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Predictors of Muscle Protein Synthesis after Severe Pediatric Burns

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Abstract

Background—Following a major burn, skeletal muscle protein synthesis rate increases, but is often insufficient to compensate for massively elevated muscle protein breakdown rates. Given the long-term nature of the pathophysiologic response to burn injury, we hypothesized that muscle protein synthesis rate would be chronically elevated in severely burned children. The objectives of

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Subject stratification to prevent confounding and interaction may be needed in future isotopic studies.

Level of evidence: IV

Study type: Descriptive (post-hoc analysis of the placebo arm of larger prospective randomized control trials).

Authors' Contribution

Eva C. Diaz, David N. Herndon, Labros S. Sidossis, Oscar E. Suman and Elisabet Børsheim conceptualized and designed the study, reviewed, revised and approved the final manuscript as submitted.

Eva C. Diaz and Craig Porter analyzed and interpreted the data, drafted the initial manuscript, reviewed, revised and approved the final manuscript as submitted.

Jinhyung Lee carried out the statistical analyses and interpretation of the data. Dr. Lee reviewed, revised and approved the final manuscript as submitted.

Matthew Cotter assisted in the acquisition and analysis of the data, critically reviewed the manuscript and approved the final manuscript as submitted.

this study were to characterize muscle protein synthesis rate of burned children over a period of 24 months post-injury, and identify predictors that influence this response.

Study design—87 children with 40% total body surface area (TBSA) burn were included. Patients participated in stable isotope infusion studies at 1, 2 and ~ 4 weeks post-burn, and at 6, 12 and 24 months post-injury to determine skeletal muscle fractional synthesis rate. Generalized estimating equations with log link normal distribution were applied to account for clustering of patients and control for patient characteristics.

Results—Patients (8 ± 6 years) had large (62, 51–72% TBSA) and deep ($47\pm 21\%$ TBSA third degree) burns. Muscle fractional synthesis rate was elevated throughout the first 12 months post-burn compared to established values from healthy young adults. Muscle fractional synthesis rate was lower in boys, children >3 years old, and when burns were >80% TBSA.

Conclusions—Muscle protein synthesis is elevated for at least one year after injury, suggesting that greater muscle protein turnover is a component of the long-term pathophysiological response to burn trauma. Muscle protein synthesis is highly affected by gender, age and burn size in severely burned children. These findings may explain the divergence in net protein balance and lean body mass in different populations of burn victims.

Background

Severe injury results in dramatic loss in whole body protein. While this response is present in most critically ill patients, its magnitude and debilitating nature appears more pronounced in patients with severe burns. (1) Given that skeletal muscle is the major reservoir of amino acids *in vivo*, it is the major site from which proteins are lost.

Under normal conditions, muscle mass is maintained through a balanced interaction between protein synthesis and breakdown rates. Following a major thermal injury this balance is lost resulting in erosion of muscle mass. (2) In some populations of severely ill patients, the loss of skeletal muscle may be caused by increased proteolysis with unchanged synthesis. (3) In major burns however, both parameters of protein turnover are increased. Elevated rates of skeletal muscle protein synthesis (MPS) have been reported immediately following thermal injury in both rodents (4) and humans. (5) However, this elevation does not match the rate of muscle protein breakdown (MPB), even in the postprandial state, resulting in net loss of muscle mass. The differing response of MPS seen in burn trauma patients can be explained by an increased availability of intracellular amino acids deriving from extremely high rates of bound protein breakdown. (6, 7) In other words, in burn patients MPB becomes the main supplier of precursor amino acids and thus an essential regulator of MPS. In consequence, we believe that elevated rates of MPS in the setting of burn trauma closely reflect a high muscle protein turnover.

Evidence shows that MPS is more likely to respond to anabolic stimuli than MPB in both healthy (8) and burned subjects. For instance, drugs such as oxandrolone, growth hormone, insulin, propranolol and metformin improve muscle protein balance mainly by increasing MPS or protein synthesis efficiency (reutilization of amino acids released from MPB) without significantly affecting MPB rates in burned patients. (6, 9–14) Since protein synthesis is the parameter of muscle protein turnover more likely to improve skeletal muscle

net balance when pharmacological interventions are in place, understanding the impact of burn trauma on MPS in both the acute and long-term setting is of clinical relevance. Although the measurement of net balance through the concurrent estimation of MPS and MPB rates is preferable to MPS alone, the estimation of MPB is limited by methodological challenges.

No study has assessed the chronic impact of burn trauma on MPS. Given that the pathophysiology of burn injury is long lasting, (15) we hypothesized that skeletal MPS and thus protein turnover remain chronically elevated in severely burned children. Our primary objective was to characterize and evaluate the time course of skeletal MPS rate encompassing both the acute hospitalization period and extending to one and two years post-injury. A secondary aim was to identify clinical and demographic predictors of MPS in severely burned children.

Methods

Patients

Eighty-seven severely burned children who underwent treatment at the Shriners Hospitals for Children – Galveston from 1999 to 2008 were included in this study. These subjects were in the placebo arm of larger prospective randomized control trials and participated in stable isotope infusion studies as part of these. The experimental protocol was approved by the Institutional Review Board at the University of Texas Medical Branch.

Stable isotope infusion trials

Stable isotope infusion studies were typically performed at approximately 1 and 2 weeks post-burn, at discharge from the ICU (~ 4 weeks post-burn, wounds 95% closed), 6, 12, and 24 months post-burn. Patients received either a constant infusion or bolus injection of isotopically labeled phenylalanine to determine muscle protein fractional synthetic rate (FSR). (16, 17) These two approaches are comparable when used to determine FSR. (18) Two muscle biopsies from the muscle vastus lateralis were collected while the subject was under intravenous conscious sedation with ketamine and local anesthesia with bupivacaine.

When studied acutely, patients were fed enterally with Vivonex TEN (Nestlé Health Care Nutrition, Nestlé S.A., Vevey, Switzerland; 2% fat, 15% protein, 83% carbohydrate). During the first week of admission, the caloric intake was estimated using the formula 1500 kcal/m^2 total body surface area + 1500 kcal/m^2 total body surface area burned. After this period and throughout the entire hospitalization period the caloric intake was modified to 1.4 times the weekly measured resting energy expenditure. After discharge from the ICU, patients' diets were supplemented three times a day with Boost (Nestlé Health Care Nutrition, Nestlé S.A., Vevey, Switzerland; 41 g of carbohydrate, 10 g of protein, and 4 g of fat). Once the regular diet met the patient's caloric requirements of 1.4 times the resting energy expenditure, all supplementations were discontinued. For studies at the discharge time point or later, patients received a constant amino acid infusion of 10% Travasol (Clintec Nutrition, Deerfield, IL; containing 100 mg/ml of amino acids) at a rate of 1.35 ml/kg per hour. Thus, the reported FSR values are considered post-prandial values.

Sample analyses and calculations

Frozen muscle tissue samples were processed as previously described. (19) Skeletal muscle FSR was calculated according to the precursor product method (16) where the precursor is the mean enrichment of the intracellular pool (E_{IC}) of biopsy 1 and 2 for constant infusion studies, and the area under the curve of E_{IC} for bolus injection studies. The product is the difference in enrichment of the bound protein pools of the two muscle biopsies. Skeletal muscle FSR is expressed as percent per hour (%/h).

Data and statistical analysis

Normally distributed data are reported as means $\pm$ standard deviations. Non-normally distributed data are reported as medians, (Quartile 1, Quartile 3). Frequency data are expressed as percentages and counts. The one-sample Wilcoxon signed rank test was used to assess differences between burned children and a reference group. The latter was comprised of seven healthy young adults (26.7 ± 1.0 years) with a mean weight of 73.0 ± 5.0 kg, height 175 ± 15 cm and BMI 23.7 ± 0.6 kg/m² whose post-prandial FSR values (0.076 ± 0.003 %/h) have been published elsewhere. (20)

To examine the relationship between FSR with other variables, we applied generalized estimating equations (GEE) with log link normal distribution. For the model selection, we tested quasi-likelihood under the independence model criterion (QIC) that accommodates all the distribution and link functions and correlation structures available and chose the independent variance model with the smallest QIC among many possible variances including exchangeable, independent, fixed, stationary, non-stationary, and unstructured. (21) The GEE approach is commonly used to analyze longitudinal data in the context of independent variables. (22, 23) These models accounted for the clustering of patients and controlled for patient characteristics including sex, age (0–2 years, 3–11 years, and 12–18 years), total body surface area (TBSA) burned (40–59%, 60–79% and 80–100%), percent of TBSA third degree burns (<40% and 40%), early vs. delayed care (immediate admission vs. admission after seven days post-injury), inhalation injury and time post-burn (1, 2, 4 weeks, and 6, 12, and 24 months post-burn).

Results

Patient characteristics

Patient characteristics are presented in Table 1. A total of 209 stable isotope infusion studies were included in the present analyses. In total, 41 and 36 patients were studied at the 1 week and 2 week time points, respectively. Forty-three and 39 patients were studied at the discharge and 6 month time-points. At 12 months post-burn, studies were performed in 43 patients. Data from 7 patients was collected at 24 months post-injury. In other words, not all children have FSR values at every time-point. Thirty-three children have FSR values at one time-point only, 15 have data at two time-points, 15 have data at three time-points, whereas 24 kids have data at four or more time-points. There are several reasons not all children have FSR values at each stage of the study, the most common being lack of return for follow-ups, as most patients live far away and even out of the country. Hurricane Ike also led to power outage with thawing of samples, thus, these were not included in this study. Due to the

above, we considered missing values as missing completely at random and as such our longitudinal analyses should have yielded valid conclusions (24). Further, to demonstrate missing at random diagnosis we have compared the characteristics of the population complete data vs. those lost to follow up (SDC 1).

Resting energy expenditure and body composition data of these subjects have been published elsewhere. (25, 26)

Skeletal muscle FSR

Skeletal muscle FSR data are presented in Figure 1 and 2 (mean $\pm$ SD). A fed state FSR value from young healthy adults is also presented to serve as a reference value (20), since to our knowledge no values for FSR in healthy children have been reported in the literature. Skeletal muscle FSR in these healthy controls one hour after consuming a meal containing 44 g of protein was 0.076 $\pm$ 0.003 %/h. Relative to the healthy reference group, skeletal muscle FSR was significantly elevated up to 12 months post-burn ($P<0.05$). Muscle FSR values at one and two weeks post-burn were 0.147 $\pm$ 0.131 %/h and 0.152 $\pm$ 0.101 %/h respectively. The highest peak occurred at the time of discharge from the ICU (0.199 $\pm$ 0.164 %/h). At 6 months and one year post-burn, FSR values were 0.184 $\pm$ 0.146 %/h and 0.127 $\pm$ 0.109 %/h respectively. At two years post-burn, FSR (0.086 $\pm$ 0.048 %/h) was not significantly different from healthy subjects.

In the GEE model, male sex, age, % TBSA burn, and time post-burn were all negatively associated with muscle FSR (table 2). Relative to females, FSR values were 35% lower in males ($P<0.001$). Adjusted values for girls vs. boys are presented in Figure 3. FSR was 33% and 43% lower in children ages 3 to 11 years ($P=0.006$) and 12–18 years ($P=0.006$), respectively, compared to children <3 years old. This was mainly caused by very high FSR values in the acute period in the youngest age group. From the 1st week to 12 and 24 months post-burn, children in the <3 year age group exhibited the largest drop in FSR, and at these later time-points, their FSR values were also lower ($P<0.05$) than those in children 3–11 and 12–18 years old (Figure 4). Also, relative to burns covering 40–60% of the TBSA, FSR values were 35% lower in patients with burns 80% of the TBSA ($P=0.022$).

Discussion

Burn trauma results in acute alterations in muscle protein turnover. However, the long-term impact of severe burns on skeletal muscle protein turnover remains unknown. The principle findings of this study were that skeletal MPS is chronically elevated for at least one year post-injury indicating that high rates of protein turnover is also a component of the long-term stress response to burns. In addition, age, sex, % TBSA burned, % third degree TBSA burned, and simultaneous inhalation injury have a significant impact on skeletal MPS following severe burn injury.

Skeletal muscle protein turnover is profoundly elevated in patients with severe burns (27). Unlike other myopathies associated with muscle wasting, such as sarcopenia and disuse atrophy, (28, 29) muscle wasting following burn injury is largely the result of elevated skeletal MPB. It has been postulated that the profound elevations in MPB following burn

injury result in amino acids saturating the intracellular pool of amino acids, which in turn leads to elevated rates of skeletal MPS. (27) Indeed, burn injury results in concurrent elevations in MPB and MPS (27, 30, 31) where the former outpaces the latter, leading to a net loss of protein from skeletal muscle. As children with severe burns have stunted growth and less lean mass than their healthy peers, (32, 33) elevated skeletal MPS post-burn is more likely a compensatory response mediated by protein breakdown and thus amino acid turnover within muscle.

Our data show that following a major burn, skeletal MPS remains elevated for 12 months, returning to normal values between 12 and 24 months post-burn. Interestingly, MPS rates appear to peak at the time of discharge from the ICU, suggesting that protein turnover increases until wounds are largely closed, underscoring the importance of early wound closure and strategies that accelerate wound healing in mitigating the pathophysiologic response to burn injury. These data show that much like the hypermetabolic inflammatory response to burn injury, (34, 35) skeletal MPS and thus protein turnover, are chronically elevated for at least one year following burn injury. Indeed, whole body protein synthesis is thought to account for as much as 30% of the resting energy expenditure in healthy individuals. (36) Our finding of increased MPS for at least one year post burn suggests that elevated protein turnover likely contributes to long-term hypermetabolism in burn survivors. (15)

We have previously shown that age, burn size, male sex, time from injury to excision, resting energy expenditure, sepsis, height and serum creatinine significantly correlate with negative muscle protein net balance acutely after injury. (1) Interestingly, in the aforementioned study children <2.5 years were the least catabolic, consistent with our observation that younger children have a different response to burns with regards to skeletal MPS. To the best of our knowledge, the current study is the first to show age-related long-term differences in skeletal MPS within the pediatric burn population. Children <3 years of age exhibited FSRs that were ~ 65% higher than those found in children 3–11 and 12–18 years old, which may explain why this age group is less catabolic than older burned children and adults in the acute phase of the trauma, as demonstrated previously (1).

Infancy is a period of rapid growth. The anabolic potential of infants and toddlers is evidenced by their ability to double their birth weight by 4.5 month of age while a three-fold and four-fold increase in weight is seen by 12 and 36 months, respectively (37). Moreover, early isotopic studies reported whole body protein synthetic rates two and three times higher in healthy infants when compared to young adults and the elderly (38, 39), showing that the capacity for protein accretion is lost with maturation. Furthermore, we have recently demonstrated that among burn patients, adults are more catabolic than their pediatric counterparts, which is largely attributable to higher rates of muscle protein synthesis in children (10). Although in the previous study differences in protein synthesis were not found within the pediatric group, their age groups significantly differ from those of the current study. We also found that at 12 and 24 months after burn injury, children < 3 years old exhibited the largest drop in FSR values. The long-term impact of burn trauma on body composition when the insult occurs at different stages of development during childhood remains to be determined.

A limitation of our study is that older children were not stratified based on Tanner stages. Consequently, the impact of burn trauma on MPS could not be determined with more precision during the pubertal years. As puberty is the only period after infancy characterized by rapid growth, higher FSR values could be expected in the 12–18 year group compared to younger children. However, we cannot rule out that some of the patients in the 3–11 year group were undergoing puberty and this overlap may have influenced the results.

We have previously reported sexual dimorphism with regards to the pathophysiologic stress response to major burn trauma. (40) Girls with severe burns not only have shorter ICU stays compared to boys, but also are less hypermetabolic, (40) with higher levels of anabolic hormones such as growth hormone and Insulin-like growth factor-1 (IGF-1) up to 12 months post-burn. (41) In agreement with this, MPS rate was higher in girls than in boys in the current study (Figure 3). Although the skeletal MPS response to burn seems to be sex specific, no sex differences in acute or long term LBM changes have been reported. (41)

The % TBSA burned is one of the most important determinants of acute muscle catabolism. (1) Although increments in burn size >40% TBSA is not predictive of a more negative muscle protein balance as assessed by the two pool arterial-venous dilution model, MPS seems to be depressed in burns >80% of the TBSA. Children with such large wounds are the most critically ill and thus sepsis and multi-organ failure are more likely to occur. This might attenuate all ATP consuming processes, including MPS. We did not control for sepsis and multi-organ failure, and this should be taken into account when interpreting the results. Puthucherry *et al.* (3) found that 40% of ICU non-burn adults exhibit myofiber necrosis with concomitant reduction in MPS. Our results show that the synthetic capacity of the skeletal muscle is hampered in children with large wounds.

Inhalation injury was the only variable with a positive association with MPS. Statistical significance was not achieved ($P=0.056$) with the current power, but simultaneous injury of the airway lining epithelium and skin may possibly trigger higher rates of muscle protein turnover than burn to the skin only, which in turn would lead to a greater demand for amino acids from skeletal muscle for repair.

Data on body composition and resting energy expenditure from this cohort of patients have been reported elsewhere. (25, 26) We have previously shown that very little accretion of LBM occurs during the first year post-burn. (32) Although deposition of LBM is greater during the second year after trauma, it is not sufficient to match LBM of healthy children at three years post-burn. (15, 32) Long term administration of anabolic drugs, e.g., growth hormone and oxandrolone plus exercise, greatly improves LBM in burned children. (25, 42, 43) However, whether accretion of LBM with these drugs is primarily mediated through elevation of MPS or MPS efficiency, as it occurs early after trauma, remains unknown.

In this study, skeletal MPS was determined during the infusion of either Vivonex or an amino acid solution (Travasol). We have shown that infusion of Travasol does not increase FSR above basal levels in severely burned children, and this anabolic resistance to amino acids can persist for a year post-burn (19). A limitation of the current study is the lack of concurrent direct measures of MPB. Development of less invasive methods (without femoral

catheters), that do not rely on the estimation of blood flow rates are needed to precisely measure also muscle protein fractional breakdown rates in humans.

Conclusion

FSR values in severely burned children are not different from healthy control values at two years post-burn. Chronically elevated rates of MPS indicate that high rates of muscle protein turnover constitute part of the long-term pathophysiological stress response to severe thermal injury. Further, MPS in burn children is highly influenced by sex, age, and % TBSA burned. This may explain the divergence in net muscle protein balance and LBM in different populations of burn victims. The findings in this study underscore the need for focused and prolonged rehabilitative strategies aimed at maintaining or increasing muscle mass and functional capacity in burned patients. Given the heterogeneous response of MPS to burn trauma in children, subject stratification may be needed in future isotopic studies of muscle protein kinetics in order to minimize confounding and interactions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Skeletal muscle FSR (mean±SEM) from 1 week (wk) to 24 months (mo) post-burn. n = 33 (1 wk), 37 (2 wk, 4wk, 6 mo), 40 (12 mo), and 7 (24 mo). The gray line represents postprandial FSR values for seven healthy young adults(20). FSR= fractional synthesis rate.

Figure 2.

Adjusted (showing the effect of time on FSR post-burn) and unadjusted FSR values up to 24 months post-burn. The dotted line represents muscle FSR values after adjusting for age, sex, % TBSA burned, % TBSA third degree burned, smoke inhalation injury, and delayed care. FSR= fractional synthesis rate; TBSA= total body surface area.

Figure 3.

Effect of sex on FSR values after major burn-injuries in children. FSR values are adjusted for age, TBSA burned, % TBSA third degree burned, smoke inhalation injury, and delayed care. FSR= fractional synthesis rate; TBSA= total body surface area.

Figure 4.

The impact of age on skeletal muscle FSR after burn. FSR values are adjusted for sex, % TBSA burned, % TBSA third degree burned, smoke inhalation injury, and delayed care. FSR= fractional synthesis rate; TBSA= total body surface area.

Table 1

Patient demographics

Variable	N=87
Age (Years)	8 ± 6
Male, No. (%)	58 (68)
Ethnicity, No. (%)	
Hispanic	77 (90)
White, non-Hispanic	4 (5)
Black, non-Hispanic	3 (4)
Etiology, No. (%)	
Flame	68 (78)
Scald	10 (12)
Electrical	8 (9)
Other	1 (1)
Burn to admission (days), Median (Q1, Q3)	2 (1, 5)
Delayed admissions, No. (%)	14 (16)
% TBSA burned, Median (Q1, Q3)	62 (51, 72)
% Third degree TBSA burned	47 ± 21
Inhalation Injury, No. (%)	32 (41)
Length of stay in the ICU (days), Median (Q1, Q3)	34 (25, 50)

Data are expressed as means ± SD, or medians (Q1, Q3), counts and percentages. TBSA=Total Body Surface Area. Delayed admission: >7 days post-burn

Table 2
Relationship between skeletal muscle FSR with clinical and demographic variables

FSR	Coef.	Std. Err	z	P>z	95%	Conf. Interval
Male vs. Females	−0.435	0.123	−3.550	0.000	−0.676	to −0.195
Age 3 to 11 vs. <3 yrs	−0.403	0.145	−2.770	0.006	−0.688	to −0.118
Age 12 to 18 vs. <3 yrs.	−0.469	0.171	−2.740	0.006	−0.804	to −0.134
% TBSA 60 to 79 vs. 40 to 60%	−0.168	0.127	−1.320	0.187	−0.418	to 0.082
% TBSA 80 to 100 vs. 40 to 60%	−0.615	0.268	−2.290	0.022	−1.141	to −0.089
% 3rd TBSA 40 vs. <40%	−0.317	0.306	−1.040	0.299	−0.916	to 0.282
Delayed admission	0.159	0.220	0.720	0.471	−0.272	to 0.590
Smoke inhalation injury	0.248	0.130	1.910	0.056	−0.007	to 0.504
2 weeks vs. 1 st wk. post-burn	0.062	0.152	0.410	0.683	−0.236	to 0.361
4 weeks vs. 1 st wk. post-burn	0.324	0.173	1.870	0.061	−0.015	to 0.664
6 months vs. 1 st wk. post-burn	0.121	0.178	0.680	0.498	−0.228	to 0.470
12 months vs. 1 st wk. post-burn	−0.353	0.212	−1.670	0.096	−0.768	to 0.062
24 months vs. 1st wk. post-burn	−0.529	0.201	−2.630	0.009	−0.924	to −0.135
Cons	−1.027	0.298	−3.45	0.001	−1.609	to −0.444

TBSA=Total Body Surface Area. Delayed admission: >7 days post-burn