ALBUMIN SYNTHESIS IN PROTEIN ENERGY MALNUTRITION

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Abstract
The dietary treatment of protein-energy malnutrition (PEM) has been designed on an empirical basis, with outcomes for successful management including body weight gain and resolution of apathy. We propose using the measurement of protein synthesis as a more objective measure of renourishment. We will therefore randomize a group of malnourished children (weight-for-height Z score < -2.0) to receive either a standard (10% of calories as protein) or increased (15%) amount of dietary protein early in their recovery phase. We will calculate albumin synthesis rates via the flooding dose technique, using ¹³C-leucine and serial measurements of ¹³C-enrichment of albumin. Isotope infusions will be performed on days one and three, following a standard three hour fast. Since albumin synthesis is reduced under the influence of cytokines which mediate the inflammatory response, results will be stratified according to the presence or absence of clinically apparent infections. We hypothesize that the provision of added dietary protein will optimize albumin synthesis rates in PEM as well as attenuate the reduction in albumin synthesis seen in the presence of infections.

1. SCIENTIFIC BACKGROUND

Protein-energy malnutrition (PEM) is one of the most common nutritional deficiency syndromes in the world, affecting approximately 100 million children less than age five in developing countries [1]. It is responsible for up to 50% of childhood mortality in such populations, being closely entwined with the concurrent morbidities of enteric and respiratory infections. Despite extensive scientific study of the multiple metabolic derangements in PEM [2], nutritional repletion of these patients has been designed largely on an empirical basis. In general, after correction of the several acute complications of PEM (dehydration, hypoglycaemia, hypothermia, and sepsis), re-feeding is often done by gradual advancement of a milk-based diet. The rapidity of this process is dictated by gastrointestinal tolerance of the volume and strength of feeds. The ultimate success of nutritional repletion is usually judged by important but imprecise measures of well-being: weight gain, return of appetite, and resolution of apathy/return of playfulness [3].

Current recommendations for repletion reflect this state of affairs: a broad range of calories (100 - 150 kcal/kg/day) and protein (2 - 3 g/kg/day) is suggested, and no rational basis is given for a plan of dietary advancement. Basic questions regarding the proper nutritional management of malnourished patients remain incompletely answered, such as the ideal quantity of calories and protein in their diet, the optimal ratio of calories
to protein, the quality of protein, and the desired amounts of micronutrients (vitamins and minerals). In practice, the adequacy of caloric intake is usually assessed by weight gain, but there is evidence that lean body mass is not always optimized by such diets [4]. Assessment of protein status can be done by nitrogen balance techniques, but this method is both cumbersome and prone to inaccuracies [5]. Another common measurement of protein status is serum albumin, but serum concentrations of proteins depend on rates of synthesis, degradation, and redistribution among body compartments [6]. A more sensitive assessment of protein nutriture would be the measurement of protein synthesis rates.

Since PEM has classically been conceptualized as either hypoalbuminemic malnutrition (kwashiorkor) or normoalbuminemic malnutrition (marasmus), the focus of many studies has been on albumin metabolism. In one of the earliest such studies, Cohen and Hansen [7] injected radioactively labelled albumin and followed decay curves to demonstrate that children with kwashiorkor had markedly reduced albumin synthesis rates (mean 0.84 gram/day). Upon nutritional recovery, these rates rose significantly (mean 2.49 grams/day). Experimentally-induced protein depletion was also shown to reduce albumin synthesis and catabolism rates [8].

James and Hay [9] further defined the response of malnourished children to dietary intake by measuring albumin synthesis and catabolism, again using $^{131}I$. Both malnourished and recovered children were fed 2.0 - 4.8 g/kg/d of protein for 7 - 10 days before being given a low protein diet (0.7 - 1.0 g/kg/d). Albumin synthesis rates during the latter period averaged 101 mg/kg/d in the malnourished group versus 148 mg/kg/d in the recovered group. Of note, when a high protein diet was re-introduced, synthesis rates jumped up quickly (to 288 and 236 mg/kg/d, respectively), while catabolic rates lagged in their response. These data strongly suggest that albumin synthesis rates change rapidly with regard to amino acid availability, whereas changes in albumin catabolism are more slowly made and thus likely regulated by alternative mechanisms.

The advent in the 1960’s of isotopic labelling of amino acid precursors made it possible to directly measure albumin metabolism [10]. Studies in rats using $^{14}C$ to measure albumin synthesis further delineated the metabolic changes of protein depletion. Kirsch, et al. [11] showed a gradual reduction in albumin synthesis rates over 12 days on a protein-free diet, with a dramatic return to normal or supra-normal rates in the first 24 hours of re-introduction of dietary protein. Albumin catabolic rates returned to normal more slowly, over 3 to 6 days. Further studies in protein depleted rats [12, 13, 14] showed that hepatic microsomal albumin content (a measure of synthesis) increased by 50% within 30 minutes of re-feeding, and that as little as 18 hours of fasting lead to a 40% - 50% reduction in synthesis rates.

Unfortunately, several aspects of these early isotopic tests limit their usefulness in human research. Constant rate infusions of the label over 24 hours or longer are needed, and measurement times are also prolonged (4 to 6 hours). In conditions where albumin metabolism may be in flux, such methods may obviously not be optimal. In addition, the use of radioactive isotopes in children is now generally thought to be unethical. Non-radioactive (so-called stable) isotopes have been devised to solve these problems. By injecting a "flooding dose" [15] of $^{13}C$-labelled leucine and measuring subsequent enrichment of plasma albumin, measurements of albumin synthesis rates can be performed.
in 90 minutes. Reports in the literature include data from adult men [16] and neonates but not children with PEM.

By using the technique of stable isotope infusion, we will be able to more clearly delineate dietary protein requirements among recovering malnourished children. By comparing refeeding regimens differing in protein content, we will be able to show how dietary changes affect serum protein synthesis. In conjunction with standard assessments of nutritional adequacy (i.e., weight gain, anthropometric status), evaluation of albumin synthesis rates will allow the design of the optimal recovery regimen. In addition, we will be able to assess the influence of age, nutritional status, and presence of infection as co-factors in determining protein requirements.

2. METHODS

2.1. Site and Patient Population

A prospective study among inpatients at the Instituto de Investigación Nutricional is proposed. The Instituto is an independent nutritional research unit in Lima, Peru which specializes in the care of patients with chronic diarrhoea and malnutrition. Children between the ages of 6 - 24 months with growth failure due to PEM (weight-for-length Z score < - 2.0) will be eligible for study participation. Both males and females will be eligible. Since abnormal extravascular collections or losses of albumin may invalidate the measure of albumin synthesis, children with ascites, severe oedema, or proteinuria will be excluded. Patients with signs of hepatic dysfunction (jaundice or elevation of serum transaminases to more than twice the upper limit of normal) will be ineligible. In addition, any condition in which the proposed study might jeopardize a patient’s health or would interfere with the conduct of the study will be an exclusion criterion. Children with fever, diarrhoea, or vomiting will not be excluded; these are often concomitant findings in PEM and we are interested in defining a range of values for albumin synthesis in these patients.

2.2. Clinical and Biochemical Considerations

Since animal models and prior human experience suggest that albumin synthesis rates can vary with many factors, standardization and measurement of them will occur as follows:

Age

Age will be recorded in days and confirmed by documentation brought by the caretaker.

Nutritional Status

Anthropometric measurements will be used to categorize the extent of malnutrition, using weight-for-height, as well as weight-for-age and length-for-age of NCHS standards. Mid-upper arm circumference and triceps skinfold measurements will also be performed.
**Presence of Infection**

Since even subclinical infections can adversely affect nitrogen balance, albumin synthesis rates may well be depressed by the presence of infections. In addition, many children with PEM suffer from diarrhoea, otitis media, pneumonia, and other infections. Therefore, evidence for the presence or absence of infections will be diligently sought. Admitting laboratory tests will include complete blood count with differential, erythrocyte sedimentation rate, chest x-ray, and cultures of urine, blood, and stool. The admitting history and physical exam and subsequent hospital course will determine whether clinically apparent infections exist, and these diagnoses will be documented. The clinical judgement of the admitting physician will determine the need for parenteral or oral antibiotics. Final results will be stratified according to the presence or absence of infections.

**Growth Rate**

Daily weights will be obtained on all subjects for the duration of their hospitalization. Absolute (g/day) and proportional rates (g/kg/day) of weight gain will be measured. Body weights will be obtained on a digital scale each morning. Lengths will be measured by a recumbent board and sliding foot piece.

**Acute Dietary Intake**

Since rates of protein synthesis vary greatly with recent food intake, a standard three hour fast will precede all isotope infusions. Total volume eaten at each feeding will be recorded and the measurements used to calculate total nutrient intake.

**Dietary Composition**

Current guidelines for re-feeding children at the Instituto call for a rice and milk-based diet, beginning at 75 kcal/kg and advancing as tolerated. We propose randomizing patients between two groups: a standard protein intake (10% of calories) and a high protein intake (15% of calories). A vitamin and mineral supplement will be provided to all subjects.

<table>
<thead>
<tr>
<th>Standard Diet:</th>
<th>Days</th>
<th>Energy (kcal/kg)</th>
<th>Protein (g/kg)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>75</td>
<td>1.9</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>125</td>
<td>3.2</td>
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</tr>
<tr>
<td>3'</td>
<td>125</td>
<td>3.2</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>High Protein Diet:</th>
<th>Days</th>
<th>Energy (kcal/kg)</th>
<th>Protein (g/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>75</td>
<td>2.8</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>125</td>
<td>4.7</td>
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</tr>
<tr>
<td>3'</td>
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<td>4.7</td>
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'first bottle of the day only
Intolerance to dietary advancement characteristically presents itself by high stool volumes. Therefore, if stool output exceeds 100 g/kg/day, dietary intake will not be advanced as outline above. If high stool output leads to dehydration, the patient will be treated with oral or intravenous fluids as thought necessary.

*Serum albumin and osmolarity*

These will be measured on days one and three.

2.3. Data Collection

Determination of albumin synthesis rates will be done via $^{13}$C-labelling of leucine as described by Ballmer et al. [16] on the first and third days of hospitalization. Infusion of the stable isotope will be done via a peripheral vein. If an intravenous line is needed for fluid support or antibiotics, this IV may be used. Alternatively, a butterfly needle can be introduced temporarily. Blood samples (1 mL each) will then be drawn at time 0, 15, 30, 60, and 90 minutes after infusion. Serum will be spun down, and samples stored for later analysis via mass spectrometry at MIT.

2.4. Measurements and Calculations

Isolation of $^{13}$C-enriched albumin for mass spectrometry will be accomplished as follows: After the serum is thawed, albumin will be extracted by differential solubility in absolute ethanol from trichloroacetic acid-precipitated protein [17]. The purity of the protein preparation will be confirmed by high-resolution polyacrylamide electrophoresis. The precipitates will be hydrolyzed in 6 M HCl for 24 hours and the hydrolysates derivitized with N-Methyl-N-(tert-butyl)dimethylsilyl)-trifluoroacetamide chromatography. The leucine will be decarboxylated with ninhydrin and the $^{13}$C enrichment of the released $^{13}$CO$_2$ will be measured by gas isotope ratio mass spectrometry.

The rate of incorporation of labelled leucine into albumin can be expressed by the following steady state equation [18]:

$$\frac{dP}{dt} = k_{syn}Fdt - (k_s + k_e)P dt$$

(1)

where $P$ represents the isotopic enrichment of the product pool (albumin) and $F$ the enrichment of free amino acid in the precursor pool. $k_s$ and $k_e$ are first order rate constants signifying the rate of albumin synthesis and transcapillary escape, respectively, both being expressed as fractions of the intravascular pool. However, calculations from Ballmer et al. [16] have shown that transcapillary escape may be safely ignored, given the brief time course of the infusion and measurements. Thus:

$$\frac{dP}{dt} = k_{syn}Fdt$$

(2)
Integrating and rearranging, we have

\[ k_s = \frac{P_2 - P_1}{\int Fdt} \] (3)

where the denominator is equivalent to A, the area under the precursor enrichment time curve between appropriate times. The time points \( t_1 \) and \( t_2 \) for measuring the increase in albumin enrichment are chosen where incorporation of the label is close to linear (fig. 1).

The final form of the equation used to calculate albumin synthesis is therefore:

\[ k_s = FSR = \frac{(P_2 - P_1)}{A} \times 100\% \] (4)

where FSR is the fractional rate of albumin synthesis (expressed as percent of whole body pool per day).

3. RESULTS

Enrollment in the protocol began in September 1993 and is ongoing. Serum samples are being collected and stored for later analysis via mass spectrometry.

4. PLANS FOR FUTURE WORK

The technique described above may easily be adapted to measure the synthesis rates of hepatic export proteins other than albumin. Other proteins, such as the acute phase reactants alpha-1-antitrypsin and C-reactive protein, could be measured in a similar fashion. In addition, the synthesis of retinol binding protein, transferrin, and other carrier proteins could be assessed. Whether and how micronutrient deficiencies and/or supplementation bear upon the synthesis of their respective carrier proteins would clearly be of interest.

In addition, further studies of recovery diets in PEM may be made, using protein synthesis rates as a standard by which to measure these diets. Our current work compares protein quantity, but protein quality could also be assessed by these methods. More precise estimates of amino acid requirements in the setting of recovery from PEM may also be made in this manner [19]. Finally, the quantification of protein synthesis rates in the presence of infections holds the promise of improving our understanding of how nutritional support may modify the body's response to infections [20].
REFERENCES


