Published in final edited form as:

Ann Surg. 2011 March ; 253(3): 592–597. doi:10.1097/SLA.0b013e31820d9a63.

Skeletal Muscle Is Anabolically Unresponsive to an Amino Acid Infusion in Pediatric Burn Patients 6 Months Postinjury

Demidmaa Tuvdendorj, MD, PhD^{*,‡}, David L. Chinkes, PhD^{*,‡}, Xiao-Jun Zhang, MD^{*,‡}, Melinda Sheffield-Moore, PhD[†], and David N. Herndon, MD^{*,‡}

^{*}Department of Surgery, University of Texas Medical Branch

[†]Department of Internal Medicine, University of Texas Medical Branch

[‡]Shriners Hospitals for Children, Galveston, TX

Abstract

Objective—To evaluate leg muscle, whole-body muscle, and whole-body non-muscle protein response to anabolic signaling of amino acids in pediatric burn patients at 6 months after injury.

Background—Burn injury is associated with a catabolic state persisting years after the injury. The tissue response to nutritional signaling (eg, amino acids) plays a critical role in tissue protein net balance via coordination of protein synthesis and breakdown mechanisms.

Methods—A total of 10 patients (7.4 ± 3.8 years; 27.4 ± 14.7 kg) and 5 healthy young males (22 ± 3 years; 76 ± 15 kg) underwent an 8-hour stable isotope infusion study. During the last 3 hours, an amino acid solution (10% Travasol, Clintec Nutrition, Deerfield, IL) was infused. Femoral arterial and venous blood samples and muscle biopsy samples were collected throughout the study. A *P* value of less than 0.05 was considered statistically different.

Results—During amino acid infusion, leg muscle protein synthesis rate significantly increased ($P < 0.05$) in both groups, however, in the burn group, protein breakdown also increased, although nonsignificantly. As a result, protein net balance remained negative. In the control group, breakdown nonsignificantly decreased resulting in a significant increase ($P < 0.05$) in muscle protein net balance. Whole-body protein breakdown was significantly higher in the burn patients.

Conclusion—In pediatric burn patients at 6 months postinjury, leg muscle protein net deposition is unresponsive to amino acid infusion; and whole-body protein breakdown is significantly higher than in the control group.

Stressed pathophysiological conditions, for example, burn, result in a hypermetabolic and catabolic state leading to muscle wasting. In burns, the most common determinants for muscle catabolism are subject weight, burn size, time from injury to surgical treatment, resting energy expenditure, and sepsis.¹ However, long lasting hospitalization with prolonged bed rest and major surgeries are contributors to loss of lean body mass. Furthermore, muscle catabolism is correlated with morbidity during acute burn treatment.^{2–4}

It is well-established that pediatric burn patients are hypermetabolic and catabolic for more than 12 months postinjury.⁵ They also demonstrate delayed linear growth up to 2 years postinjury.⁶ Recent studies have shown that in a pediatric burn patient population, muscle catabolism persists up to 9 months beyond the injury.⁵ In this patient population, persistent

muscle catabolism has multiple negative effects. First, children are in the physiological process of growing and for normal growth to occur, the catabolic condition must be reversed, that is, the rates of protein synthesis are greater than the rates of proteolysis leading to a higher net protein deposition. Second, burn victims spend days and/or months in intensive care unit during the acute phase after injury. This results in loss of mobility, loss of lean body mass, and delayed start of rehabilitation. Third, burn patients undergo numerous reconstructive surgeries in years after wound healing has occurred. Major surgeries are known stressors for the catabolic state. The combination of the acute burn injury, prolonged intensive care unit stays, and years of reconstruction surgery render the skeletal muscle persistently catabolic, making amelioration of muscle wasting an important consideration during the treatment of pediatric burn patients.

Studies have shown that the tissue response to nutritional signaling (eg, amino acids) plays a critical role in protein net deposition via coordination of protein synthesis and breakdown mechanisms. In normal conditions, amino acid abundance increases muscle protein synthesis with no change or decrease in the breakdown rate, resulting in an increased net protein deposition.^{7–10} In critical or catabolic conditions, this depends on the severity of the condition. In less severe critical illness, there are milder changes in both protein synthesis and breakdown rates.^{11–13} Whereas in severe cases, namely acute phase after burn injury, amino acid availability increases protein synthesis but this increase is not sufficient to match the increase in breakdown and prevent the protein loss.¹⁴ It is not known, however, how the skeletal muscle of pediatric burn patients responds to amino acid supplementation at later periods after burn injury, for example, 6 month postinjury.

Therefore, in this study we examined the effect of a steady state amino acid (AA) infusion (Travasol) on leg muscle protein turnover in pediatric burn patients at 6 month postinjury compared with that of healthy subjects. We also examined whole-body and whole-body nonmuscle basal and AA affected protein breakdown rates. Nonmuscle protein turnover represents protein turnover from tissues other than muscle, such as liver. We hypothesize that in burn victims, protein turnover is unresponsive to the anabolic signaling of AAs.

METHODS

Patients

A total of 10 pediatric patients [7.4 ± 3.8 years; 27.4 ± 14.7 kg; 120 ± 24.2 cm, mean $\pm$ SD] that had acute burn treatment at Shriners Hospital for Children, Galveston, TX, and returned for their 6-month follow-up/reconstructive surgery visit participated in a stable isotope tracer study. This project was a part of a larger clinical trial evaluating the effect of anabolic agents on muscle mass preservation after burn injury. These patients were randomized to a control group and received only standard of care during acute burn treatment period and long term follow up. The study was performed under a University of Texas Medical Branch (UTMB) institutional review board approved protocol. Child assent and parental permission were obtained from each patient and patient's guardian before enrollment into the study.

Control Subjects

Five healthy males [22 ± 3 years; 76 ± 15 kg; 176 ± 5 cm, mean $\pm$ SD] were studied at the General Clinical Research Center at UTMB, Galveston, TX. Portions of these data were previously presented elsewhere.¹⁰ Before participation in the study, all subjects gave informed written consent according to guidelines established by the institutional review board at UTMB.

All stable isotope studies were performed in the postabsorptive state (fasted overnight, 6–12 hours). After baseline blood samples for background amino acid enrichment were drawn, a

primed continuous infusion of L-[*ring*- $^2\text{H}_5$]-phenylalanine (Cambridge Isotope laboratories, Andover, MA) was initiated at a prime dose of 3.2 and 2 $\mu\text{mol}\cdot\text{kg}^{-1}$ and an infusion rate (IR) of 0.08 and 0.05 $\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ for burn patients and controls, respectively.

Stable Isotope Study

All subjects were admitted into the hospital the night before the tracer study. Before the tracer study, dual 3F polyethylene catheters (Cook, Inc, Bloomington, IN) were inserted under local anesthesia (1% lidocaine) into the femoral artery and vein of one leg. In addition, pediatric burn patients were sedated with ketamine (4 mg/kg intramuscularly) before the catheter placement procedure. In all burn patients, both legs had healed burn or donor harvest site wounds, so there were no differences between limbs in measurement of protein kinetics. Femoral catheters were used for arteriovenous blood sampling and infusion of indocyanine green for determination of leg blood flow. In addition, indwelling catheters were placed in the ante-cubital vein of each arm for infusion of isotopes and blood sampling for blood flow measurements.

Blood samples to measure plasma phenylalanine enrichment were collected simultaneously from the femoral artery and vein during the last hour of each period—postabsorptive or AA infusion (Fig. 1).

Biopsies of the *Vastus lateralis* for calculation of muscle protein fractional synthesis rate (FSR) were obtained at 2, 5, and 8 hour of tracer infusion using a 5-mm Bergström needle (Stille, Stockholm, Sweden) (Fig. 1). Tissue was immediately frozen in liquid nitrogen and stored at -80°C until further analysis.

A continuous infusion (IR = 0.05 mg/min) of indocyanine green (Akron, Buffalo Grove, IL) was used for leg blood flow measurements (Fig. 1) as previously described.^{10,15}

After the basal period (0–5 h), a primed (prime dose = 0.45 mL/kg) continuous (IR = 1.35 $\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) infusion of 10% Travasol (Clintec Nutrition, Deerfield, IL; total amino acids = 100 mg/mL) was initiated and maintained until 8 h. One hundred milliliter of Travasol solution contains 730-mg leucine, 600-mg isoleucine, 580-mg lysine, 580-mg valine, 560-mg phenylalanine, 480-mg histidine, 420-mg threonine, 400-mg methionine, 180-mg tryptophan, 2.07-g alanine, 1.15-g arginine, 1.03-g glycine, 680-mg proline, 500-mg serine, and 40-mg tyrosine.

At the end of the study, peripheral lines and femoral catheters were removed or for the burn patients, were left in place for use at the next reconstructive surgery as required.

ANALYTICAL METHODS

Blood

Blood samples for the measurement of phenylalanine enrichment and concentration were collected as previously described.^{10,15} Briefly, arteriovenous blood samples were collected in preweighed tubes containing 15% sulfosalicylic acid. A known internal standard was added to the blood samples (100 $\mu\text{L}/\text{mL}$ of blood) for the measurement of blood phenylalanine concentration. After deproteinization blood amino acids were separated using cation exchange chromatography.¹⁵ The enrichments (tracer-to-tracee ratio) in arterial and venous blood samples were determined on their tert-butyldimethylsilyl derivatives using gas chromatograph-mass spectrometry [GC-MS) HP 5989; Hewlett-Packard, Palo Alto, CA] with electron impact ionization.

Serum indocyanine green concentration was measured by means of a spectrophotometry at $\lambda = 805 \text{ nm}$.

Muscle

Muscle samples were weighed and protein precipitated with 800 μL of 10% perchloric acid. The supernatant was collected after homogenization of the tissue and centrifugation. The amino acids in the supernatant were then separated using cation exchange chromatography.¹⁵ The isotopic enrichment of the intracellular phenylalanine was determined on their tertbutyldimethylsilyl derivatives by GC-MS in electron impact mode. The remaining pellet was washed as previously described,^{10,15} dried at 50°C overnight, and hydrolyzed in 6 N HCl at 110°C for 24 hour. The hydrolysate was then passed over a cation exchange column and phenylalanine enrichment was measured by GC-MS in the same manner as the intracellular portion.¹⁵

CALCULATIONS

Leg Muscle Protein Kinetics

As phenylalanine is neither synthesized nor oxidized in the periphery, the rates of appearance (R_a) and disappearance (R_d) of this amino acid in the femoral arterial and venous plasma pools reflect leg skeletal muscle protein breakdown and synthesis, respectively. The assumptions and limitations of the kinetic model have been described elsewhere.^{10,15} Because calculation of the model kinetics depends on the maintenance of steady-state physiological conditions, we primed the amino acids and infused at a constant infusion rate. Finally, arte-riovenous sampling took place in the last hour of the 3-hour amino acid infusion once blood amino acid concentrations and enrichments achieved steady state. The phenylalanine kinetic rates within the leg were calculated and standardized for leg volume.

Whole-Body Protein Kinetics

Whole-body amino acid R_a represented by phenylalanine plasma kinetics was calculated according to the standard equation, that is, tracer infusion rate divided by arterial enrichment. Because subjects were fasted, no correction for protein intake was needed. For the amino acid infusion period whole-body R_a was corrected for the amount of the infused phenylalanine. Whole-body muscle amino acid R_a was calculated from leg muscle amino acid R_a , corrected for the whole-body muscle mass using an assumed value of 4.5.¹⁵ Whole-body nonmuscle amino acid R_a was calculated by subtracting whole-body muscle R_a from total whole-body R_a .

Muscle Protein Fractional Synthesis Rate

Calculations of the muscle protein FSR were based on the precursor-product method, using intracellular free and bound protein tracer enrichments in the muscle biopsy as precursor and product, respectively.

The specific equations for the calculations described above are:

1. Leg muscle protein net balance: $\text{NB} = (C_A - C_V) \times \text{BF}$
2. Leg muscle protein rate of disappearance: $\text{IR}_d = (C_A \times E_A - C_V \times E_V) / E_A \times \text{BF}$
3. Leg muscle protein rate of appearance: $\text{IR}_a = R_d - \text{NB}$
4. Transport into the leg: $F_{\text{IN}} = C_A \times \text{BF}$
5. Inward transport: $F_{\text{M,A}} = \{[(E_M - E_V) / (E_A - E_M)] \times C_V + C_A\} \times \text{BF}$
6. Outward transport: $F_{\text{V,M}} = \{[(E_M - E_V) / (E_A - E_M)] \times C_V + C_V\} \times \text{BF}$

7. Shunting: $F_{V,A} = F_{TN} - F_{M,A}$
8. Whole-body protein rate of appearance: $wbR_a = IR/E_A$
9. Whole-body muscle protein rate of appearance: $wbmR_a = \text{Leg } R_a \times \text{Leg Volume} \times 4.5/\text{Weight}$
10. Whole-body nonmuscle R_a : $wbeR_a = wbR_a - wbmR_a$
11. Fractional synthesis rate: $FSR = [(Em_{t2} - Em_{t1})/(Ef \times t)] \times 60 \times 100 \times 24$

where NB is net protein deposition in the leg muscle; lR_d and lR_a are the rates of disappearance and appearance of phenylalanine within the leg, respectively; C_A and C_V are the blood free phenylalanine concentrations of the femoral artery and vein; E_A and E_V are the blood free phenylalanine enrichments (tracer/trace ratio) in the femoral artery and vein; and BF is leg blood flow in mL/min/100 mL leg; wbR_a is whole-body rate of appearance of phenylalanine; IR-tracer phenylalanine infusion rate; $wbmR_a$, whole-body muscle rate of appearance of phenylalanine; $wbeR_a$, whole-body endogenous rate of appearance of phenylalanine; FSR is fractional synthesis rate of muscle protein; E_f and E_M are the enrichments of free and bound amino acid respectively, and t is the time interval in minutes between 2 sequential biopsies. Leg and whole-body protein R_d , R_a , and NB are expressed in nmol Phe/min/100 mL leg volume and $\mu\text{mol Phe/min/kg}$, respectively. Fractional synthesis rate was expressed in units of percent per hour (%/hour).

Data Presentation and Statistical Analysis

Data are presented as Means $\pm$ SE, unless otherwise stated. A 2-way repeated-measures analysis of variance with the factors time and treatment with post hoc Tukey correction was used to assess differences in measurements. A P value less than 0.05 was considered statistically different.

RESULTS

General demographics and phenylalanine kinetic parameters are presented in Table 1.

Phenylalanine enrichments and concentrations in the femoral artery (FA) and vein (FV) were in steady-state conditions over the last 1 hour of each study period in both groups. After start of AA infusion, phenylalanine enrichments in the FA and FV significantly decreased ($P < 0.05$), (FA: Burn: 0.078 ± 0.027 vs 0.044 ± 0.012 ; Control: 0.064 ± 0.005 vs 0.039 ± 0.004 ; FV: Burn: 0.074 ± 0.029 vs 0.042 ± 0.011 ; Control: 0.049 ± 0.003 vs 0.035 ± 0.003 , Basal vs AA, respectively) whereas phenylalanine concentration significantly increased ($P < 0.05$), (FA: Burn: 66 ± 12 vs 113 ± 31 ; Control: 62 ± 3 vs 113 ± 12 , FV: Burn: 67 ± 11 vs 133 ± 30 ; Control: 70 ± 3 vs 103 ± 9 , Basal vs AA, respectively). During AA infusion, FV phenylalanine concentration was significantly ($P < 0.05$) higher in the burn patients than in the control groups. The rates of tracer infusion were greater in the patients than in the controls (see Methods); thus the values of AA enrichments cannot be directly compared. Nonetheless, the ratio between AA enrichments in muscle and the artery were not significantly different between the groups and periods (Burn: 0.63 ± 0.08 vs 0.81 ± 0.05 ; Control: 0.63 ± 0.04 vs 0.77 ± 0.05 , Basal vs AA, respectively, $P > 0.05$). In the control group, muscle intracellular enrichment significantly decreased at the end of the AA period compared with the basal period (Basal vs AA, 0.039 ± 0.004 vs 0.03 ± 0.004 , $P < 0.05$), in the burn group although the enrichment decreased, the difference did not reach statistical significance (Basal vs AA, 0.041 ± 0.007 vs 0.034 ± 0.003 , $P = 0.085$).

Phenylalanine kinetic data are presented in Table 1. The rate of leg blood flow was higher in burn patients but the difference did not reach statistical significance because of the

variability in the burn group (Burn: 9 ± 5 vs 12 ± 13 mL/min per 100 mL leg volume; Control: 4 ± 2 vs 5 ± 2 , Basal vs AA, respectively). Consequently, the rate of phenylalanine delivery to the leg was significantly higher ($P < 0.05$) in the burn group in both periods (Table 1). It was also significantly increased ($P < 0.05$) in the burn group by AA infusion. Inward transport was also significantly increased in both groups during AA infusion; however, there was no difference between the groups during AA infusion. Outward transport was not different between the groups and periods. During AA infusion phenylalanine shunting significantly increased ($P < 0.05$) in the burn patients but not in the control subjects. And, this increase in shunting in burn patients was significantly higher ($P < 0.05$) than the increase in the control group with AA infusion.

Leg muscle protein kinetic data are presented in Figure 2. There was no significant difference in postabsorptive net protein deposition in burn patients compared to healthy young controls. Amino acids infusion significantly increased ($P < 0.05$) muscle protein synthesis, represented by (R_d) in both groups (Fig. 2A) compared to the basal period. Amino acids infusion did not affect significantly leg muscle protein breakdown, represented by rate of appearance (R_a) in either group (Fig. 2B); however, in the control group it decreased by 21% ($P > 0.05$) whereas in the burn group it increased by 36% ($P > 0.05$). As a result, during AA infusion muscle protein net deposition did not change in the burn group but significantly increased in the control group ($P < 0.05$) (Fig. 2C).

Muscle protein FSR increased in both groups during AA infusion (Basal vs AA, 0.115 ± 0.037 vs 0.161 ± 0.045 and 0.057 ± 0.004 vs $0.11 \pm 0.012\%/h$, Burn and Control, respectively), however, it did not reach statistical significance ($P = 0.089$ and $P = 0.074$; Burn and Control, respectively).

Whole-body protein turnover data are presented in Figure 3. Basal whole-body protein R_a was significantly higher ($P < 0.05$) in burn patients than in the control group (Fig. 3A). During AA infusion in burn patients whole-body protein R_a did not change unlike in controls (Fig. 3A), where it was significantly suppressed ($P < 0.05$) compared to the basal level within the group and the AA period of the burn group. There was no difference in whole-body muscle R_a between the groups (Fig. 3B) during both study periods. Basal whole-body nonmuscle protein R_a was 189% higher in the burn group than in the control group (Fig. 3C) but the difference did not reach statistical significance ($P = 0.13$). Similarly, AA stimulated whole-body nonmuscle protein R_a was 300% higher in the burn group but again the difference did not reach statistical significance ($P = 0.07$) (Fig. 3C).

DISCUSSION

The first principal finding of this study is that in the pediatric burn patient population at 6 months postburn injury leg muscle protein net deposition is unresponsive to AA infusion (Fig. 2). When AAs were infused, leg muscle protein synthesis (R_d) significantly increased ($P < 0.05$) in both groups. However, in the burn group there was a simultaneous (although nonsignificant) increase in protein breakdown (R_a), resulting in no change in protein net deposition (NB). This is unlike healthy young adults, in whom the breakdown rate decreased (although nonsignificantly), resulting in a significant increase ($P < 0.05$) in protein NB (Fig. 2).

Delivery of phenylalanine to the leg was significantly increased by burn injury (Table 1). Phenylalanine kinetic data demonstrate that the AAs transport system is not affected, but arterio-venous shunting is increased in burns. One speculation to explain this phenomenon is that due to an increase in protein breakdown muscle intracellular phenylalanine concentration was elevated therefore the inward transport was working against a greater

concentration gradient between blood and intracellular space, so did not increase even though there was greater phenylalanine delivery to the leg. Perhaps the protein synthesis rate is at a maximal level, but is not sufficient to counteract the massive increase in protein breakdown.

Critical conditions or stress (eg, exercise training) increase both protein synthesis and breakdown rates; but the degree of change depends on the degree of the severity of one's condition. For example, exogenous AAs enhance muscle anabolism after exercise by increasing muscle protein synthesis and suppressing the exercise-induced breakdown.^{7,16} In patients with less severe critical illness than burn injury changes in protein breakdown are milder along with suppressed rates of synthesis.^{11–13} Our current data demonstrate that AA availability can stimulate muscle protein synthesis in burns. However, the synthesis in response to extra AA availability may be at the maximal level but not sufficient to match the breakdown and consequently prevent the severe protein loss in burns. Given that our patients are pediatric patients who are supposed to be more anabolic than the healthy control adults, we cannot exclude the possibility that synthesis might have been higher if the children were uninjured.

The second principal finding of this study is that whole-body protein breakdown is significantly higher in burn patients and also unresponsive to AA infusion (Fig. 3). Whole-body protein breakdown includes breakdown from muscle tissue and all other organs including liver, gut, and skin. These data are again different from data in healthy adult subjects, where a significant decrease is observed ($P < 0.05$), but consistent with other observations from catabolic conditions.^{17–20} Also, splanchnic tissue protein breakdown is the main contributor in exercise- and insulin-induced increase in whole-body protein R_a .^{18–20} These data demonstrate that splanchnic protein metabolism is readily and significantly stimulated and can be affected by different kinds of stress including burn injury. In the current study we did not measure whole-body and whole-body nonmuscle protein synthesis. Our postulation is that most probably splanchnic protein synthesis also increased during AA infusion. However, evidences suggest that during interventions AAs released from gut are redirected into muscle protein synthesis.¹⁸

Skin is the largest nonmuscle organ and the main organ that is affected in burn injury. Therefore, it is reasonable to question if skin protein breakdown is the main contributor to the increased whole-body protein breakdown. The current studies were conducted 6 months after the injury when the skin wounds are completely healed and mostly hypertrophic scars are developing, therefore, we presume that skin protein synthesis is increased but the breakdown is suppressed. Therefore, we do not think that skin wound protein breakdown is the main contributor in this case; however, future studies to evaluate this possibility would be of interest.

Basal and AA-stimulated whole-body nonmuscle protein breakdown was 189 and 300% higher in the burn group than in the control group, however, the difference did not reach statistical significance ($P = 0.13$ for the basal; and $P = 0.07$ for the AA period between the groups). Sample size analysis demonstrated that 25 patients per group would have been required to reach statistical significance with power of 0.8 and $\alpha = 0.05$. Nevertheless, we conclude that burn pediatric patients are catabolic with elevated protein degradation at whole body level.

A comparison of data from burn pediatric patients to the results from young adults is potentially problematic because differences may be due to age differences rather than burn injury. However, because children are growing, their protein kinetics would be expected to be more anabolic, rather than catabolic. Therefore, the results here may underestimate the

true extent of catabolism caused by burns. Unfortunately, it was not possible to obtain data from an age-matched pediatric population; however, several whole-body protein turnover studies have concluded that children and young adults have comparable protein kinetics.^{21–24}

Although this is the first study to examine the effects of amino acid infusion in burn patients after recovery, the effects of amino acids in burn patients in the intensive care unit as well as other clinical populations have been studied. van Waardenburg et al,²⁵ using ¹⁵N-glycine measured whole-body protein turnover in healthy (fasting or fed) children and children with meningococcal septic shock (fed). Healthy children demonstrated significantly suppressed whole-body protein breakdown during feeding; children with meningococcal septic shock demonstrated twice as high protein breakdown rate ($P < 0.05$) compared with healthy fed children and almost 7-fold increase compared with the fasting children ($P < 0.05$). Children with meningococcal septic shock also demonstrated significantly increased whole-body protein synthesis rate. Similar results have been reported from patients with cystic fibrosis and patients undergoing elective surgeries.^{24,26} The main conclusions were that breakdown is largely an obligatory response to the severity of the trauma, whereas synthesis responds to substrate availability. Yu et al,²⁷ showed that feeding significantly increased whole-body protein synthesis and breakdown rates in burn patients, and those values were almost 2-fold higher than the values reported from healthy controls. These results are consistent with our current findings.

The increase in FSR was consistent with the increase in leg muscle protein R_d , however, by 2-way repeated-measures analysis of variance analysis the results did not reach statistical significance. In our previous report on the control subjects,¹⁰ muscle protein FSR was significantly increased ($P < 0.05$, t test) with AA infusion. The explanation on the current nonsignificant result might be because of the results from the burn group. One of the patients demonstrated decreased FSR at AA period compared to the postabsorptive state. If we exclude FSR data from this particular patient from the analysis, P values become 0.003 and 0.015 for burn and control groups, respectively, demonstrating that AA significantly increases muscle protein FSR in both groups. Unfortunately, we do not have an explanation why the FSR data from that patient is inconsistent with others.

In conclusion, at 6-month postinjury in a pediatric patient population tissue protein turnover is unresponsive to AA anabolic signaling and the patients are in more catabolic state rather than anabolic. This can be supported by previous reports that burn injury results in long-lasting increase in catabolic hormones and insulin resistance.^{28–30} Thus, it maybe that nutrition alone is not sufficient for burn children to resume normal growth, and additional interventions (anabolic hormones, exercise) should be indicated. Future studies on the effect of anabolic stimulus to restore tissue protein response to nutrient's anabolic signaling in burns would be of interest.

Acknowledgments

Supported by NIH P50 GM 60338, Shriners Mass Spectrometry Core Grant 8490, NIH M01 RR-00073, 5-R01-DK-38010-11, and R01-GM-57295-01.

The authors thank the study volunteers and their families for their patience and dedication, and the clinical and research staff of Shriners Hospital for Children (SHC) and General Clinical Research Center, UTMB, Galveston, TX for their help with conducting the clinical portion of the study; thanks to Cindy Locklin and Deb Benjamin for performance of the metabolic infusion protocol of the study; Tara Cocke, Christopher Danesi, Ann Hightower for excellent technical performance with sample processing; Guy Jones and Jariwala Guarang for performing GC-MS analyses; and Steve Schuenke for an excellent technical assistance with the manuscript preparation.

References

1. Hart DW, Wolf SE, Chinkes DL, et al. Determinants of skeletal muscle catabolism after severe burn. *Ann Surg.* 2000; 232(4):455–465. [PubMed: 10998644]
2. Moore, FD. Response to starvation and stress. In: Moore, FD., editor. *Metabolic care of the surgical patient.* Philadelphia: WB Saunders; 1959. p. 202-275.
3. Newsome T, Mason A, Pruitt B. Weight loss following thermal injury. *Ann Surg.* 1973; 178:215–220. [PubMed: 4198796]
4. Mason AD Jr. Weight loss in burned patients. *J Trauma Inj Infect Crit Care.* 1979; 19(11 suppl): 903–904.
5. Hart DW, Wolf SE, Mlcak R, et al. Persistence of muscle catabolism after severe burn. *Surgery.* 2000; 128:312–319. [PubMed: 10923010]
6. Rutan RL, Herndon DN. Growth delay in postburn pediatric patients. *Arch Surg.* 1990; 125:392–395. [PubMed: 2306187]
7. Biolo G, Tipton KD, Klein S, et al. An abundant supply of amino acids enhances the metabolic effect of exercise on muscle protein. *Am J Physiol Endocrinol Metab.* 1997; 273(36):E122–E129.
8. Volpi E, Ferrando AA, Yeckel CW, et al. Exogenous amino acid stimulate net muscle protein synthesis in the elderly. *J Clin Invest.* 1998; 101(1):2000–2007. [PubMed: 9576765]
9. Katsanos CS, Kobayashi H, Sheffield-Moore M, et al. A high proportion of leucine is required for optimal stimulation of the rate of the muscle protein synthesis by essential amino acids in the elderly. *Am J Physiol Endocrinol Metab.* 2006; 291(2):381–387.
10. Sheffield-Moore M, Wolfe RR, Gore DC, et al. Combined effects of hyper-aminoacidemia and oxandrolone on skeletal muscle protein synthesis. *Am J Physiol Endocrinol Metab.* 2000; 278:E273–E279. [PubMed: 10662711]
11. Mansoor O, Cayol M, Gachon P, et al. Albumin and fibrinogen syntheses increase while muscle protein synthesis decreases in head-injured patients. *Am J Physiol Endocrinol Metab.* 1997; 273(36):E898–E902.
12. Essen P, McNurlan MA, Wernerman J, et al. Uncomplicated surgery, but not general anesthesia, decreases muscle protein synthesis. *Am J Physiol Endocrinol Metab.* 1996; 262:E253–E260.
13. Tjader I, Essen P, Thorne A, et al. Muscle protein synthesis rate decreases 24 hours after abdominal surgery irrespective of total parenteral nutrition. *J Parenter Enteral Nutr.* 1996; 20:135–138.
14. Jeschke MG, Chinkes DL, Finnerty CC, et al. Pathophysiologic response to severe burn injury. *Ann Surg.* 2008; 248(3):387–401. [PubMed: 18791359]
15. Wolfe, RR.; Chinkes, DL. *Isotope Tracers in Metabolic Research.* New Jersey, NJ: John Wiley & Sons, Inc; 2005.
16. Felig P, Wahren J. Amino acid metabolism in exercising man. *J Clin Invest.* 1971; 50:2703–2714. [PubMed: 5129318]
17. Melville S, McNurlan MA, McHardy KC, et al. The role of degradation in the acute control of protein balance in adult man: failure of feeding to stimulate protein synthesis as assessed by L-[1-13C]Leu infusion. *Metabolism.* 1989; 38:248–255. [PubMed: 2493121]
18. Williams BD, Wolfe RR, Bracy DP, et al. Gut proteolysis contributes essential amino acids during exercise. *Am J Physiol Endocrinol Metab.* 1996; 270:E85–E90.
19. Biolo G, Maggi SP, Williams BD, et al. Increased rates of muscle protein turnover and amino acid transport after resistance exercise in humans. *Am J Physiol Endocrinol Metab.* 1996; 268(31):E514–E520.
20. Hutson NJ, Mortimore GE. Suppression of cytoplasmic protein uptake by lysosomes as the mechanism of protein regain in livers of starved-refed mice. *J Biol Chem.* 1982; 257:9548–9554. [PubMed: 7107581]
21. Motil KJ, Bier DM, Matthews DE, et al. Whole body leucine and lysine metabolism studied with [1-13C]leucine and [alpha-15N]lysine: response in healthy young men given excess energy intake. *Metabolism.* 1981; 30:783–791. [PubMed: 6790902]
22. Hourani H, Williams P, Morris JA, et al. Effect of insulin-induced hypoglycemia on protein metabolism in vivo. *Am J Physiol Endocrinol Metab.* 1990; 259:E342–E350.

23. Denne SC, Karn CA, Liechty EA. Leucine kinetics during feeding in normal newborns. *Pediatr Res.* 1991; 30:23–27. [PubMed: 1891278]
24. Kien CL, Zipf WB, Horswill CA, et al. Effects of feeding on protein turnover in healthy children and in children with cystic fibrosis. *Am J Clin Nutr.* 1996; 64:608–614. [PubMed: 8839507]
25. van Waardenburg DA, Deutz NE, Hoos MB, et al. Assessment of whole body protein metabolism in critically ill children: can we use the [15N]glycine single oral dose method? *Clin Nutr.* 2004; 23(2):153–160. [PubMed: 15030954]
26. Clague MB, Keir MJ, Wright PD, et al. The effects of nutrition and trauma on whole-body protein metabolism in man. *Clin Sci.* 1983; 65(20):165–175. [PubMed: 6861447]
27. Yu YM, Wagner DA, Walesreswski JC, et al. A kinetic study of leucine metabolism in severely burned patients. *Ann Surg.* 1988; 207(4):421–429. [PubMed: 3128190]
28. Thomas S, Wolf SE, Chinkes DL, et al. Recovery from the hepatic acute phase response in the severely burned and the effects of long-term growth hormone treatment. *Burns.* 2004; 30:675–679. [PubMed: 15475141]
29. Finnerty CC, Jeschke MG, Herndon DN, et al. Investigators of the Inflammation and the Host Response Glue Grant. Temporal cytokine profile in severely burned patients: a comparison of adults and children. *Mol Med.* 2008; 14:553–560. [PubMed: 18548133]
30. Gauglitz GG, Herndon DN, Kulp GA, et al. Abnormal insulin sensitivity persists up to three years in pediatric patients post-burn. *J Clin Endocrinol Metab.* 2009; 94:1656–1664. [PubMed: 19240154]

FIGURE 1.
Stable isotope infusion protocol.

FIGURE 2.

Leg muscle protein kinetics. Data are presented as Means $\pm$ SE. A, Rate of disappearance (R_d); B, Rate of appearance (R_a); C, Net protein deposition (NB). * $P < 0.05$, significantly different from basal period; † $P < 0.05$, significantly different between the groups within the period.

FIGURE 3.

Whole-body endogenous protein kinetics. Data are presented as Means $\pm$ SE. A, Whole-body protein rate of appearance; B, whole-body muscle protein rate of appearance; C, whole-body nonmuscle protein rate of appearance. * $P < 0.05$, significantly different from burn within the period; † $P < 0.05$, significantly different from the basal within the group; ‡, significantly different between groups within the period.

TABLE 1

Demographics and Phenylalanine Kinetic Data *

	Burn Patients	Healthy Controls
Age, y	7.4 ± 3.8	22 ± 3 [†]
Weight, kg	27.4 ± 14.7	76 ± 15 [†]
Height, cm	120.3 ± 24.2	176 ± 5 [†]
Gender (males/females)	4/6	5/0
Postburn, mo	6.1 ± 0.6	...
TBSA, %	58.4 ± 18.3	...
Type of burn	Flame 8/Scald 2	...
Delivery to the leg		
Basal	460 ± 121	263 ± 103
AA	905 ± 242 [†]	591 ± 185 ^{†,‡}
Inward transport		
Basal	108 ± 34	145 ± 39
AA	244 ± 135 [†]	294 ± 130 [†]
Outward transport		
Basal	114 ± 34	175 ± 33
AA	227 ± 169	249 ± 143
Shunting		
Basal	352 ± 108	118 ± 78
AA	661 ± 315 [†]	297 ± 275 [†]

* Data are presented mean ± SD.

[†] $P < 0.05$ compared to the basal period.

[‡] compared to burn group within the period.

TBSA indicates total burn surface area.