Are Visceral Proteins Valid Markers for Nutritional Status in the Burn Intensive Care Unit?

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The aim of this study was to determine whether visceral protein levels increase under positive nitrogen balance during times of decrease in acute-phase reactant levels in patients with burn injury. This was a post hoc analysis of a prospective, interventional study approved by the local institutional review board. A total of 10 subjects between the ages of 18 and 72 with ≥20% total body surface area burn were enrolled over a 14-month period. Data were collected for five subjects (average age of 28 ± 8 years and total body surface area burn of $69 \pm 15\%$) who met the inclusion criteria. Changes in visceral protein levels were examined along with nitrogen balance and acute-phase reactants when the subjects were on enteral nutrition, and the proteins were not examined during times of acute kidney injury. Descriptive statistics were performed, and linear regression was used to analyze the association of visceral proteins and nitrogen balance during times that acute-phase reactant levels were decreasing. The subjects received an average of 3044 ± 1613 kcal/day (39 ± 20 kcal/kg), meeting 72% of caloric goals and achieving positive nitrogen balance during 68% of the 40 weekly measurements, with 174 ± 85 g of protein intake per day (2.2 ± 1.1 g/kg). There was a weak relationship between nitrogen balance and changes in visceral protein levels during times that the acute-phase reactant levels were decreasing ($P > .05$). Visceral proteins were found to be poor markers of nutritional status. This study is unique because the subjects were able to achieve positive nitrogen balance despite severe burns. (J Burn Care Res 2014;XXX:00–00)

Nutrition plays an important role in improving outcomes for patients suffering from thermal injury.[1,2] Resting energy expenditure (REE) increases twofold and protein loss increases threefold compared with preinjury levels.[3,4] Providing adequate nutrition is essential to decrease infection rates and to promote wound healing.[2] Monitoring nutritional adequacy is difficult for many reasons. Edema following fluid resuscitation limits the use of anthropometric measures. Nutritional markers are commonly used in majority of burn intensive care units, yet these are unreliable because of the inflammatory response. Production of hepatic proteins is reprioritized after thermal injury. Positive acute-phase reactant levels, such as C-reactive protein (CRP), haptoglobin, and α-1-antitrypsin, are increased, whereas visceral protein levels (negative acute-phase reactants), such as albumin, prealbumin, transferrin, and retinol-binding protein, are decreased. Therefore, many researchers question the use of nutritional markers in trauma patients.[5] In fact, their reliability in any disease process is considered controversial.[6] Graves et al found that 85% of dietitians at burn care centers use visceral proteins for nutritional assessment,[7] but the

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Society of Critical Care Medicine and the American Society for Parenteral and Enteral Nutrition[8] 2009 guidelines state that visceral proteins have not been validated for use in nutritional assessment for critically injured patients.

Manelli et al[9] reported that the level of visceral proteins is inversely proportional to the level of acute-phase reactants in the recovery period postburn and also stated that evaluating the ratios of these proteins over time is a more reliable assessment of nutritional adequacy. They propose that decrease in acute-phase reactant levels with an increase in visceral protein levels indicates an adequate nutritional regimen for patients with major burns, whereas a decrease in acute-phase reactant levels without an increase in visceral protein levels indicates an inadequate nutritional regimen. However, these findings were not correlated to nutritional intake. Carlson et al[10] examined the correlation between nutritional intake and visceral proteins in patients with burn injury. They found that the level of visceral proteins is not good indicators of nutritional status, but they did not evaluate the impact of the acute-phase reactants.

The aim of this study was to determine the relationship between nutritional status and changes in visceral protein levels with regard to changes in acute-phase reactant levels

Methods

This is a post-hoc analysis on the relationship between nutritional status and changes in visceral protein levels with regard to changes in inflammatory markers. The subjects who were able to take an oral diet, required total parenteral nutrition or continual renal replacement therapy, or were in acute kidney injury stage 2 or higher were not included in the study.

During a 14-month period, subjects between the ages of 18 and 72 with at least 20% total body surface area (TBSA) burn sustained within 14 days of admission were enrolled in this prospective, interventional study on high-dose insulin. The study was approved by the local institutional review board. The primary objective of the study was to examine the long-term therapeutic results of a high-dose insulin infusion with the aim to decrease protein catabolism, as described by Ferrando et al.[11] Insulin was administered at the rate of 1.5 mU/kg/min, and 20% dextrose was intravenously administered to maintain the blood glucose between 80 and 110 mg/dL. The Carlson[12] or Milner[13] equations were used to determine the REE. We found that these equations most accurately predict the REE.[14] During this 14-month period, we used the Carlson equation for the first 30 days after the burn and the Milner equation thereafter if we were unable to perform indirect calorimetry. Indirect calorimetry was performed to measure the REE when clinically available. An activity factor of 1.2 and 1.4 was used clinically with the Carlson or Milner equations and with indirect calorimetry because these levels have been found to increase the lean body mass and maintain the body weight, respectively.[15]

Visceral proteins (serum prealbumin and transferrin) and acute-phase reactants (serum CRP, haptoglobin, and α-1-antitrypsin) were measured weekly. Serum creatinine was measured daily. Urinary urea nitrogen (UUN) was measured weekly using 24-hour urine collections. Nitrogen losses were calculated weekly (using UUN × 1.25) to estimate the total urinary nitrogen excretion.[16] Insensible nitrogen losses were estimated to be 4 g/day, and the losses through open wounds were estimated by the Waxman equation.[17]

$$\text{Nitrogen losses per day through open wounds}$$
$$(g) = 0.1 \times BSA \times \%TBSA \text{ open wound} \times 100$$

Data on protein intake (including both enteral nutrition and protein-containing intravenous fluids) were obtained from the medical record, and protein intake was divided by a factor of 6.25 to convert to grams of nitrogen consumption. Nitrogen balance for each day was calculated by total nitrogen intake minus total estimated nitrogen loss. Nutrition status was determined by nitrogen balance.

Descriptive data were obtained from the medical record. The required nutritional intake and actual nutritional intake were expressed as mean ± standard deviation. Changes in visceral protein levels between weekly measurements were expressed as median, range, and interquartile range. A decrease in acute-phase reactant levels between consecutive weekly measurements was examined along with nitrogen balance data and changes in weekly measurements of visceral proteins. Sex and mortality were expressed as percentages. Age, burn size, length of stay in the burn intensive care unit, height, and preinjury weight were reported per subject. Preinjury weight and height were used to calculate energy intake goals and nitrogen balance. Linear regression was used to

Table 1. Subject characteristics, *N* = 5

	1	2	3	4	5
Age, years	23	26	25	23	42
Total body surface area burn, %	69	56	54	92	74
Stay in intensive care unit, days	155	97	77	26	62
Height, cm	180	173	185	173	165
Weight, kg	76	78	109	67	70

Table 2. Nitrogen intake, losses, and balance data, mean ± standard deviation

Subject	Weeks	Nitrogen Intake	Wound Losses per Waxman Equation	Urinary Urea Nitrogen	Total Nitrogen Loss	Nitrogen Balance	% of Weeks in Positive Nitrogen Balance
1	18	24.6 ± 7.6	5.3 ± 4.9	11.1 ± 3.3	23.2 ± 12.1	1.3 ± 10.8	61
2	5	26.1 ± 8.6	3.5 ± 1.0	11.1 ± 2.9	21.4 ± 4.1	4.7 ± 7.9	80
3	6	38.4 ± 4.7	6.9 ± 2.5	15.3 ± 6.1	30.0 ± 9.9	8.5 ± 8.5	83
4	2	40.1 ± 11.5	18.8 ± 0.3	16.6 ± 2.6	43.5 ± 3.0	-3.2 ± 14.4	50
5	9	29.9 ± 7.6	6.0 ± 1.8	13.9 ± 4.8	27.3 ± 7.3	2.6 ± 3.7	67

analyze the association of visceral proteins and average daily nitrogen balance during times that CRP was decreasing. Correlation coefficient ranges were defined as follows: $r^2 < .2$, weak; $.7 \geq r^2 \geq .2$, moderate; and $r^2 > .7$, strong. P values of less than .05 were considered statistically significant. Each acute-phase reactant was evaluated against prealbumin and transferrin, during the times that acute-phase reactant levels were decreasing and the subjects were in positive nitrogen balance. These analyses were conducted with Microsoft Excel 2007 (Microsoft, Redmond, WA) and SAS (v. 9.2).

RESULTS

A total of 10 subjects were enrolled in the study. One subject died prior to getting the required laboratory results, and four subjects had their diets advanced and did not require supplemental enteral nutrition. The remaining five subjects were examined in this post hoc analysis.

All of the five subjects included in this analysis were in the insulin arm. A total of 40 weekly measurements were performed for these five subjects, with a median and an interquartile range of 6 and 5–9, respectively. Demographic characteristics of the subjects are shown in Table 1.

The mortality rate of the included subjects was 100%, with 110 ± 42 days in the burn intensive care unit after injury. The estimated total energy expenditure (per indirect calorimetry or the Carlson equation, both with an activity factor of 1.4) was 4241 ± 748 kcal/day. To measure the REE, indirect calorimetry was performed

for four of the five subjects. The estimated REE was 96 ± 19% for the Carlson equation. The average actual intake was 3044 ± 1613 kcal (39 ± 20 kcal/kg) and 174 ± 85 g of protein (2.2 ± 1.1 g/kg preinjury wt) per day. The subjects met 72% of the estimated total energy expenditure and were at required protein intake (positive nitrogen balance) for 68% of the weekly measurements (Table 2). An average of 8 ± 6 nitrogen balance studies were performed per subject, with a total of 40 weekly measurements. The percentage of weeks that subjects were in positive nitrogen balance ranged from 50% (n = two measurements) to 83% (n = six measurements).

The nitrogen balance data along with changes in visceral protein and acute-phase reactant levels for all subjects were pooled for the remainder of the analysis. The changes in visceral protein and acute-phase reactant levels are shown in Table 3. Changes in visceral protein levels during downward trends in the CRP level were evaluated along with nitrogen balance and were expressed using linear regression (r^2 values) in Table 4. Nitrogen balance correlated poorly with protein markers, as shown by $r^2 < .10$ (far right column, Table 4). Moderate correlations were observed between prealbumin and CRP (negative), prealbumin and transferrin (positive), and haptoglobin and α-1-antitrypsin (positive) levels.

The level of CRP, haptoglobin, and α-1-antitrypsin showed a decreasing trend in 40–50% of the 40 weekly measurements (Table 5). When any of the acute-phase reactant levels were of a decreasing trend and nitrogen balance was positive, neither prealbumin nor transferrin level increased as hypothesized by

Table 3. Changes in acute phase reactants along with visceral proteins between weekly measurements

Hepatic Protein (mg/dL)	Median	Minimum	Maximum	Interquartile Range
C-reactive protein	0	−20	+21	14
Prealbumin	4.4	−11	+108	5
Transferrin	0	−68	+58	36
Haptoglobin	-8	−236	+226	100
α-1-antitrypsin	-2	−221	+278	81

Table 4. Linear regression r^2 values of data during times that C-reactive protein was decreasing

Hepatic Protein	Δ C-Reactive Protein	Δ Prealbumin	Δ Transferrin	Δ Haptoglobin	Δα-1-Antitrypsin	Nitrogen Balance
Δ C-reactive protein	X	0.34*	0.14	0.02	<0.01	0.04
Δ prealbumin		X	0.41*	<0.01	0.03	<0.01
Δ transferrin			X	0.04	0.02	<0.01
Δ haptoglobin				X	0.11*	0.07
Δ α-1-antitrypsin					X	<0.01
Nitrogen balance						X

*$p < .05$

DISCUSSION

the trending theory (Table 5). When the CRP level was of a decreasing trend and subjects were in positive nitrogen balance, only 50% of incidences of prealbumin showed an increasing trend (Figure 1).

This study examined nutritional status along with changes in both visceral protein acute-phase reactant levels in patients with burn injury. The majority of burn care centers use visceral proteins as markers of nutritional status,[7] even though this has not been studied along with nutritional intake.

Carlson et al[10] evaluated visceral protein levels along with nitrogen balance and found that changes in nitrogen balance were not associated with changes in visceral protein levels, which is similar to the results of this study. We found that changes in visceral protein levels were not related to nitrogen balance, even during times that acute-phase reactants were decreasing. Our results do not support the theory of trending visceral proteins along with acute-phase reactants to determine nutrition status (as proposed by Manelli et al).[9] Our results do agree with Manelli et al's results that prealbumin and CRP have an inverse relationship.

We found the visceral proteins to be poor markers of nutritional status, even in the subjects with positive nitrogen balance. The clinical use of these markers in the burn intensive care unit has not been proven despite several attempts to do so and may provide inaccurate information to clinicians regarding the patient's nutritional status. If visceral protein levels are not increasing,

a decision to increase feeding could be made, which could lead to overfeeding. Blood loss associated with weekly assessment can be prevented, and the resources required to run and analyze these tests can be conserved. Clinical nutritional evaluation should be focused on achieving nitrogen and caloric balance.

Although the underlying mechanisms remain to be explored, this study demonstrated that high levels of protein intake are not associated with increases in visceral protein levels. Visceral proteins can be affected by several factors, such as endogenous synthesis, intravascular consumption or breakdown, extravascular leakage, and plasma volume fluctuations. The lack of increase in visceral protein levels under positive nitrogen balance does not necessarily reflect changes in endogenous production of these proteins. In fact, in patients with 48% ± 4% TBSA burn at 13 days post-burn, we found that albumin synthesis (4.6 ± 0.2 mg/kg/h) was higher than that in the control (2.2 ± 0.2 mg/kg/h), although albumin level (1.1 ± 0.1 g/dL) was much lower than that of the control(3.8 ± 0.1 g/dL).[18] It is possible that extravascular leakage of albumin and other visceral proteins is very high to be compensated by an increased synthesis, which may or may not be affected by nutrition intake.

The experimental design reported here is unique because subjects were able to achieve positive nitrogen balance despite severe burns and because it allowed to compare protein intake to changes in visceral protein and acute-phase reactant levels. As a result, we were able to show that positive nitrogen balance does not promote an increase in visceral

Table 5. Changes in visceral proteins with decreasing acute-phase reactants between weekly measurements and during times of positive nitrogen balance

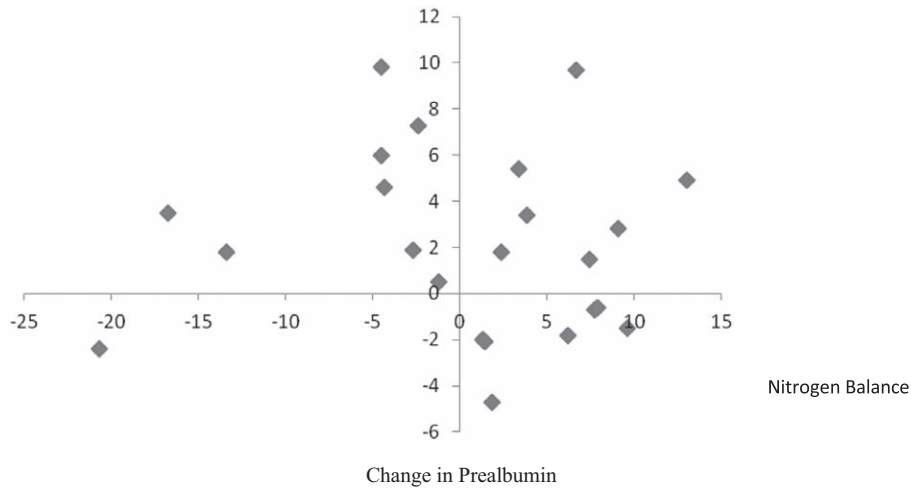| Acute Phase Reactant	% of Measurements with Acute Phase Reactant Downtrending	% of Prealbumin Increase	% of Transferrin Increase
C-reactive protein	43%	50	50
Haptoglobin	40%	13	19
α-1-antitrypsin	50%	16	25

Figure 1. Prealbumin and nitrogen balance values only including those when CRP are decreasing

protein levels in severely burned patients during their time in the burn intensive care unit, even when acute-phase reactants are decreasing.

Another strength of this study was that we were able to collect a total of 40 weeks of acute-phase reactants and visceral protein trends along with nitrogen balance during the time in the burn intensive care unit. The level of illness is evident from the mortality rate as well as the length of stay in the burn intensive care unit. Average time to death for patients with burn injury at our facility is approximately 2 weeks.[19] The subjects in this study survived an extended period of time, with a median and an interquartile range of 6 and 5–9 weeks of measurements. We were able to achieve 72% of the caloric goal and achieve nitrogen balance in 68% of the weekly measurement. This makes our study distinctive, as average intake during critical illness for surgical patients is 33% of caloric goal.[20]

All subjects we examined received high-dose insulin infusions. Insulin has been found to decrease protein catabolism in patients with major burn injury,[11] but its effects on acute-phase reactant and visceral protein metabolism are not known. The insulin infusion may have decreased the urinary nitrogen losses, aiding in positive nitrogen balance; therefore, limiting the conclusions made from the results of this study. In this study, there were examples of subjects in both positive and negative nitrogen balance. Trends in visceral proteins and acute-phase reactants were examined, yet no correlations were found between these proteins and nutritional intake. Only moderate correlations were noted between the proteins themselves.

The limitations of the study include a small but severely burned patient population. Population demographics, the availability of visceral proteins and acute-phase reactants, and the fact that this study is a post-hoc analysis are also limitations. All of the subjects were men, and because of their high degree of injury, none survived.

A prospective study comparing results between treatment arms targeting several different daily caloric goals may uncover an association that is not identified in this study; however, our data does not support that supposition. We measured prealbumin, transferrin, CRP, haptoglobin, and α-1-antitrypsin. It is possible that different biological markers may exhibit better correlation. Other visceral protein and acute-phase reactants will be studied continually, as the use of a protein marker that reflects changes in nutritional status is unquestionable, but further research on nutritional status should focus on outcomes, such as healing time, lean body mass retention, strength, etc.

In summary, we examined the validity of the theory that with a decrease in acute-phase reactant levels, visceral protein levels will increase with adequate protein intake (defined as positive nitrogen balance) and will decrease with inadequate protein intake. We found visceral proteins to be poor markers of nutritional status in this population, even during times that levels of acute-phase proteins are decreasing. This study is unique in that subjects were able to achieve a positive nitrogen balance despite severe burns.

ACKNOWLEDGMENTS

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