery from exercise (8), burns (9), sepsis (1,4), liver diseases (10), and acute uremia (11). Therefore, Leu has been proposed to be an important regulator of protein metabolism (5,12,13).

Whereas the anabolic effect of Leu on protein metabolism at the whole body level (6,7,10) and at the level of some tissues such as muscle and liver (8,9,11) has been reported, its effect on protein metabolism in skin wounds is not clear. Because healing of a skin wound requires deposition of new proteins (14,15), an improvement in protein net balance in the wound should facilitate the healing process. The present experiment was designed to investigate the effect of Leu supplementation on protein kinetics in the thermally injured skin. Our previous experiment showed that infusion of a commercially available AA solution (100 g/L; Travasol, Baxter Healthcare), in which Leu accounts for 5% (mol/mol) of total nitrogen, was not sufficient to reduce net protein loss in the skin wound (16). In the present study we tested the effect of the infusion of Leu, either alone or with the Travasol solution, on protein metabolism in the skin wound. Further, muscle catabolism is a feature of the response to burn injury (17); an optimal nutritional formula should not only enhance wound healing but also ameliorate muscle loss. Therefore, we also measured the responsiveness of the skeletal muscle to Leu supplementation.

METHODS

Animals. Male New Zealand White rabbits (Myrtle's Rabbitry), weighing 4–5 kg, were used for this study. The rabbits were housed in individual cages and were given 150 g/d of Lab Rabbit Chow No. HF 5326 (Purina Mills) for weight maintenance. This protocol complied with NIH guidelines and was approved by the Animal Care and Use Committee of The University of Texas Medical Branch at Galveston.

Isotopes. L-[ring-$^{13}C_6$]phenylalanine (Phe, 99% enriched), L-[ring-$^{2}H_5$]Phe (98% enriched), and L-[$^{2}H_7$]proline (97–98% enriched) were purchased from Cambridge Isotope Laboratories. L-[ring-$^{13}C_6$]Phe was used as the tracer for intravenous infusion. L-[ring-$^{2}H_5$]Phe and L-[$^{2}H_7$]proline were used as internal standards for measurement of Phe and proline concentrations in the blood.

Skin wound. A partial-thickness thermal injury was created on the left ear by submerging the ear in 72°C water for 3 s under general anesthesia (16,18). Immediately after the scald injury, a single dose of antibiotic (Bicillin, 50 kU/kg; Wyeth Laboratories) was injected i.m. to prevent wound infection. When the rabbits awakened from anes-

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[4] Abbreviations used: AA, amino acid; a-v, arteriovenous, BCAA, branched chain AA; Leu, leucine; *m/z*, mass-to-charge; NAP, *N*-acetyl, *n*-propyl ester; Phe, phenylalanine; TBDMS, *t*-butyldimethylsilyl; 25% Leu/AA, leucine accounts for 25% of AA nitrogen; 35% Leu/AA, leucine accounts for 35% of AA nitrogen.

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thesia, buprenorphrine (0.03 mg/kg) was injected i.m. twice a day for 3 d as an analgesic.

Experimental design. There were 4 groups: AA ($n = 7$), Leu ($n = 6$), 25% Leu/AA ($n = 7$), and 35% Leu/AA ($n = 7$). In the AA group, Travasol (100 g/L) was infused at 1.5 mL/(kg · h) to deliver AA nitrogen at 1638 μmol/(kg · h) [including Leu at 83.4 μmol/(kg · h)]. In the Leu group, only Leu was infused at 525 μmol/(kg · h). In the 25% Leu/AA group, Leu was infused at 350 μmol/(kg · h) and Travasol was infused at 1.18 mL/(kg · h) [including Leu at 65 μmol/(kg · h)] so that Leu accounted for 25% of total AA nitrogen. In the 35% Leu/AA group, Leu was infused at 520 μmol/(kg · h) and Travasol was infused at 1.0 mL/(kg · h) [including Leu at 57 μmol/(kg · h)] so that Leu accounted for 35% of total AA nitrogen (**Table 1**).

The isotope infusion study was performed on d 7 after injury, as in our previous experiments (16,18). The anesthesia and surgery were described in detail in our previous publications (16,18–20). In brief, after an overnight food deprivation, the rabbits were anesthetized with ketamine and xylazine. Catheters were inserted in the right femoral artery and vein through an incision on the groin. The arterial catheter was used for blood collection and monitoring of heart rate and mean arterial blood pressure; the venous catheter was used for infusion. A tracheal tube was placed via tracheotomy. The free end of the tracheal tube was placed in an open hood that was connected to an oxygen supply line so that the rabbits spontaneously breathed oxygen-rich room air. The central artery of the scalded ear and the left femoral artery were isolated for placement of flow probes (Model 1 RB for ear artery and Model 1.5 RB for femoral artery; Transonic Systems). The flow probes were connected to a small animal blood flowmeter (Model T106; Transonic Systems) for measurement of blood flow rates. The ipsilateral carotid artery was isolated, and a bulldog clamp was placed on the artery to control the blood flow rate in the scalded ear at 4- to 5-fold the rate in normal skin (16). This procedure ensured accurate measurement of arteriovenous (a-v) difference of Phe enrichment and would not cause nutritional deficiency in the wound (16). After completion of the surgical procedures, a blood sample was taken from the arterial line, and skin and muscle samples were taken from the right leg for measurement of background enrichment.

The infusion of L-[ring-$^{13}C_6$]Phe [infusion rate, 0.12–0.18 μmol/(kg · min); prime, 4.8–7.2 μmol/kg] was started 1 h after the start of Travasol and/or Leu infusion to ensure a stable nutritional condition during the tracer infusion period. During the 150–240 min of the tracer infusion, 4 pairs of arterial and ear venous and 4 pairs of arterial and femoral venous blood were collected in an alternating manner. The arterial blood was drawn from the catheter placed in the right femoral artery. The venous blood was drawn by directly penetrating the left femoral vein or the left marginal ear vein. The blood flow rate in the ear center artery or in the left femoral artery was recorded at each a-v sampling. At 240 min, a muscle sample was taken from the adductor muscle (predominantly slow-twitch fibers) of the left leg and a skin sample was taken from the ventral side of the scalded ear. The tissue samples were immediately frozen in liquid nitrogen and stored at −80°C for later processing. Additional arterial blood was taken for measurement of plasma AA and insulin concentrations and blood gas analysis. Finally, both ears were resected at the skin fold between the base and auricle to measure the ear weight (16,18,19).

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

Sample analyses

Blood samples. The blood samples were deproteinized by sulfosalicylic acid solution containing L-[ring-$^2H_5$]Phe as an internal standard for calculation of Phe concentration (16). After centrifugation, the supernatant was processed to make the *t*-butyldimethylsilyl (TBDMS) derivatives of AAs (21). Plasma insulin concentration was determined by an RIA kit (Diagnostic Products). Blood gas was analyzed on an Atat Profile 5 Analyzer (Nova Biomedica). The concentrations of AAs in the plasma and in the free AA pools in the skin wound and muscle were measured on a Waters 2690 HPLC system (Waters) (16). Because the HPLC system did not measure proline concentration, we used the internal standard method with mass spectrometry to measure proline concentration in the arterial blood (16,22).

Tissue samples. Tissue samples of ~50 mg were homogenized in 10% (w:v) perchloric acid and the supernatant was processed to make the TBDMS derivatives for measurement of Phe enrichment in the tissue-free pools (16). The protein precipitates of muscle samples were thoroughly washed to remove free AAs and lipids and dried in an oven at 80°C (19). The dry protein pellets were hydrolyzed in 6 mol/L HCl at 110°C for 24 h and processed for the *N*-acetyl, *n*-propyl ester (NAP) derivatives of AAs (22).

Measurement of isotopic enrichment. The isotopic enrichments in the blood and tissue supernatants were determined on a Hewlett-Packard 5973 GC-MS; ions were selectively monitored at mass-to-charge (*m/z*) ratios of 234, 235, 239, and 240 for Phe enrichment (in TBDMS derivative) and at *m/z* ratios of 200 and 207 for proline enrichment (in NAP derivative). L-[ring-$^{13}C_6$]Phe enrichment was corrected for the contribution of the abundance of isotopomers of lower weight to the apparent enrichment of isotopomers with larger weight, and a skew correction factor was applied (23). L-[ring-$^{13}C_6$]Phe enrichment in the muscle protein hydrolysate was determined on a GC-combustion-isotope ratio MS (Finnigan, MAT). The measured $^{13}CO_2$ enrichment was converted to Phe enrichment by multiplying by 14/6 to account for the dilution of 6 labeled carbons with total 14 carbons in the derivatized Phe.

Calculations. Protein and Phe kinetics in the scalded skin and muscle were calculated by the 3-pool model, which was originally developed in leg muscle (24) and then applied to ear skin (19). The equations are as follows.

TABLE 1

Infusion rates of amino acids in rabbits during stable isotope infusion period[1]

Group	*n*	Infusion rate					% of Leu in AA solution
		Leu	10% Travasol	Leu in Travasol	Total Leu	Total nitrogen	
		μmol of amino acid nitrogen · kg^{-1} · h^{-1}					
AA	7	0	1638	83.4	83.4	1638	5
Leu	6	525	0	0	525	525	100
25% Leu/AA	7	350	1288	65	514	1638	25
35% Leu/AA	7	520	1118	57	574	1638	35

[1] One hundred milliliters of 10% Travasol solution contains 730 mg L-leucine, 600 mg L-isoleucine, 580 mg L-lysine hydrochloride, 580 mg L-valine, 560 mg L-phenylalanine, 480 mg L-histidine, 420 mg L-threonine, 400 mg L-methionine, 180 mg L-tryptophan, 2.07 g L-alanine, 1.15 g L-arginine, 1.03 g glycine, 680 mg L-proline, 500 mg L-serine, and 40 mg L-tyrosine.

$$\text{Inflow} = C_A \times BF \quad (1)$$

$$\text{Inward transport} = \{[(E_T - E_V)/(E_A - E_T)] \times C_V + C_A\} \times BF \quad (2)$$

$$\text{a-v shunting} = \{-[(E_T - E_V)/(E_A - E_T)] \times C_V\} \times BF \quad (3)$$

$$\text{Outward transport} = \{[(E_T - E_V)/(E_A - E_T)] \times C_V + C_V\} \times BF \quad (4)$$

$$\text{Outflow} = C_V \times BF \quad (5)$$

$$NB = (C_A - C_V) \times BF \quad (6)$$

$$\text{Synthesis} = [(E_A \times C_A - E_V \times C_V)/E_T] \times BF \quad (7)$$

$$\text{Breakdown} = \text{synthesis} - NB \quad (8)$$

where E_A, E_V, and E_T are Phe enrichment in the arterial blood, ear or femoral venous blood, and tissue (skin or muscle)-free AA pool, respectively. C_A and C_V are Phe concentration in the arterial blood and ear or femoral venous blood, respectively. BF is the blood flow rate in the scalded ear or hindlimb; and NB is net Phe balance. Inflow is the rate of Phe entering the ear or limb via the artery; inward transport is the rate of delivery from the artery to the tissue free pool; a-v shunting is the rate of delivery directly from artery to vein; outward transport is the rate of delivery from the tissue free pool to vein; and outflow is the rate of Phe exit via vein. Because Phe is neither synthesized nor degraded in the peripheral tissues, its rate of appearance represents protein breakdown and rate of disappearance represents protein synthesis.

The fractional synthetic rate (FSR) of muscle protein was calculated by means of the tracer incorporation method (22). Tissue-free Phe enrichment was used as the precursor for protein synthesis. The acceptability of this surrogate of the true precursor enrichment is supported by in vivo (25) and in vitro (26) experiments.

Statistical analysis. Data are expressed as means ± SD. Differences among the 4 groups were evaluated using one-way ANOVA. Post hoc testing was accomplished using Tukey's test for equal variance test. If the equal variance test failed, the Kruskal-Wallis one-way ANOVA on rank was used to test the differences and Dunn's method was used to accomplish post hoc testing. A P value < 0.05 was considered significant.

RESULTS

The general conditions of the rabbits, including body weight and food intake, were comparable among groups before the stable isotope infusion and rectal temperature, heart rate, mean arterial blood pressure, and arterial oxygen saturation during the infusion period (data not presented here). The blood flow rate recorded from the ultrasonic flowmeter was in mL/ear or leg/min. In order to convert this unit to mL/(100 g tissue · min), the tissue weight in the ear or leg was needed. The weight of the scalded ear poorly reflected tissue mass because of variable edema. In our previous experiment, we found that the dry weight of ear skin in the scalded ear was not different from that in the contralateral normal ear (16). We therefore used the contralateral normal ear to estimate the ear skin weight. The ear skin weight was estimated by ear weight × 78% (w:w, the rest is ear cartilage), which was derived from dissection of 10 normal ears in our previous experiment (20). The muscle mass in the hindlimb was calculated by body weight × 4.8% (w:w), which was derived from the dissection of 10 legs (20). After conversion, the blood flow rates were 32–38 and 5.8–6.8 mL/(100 g tissue · min) in the skin wound and leg muscle ($P = 0.18$ and $P = 0.36$, respectively).

Plasma glucose concentrations in the 4 groups did not differ (11.3–13.9 mmol/L; $P = 0.15$). Plasma insulin concentration in the Leu group (15 ± 11 pmol/L) tended to be lower than in the AA (40 ± 22 pmol/L; $P = 0.09$), 25% Leu/AA (37 ± 37 pmol/L; $P = 0.57$), and 35% Leu/AA groups (39 ± 23 pmol/L; $P = 0.12$). The lack of statistical significance likely was due to the large within-group variability. The majority of plasma AAs had lower concentrations in the Leu group than in the AA group ($P < 0.05$, **Table 2**). Plasma Leu concentrations were

TABLE 2

Plasma amino acid concentrations in AA, Leu, 25% Leu/AA, and 35% Leu/AA groups of rabbits at the end of tracer infusion[1,2]

	AA (n = 7)	Leu (n = 6)	25% Leu/AA (n = 7)	35% Leu/AA (n = 7)
			μmol/L	
AsX	70 ± 16[a]	37 ± 6[b]	39 ± 19[b]	57 ± 28[a,b]
GlX	970 ± 42	698 ± 111	1226 ± 563	964 ± 344
Serine	739 ± 299[a]	145 ± 50[b]	330 ± 115[b]	391 ± 160[b]
Histidine	236 ± 61[a]	105 ± 16[b]	159 ± 35[a,b]	238 ± 170[a]
Glycine	2175 ± 440[a]	577 ± 397[c]	1510 ± 617[a,b]	1260 ± 442[b,c]
Arginine	510 ± 72[a]	105 ± 32[b]	352 ± 74[a,b]	501 ± 180[a]
Alanine	687 ± 175[a]	129 ± 26[b]	376 ± 120[c]	312 ± 87[c]
Tyrosine	42 ± 12	21 ± 4	32 ± 13	36 ± 28
Proline	389 ± 90[a]	141 ± 40[b]	306 ± 53[a]	296 ± 50[a]
Threonine	360 ± 108[a]	117 ± 29[b]	227 ± 80[a,b]	237 ± 121[a,b]
Valine	443 ± 41[a]	187 ± 40[b]	367 ± 68[a,b]	460 ± 237[a]
Methionine	112 ± 28[a]	18 ± 8[b]	66 ± 23[c]	56 ± 7[c]
Tryptophane	47 ± 19[a]	21 ± 2[b]	38 ± 3[a,b]	39 ± 10[a]
Phenylalanine	100 ± 30[a]	43 ± 11[b]	76 ± 17[a]	80 ± 16[a]
Isoleucine	214 ± 26[a]	62 ± 18[b]	177 ± 32[a,b]	235 ± 122[a]
Leucine	335 ± 48[c]	1289 ± 281[b]	1183 ± 188[b]	1790 ± 304[a]
Lysine	378 ± 43[a]	141 ± 46[b]	306 ± 103[a]	319 ± 100[a]
BCAA	992 ± 112[b]	1538 ± 307[a,b]	1726 ± 250[a,c]	2402 ± 599[a]
EAA	1988 ± 233[a]	1877 ± 350[a]	2439 ± 408[a,b]	3134 ± 800[b]
NEAA	5818 ± 1245[a]	1956 ± 486[c]	4331 ± 1185[a,b]	3970 ± 1246[b]
TAA	7806 ± 1376[a]	3833 ± 508[b]	6770 ± 1491[a]	7104 ± 1898[a]

[1] Values are means ± SD; means without a common superscript letter within a row differ, $P < 0.05$.

[2] Abbreviations used: AsX, sum of asparagine and aspartic acid; EAA, essential AA; GlX, sum of glutamine and glutamic acid; NEAA, nonessential AA; TAA, total AA.

higher in the Leu, 25% Leu/AA, and 35% Leu/AA groups than in the AA group ($P < 0.01$, Table 2). Consistently, in the free AA pools in skin wound and muscle Leu concentrations were also higher in the Leu-supplemented groups than in the AA group ($P < 0.01$, **Fig. 1**).

Protein kinetics and Phe transport in skin wound and muscle were calculated from Eqs. (1–8) using Phe enrichment and concentration in the arterial and venous blood and Phe enrichment in the free AA pools in tissues (data not presented here) as well as the blood flow rates. The skin wound data in the AA group were reported in our previous publication (16). The rates of protein synthesis in the skin wound increased by 22–28% in the 25% Leu/AA and 35% Leu/AA groups in comparison with the AA group ($P = 0.37$ and $P = 0.61$, respectively) (**Table 3**). The rate of protein synthesis in the muscle was greater in the 35% Leu/AA group than in the AA group ($P < 0.05$). In comparison with the AA group, the rates of protein net balance in the skin wound of the 35% Leu/AA group and in the muscle of both the 25% Leu/AA and the 35% Leu/AA groups were increased ($P < 0.05$) (Table 3).

In the skin wound, the rates of Phe transport were generally slower in the Leu group than in the other groups receiving Travasol infusion (**Table 4**). The ratio of inward transport/total intracellular R_a was smaller in the Leu group than in the AA and 25% Leu/AA groups ($P < 0.05$). In contrast, the ratio of synthesis/total R_a, a refection of efficiency of protein synthesis, was greater in the Leu group than in the AA group ($P < 0.05$). The 35% Leu/AA group had a slower rate of outward transport than the AA group, a smaller ratio of inward transport/total R_a than the AA and 25% Leu/AA groups, and a greater ratio of synthesis/total R_a than the AA and 25% Leu/AA groups ($P < 0.05$). In the muscle, the Leu group had a slower rate of inflow than the AA and 25% Leu/AA groups and a smaller rate of a-v shunting than the AA and 25% Leu/AA group ($P < 0.05$) (Table 4). The 35% Leu/AA group had a smaller rate of a-v shunting than the AA group ($P = 0.013$). The ratios of synthesis/total R_a tended to increase in the Leu-supplemented groups compared to the AA group (Table 4).

The FSR of muscle protein, which was measured from the tracer incorporation method, was greater in the 35% Leu/AA group than in the AA and Leu groups ($P < 0.01$, **Fig. 2**).

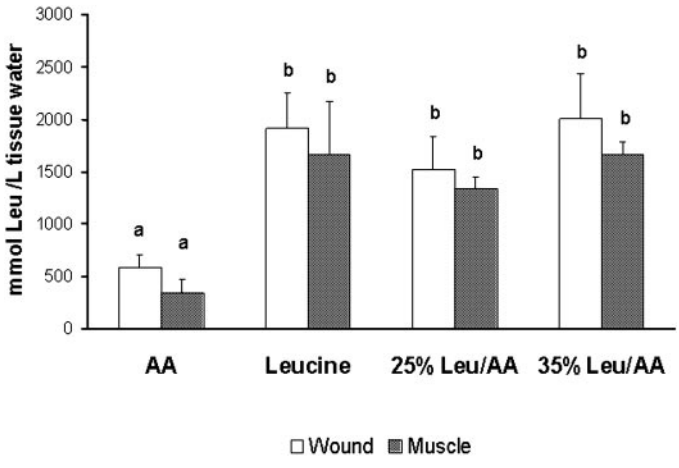

FIGURE 1 Concentrations of leucine in the free pools in rabbit skin wound and muscle in AA, Leu, 25% Leu/AA, and 35% Leu/AA groups. Values are means ± SD, $n = 6$ or 7. Means without a common letter within skin wound or muscle differ, $P < 0.01$.

TABLE 3

Protein kinetics in rabbit skin wound and muscle in AA, Leu, 25% Leu/AA, and 35% Leu/AA groups calculated from the 3-pool model[1]

	n	Synthesis	Breakdown	Net balance
		μmol phenylalanine · 100 g tissue^{-1} · h^{-1}		
Ear balance				
AA	7	40.4 ± 10.2	47.1 ± 13.5	−6.7 ± 6.2[b]
Leu	6	44.1 ± 15.2	48.2 ± 18.8	−4.1 ± 4.6[a,b]
25% Leu/AA	7	49.3 ± 9.8	50.0 ± 10.9	−0.6 ± 2.5[a,b]
35% Leu/AA	7	51.9 ± 20.3	51.0 ± 20.5	0.9 ± 3.7[a]
Leg balance				
AA	7	4.3 ± 1.6[b]	8.7 ± 2.1	−4.4 ± 2.4[b]
Leu	6	6.9 ± 1.7[a,b]	9.6 ± 1.6	−2.7 ± 0.7[a,b]
25% Leu/AA	7	6.9 ± 2.2[a,b]	8.6 ± 2.6	−1.7 ± 0.9[a]
35% Leu/AA	7	7.1 ± 1.8[a]	8.1 ± 1.2	−1.0 ± 1.1[a]

[1] Values are means ± SD; for each measured variable in the skin wound or muscle, means without a common superscript letter within a column differ, $P < 0.05$.

DISCUSSION

The present experiment demonstrated that Leu supplementation had an anabolic effect on protein metabolism in both skin wound and muscle. The anabolic effect was due to an increase in protein synthesis. There was a significant increase in muscle protein synthesis in the 35% Leu/AA group in comparison with the AA group measured by both the leg a-v balance method (Table 3) and the tracer incorporation method (Fig. 2). In the skin wound, whereas the increase in synthesis in the 35% Leu/AA group did not reach a statistical significance, the ratio of synthesis/total R_a was significantly greater in the 35% Leu/AA group than in the AA group (Table 4), suggesting a greater efficiency of protein synthesis. The rate of protein synthesis in the d 7 wound was 2.6-fold that in normal skin (20). This may explain the limited increase in wound protein synthesis in response to Leu/AA infusion. Because the AA, 25% Leu/AA, and 35% Leu/AA groups received the same dose of AA nitrogen (Table 1), the anabolic effect in the 35% Leu/AA group is reasonably attributed to the supplemental Leu.

Leu is potentially an insulin secretagogue. However, the plasma insulin concentrations were not significantly different among groups. This does not exclude the possible effect of Leu on plasma insulin levels. In the present experiment all groups received some Leu infusion, either alone or with other AAs (Table 1). Other AAs such as isoleucine and valine (27) and arginine (28) can also stimulate insulin release, thereby contributing to the measured insulin concentrations. Nonetheless, the fact that the 35% Leu/AA group did not have a higher plasma insulin concentration than the AA group supports the idea that most likely Leu supplementation may have a direct effect on protein metabolism in skin wound and muscle, which is consistent with the finding that Leu administration stimulated protein synthesis at the level of initiation of mRNA translation (5,29).

The AA transport data showed that in the 35% Leu/AA group the ratio of synthesis/total R_a increased in skin wound or tended to increase in muscle, suggesting a greater efficiency of protein synthesis. However, it is likely that increased efficiency can only result in increased protein synthesis if there is a

TABLE 4

Phenylalanine transport in rabbit skin wound and muscle in AA, Leu, 25% Leu/AA, and 35% Leu/AA groups calculated from the 3-pool model[1,2]

		Phenylalanine						
	n	Inflow	Inward transport	a-v shunting	Outward transport	Total R_a	Inward transport/ Total R_a	Synthesis/ Total R_a
		μmol 100 g tissue^{-1} · h^{-1}						
Ear skin wound								
AA	7	222 ± 59[a]	33 ± 15[a]	190 ± 59[a]	39 ± 18[a]	80 ± 26	0.40 ± 0.07[a]	0.52 ± 0.08[c]
Leu	6	106 ± 12[b]	15 ± 3[b]	91 ± 13[b]	19 ± 7[b]	63 ± 21	0.25 ± 0.06[b]	0.70 ± 0.06[a,b]
25% Leu/AA	7	239 ± 48[a]	33 ± 11[a]	205 ± 43[a]	34 ± 12[a,b]	82 ± 22	0.39 ± 0.04[a]	0.60 ± 0.06[b,c]
35% Leu/AA	7	200 ± 39[a]	20 ± 8[a,b]	180 ± 36[a]	19 ± 9[b]	71 ± 26	0.28 ± 0.08[b]	0.73 ± 0.08[a]
Leg muscle								
AA	7	44 ± 13[a]	14 ± 5	30 ± 12[a]	18 ± 5	22 ± 5	0.60 ± 0.12	0.20 ± 0.09
Leu	6	24 ± 5[b]	15 ± 5	9 ± 4[c]	18 ± 5	25 ± 5	0.59 ± 0.10	0.29 ± 0.09
25% Leu/AA	7	38 ± 6[a]	16 ± 5	21 ± 4[a,b]	18 ± 6	25 ± 5	0.65 ± 0.11	0.29 ± 0.11
35% Leu/AA	7	32 ± 7[a,b]	15 ± 3	17 ± 6[b,c]	16 ± 4	23 ± 3	0.64 ± 0.08	0.32 ± 0.11

[1] Values are means ± SD; for each measured variable in ear skin wound or leg muscle, means without a common superscript letter within a column differ, $P < 0.05$.

[2] Abbreviations used: inflow, rate of delivery into the ear or leg via artery; inward transport, rate of delivery from artery to the free pool in tissue; a-v shunting, rate of delivery directly from artery to vein; outward transport, rate of delivery from the free pool in tissue to vein; total R_a, rate of appearance in the free pool in tissue from inward transport and protein breakdown.

sufficient availability of other AAs. In the Leu group, the concentrations of most of plasma AAs were lower than in the AA group (Table 2), which was associated with lower rates of AA transport (Table 4). This may explain the fact that an anabolic effect was not achieved, despite increased efficiency of protein synthesis. In other words, the high concentration of Leu might have acted as an anabolic agonist; however, an anabolic effect could not be achieved in the face of a relative deficiency in plasma AAs. This notion is consistent with the results of our previous experiment in which insulin infusion alone was not sufficient to improve net protein balance in the skin wound (16), presumably because of a drop in AA availability. This notion is also consistent with the observation that oral Leu administration stimulated muscle protein synthesis in rats deprived of food overnight (29). Because in that experiment the flooding dose technique was used to measure muscle protein FSR during the first 60 min after the Leu load, it is unknown how long the stimulatory effect of Leu lasted and whether the increase in protein synthesis resulted in an anabolic response in the absence of measurement of the rate of protein breakdown. Skeletal muscle is the only source of protein that can be mobilized to provide free AAs under catabolic conditions such as in the postabsorptive state (20,24). Therefore, in order to achieve anabolic responses in both skin wound and muscle, it is necessary to provide exogenous AAs.

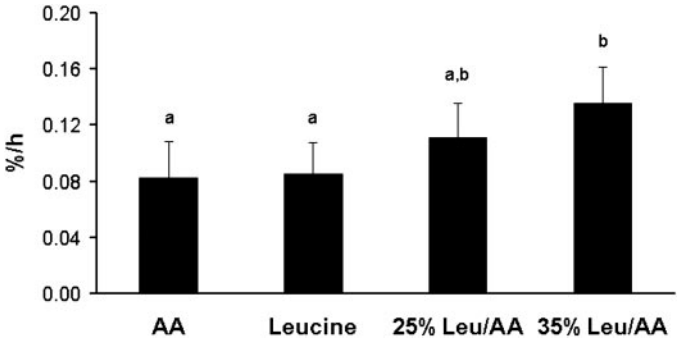

FIGURE 2 FSR of protein in rabbit muscle measured from the tracer incorporation method in AA, Leu, 25% Leu/AA, and 35% Leu/AA groups. Values are means ± SD, $n = 6$ or 7. Means without a common letter differ, $P < 0.01$.

The function of Leu as a nutrient signal provides a potential approach in the treatment of catabolic patients. In this respect, a key point is to determine the effective dosage. In the 35% Leu/AA group, plasma Leu concentration was 1790 ± 304 μmol/L (Table 3), which is 10.6-fold greater than the postabsorptive value (154 μmol/L) (16). Leu concentrations in the free AA pools in skin wound and muscle were correspondingly increased (Fig. 1). Various doses of Leu have been previously reported to stimulate protein synthesis in muscle. In 18-h food-deprived rats, an oral Leu dose of 1.35 g/kg, which had a peak plasma leucine concentration of 2237 μmol/L, was effective in stimulating muscle protein synthesis (5,29). In free-living rats, chronic oral Leu supply via drinking water to increase plasma leucine by ~50% induced an anabolic response of muscle protein (30). In healthy humans oral intake of a branched-chain AA (BCAA) solution (45% Leu, 30% valine, and 25% isoleucine) to raise plasma BCAA concentration to 2.3-fold of the basal level stimulated muscle protein synthesis during recovery from exercise (8). Thus, the effective dose of Leu may vary according to experimental conditions, and a larger dose may be required to counteract a catabolic stimulus such as in the anesthetized rabbits (31).

In summary, the present experiment for the first time demonstrated that Leu supplementation had an anabolic effect on protein metabolism in skin wound and muscle when other AAs were provided. Wound protein anabolism may promote the healing process because new proteins must be synthesized to repair the tissue defect and cellular activities such as cell migration and cell differentiation require synthesis of various proteins. At the same time, an improvement in muscle protein balance should facilitate preservation of muscle mass for a better recovery from injury. Therefore, the anabolic effect of

Leu supplementation may have clinical implications in the treatment of burn patients.

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