THE LIMITS OF ADAPTATION OF FUNCTIONAL PROTEIN SYNTHESIS TO SEVERE UNDERNUTRITION

F. JAHOOR[1], S. BHATTIPROLU[1], P. REEDS[1], T. FORRESTER[2], M. BOYNE[2]

[1]Children's Nutrition Research Centre, Baylor College of Medicine, Houston, Texas, [2]Tropical Metabolism Research Unit, University of the West Indies, Mona, Kingston-7, Jamaica, WI.

Abstract

Our goal is to determine how the stress of infections alters the adaptation to reduced food intake in children. We think that an important element is the need for hepatic synthesis of rapidly turning over acute-phase proteins, a critical factor in overall maintenance of host defenses. When the child's prior intake has been adequate, even though growth may temporarily cease, the presence of adequate amino acid stores in tissues allows the hepatic response to stress to be maintained at the same time as an adequate rate of synthesis of nutrient transport proteins. However, when the immune system is activated in a child whose nutrition is already suboptimal, the ability of the liver to synthesize nutrient transport proteins is compromised thereby further impeding nutrient utilization. We will use stable isotope tracer methodology to determine the effects of severe protein energy malnutrition, with and without infection, on the rates of synthesis of nutrient transport proteins and acute-phase proteins in undernourished children at three time points during treatment; in the early resuscitative period, after appetite has returned, and at the end of the catch-up growth phase when normal growth has resumed.

1. SCIENTIFIC BACKGROUND AND SCOPE

Severe malnutrition is a significant health care problem in developing countries, because it is a major contributor to the high mortality and morbidity of early childhood [1-4]. When food intake is reduced, the body adapts by sacrificing redundant capacities of organ and functional systems to bring nutrient demand in line with nutrient supply. Infection, however, disturbs this reductive adaptation by increasing demand, thereby upsetting a fragile equilibrium, and precipitating the classical syndromes of severe Protein Energy Malnutrition (PEM). Delineation of the interaction between the processes of reductive adaptation and the response to the stress of infection is necessary in order to quantify the impact on nutrient needs. Such information can be used in selecting strategies to prevent severe PEM in marginally nourished populations who are "at risk" to recurrent infections.

Both inadequate nutrition and the stress associated with even minor infections have a deleterious effect on normal growth. The stress of infections alters the nutrient requirements of the child in ways which have not been quantified. The process leading to severe childhood PEM often involves repeated episodes of infections, with very little recovery in the intervening periods [5]. The cumulative demand for more nutrients can exceed the body's ability to supply from stores, especially in the presence of reduced food

intake. This state of inequality marks the point at which loss of adaptation occurs. While the importance of the interaction between nutrition and the immune system is well recognized, the mechanisms whereby they interact are poorly understood. Kwashiorkor is a syndrome at the upper extreme of severity along the spectrum of childhood PEM, in which poor nutritional status and infection interact [1,3,5]. For this reason, we propose to use it as a model to investigate the limits of adaptation to reduced nutrient intakes which might be imposed by the demands of the stress response to infection.

With repeated episodes of stress, the increase in obligatory nutrient needs compromises the ability of the child to adapt successfully to reduced food intake. This extreme condition is characterized by suboptimal liver function [6], and a substantial reduction in the levels of plasma proteins that are responsible for the transport of absorbed nutrients to sites of utilization, thereby further impeding nutrient utilization [5,7]. Although the underlying mechanism(s) of the impact of stress on nutrient requirements is poorly characterized we are proposing that an important element is the need for the hepatic synthesis of rapidly turning over acute-phase proteins, a critical factor in overall maintenance of host defenses. When the child's prior intake has been adequate, even though growth may temporarily cease, the presence of adequate amino acid stores in tissues allows the hepatic response to stress to be maintained at the same time as an adequate rate of plasma protein synthesis. However, when the immune system is activated in a child whose nutrition is already suboptimal, the ability of the liver to synthesize nutrient transport proteins is compromised to the ultimate detriment of the individual. In this project we propose to test three hypotheses:

(1) That PEM leads to a reduction in the rate of synthesis of nutrient transport proteins.

(2) That the synthesis of these proteins, although lower, is adequate for the maintenance of a successful metabolic adaptation unless the child is also subjected to the stress of infection(s).

(3) That the extent to which synthesis of transport proteins is impaired, is related to the degree of increase in rate of synthesis of acute-phase proteins.

Specific experiments will be performed in undernourished children before, during and after recovery from severe PEM:

(1) To determine that the plasma levels of nutrient transport proteins, albumin, retinol binding protein, transtyretin, transferrin, VLDL-apoB-100 and HDL-apoA-1 are decreased in kwashiorkor because rates of synthesis are suppressed, whereas in marasmics, the plasma levels are within the normal range because rates of synthesis are maintained constant.

(2) To determine that the normal relationship between the rates of synthesis of the nutrient transport proteins, albumin, retinol binding protein, transtyretin, VLDL-apoB-100 and HDL-apoA-1 and the acute-phase proteins, fibrinogen, α1-antitrypsin, α1-acid glycoprotein, and haptoglobin is reversed in kwashiorkor.

Experiment (1) will also serve to validate a new technique to measure rate of synthesis of hepatic synthesized plasma proteins using U-^{13}C-glucose as tracer.

2. METHODS

2.1. Study description

During the next year we plan to study two groups comprising 6 marasmic and 6 kwashiorkor patients at 3 time points during hospitalization.

The first study (study 1) will be performed during the acute resuscitative period, post-admission day 1 or 2, when the patient is evaluated as being clinically stable and starts tolerating a maintenance diet of 0.75 g protein and 100 Kcal/kg/d.

The second study (study 2) will be performed at the time when appetite returns, usually 3 to 6 days post-admission, the patient will still be on the maintenance diet. He/she will have lost oedema, (may be free of infections), and have a stable body weight. This study will enable us to determine underlying differences between the two syndromes when homeostasis has returned. That is, using the return of appetite as an indicator of physiological wellness. If appetite does not return by 7 days, the patient will be studied anyway on day 7, and again when appetite has returned.

The third study (study 3) will be done at the end of the period of accelerated growth when the patient's growth rate returns to normal, ~3-4 g/kg/day, and body weight for height is > 90 % of the reference median. At this point, the patient will be classified as fully recovered.

For all studies, the patient will be on the same diet of 0.75 g protein and 100 Kcal/kg/d. In study 2 and 3 the patient will be placed on this diet for 3 days. The experiment will be performed on the last day.

Although it may not be feasible to perform all three studies in the same patient, attempts will be made to conduct at least the first two.

2.2. Isotope Infusion Protocol

In the first series of studies the stable isotopes U-^{13}C-glucose and $^{2}H_3$-leucine will be used to measure rate of synthesis of hepatic secreted nutrient transport and acute phase proteins. The rationale is explained in the project statement.

The isotope will be infused for eight hours during which time the patient will receive equal hourly feeds or continuous feeding through a nasogastric tube (Fig.1). One 22-gauge, 2.5 cm long plastic catheter (Angiocath, Deseret Medical Inc.) will be placed in a peripheral vein and used for blood sampling. The sampling catheter will be kept patent with a saline drip. After a 2 ml baseline blood sample is drawn, a primed-constant infusion of the U-^{13}C-glucose (prime = 160 μmol/kg; infusion rate = 120 μmol/kg/h) and $^{2}H_3$-leucine (prime = 45 μmol/kg; infusion rate = 45 μmol/kg/h) will be started and maintained for eight hours via the nasogastric tube.

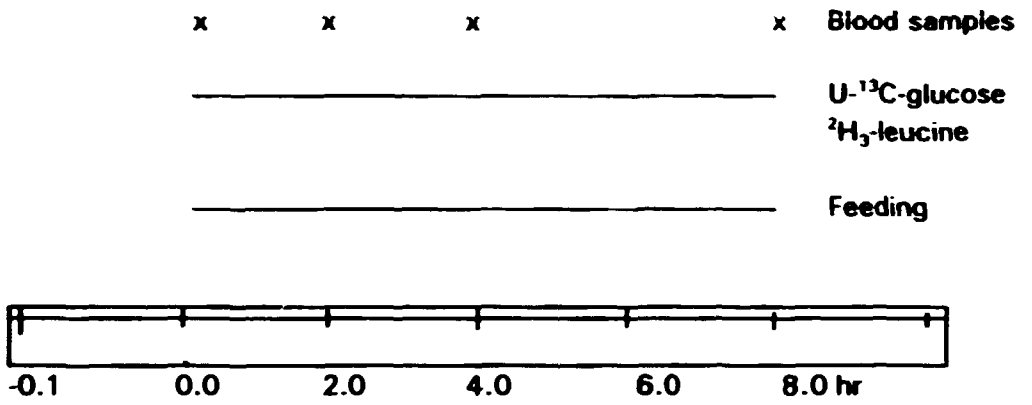

Figure 1. Diagram of isotope infusion protocol.

Three more blood samples will be drawn at 2.0, 4.0, and 8.0 hours after the infusion starts, immediately centrifuged at 0-4 °C, plasma separated and stored in plastic tubes at -40°C for later analysis.

2.3. Sample Analysis

2.3.1. Sample Collection

Blood will be drawn in pre-chilled tubes containing Na_2EDTA, and a cocktail of sodium azide, merthiolate, and soybean trypsin inhibitor, in protocols 3 and 4. The samples will be immediately centrifuged at 4 °C, the plasma removed and stored on ice until the end of the infusion. The samples will be stored at -70 °C for later analysis.

2.3.2. Plasma Proteins Isolation and concentration

The volume of each plasma sample available for analysis will be ~ 1 ml. First, the concentration of the proteins will be determined by radial immunodiffusion techniques only on the background sample . This will require a total of 100 μl of plasma. The rest of the plasma will then be used to isolate the proteins. First, fibrinogen will be isolated as fibrin from 0.2 ml of the plasma by thrombin precipitation. The remaining serum will be re-combined with the rest of plasma. Next, albumin will be extracted with acidified ethanol from 5μl of the remaining plasma. The rest of the plasma will be divided into two aliquots of 0.6 ml and 0.4 (or 0.3) ml, and used to isolate VLDL-ApoB-100, HDL-ApoA-1, α1-antitrypsin, α1-acid glycoprotein, transferrin, prealbumin and retinol binding protein. VLDL will be removed from 0.6 ml of serum by ultracentrifugation at a density of 1.006 g/ml, and apoB-100 will be extracted with isopropanol as previously described [8]. The density will be adjusted to 1.21 g/ml, ultracentrifuged, and HDL-ApoA-1 isolated by SDS-PAGE [9]. The supernatant remaining after ultracentrifugation, will be used to extract plasma-free alanine and α-KICA. The 0.4 ml aliquot of plasma will be used to isolate the remaining proteins by immunoprecipitation followed by SDS-PAGE. After staining with Coomasie Blue, the band corresponding to the particular protein standard will be cut out, washed several times, and lyophilized for acid hydrolysis.

2.3.3. Isotopic enrichment of leucine, alanine, and alpha keto-isocaproic acid

The dried protein precipitates and lyophilized protein-gel bands will be hydrolysed in 6 mol/L HCl at 110 °C for 24 hours, and the released amino acids will be purified by cation exchange chromatography. The isotopic enrichment (tracer/tracee ratio) of leucine and alanine will be determined by negative chemical ionization gas chromatography-mass spectrometry as previously described [10]. The amino acid will be converted to the n-propyl ester, heptaflourobutyramide derivative, and the isotope ratio determined by monitoring ions at m/e 307 to 310, and 349 to 352. The isotopic enrichment (tracer/tracee ratio) of plasma α-KICA will be determined by negative chemical ionization gas chromatography-mass spectrometry on the pentafluorobenzyl derivative by monitoring ions at m/e 129 to 132.

3. CALCULATIONS AND THEORETICAL CONSIDERATIONS

3.1. Plasma protein kinetics

A new approach employing the infusion of U-^{13}C-glucose will be used with a more conventional approach to measure the rates of synthesis of the different hepatic-synthesized proteins. The U-^{13}C-glucose will be used to indirectly produce labelled alanine from labelled pyruvate via the alanine aminotransferase reaction. Rate of synthesis of a plasma protein will then be obtained from the rate of incorporation of labelled alanine, using the plasma alanine steady-state enrichment as an estimate of the actual enrichment of the intra-hepatic alanine pool from which the protein was synthesized. The rationale is based on previous findings in this laboratory that the plateau enrichment of hepatic synthesized VLDL-apoB-100 alanine and plasma alanine (and pyruvate) were identical [11], suggesting that the alanine used to synthesize export proteins in the liver is largely derived from plasma pyruvate. The standard precursor-product equation will be used to calculate fractional protein synthesis rate (%/hour):

$$FSR = \frac{PEt_1 - PEt_0}{Epl} \times \frac{100}{t_1 - t_0}$$

where $PEt_1 - PEt_0$ is the increase in enrichment of protein-bound alanine or leucine over the period of tracer infusion $t_1 - t_0$, and *Epl* is the plateau enrichment of plasma alanine or ApoB-100- leucine.

$$\text{Absolute I.V R.S} = \text{Total I.V. Protein Mass} \times \text{FSR}$$

where the intravascular protein mass is calculated by multiplying the plasma volume (estimated as 45 ml/kg) by the plasma concentration of the particular protein.

Although three isotopomers (1-^{13}C-, 1,2-^{13}C-, 1,2,3-^{13}C-) of labelled alanine (produced via pyruvate) are produced from U-^{13}C-glucose, we will use only the uniformly labelled species as tracer, because the steady-state enrichment of the m + 3 isotopomer is the least likely to be affected by recycling of isotope through glucose. First, the likelihood of two uniformly labelled (phosphoenol) pyruvate molecules coming together to form a new molecule of U-^{13}C-glucose will be negligible, because most pyruvate produced

will be unlabelled, having been produced from unlabelled glucose [12]. This is so because the infused U-^{13}C-glucose will make only a minor contribution to the total amount of glucose produced. Second, only ~ 14% of the labelled glucose at most will be recycled as 3-carbon intermediates back to glucose [12].

4. STATISTICAL ANALYSIS

All data will be analyzed by standard statistical methods. When the same subjects are studied more than once, the paired t-test will be used to compare values obtained at different times during treatment. When different patients are studied, comparisons will be made using analysis of variance All statistical analyses will be done in close collaboration with a statistician.

5. PRELIMINARY RESULTS

Infusions were performed in a kwashiorkor child on post-admission day 3, and in a child during the catch-up growth phase of recovery from marasmic-kwashiorkor. Both children were fed a maintenance diet of 0.75 g protein and 100 Kcal/kg/d by constant intra-gastric infusion during the study. Preliminary results suggest that the fractional rates of synthesis of most of the transport proteins are markedly reduced in the malnourished child when compared to the recovering child (Table I). The exceptions were HDL-apoA-1 and transferrin. On the other hand FRS of the positive acute-phase proteins are higher in the malnourished child.

6. PLANS FOR FUTURE WORK

Work on this project has just started in August of this year. Our plans are to complete the studies described in the present proposal over the next year.

REFERENCES

[1] MCLAREN, D.S., A fresh look at protein-calorie malnutrition, Lancet ii (1966) 485-488.

[2] ALLEYNE, G.A.O., HAY, R.W., PICOU, D.I., STANFIELD, J.P , WHITEHEAD, R.G., In: Protein-energy malnutrition. Edward Arnold, London, (1977) 13.

[3] JACKSON, A.A., Severe undernutrition in Jamaica, Acta. Paediatr. Scand. m. suppl. **323** (1986) 43-51.

[4] WATERLOW, J.C., In: Protein Energy Malnutrition. Edward Arnold, div. of Hodder Stoughton Ltd., London, 1992.

[5] FROOD, J.D.L., WHITEHEAD, R.G., COWARD, W.A., Relationship between pattern of infection and development of hypoalbuminemia and hypo-b-lipoproteinemia in rural Uganda children, Lancet ii (1971) 1047-1049.

[6] McLEAN, A.E.M., Hepatic failure in malnutrition, Lancet ii (1962) 1292-1294.

[7] DHANSAY, M.A., BENADÉ A.J.S., DONALD, P.R., Plasma lecithin-cholesterol acyltransferase activity and plasma lipoprotein composition and concentrations in kwashiorkor, Am. J. Clin. Nutr. **53** (1991) 512-519.

[8] EGUSA, G., BRADY, D.W., GRUNDY, S.M., HOWARD, B.V., Isopropanol precipitatic.. method for the determination of apolipoprotein B specific activity and plasma concentrations during metabolic studies of very low density lipoprotein and low density lipoprotein apolipoprotein, B. J. Lipid Res. **24** (1983).

[9] PATTERSON, B.W., HACHEY, D.I., COOK, G.L., AMANN, J.M., KLEIN, P.D., Incorporation of a stable isotopically labelled amino acid into multiple human apolipoproteins, J. Lipid Res. **32** 1063-(1991) 1072.

[10] REEDS, P.J., HACHEY, D.L., PATTERSON, B.W., MOTIL, K.J., KLEIN, P.D., VLDL apolipoprotein B-100, a potential indicator of the isotopic labelling of the hepatic protein synthetic precursor pool in humans: Studies with multiple stable isotopically labelled amino acids, J. Nutr. **122** (1992) 457-466.

[11] JAHOOR, F., BURRIN, D.G., REEDS, P.J., FRAZER, M., Measurement of plasma protein synthesis rate in the infant pig: An investigation of alterative tracer approaches, Am. J. Physiol., Submitted, 1993.

[12] TSERNG, K.Y., KALHAN, S., Estimation of glucose carbon recycling and glucose turnover with [U-^{13}C]glucose, Am. J. Physiol. **245** (1983) E476-E482.

TABLE I. FRACTIONAL SYNTHESIS RATE (%/H) OF ACUTE PHASE AND NUTRIENT TRANSPORT PROTEINS IN A KWASHIORKOR AND A RECOVERING CHILD

Proteins	Recovering	Kwashiorkor
Transport	FSR (%/h)	
Albumin	0.61	0.52
RBP	4.42	1.85
Prealbumin	1.82	1.0
VLDL-apoB-100	21.0	14.5
HDL-apoA-1	1.75	2.0
Transferrin	0.86	1.07
Acute Phase		
Fibrinogen	1.56	2.0
Haptoglobin	1.98	4.46
α1-acid Glycoprotein	1.25	2.04
α1-Antitrypsin	--	--