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The Contribution of Muscle to Whole Body Protein Turnover Throughout the Course of Burn Injury in Children

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Abstract

The physiologic response to trauma results in the efflux of large amounts of amino acids from skeletal muscle. This is extreme in large burn injuries. Protein kinetic studies, although useful in determining the rates of protein synthesis and breakdown, do not provide information about muscle loss. This study determined the contribution of muscle protein to whole-body protein breakdown in children throughout their course of burn injury. Children aged 0 to 18 years with initial burn size 30% TBSA underwent ¹⁵N glycine and 3 methylhistidine (3MH) analysis during three phases of care: A, early acute; B, wound closure; and C, convalescence. Muscle protein breakdown was estimated using a factor of 4.2 μmol 3MH per 1 g of mixed protein. Twenty-two patients with a mean of 54.5 ± 20.1% TBSA burn were studied. Protein balance did not change remarkably and remained positive by 2 g/kg during hospitalization. However, muscle protein breakdown dropped from 1.1 to 0.6 g/kg with wound closure ($P < .0001$), representing a decrease in the contribution of muscle protein to whole-body protein breakdown from 20 to 7%. Ten patients returned for a third measurement after discharge. Although protein turnover was high, muscle breakdown was consistent with 3MH values reported in healthy children. Serial determination of 3MH excretion is a simple way to track muscle catabolism throughout burn injury. Our data suggest that despite accelerated protein turnover, muscle catabolism significantly decreases with wound closure and begins to normalize around discharge. In convalescence, 3MH excretion is comparable with normal children.

Introduction

The physiologic response to trauma results in the efflux of large amounts of amino acids from skeletal muscle. This increased availability of free amino acids is a necessary step for wound repair, acute phase protein production, and gluconeogenesis.^{1, 2} In most cases, the response are short-lived without significant impact on outcome. However, when hospital recovery is drawn out, as is the case with large burn injury, muscle wasting can be extreme.

Numerous factors surrounding burn injury exacerbate protein breakdown and resultant muscle loss during hospitalization. Whole body and muscle protein turnover studies have

elucidated the most prominent contributors among these including the magnitude of burn injury itself, and the ensuing inflammatory state. Delay in surgical treatment and sepsis are also significant contributors to skeletal muscle breakdown.³ Consequently, early excision and grafting of burn wound by reducing metabolic rate, protein losses, and the onset of sepsis ameliorates the extent of muscles wasting that can occur early in the course of recovery. Prompt, uninterrupted nutritional support during this phase is also a useful adjunct for sustaining protein requirements for wound healing that would otherwise come at the expense of skeletal muscle.

During the second phase of burn recovery, namely rehabilitative care, many patients demonstrate an apparent delay in anabolism and weight gain. One theory is that this is the result of unremitting hypermetabolism, which persists despite wound closure.^{4,5} However, the demands of physical therapy and normal activities of daily living, which may require greater than usual amounts of energy to perform, can also increase nutritional requirements. Ironically, in attempt to transition the patient to normal nutrition, supplemental nutrition support is often weaned at this time. Sustaining calorie and protein balance on an oral diet alone, despite anorexia due to medications or poor emotional adjustment, or difficulty in feeding is a clinical challenge, making it difficult for patients to regain their pre-injury nutritional status prior to discharge.

For many patients, even upon discharge home, growth velocity is subnormal, and phenomenon that appears to be most pronounced with severe burn injury.^{6,7} Several factors are likely to play a role in disrupting catch-up growth in these children including immobility due to complications of the burn injury, poor nutrition or anorexia, recurrent infections, and difficult emotional adjustment. Yet because gains in skeletal muscle precede growth spurts, a continued negative protein balance may hinder growth during convalescence.

Protein kinetic studies can be used to determine whether a hypercatabolic state is a factor interfering with the return of normal nutritional status in burn children throughout the various stages of recovery. Unfortunately, the relative response of muscle to whole body protein turnover throughout the course of injury can not be differentiated by protein kinetic studies alone, making it difficult to relate changes in turnover to changes in body composition, namely muscle mass. It is possible however, and relatively simple to measure the contribution of muscle protein to the rate of whole body protein breakdown by incorporating measures of urinary 3 methyl-histidine (3MH). Because it exists almost exclusively in skeletal muscle protein, and is not reutilized, 3MH a good adjunct to protein turnover studies. Combined with ¹⁵N-glycine for determination of protein turnover, the contribution of muscle to protein breakdown can be measured with a basic urine collection throughout the course of recovery.

The purpose of this study was to measure whole body protein kinetics using ¹⁵N-glycine and muscle protein breakdown by 3MH throughout the course of burn injury-from the acute phase of care through convalescence. Secondly, the effects of wound closure and nutrition therapy, (mode of feeding and protein intake) on protein dynamics, and the contribution of muscle to protein breakdown were also identified.

Methods

Subjects

Twenty-two patients aged 0–18 years and admitted to the acute burn unit in our hospital with initial burns of greater than 30% total body surface area (TBSA) were enrolled into this study. Additional inclusion criteria were that patients were hemodynamically stable and without underlying metabolic conditions. None of the patients received pharmacologic intervention to promote anabolism.

Study Design

This study was a prospective, descriptive study designed to have three measurements based on the patient's clinical course. The first measurement was obtained during the acute phase, when the patient's open wounds were at least 30% or greater (A). Then, a second measurement was obtained following definitive wound closure (greater than 95% closed, as defined by the attending surgeon for all patients). This also represents the time of patient's entry into the rehabilitative phase (B). A third measurement, representing the convalescent phase (C) was taken after the patients were discharged home and returned to the hospital for follow-up medical care. At each measurement phase, ^{15}N -glycine, 3MH, and protein intake were measured.

Diet

During the acute phases (A and B), nutrition support for these patients consisted of our usual therapy of combined enteral and parenteral nutrition support. Once patients tolerate 50% or greater of their enteral target volume, parenteral feedings are weaned, as the enteral diet is further advanced. Our parenteral nutrition solution is a lipid-free solution of dextrose (20%) and amino acid (7.4%). Energy intake was aimed to meet the patients' basal metabolic rate with a 30 to 70% increment based on their predicted degree of metabolic stress and level of pain or activity. Protein targets were set at 2.5 to 3.5 g/kg/d. These guidelines for intake are consistent with our standard of care based on previously gathered outcomes data.^{8,9} Energy and protein targets remained the same for measurements A and B or, in other words, until the time of wound closure. Actual weight on admission was used for these estimates. For patients with fluid overload on admission, the patient's presumed dry weight (from history or interview) was used for calculations. No patients required use of an adjusted weight. For phase C, patients were on a regular house diet appropriate for their age, with no specifically established energy and protein goals.

Measure of Whole Body Muscle and Protein Turnover

On the day of tracer study, each patient provided a urine sample to determine the basal level of N^{15} enrichment in urea and ammonia. Patients then received a dose of ^{15}N -glycine ($4\text{mg}\cdot\text{kg}^{-1}$ dissolved in 20 ml of saline) mixed in 50 cc of apple juice, or other clear liquid beverage (protein-free), followed by a 50 cc wash of water. A 24-hour urine collection commenced immediately for the determination of urinary urea nitrogen, total nitrogen, creatinine, and ammonia contents. The enrichments of ^{15}N -urea and ^{15}N -ammonia in urine were determined for evaluation of whole body nitrogen flux.

3MH Chemical analysis of the samples

On the same day of the ^{15}N -glycine study, 24-hour urine samples were collected for three consecutive days. During this time, patients were on a consistent, meat-free diet, ensuring that endogenous 3MH was measured. The quantification of 24-hour 3MH was done by negative chemical ionization gas chromatography mass spectrometry at Shriners Hospitals for Children® Boston. This method has been previously validated and has excellent linearity. Its sensitivity allows for the detection of 6.8 pmol of urinary 3MH with a precision of 9.8%.¹⁰ The mean 3MH obtained by averaging the three consecutive 24-hour urine samples was used for calculating skeletal muscle protein breakdown. Skeletal muscle protein breakdown was estimated using a factor of 4.2 μmol 3MH per 1 g of mixed protein. A factor of 6.25 was used to convert gram of nitrogen to gram of protein. Values are presented as 3-day averages in units of milligrams per day or milligrams per kilogram.

Data Analysis

Differences among the three phases were compared by repeated-measures analysis of variance (ANOVA) and paired *t*-test for differences related to open and closed wound status during hospitalization. Data are expressed as mean (SD) unless otherwise indicated.

Results

Table 1 shows demographics of the 22 patients enrolled into this study. Their initial mean burn size was $54.5 \pm 20.1\%$ TBSA. Current open wound size at the time of study was $47.8 \pm 22.5\%$ TBSA. This estimate included open wound area that still needed surgical autograft placement and open donor wound. Patients were studied for an average of 14.4 ± 11.5 days postburn for their acute measurement (phase A). The wide range of days for patients to receive their first measurement reflected primarily late transfers to our hospitals from other facilities. Seventeen of the 22 patients were mechanically ventilated at the time of measurement A. The second measurement was taken at the time of wound closure (phase B), averaging 41.9 ± 24.6 days postburn. Only one patient continued to require ventilatory support at this time. The average number of days between measurements A and B was 28. One patient had only a 1-week time lapse between measurements A and B. This patient had the smallest burn wound size, requiring only one surgical visit. The patient received the second measurement soon after wound closure before his discharge. Only 10 patients returned to participate in the third measurement (phase C), representing their convalescent phase of care. The third measurement averaged 388.4 ± 224.0 days after the initial burn injury. This also represents a time span of 0.9 ± 0.7 (0.8–1.8) years after the hospitalized rehabilitative phase of care (phase B).

Change in protein and muscle breakdown throughout the course of injury

Figure 1 depicts whole-body protein breakdown, synthesis, balance, and muscle protein breakdown in all 22 patients before and after wound closure (phase A and B). Both protein synthesis and breakdown increased. Protein balance (defined as the difference between protein synthesis and breakdown) did not change remarkably and remained positive by >2 g of protein/kg throughout hospitalization as represented by phases A and B. However, muscle contribution to whole-body protein breakdown did show considerable change with wound

closure. After wound closure, muscle protein breakdown dropped from 1.1 to 0.6 g/kg, a significant decrease ($P < .0001$). This decrease represented an alteration in the contribution of muscle protein to whole-body protein breakdown from 20 to 7%.

Figure 2 shows protein and muscle breakdown throughout the course of injury in those 10 patients who returned for the third measurement after convalescence. After discharged home, muscle protein breakdown continued to decline to an average of 0.4 g/kg (approximately 4% of whole-body protein breakdown). Although the change in muscle contribution was statistically significant, there was no significant difference in the change between phases B and C by post hoc analysis (ANOVA; $P = .004$; $F = 3.37$). Muscle breakdown as represented by 3MH excretion during convalescence was consistent with normal values found in children.¹¹

The effect of mode of nutrition and dietary protein intake on muscle and whole body protein breakdown during hospitalization

Table 2 shows whole-body protein and muscle breakdown according to mode of feeding and dietary protein intake. Patients were stratified according to parenteral ($n=10$) vs. enteral ($n=12$) intake. The mode of administration was determined by patients' ability to achieve adequate intake. This related to gastrointestinal tolerance, operating schedule, and other clinical interventions that might indicate the need for suspending enteral intake for a significant time. All enterally fed patients required tube feedings to meet their daily requirement. A small number of patients ($n=4$) were also eating small amounts of food. There was no difference in protein intake, synthesis, breakdown, or balance among patients fed enterally or parenterally. The contribution of muscle to protein breakdown was nearly twice as high in the enterally fed group vs. the parenteral group; however, this was not statistically significant.

There was a significant correlation between dietary protein intake and protein and muscle kinetic data during hospitalization. Dietary protein showed a significant inverse correlation with muscle protein breakdown ($R = .50$; $P < .003$). We stratified the patients into groups according to protein intake, using 2.5 g/kg as a cutoff. This cutoff was selected based on previous outcome studies demonstrating that when patients achieved above 2.5 g/kg of protein, efficient protein utilization for recovery was achieved.⁹ In this study, dietary protein intake at this level improved protein synthesis during phase A (13.2 vs. 9.06; $P < .03$). A higher protein diet also resulted in increased overall whole-body protein balance. In addition, the contribution of muscle to whole-body protein breakdown was substantially less in the high protein group (13 vs. 25% in those with low protein intake). In phase B, the mechanism of benefit of dietary protein on protein economy changed. Dietary protein intake above 2.5 g/kg did not improve protein synthesis; however, it did reduce protein breakdown. There was no influence of dietary protein on muscle breakdown or the contribution of muscle to whole-body protein breakdown.

Discussion

The physiology of burn injury includes a distressing degree of skeletal muscle catabolism.⁵ It is presumed that this response evolved to support recovery from severe injury by

increasing the pool of protein available for wound healing. However, in the setting where protein can be supplied exogenously through nutritional support, an endogenous source of protein is not essential for recovery and may even be harmful. Certainly, the muscle wasting that accompanies the liberation of endogenous protein may slow rehabilitation through general weakness, as has been our clinical experience working with burned children.

Prompt and effective wound closure is the most effective way to truncate this physiology.^{3,12} Modification of this biology is being explored through pharmacologic agents, namely anabolic hormones and beta-adrenergic blockade.¹³⁻¹⁵ However, at present, the cornerstone of nutritional management involves providing protein and caloric support beyond that usually required by uninjured persons. Furthermore, pharmacologic therapy may not be an option for all patients demographically. Our study describes the efficacy of protein feeding alone without pharmacologic intervention throughout the course of burn in-whole-body and muscle protein metabolism together, the relative contribution of muscle to whole body turnover, particularly in response to nutritional support, was determined.

During acute hospitalization, the process of wound closure alone did not significantly change whole body protein turnover (both synthesis and breakdown). In fact, protein turnover rates accelerated to some degree from the time of measurement A to B. However, wound closure was associated with a significant decrease in muscle protein breakdown, as demonstrated by the 3MH data. The disassociation between protein turnover and muscle breakdown suggests that factors other than wound closure contribute to whole-body protein turnover. Determining what these factors are and the relevance of this from an outcome perspective requires further exploration.

During the early acute phase of care, dietary protein improved protein synthesis rate but had no significant effect on whole-body protein breakdown. Although the difference in muscle loss by weight was negligible, the contribution of muscle to whole-body protein breakdown was diminished by 2-fold in the group fed with high-protein diet. More specifically, the percent of muscle contributing to whole-body protein breakdown in patients with protein intakes of less than 2.5 g/kg was 25% as opposed to 13% in patients fed greater than 2.5 g/kg/d, which is clinically significant.

Another interesting observation relates to mode of nutritional therapy. In our studies, despite similar protein intakes among patients receiving enteral vs. parenteral, the contribution of muscle to whole-body protein breakdown was considerably less with parenteral. This discovery cannot be explained by means of this descriptive study; however, the finding may relate to preferential uptake of protein in the gut, as opposed to availability of parenteral amino acids for skeletal muscle. Additional studies on protein utilization and the effect on muscle of patients receiving either mode of nutritional support alone would be of interest.

During rehabilitation, a high-protein diet did not improve protein synthesis. Intakes greater than 2.5 g/kg did significantly decrease whole-body protein breakdown, suggesting that this level is important for minimizing obligatory protein losses. Muscle breakdown was not affected by diet during this phase. In fact, muscle protein breakdown during the

rehabilitation phase was close to that of normal children and only decreased slightly more during the convalescent phase.

Our convalescent phase has a wide range, approximating 1 month to just below 2 years in the 10 who returned to our facility for other treatment. Unfortunately, the remaining patients received care elsewhere or were not admitted to our facility for long enough to complete the third measure of study. There was no correlation between days postburn or days post discharge and degree of catabolism. However, it is worth noting that the greatest value for rate of muscle protein breakdown in any one patient was 0.8 g/kg. This was observed in a 7-year-old patient with an initial burn size of 85% TBSA ~ 1.5 years postburn. So, although our findings suggest that burned children in general should not be presumed catabolic on discharged home, some patients may exhibit a small degree of muscle breakdown beyond normal values.

In conclusion, serial determination of 3MH excretion may be a noninvasive and simple way to track muscle catabolism over the course of burn injury. Our data suggest that muscle catabolism significantly decreases with wound closure. Provision of dietary protein above 2.5 g/kg during the acute phase helps maintain positive protein balance and seems to diminish muscle loss. However, whether this level of protein intake is minimally sufficient for burned children requires further study. After wound closure, muscle breakdown as defined by 3MH excretion decreases to near normal levels in burned children before their discharge to home and is within normal limits during convalescence.

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Figure 1. Whole Body Protein Turnover and Muscle Breakdown in 22 Burned Children Before and after Wound Closure. Following wound closure, muscle protein breakdown significantly decrease (*p<0.0001).

Figure 2. Protein Turnover and Muscle Breakdown During Acute, Rehabilitative, and Convalescent Phase of Burn Care in 10 Patients. The overall change in muscle contribution was statistically significant, however there was no significant in the change between phase B and C by post hoc analysis (ANOVA; $p=0.004$; $F=3.37$).

Table 1

Demographics and Study Characteristics in 22 Enrolled Burned Patients

Variable	Mean (SD)	Range
Age (yr)	8.7 (4.5)	0.8–16
Gender	14 M / 8 F	
Ventilator Days (d)	22.4 (17.6)	(0–72)
Initial Burn Size (%TBSA)	54.5 (20.1)	30–95
Current Open Wound at Study (%TBSA)	47.8 (22.5)	10–95
Time of Measurement (Days Post Burn)		
<i>Phase A</i>	14.4 (11.5)	1–42
<i>Phase B</i>	41.9 (24.6)	7–121
<i>Phase C</i>	388.4 (224.0)	63–715
Length of Stay (d)	108.1 (93.3)	18–439

Effect of Clinical Phase, Mode of Nutrition and Protein Intake on Different Aspects of Protein Metabolism During Hospitalization

Table 2

	Protein Intake (g/kg)	Protein Synthesis (g/kg)	Protein Breakdown (g/kg)	Protein Balance (g/kg)	Muscle Breakdown (g/kg)(% WBP Breakdown)*
Wound Status					
Open	2.6 ± 1.6	11.4 ± 4.0	9.1 ± 5.5	2.3 ± 1.5	1.1 ± 0.6 (20) [†]
> 95% Closed	2.3 ± 1.2	12.9 ± 4.4	10.7 ± 4.2	2.2 ± 1.6	0.6 ± 0.4 (7)
Mode of Diet Therapy					
Enteral	3.0 ± 1.6	11.6 ± 5.8	9.0 ± 5.0	2.6 ± 1.7	1.0 ± 0.6 (23)
Parenteral	2.8 ± 1.3	11.7 ± 3.2	9.1 ± 2.8	2.6 ± 1.3	0.8 ± 0.1 (14)
Protein Diet Group[‡]					
Phase A					
Low	1.6 ± 0.7	9.6 ± 3.8	8.2 ± 4.0	1.4 ± 0.7	1.1 ± 0.7 (25)
High	3.8 ± 1.2	13.3 ± 4.7*	9.7 ± 3.9	3.5 ± 1.2	1.1 ± 0.6 (13)
Phase B					
Low	1.2 ± 0.8	14.4 ± 5.7	13.5 ± 5.5	0.9 ± 1.0	0.7 ± 0.4 (6)
High	3.1 ± 0.4	13.1 ± 4.2	9.7 ± 4.2 [†]	3.4 ± 1.3	0.7 ± 0.6 (8)

Data are expressed as mean and standard deviation.

* % WBP Breakdown = muscle breakdown / whole body protein breakdown

[†] Significantly different by Student's paired t test; p<0.05

[‡] Protein diet groups defined as follows: low, <2.5 g/kg protein; high, >2.5 g/kg