The Metabolic Effects of Infection on Nutritional Status

Carla R. Fjeld

Fjeld and Associates, Bethesda, Maryland, USA

WHY FOCUS ON AMINO ACIDS AND FUNCTIONAL PROTEINS?

Stunted growth in many children from developing countries is caused by the synergistic effects of infection or inflammation and malnutrition. As has been shown many times over, children’s growth can be impeded through the effects of infection on intake, metabolism, and partitioning of nutrients. The impact of infectious disease on nutritional status is so profound that infections are thought to be responsible for as much of the malnutrition in children in developing countries as the lack of suitable food (1–4). Moreover, the cumulative effects on nutritional status of chronic or multiple infections without full recovery in between probably outweigh those of single acute episodes (1).

Most of our current understanding of the synergy between infection and malnutrition was derived from studies done in patients with severe infection. However, differences in the pathogenicity of the infectious agent or the immunocompetence of the host determine whether an infectious challenge results in clinical disease. Observations made in studies of animals, and others, although fewer, in children, suggest that even those challenges that do not result in clinical disease have potentially negative effects on nutritional status and, thus, give rise to the hypothesis that chronic subclinical or unapparent infections can also compromise nutritional status, net anabolism, and growth (5).

Severe infections trigger a host of responses which, in turn, alter nutrient intake, most major metabolic pathways, and the relations between many clinical and functional assessments of nutritional status and dietary intake. The metabolic effects of infection are mediated through cytokine activation and the ensuing amplification of
host defense mechanisms. These factors favor the partitioning and redistribution of dietary and endogenous nutrients away from the maintenance of host nutritional status (6), body composition, and growth and toward support of the immune response and the increase in the hepatic synthesis of some proteins (positive acute phase reactants) and involve most major metabolic pathways. It is now more than 20 years since Beisel developed a generalized model to portray the relationship between the sequential phases of an acute infection and the major metabolic events just listed (7).

Are these metabolic events also altered during immune stimulation that is not manifested as a clinical infection? Evidence suggesting that they may be is to be found in the fact that alterations in body composition and nitrogen balance are initiated during the incubation (presymptomatic) phase, and that catabolic losses persist even when clinical signs of infection have abated and child’s appetite has been restored (1,8,9). Studies in elderly persons, even those without clinically detectable infection or inflammation, have also shown that chronic immune stimulation contributes to a deterioration in lean body mass, a process referred to as sarcopenia in the elderly (10). Such observations provide further support for the hypothesis that chronic immune stimulation caused by subclinical infection is a significant environmental cause of reduced lean body cell mass in undernourished, or inappropriately nourished, populations.

Further evidence of the metabolic effects of subclinical infection comes from studies of micronutrient nutrition. Micronutrient deficiencies that persist despite micronutrient supplementation and which are accompanied by low levels of circulating nutrient transport proteins in children with raised C-reactive protein but no clinical signs of infection, suggest that low levels of transport proteins (e.g. albumin or transferrin) lead to micronutrient diversion and sequestration in less accessible body pools (6,11), and that these alterations occur as a secondary effect of infection.

**GOAL OF THE REVIEW**

There are three general modes of action by which infection inhibits growth:

- Reduced exogenous supply of substrates through anorexia and malabsorption
- Nutrient-endocrine interaction: decreased production of insulinlike growth factor 1 (IGF-1) caused by increased interleukin (IL)-6 production
- Resetting metabolic priorities and competition for substrates: increased demand for amino acid substrates for acute phase protein synthesis leading to catabolic losses, inhibition of anabolic processes, and diversion of amino acid substrates for nutrient transport

The main purposes of this chapter is to discuss the nutrient-endocrine and substrate competition mechanisms, as food intake will be discussed in other chapters in this book. Within this framework, I will attempt to put into perspective the alterations in metabolic priorities that involve the redistribution of dietary and endogenous amino acids from growth and maintenance of body composition toward support of the immune response, including synthesis of the acute phase proteins, and to speculate how
amino acid metabolism can negatively affect nutrient status, even in subclinical infection. Although infection alters the major metabolic pathways involving most nutrients, focus is mainly on amino acids because of the multitude of nutritional roles that they play; for example, as substrates for both the positive nutrient transport and export or transport proteins (referred to here as negative acute phase proteins), and in anabolism and growth. Moreover, stable isotope methods are available for tracing specific amino acids and specific proteins that have had only limited application in children in developing countries but which would be highly useful in conducting the studies needed to increase our understanding of the relationship between infection, amino acid metabolism, micronutrient status, and growth, and also to improve our capacity to intervene.

MECHANISMS OF EFFECTS OF CYTOKINES ON MAINTENANCE OF BODY CELL MASS AND GROWTH

Chronic inflammation or recurrent infection can lead, within hours, to increased production of IL-6 and other cytokines (12), which have both systemically and locally produced effects. The cytokines that have the most documented effects on metabolism and nutrition are tumor necrosis factor (TNF), IL-1, and IL-6, partly because they were discovered earlier than the other cytokines. The acute phase response is associated with species-specific increases and decreases in the rates of secretory protein synthesis and is controlled at least in part at the transcriptional level. It involves upregulation in the synthesis and mobilization of substrates for the positive acute phase proteins (e.g., C-reactive protein, serum amyloid A, \(\alpha_1\)-acid glycoprotein, haptoglobin, fibrinogen) and decreased plasma concentrations of transport proteins (e.g., albumin, transferrin, and retinol binding protein) (13). The metabolic effects are both direct, mediated by binding of cytokines to cell receptors in the responding tissue, and indirect, elicited by stimulation of the release of cytokines, glucocorticoids, and prostaglandins, and decreased production of IGF-1.

WHAT ELEMENTS OF EXPERIMENTAL DESIGN ARE RATE-LIMITING IN DETERMINING THE NUTRITIONAL EFFECTS OF CYTOKINES?

Before discussing the evidence for the various mechanisms by which infection or inflammation affect growth, I will comment on the difficulties in determining the effects of cytokines.

1. Cytokine concentrations in the plasma do not represent their rates of synthesis or predict their effects. This is because cytokines are synthesized both locally and systemically but with different time courses; they are present in very low concentrations in blood, turn over very rapidly, and their modes of action are affected by various inhibitory mechanisms. Therefore, the impact on nutritional outcomes from circulating cytokine levels is hard to establish.
2. Stimuli for the production of early response cytokines (e.g., IL-1 and TNF) are varied and include the bacterial lipopolysaccharide of gram-negative organisms, peptidoglycan of gram-positive organisms, antigen-antibody complexes, and viral interactions with host cells. Within this complex set of stimuli, some cytokines act to induce other cytokines and some act together to cause stereotypical host responses, which are well described in an excellent review of the interactions between nutrition and infection by Keusch (14).

3. Cytokines directly and indirectly mediate a wide range of host responses which, in turn, affect or confound their nutritional significance.

4. Most of the human studies of the effects of infection on nutritional status were done during severe infections. Thus, studies in animal models involving both febrile and afebrile responses, in which any of the cytokines, crude or recombinant, were infused at either high or low dose, provide some understanding of the mechanisms whereby infection perturbs growth and body mass, but do not speak directly to the issue of chronic, and particularly chronic subclinical, infection. The latter is especially relevant, given that subclinical infection is widespread among young children in developing countries. For example, 35% (6) to 50% (15) of the children in field studies in Guatemala and Ghana, respectively, were found to have subclinical infections. Furthermore, their poor nutritional status seemed to result from metabolic alterations or rearrangements during those infections more than from inadequate intake of micronutrients.

5. Because subclinical infections are not easily detectable but may, in fact, be chronic, a fourth methodologic issue is that much of the evidence that subclinical infection is a cause of malnutrition is anecdotal. Thus, another complication of the study of the nutritional effects of cytokines is that malnutrition may blunt the acute phase response, so it is less vigorous in undernourished than in well-nourished individuals. This point is addressed in other chapters in this book.

6. How nutritional status and the nutritional outcomes are defined and measured are further challenges to clarifying the relationship between infection and nutritional status.

**CYTOKINE-INDUCED REARRANGEMENTS IN AMINO ACID METABOLISM**

**Nutrient-Endocrine Interactions**

The next step is to try to pinpoint the mechanisms of cytokine actions on growth. An endocrine-nutrient growth inhibitory role of IL-6 was discussed by De Benedetti et al. (16), who studied children with systemic juvenile rheumatoid arthritis, a chronic inflammatory disorder characterized by raised circulating levels of IL-6. In patients with this disease, IL-6 is known to be markedly increased. In the first part of that particular study, De Benedetti et al. worked with colonies of transgenic mice carrying a neurospecific enolase promoter that drives the expression of IL-6 cDNA, leading to overexpression of IL-6. The control group was a colony of wild-type mice. The pri-
mary measurements made were of IGF-1, IL-6, TNF, food intake, and weight gain. Food intake (g/g body weight) did not differ between the two mouse types. Nevertheless, the wild-type mice gained significantly (p < 0.05) more body weight between days 0 and 21 and between days 29 and 35 of the 35-day study, suggesting a growth inhibitory effect in the IL-6 transgenic mice, despite the fact that feed intake per gram of body weight did not differ between the two groups.

The next experiment was to inject a monoclonal antibody (15-A7) to neutralize the IL-6 receptor and enable further study of the mechanism of the growth suppression in the mice. The 30% to 50% reduction in body weight that the transgenic mice showed without the antibody was partially reversed by the antibody. The attribution of the effect specifically to IL-6 was strengthened by the reproduction of the effects in the wild-type mice treated with IL-6. The hypothesis that the decreased growth rate reflected a behavioral disorder leading to a decrease in food intake was ruled out by the observation of equal food intake and conversion per gram of body weight. The hypothesis that the overexpression of IL-6 had an effect on the function of the pituitary was ruled out by the observation that the distribution of the cells that produce growth hormone and thyroid-stimulating hormone, and the circulating concentrations of the two hormones, were the same in the transgenic as in the wild-type littermates.

In further studies on children with systemic juvenile rheumatoid arthritis, De Benedetti et al. reported a consistently negative correlation between serum IL-6 and plasma IGF-1 Z-scores (p = 0.004). Together, these findings strongly suggest that the IL-6-mediated decrease in IGF-1 production is a major mechanism whereby chronic inflammation limits growth.

Resetting Metabolic Priorities and Competition for Substrates

The conclusions drawn from the types of endocrine-nutrient studies just discussed do not explain the profound catabolic responses that occur with severe infections (1), of which the classical indices are increased urinary nitrogen excretion, depletion of muscle protein, nutrient wastage, and peripheral wasting (4). The description and understanding of infection-induced muscle wasting has evolved over at least the last 100 years. In 1910, a German case was cited in which the nitrogen equivalent of 2.5 kg of muscle was lost in 8 days of fever (17). By the end of the 19th Century, the nitrogen equivalent of muscle lost during a febrile episode had already been calculated, and Voit had made the observation (in dogs) that carbohydrate can reduce protein catabolic degradation during induced septic fever (18).

Reorganization of amino acid metabolism occurs to support the hepatic synthesis of acute phase proteins and the synthesis and secretion of factors involved in host defense (12). As hypothesized by Powanda and Beisel in 1982 (19), these host defense mechanisms are dependent on the ability of the host tissue or cells to provide amino acid and other substrates in sufficient quantity for the formation of the positive acute phase proteins, and deficiencies of amino acids can depress the synthesis of the proteins that contribute to host defense.
Such deficiencies can occur through reduced influx of protein and energy substrates; through anorexia or malabsorption, or both; through increased metabolic rates from hyperthermia or fever; or by the transcriptional activation and inhibition of hepatic genes involved in the synthesis of the positive and negative acute phase proteins (14). Some of the nutritional significance of limitations on the influx of nutrients, through anorexia and malabsorption, is discussed by others in this volume. However, the fact that catabolic and oxidative losses of endogenous nitrogen exceed those resulting from fasting alone and persist even when patients become afebrile and asymptomatic, resume an adequate dietary intake, and are no longer malabsorbing, speaks to the importance of the other two main types of mechanism listed at the beginning of this review, which may lead to the depletion of body nitrogen and amino acid substrates.

Scrimshaw et al. hypothesized in 1957 (20) and again in a classic monograph (21) that “the internal diversion of nutrients for the synthesis of compounds involved in the response to infection contributes importantly to the depletion of body stores.” The fact that the cytokine network modulates protein synthesis and breakdown enzymes and that it inhibits the regulatory actions of anabolic hormones on protein synthesis (22) suggests a basis for the competition for amino acid and other substrates, particularly under the stress of dietary insufficiency.

In 1976, Bostian et al. reported the changes in serum concentrations of certain proteins during typhoid fever infection, and speculated that meeting the demand for increased hepatic output of positive acute phase proteins altered protein balance (23). Other early evidence indicated that the acceleration of the synthesis of acute phase proteins altered protein balance by altering the demand for specific amino acids.

Experiments based on infusion of crude, and later recombinant, cytokines have shown induction by cytokines of muscle proteolysis in both animals and humans. Some of those have been done using skeletal muscle isolated from either animals or humans, with or without the addition of inhibitors or stress hormones; others have been done in vivo in animals and humans, again with or without other stress hormones. Some have used bolus or continuous high doses of cytokines to simulate sepsis, whereas others have used bolus or continuous lower levels of the cytokines and hormones. However, very few have looked at the combined effects of marginal malnutrition and chronic immunostimulation, such as may occur among children living in crowded, unhygienic environments, so some initial information can be gleaned from studies on the metabolic effects of sepsis.

**CYTOKINE-MEDIATED SYNTHESIS AND BREAKDOWN OF PROTEIN**

One of the earliest studies on the metabolic effects of sepsis was published by Clowes et al. (24), who conducted a series of experiments to elucidate the relations between muscle protein synthesis and breakdown in sepsis. The first step was to determine how the rates of protein synthesis and degradation in muscle tissue from septic patients differ from the rates in metabolically normal people. To do this, they obtained specimens of the rectus abdominus muscles from septic and nonseptic surgical pa-
tients. For comparison of the human with an animal model they also obtained rat muscle tissue: they first starved the rats for 3 days, then induced peritonitis by cecal ligation, after which the rats were killed and the soleus muscle of each hind leg extracted; one group of control animals was studied to assess the effect of 3-day starvation alone. Protein breakdown was measured *in vitro* in both human and rat muscle from the rate of tyrosine appearance in the incubating medium (to which no measurable contribution from free intracellular tyrosine was seen). Protein synthesis was measured from the intracellular incorporation of UL\(^{14}\)-C-tyrosine in the human and rat studies. Rates of *in vitro* protein breakdown were compared with *in vivo* rates by measuring tyrosine release from leg muscles obtained from both the septic and aseptic humans. Finally, to understand the proteolysis-inducing influence of factors in the plasma itself, plasma from patients with or without sepsis was added to the incubating medium, which contained either muscle tissue from aseptic patients or aseptic rats. The investigators concluded that sepsis induced a 212% increase in the rate of protein breakdown and a modest increase in protein synthesis in human muscles. In rats, a modest acceleration in synthesis was accompanied by a 190% increase in the rate of degradation. The rats that were only starved but not infected showed an increase in degradation of 24%, so that the calculated rate of protein degradation that could be attributed to infection alone was approximately 164% of the control value. Incubation of normal human muscle with plasma from septic patients caused a small increase in synthesis and a 192% increase in protein degradation, a finding that contributed to the isolation and identification of cachectin, as TNF was first called.

At least in sepsis, TNF has been found to stimulate muscle protein catabolism and the net release of amino acids from peripheral muscle (7,14,25–31) and may involve the release of adrenocorticotropin and cortisol (32,33) to stimulate muscle catabolism. In experiments designed to disaggregate the effects of these activators, Raina and Jeejeebhoy (33) gave 100 g TNF/kg/d to rats and compared the effects on measures of protein metabolism between those rats and rats infused with TNF and corticosterone, the most abundant circulating steroid in rats. Carcass weight was used as an index of change in lean body mass and, thus, of catabolic effects. By this index, lean body mass, as a percentage of body weight, was significantly reduced by TNF. The fact that urinary nitrogen output was not increased but that an increase in total protein content of the liver was observed suggests that amino acid substrates were redirected from peripheral muscle for acute phase protein synthesis in the liver by TNF alone, as well as by TNF plus corticosterone.

Blocking the effect of TNF in rats by giving TNF antiserum reduced mortality from 25% to 5%, had no effect on muscle protein synthesis, but reduced rates of total protein breakdown and myofibrilar protein breakdown, as shown by increases in the rates of release of tyrosine and 3-methylhistidine, respectively (34).

**LESSONS FROM THE BIRDS**

Building on the knowledge that microbial challenges, which rarely result in clinical disease produce significant immune responses in poultry, and on the long established
practice of giving antibiotics as feed supplements to poultry in insanitary environments, Klasing et al. (35–38) conducted a series of studies in poultry to explain the mechanisms and nutritional significance of subclinical infection on metabolism, specifically on the growth inhibition of the subclinical infections. For example, they found that asymptomatic chickens reared in typical unsanitary environments and fed antibiotics grew significantly better (p < 0.05) than nonantibiotic-treated chickens (38). In other words, antibiotics appeared to reverse the growth inhibition caused by immunologic stress. Importantly, the antibiotic treatment also resulted in greater weight gain per gram feed consumed than in the nontreated controls (i.e., it improved the efficiency of feed conversion: p < 0.05) (38). The overall conclusions from these and other studies done by Klasing’s team are that decreased growth, decreased feed efficiency (gram gain per gram feed), and changes in nutrient requirements all result from subclinical, asymptomatic immune stimulation. If the immune system has an especially high demand for nutrients, then animal nutritionists must consider immunity when setting dietary requirements and predicting or evaluating the efficiencies of feed conversion. Are similar paradigms relevant to understanding the net efficiencies of anabolism in children living in unhygienic environments?

SUBSTRATE COMPETITION DURING INFECTION IN CHILDREN

Referring back to the hypothesis that the growth inhibition of IL-6 is a secondary effect of its causing decreased concentrations of IGF-1 (16), Reeds et al. emphasized that the diversion of nitrogen from somatic protein to the synthesis of the acute phase proteins would result in nitrogen conservation and, thus, would not explain the increases seen in urinary nitrogen and protein oxidation, and the net losses of nitrogen from the body during infection (39). To look further into the mechanism whereby nitrogen is irreversibly lost from the body, we compared the gross amino acid composition of the major acute phase proteins synthesized by humans with those of mixed muscle (39). Four of the six acute phase proteins (C-reactive protein, amyloid A, haptoglobin, and α1-antitrypsin) contain high contents of phenylalanine, three of them (α1-acid glycoprotein, haptoglobin, and amyloid A) contain high amounts of tyrosine, and five of them (C-reactive protein, fibrinogen, α1-acid glycoprotein, haptoglobin, and amyloid A) are rich in tryptophan.

By contrast, mixed muscle protein has a relatively low content of phenylalanine, tyrosine, and tryptophan, whereas it is rich in the branched chain amino acids. Thus, if the major source of amino acids used in the synthesis of acute phase proteins is from endogenous sources (mainly muscle), then during the catabolic response the demands for the aromatics and tryptophan together would require the mobilization of substantially more muscle protein than would be gained as acute phase protein (Fig. 1).

Based on data published by Colley et al. (40) and Kushner (41), Reeds et al. (39) estimated the amino acid requirements for synthesis of a typical mix of acute phase proteins. Acute phase proteins synthesized under conditions of infection increased by 850 mg protein per kilogram body weight per day. On the basis of those results and
the amino acid composition of the acute phase proteins, we calculated the quantity of each amino acid that would have been incorporated into the acute phase protein mixture. We then calculated the quantity of muscle protein that would have to be catabolized to supply a sufficient amount of each amino acid to support acute phase protein synthesis. The figure of 850 mg was actually a conservative estimate, given Waterlow\'s estimate of a peak synthetic rate of 1.2 g acute phase proteins per kilogram body weight per day (13). In any case, by our calculations, the quantity of phenylalanine in 850 mg of the mixture of acute phase proteins represents the quantity of phenylalanine contained in 1,980 mg of muscle protein. Assuming that the phenylalanine is used completely in acute phase protein synthesis, we then calculated the difference between the quantity of each amino acid in 1,980 mg muscle protein and that in 850 mg acute phase protein. Including the needs for the aromatic amino acids, which by these calculations are the limiting amino acids when skeletal muscle provides the amino acid substrates for acute phase proteins, results in a surfeit of the branched chain amino acids because they are so much more abundant in muscle than in the acute phase proteins. This excess has to be catabolized, as it cannot be recycled into body protein because phenylalanine becomes limiting to further synthesis. The total of the nitrogen contained in this excess is 130 mg/kg body weight, which, according to Reeds et al. (39), is close to a typical daily loss of body nitrogen following uncomplicated trauma.

One of the conclusions from our study was that 2 g of muscle protein must be broken down to support the synthesis of 1 g of the mixture of positive acute phase proteins; therefore, a significant proportion of the net loss of body nitrogen results from the excessive demands for aromatic amino acids (phenylalanine, tyrosine), the limiting amino acids for synthesis of acute phase proteins when amino acids contained in muscle provide the substrate. Theoretically, these surplus amino acids would be deaminated and the carbon skeletons lost through oxidation. According to this analysis, the source of the protein oxidized and the nitrogen lost is the muscle amino acids...
that remain after the relatively greater demands for phenylalanine for synthesis of acute phase proteins have been satisfied, or the available phenylalanine runs out.

**ISOTOPIC STUDIES OF AMINO ACID OXIDATION DURING INFECTION**

In October of 1992, the International Atomic Energy Agency in Vienna released a Request for Proposals for studies relating infection to protein metabolism in marginally nourished children from developing countries. Proposals were reviewed and a coordinated research program was founded. Initially, the program involved researchers from seven developing and four industrialized countries. However, it became clear that a set of assumptions, measurements, and study design elements was common (or could be common) to each study. By an iterative process, those elements were forged into a *generic protocol* for studying the effects of subclinical infection on protein oxidation in marginally nourished, free-living children.

The team was composed of researchers from the Center for Studies of Sensory Impairment, Aging and Metabolism in Guatemala City, Guatemala; the International Centre for Diarrhoeal Disease Research, Bangladesh in Dhaka, Bangladesh; St. John’s Hospital, Bangalore, India; the Tropical Metabolism Research Unit in Kingston, Jamaica; the Instituto de Investigación Nutricional in Lima, Peru; the Queen Elizabeth Central Hospital, Balanytre, Malawi; the Children’s Nutrition Research Center, Houston, Texas, USA; Washington University School of Medicine, St. Louis, Missouri, USA; Johns Hopkins University, Baltimore, Maryland, USA; and the International Atomic Energy Agency, Vienna, Austria. The expert advice of F. Jahoor, P. Reeds, D. Halliday, and G. Keusch contributed substantially to the development of that protocol, which was published and used as the foundation for subsequent studies in most of the developing country research sites (42).

The generic protocol and, thus, each of our studies tried to achieve several things:

1. Standardize the infectious challenge and the markers thereof
2. Standardize the characterization of nutritional status
3. Preclude the substitution of exogenous protein for amino acids that, under home feeding conditions, might have been provided from exogenous supplies
4. Develop and validate a study design that could be implemented in children and would require no blood (i.e., only urine and breath)
5. Study the metabolic effects of infection at an approximately uniform point in the course of the infection or challenge

One of the main achievements of the Coordinated Research Program was to unite developing country scientists in their mutual interest in the effects of chronic immune challenges or infection on growth, a pre-eminent concern in most developing country settings. This Program also resulted in several publications (43–46) and substantial experience in the uses of stable isotopic research methods in nutrition research in developing country settings, where typically access to these kinds of methods may be limited.
The central hypothesis of each of the studies was that obtaining sufficient quantities of the aromatic amino acids for synthesis of the acute phase proteins, using skeletal muscle as the sources of the amino acid substrates, would result in a relative surplus of the branched chain amino acids, which would be oxidized after the amino acids that limited synthesis were exhausted. If true, this could explain some of the increases in amino acid oxidation that occur along with the negative nitrogen balance that is observed during infection. Our interest in the issue of nitrogen wasting was based in no small part on the hypothesis put forward by Scrimshaw in 1959 (17) and elaborated further by Solomons et al. (5): the occurrence of unapparent infections in children (i.e., no clinically apparent infection but a raised erythrocyte sedimentation rate and white cell count) is responsible for the diversion of nutrients from anabolic pathways and, thus, explains some of the growth faltering and malnutrition among children in developing countries. Thus, we were interested in the following:

1. Finding out whether a subclinical infection would result in increased oxidative losses of leucine, a branched chain amino acid present in relatively greater abundance in skeletal muscle than in acute phase proteins—as had been observed during *in vivo* infusions of crude IL-1 which resulted in increased excretion of N-methylhistidinone, hydroxyproline, and creatinine (47) and increased leucine oxidation (48);
2. The dietary modification of the oxidative losses, if indeed they were shown to occur;
3. The practicalities and accuracies of orally administered stable isotope tracers given non-invasively for these kinds of studies.

To simulate a subclinical infection, we used a diphtheria, tetanus, and pertussis (DTP) vaccine. The particular field work discussed below was carried out in Bangalore, India, by a team that combined researchers at St. John’s Medical College in Bangalore, India and at the Children’s Nutrition Research Center at the Baylor College of Medicine in Houston, Texas, USA. The research program’s interest was in evaluating the putative effects of subclinical infections on amino acid substrate partitioning, and particularly in developing practical alternatives to the intravenous tracer infusion methods to evaluate amino acid kinetics in children. The group, thus, had been working to elaborate the theoretic basis and practical application of a minimally invasive protocol (42,46).

Tracer studies of leucine metabolism were conducted using carbon-13 as a tracer of leucine and bicarbonate. A protocol in which isotopes were given orally to the children was run in parallel with a more conventional protocol for the intravenous administration of the isotopes in adults (46). The children were undernourished (weight for age Z-score, -2.3) and the adults had an average body mass index of 16.3 kg/m². Tracer protocols and breath collections performed during the fed state enabled the measurement of the rate of leucine oxidation and the rates of protein synthesis and breakdown. During the 6-hour studies, subjects were given small, frequent feeds of wheat starch biscuits (protein-free) at 30-minute intervals; breath, blood (adults only), and urine samples were collected according to the respective protocols. Our re-
suits showed significant increases in the rate of protein breakdown (p < 0.05) and a significant (17%) increase in the rate of leucine oxidation in the children following vaccination with DTP (p < 0.01). In adults, the DTP vaccination had no significant effect on whole body protein synthesis, but did cause a significant acceleration in the rates of protein breakdown, leucine flux, and leucine oxidation (21% increase; p < 0.01). The main conclusion from the study was that minor infections increase the irreversible oxidative loss of leucine, the amino acid that becomes available in disproportionately large amounts when muscle is catabolized to provide amino acid substrates. Thus, the process of redirecting amino acid metabolism appears to contribute to the irreversible loss of nitrogen and nitrogen wasting. This study was purposely controlled to preclude confounding effects of the dietary amino acid intake on the rate of leucine oxidation.

**EFFECTS OF TUMOR NECROSIS FACTOR IN DOGS**

Leucine released from the body protein mass must either be reutilized for *de novo* protein synthesis or be irreversibly lost to oxidation. Sakurai *et al.* (49) infused $^{13}$C-leucine and $^{14}$HCO$_3$ into conscious dogs along with recombinant tumor necrosis factor (rTNF) (bolus 2.5 μmol/kg plus a continuous infusion of 62.5 ng/kg/min), collected expired air through tracheostomy tubes, and took blood samples in which they measured isotopic enrichments and cytokine concentrations to study the effects of TNF on amino acid kinetics. Their observation of a 49% increase in the rate of leucine oxidation, which was significantly greater than the loss to nonoxidative disposal (*i.e.*, synthesis), along with a significant increase in the rate of urea production (p < 0.05 at each time point), suggested that net losses of leucine occurred from the body protein pool and that the leucine was irreversibly lost to oxidation. This finding supports the hypothesis that during infection or immune challenge, the body draws on the protein pool as a source of amino acids, reutilizes the amino acid substrates needed to mount the acute phase response, and oxidizes the surfeit that arises when muscle is catabolized to synthesize acute phase proteins.

**NEGATIVE ACUTE PHASE PROTEINS**

As stated, the negative acute phase proteins are the export or transport proteins, the synthetic rates of which decrease during infection (thus *negative acute phase*) through hepatic transcriptional inhibition of genes that code for these proteins (14). In this context, how might a chronic immune challenge or chronic infection or inflammation affect micronutrient status? To my knowledge, we do not know what effect leukocytes have in this relation; if cytokines are involved, however, then some effects are potentially attributable to infection. Cytokines inhibit the synthesis and release of transport proteins such as serum albumin, transthyretin, retinol-binding protein, and transferrin, among others. Those with reduced synthetic rates during the acute phase are referred to as *negative acute phase proteins*, such as transferrin and albumin. The reduction in the circulating levels of transport proteins results in decel-
erations in nutrient transport and in the redistribution of nutrients and, thus, is another mechanism whereby infection affects nutritional status.

Serum albumin concentration has been one of the tools relied on in the clinical evaluation of undernourished children in developing countries. It is thought to reflect both protein status and recent infection. One nutritionally relevant effect of TNF is a reduction in serum albumin, which can occur by two mechanisms. First, TNF decreases albumin gene transcription and steady state albumin mRNA levels (50) and, thus, reduces hepatic albumin synthesis (51). Second, the increased vascular permeability that cytokines induce leads to leaking of albumin into interstitial spaces and to loss of albumin through the gastrointestinal tract (52).

The acute phase is associated with low concentrations of plasma retinol; reduced concentrations of retinol-transport proteins, retinol-binding protein, and transthyretin; and a reduced abundance of retinol-binding protein mRNA in the liver (53). Thus, the reduced hepatic synthesis of retinol-binding protein and secretion of the retinol-to-retinol binding protein complex is a mechanism by which hyporetinolemia occurs in infection and explains the unreliability of plasma retinol concentration alone as an indicator of vitamin A status during or following an acute phase response. The mechanism whereby synthesis of acute phase proteins supersedes synthesis of transport proteins is, I believe, unknown. Recently, Mitra et al. (54) observed that serum retinol concentrations abruptly rebounded in patients during early convalescence from infection without administration of vitamin A, suggesting that hyporetinolemia is induced by the cytokine-mediated response to infection rather than being directly related to the hepatic retinol stores. In an accompanying review of the work by Mitra et al., Beisel (55) raised the question of whether the transient reduction in serum retinol during infections is a secondary consequence of cytokine-induced inhibition of retinol-binding protein and transthyretin production, or whether it should be classified, along with the effects on iron and copper, as an apparently purposeful cytokine-induced component of the acute phase reaction. The extent to which the competition for amino acid substrates under conditions of infection and an inadequate dietary intake blunts the synthesis of the nutrient transport proteins involved in vitamin A nutrition is an issue that can be explored using the isotopic methods for tracing amino acid metabolism.

Putting the research results into the challenging context of marginally nourished children living in crowded, unhygienic condition, Ruz et al. (6) postulated that some of the growth faltering in children results from the immunostimulation that occurs secondary to chronic exposure to insanitary environmental conditions, and the hypothesis has come to be known as Noel’s dirty chicken hypothesis. At issue is the extent to which the crowded unhygienic living conditions foster unapparent or occult infections in children and thereby explain part of the syndrome of malnutrition in children. Some fundamental experimental evidence for this hypothesis came from studies by Klasing et al. on the effects of antibiotic treatment on growth in chickens (35–38), showing that chickens grew more poorly when reared in unsanitary conditions than when reared under the same conditions but were given antibiotics.
The main goal of this review has been to provide further evidence for the aforementioned hypothesis by reviewing evidence for the redistribution of amino acid substrates during infection, effects that could have particular relevance to anabolism under the pressure of chronic dietary deficiency. Evidence has been provided to support the hypothesis that the diversion of anabolic substrates from the peripheral skeletal muscle to support acute phase protein synthesis increases the oxidative disposal of indispensable amino acids. Furthermore, it was shown that growth inhibition by nonpathogenic microbes is reversed in asymptomatic poultry by antibiotic treatment. An insanitary environment may trigger a sufficient acute phase response to effect a redistribution of amino acids from nutrient transport and growth to maintenance of an acute phase response, where the duration of the phase may be most of early childhood. For many children in developing countries, and for some adults in developed countries (e.g., elderly people and those in hospital) the concurrence of malnutrition and infection is a reality. Despite this, little is known about the chronic effects of cytokines on either the positive or the negative acute phase proteins in subclinical or unapparent infections. Growth faltering in asymptomatic children may result from either or both of the following factors: a diversion of anabolic substrates, and a cytokine effect on IGF-1, with a secondary effect on the growth hormone receptor. Oxidative disposal of exogenous amino acids, which cannot be used in the synthesis of acute phase proteins, partially explains the negative nitrogen balance in infection, including subclinical infection.

DIRECTIONS FOR FUTURE RESEARCH

Future research should take heed of the hypothesis made 40 years ago (20) that “the internal diversion of nutrients for the synthesis of compounds involved in the response to infection contributes importantly to the depletion of body stores.” We need to learn more about the conditions under which, and to what extent, subclinical infections can cause a reorientation of amino acid metabolism such that growth or micronutrient status are altered. This research would need to be done in the field, where the children are living, and would benefit from the use of stable isotope tracers of amino acid metabolism such as have been used for decades but not applied extensively in developing countries. Less invasive methods, which are based on the oxidation of the limiting amino acid and breath collections or oral infusions and breath collections, may make these studies more practical for developing country settings.

Specific Goals or Questions for Future Research

1. A method to detect subclinical chronic infection in children, preferably based on urinary rather than plasma measures, is needed.

2. Characterization of the magnitude and mechanisms of growth inhibition in marginally nourished children (general or pathogen-specific) particularly in terms of:
   - Decreasing synthesis of visceral proteins, especially IGF-1 (the nutrient-endocrine axis)
• Catabolism of structural proteins, enzymes, or nutrient transport proteins to provide amino acid substrates under conditions when exogenous intake cannot satisfy the priority need to make the acute phase proteins (competition for substrates mechanism)

3. Reversal of the growth inhibitory effects of chronic infection in children by giving cytokine antibodies and determining the effect on the variables listed above

4. Reversal or blunting of the growth inhibitory effects listed above

5. Testing the hypothesis that the drive to provide sufficient substrates for the synthesis of acute phase proteins competes for substrates, energy, or in related ways compromises the synthesis of nutrient transport proteins and, thus, contributes to micronutrient malnutrition and possibly growth faltering. (Jahoor’s group at the Children’s Nutrition Research Center in Houston, Texas, working with researchers at the TMRU in Kingston Jamaica, has recently published a series of papers [56–60] that provide fundamental conceptual and methodologic insights into the experimental model needed to test this hypothesis, particularly with regard to modeling nutrient transport protein kinetics.)

ACKNOWLEDGMENTS

Comments and suggestions on drafts of this manuscript provided by Drs. William Beisel, Jerry Keusch, Kirk Klasing, Noel Solomons, and Bob Suskind are greatly appreciated.

REFERENCES


**DISCUSSION**

**Dr. Keusch:** I wanted to make a comment about the dirty chicken, and the concept that antibiotics exert their growth-promoting action by an antibacterial effect. That may not be the case. Studies were carried out in the 1950s and 1960s using the dregs from tetracycline production, which have no antimicrobial activity but were fed to domestic animals for testing growth promotion. They were found to have a growth-promoting effect that was similar to the fractions that had antibiotic activity. Human experiments were also carried out by Scrimshaw in Guatemala, using a β-lactam and a protein synthesis inhibitor (tetracycline, I think). What they observed in children, which depended somewhat on the antibiotic, was some initial growth promotion and then a failure to show any effect as the experiment continued, which is
unlike what you see in animal husbandry (1). So, even though a growth-promoting effect of antibiotics may be seen in humans, it is not sustained, and we would have to try to explain that on the basis of alterations in the flora, which makes it a lot more complicated than you were implying.

This brings me to question the hypothesis about latent infection, and extrapolating from your experiment with DTP to asymptomatic infections. After all, DTP immunization is not asymptomatic; it causes an inflammatory response, typically with fever, whereas the situation that you are talking about with latent infection would be asymptomatic. Whether or not an activation of the acute phase response occurs that is sufficient to cause a sustained effect remains to be proved, I think. Your experiment does not address that, although it is very provocative in terms of mild inflammatory stimuli. But to give you the benefit of the doubt, under conditions of limiting diet, small changes might be important over the long run.

Dr. Griffin: Following on from that, we studied a vaccination model in the same way as you did in adults, using the Burroughs-Wellcome monovalent typhoid vaccine, which induces a beautiful acute phase response over about 36 to 48 hours. We applied stable isotope technology with $^{15}$N guanidino-labeled nitrogen in arginine to see if nitric oxide is part of this acute phase response. In the 12 humans we looked at, no increased conversion to $^{15}$N nitric oxide was seen in this 48-hour period. So, we can rule out nitric oxide in vaccine-induced acute phase responses as a potential for therapy.

Dr. Meydani: Can you predict whether you would see the same effect or a different effect if you were to do the same experiments in well-nourished children or adults?

Dr. Fjeld: I think the point is that if the dietary intake is not adequate to support that diversion, then the endogenous source becomes the source of substrates. So, it is a low dietary quality situation that we are most interested in, but the metabolic effect should still hold in the well-nourished individual if dietary intake is short circuited during that time.

Dr. Zoppi: You said at the beginning of your presentation that there is an impairment in growth hormone efficiency and secretion. Do you have any experience in the administration of growth hormone to infants who are infected in order to improve nutritional status and growth?

Dr. Fjeld: Reports have been made of children in developing countries where growth hormone concentrations have been measured and found to be normal. Normal growth hormone concentrations were also found in juvenile rheumatoid arthritis. It seems that the expression of the receptor on the hepatocyte is reduced by the changes in IL-6 and IGF-1 (2). I do not have any experience with the administration of growth hormone to infected children, although I do have some in relation to children with short stature. It can enhance growth in the short term, but the effect is generally not sustained in the long term.

Dr. Klish: In your DTP immunization studies, I would assume that you could affect your leucine kinetics by the nutritional status of the individual at the time the kinetics were done. You mentioned the children were on an equilibration diet, but it was only for 2 days. I would be curious to know what the equilibration diet was. Did you maintain the children in catabolism or convert them to anabolism?

As a comment, everybody who has discussed growth faltering in relation to infection has used Mata’s weight data. I have a slide in my collection of his height data, which I think tells more about the impact of negative nitrogen balance in infected children. Every time one of these children became infected, they would stop growing in height. After recovery, they would begin to grow again, but would not quite catch up to normal before they became infected again. As a result, you can see persistent height faltering over the 3- to 4-year period they were studied.
Dr. Fjeld: The people in Bangalore who did the study have excellent dietitians who measured the customary dietary intake. They then concocted a diet for the equilibration phase that would keep the subjects in energy balance but that would give them all a similar background abundance of $^{13}$C, which varies in the different foodstuffs in the diet. Thus, the diet was tailored to meet their energy needs, but gave a consistent amount of $^{13}$CO$_2$ as background.

Dr. Klish: Were 2 days enough to do that?

Dr. Fjeld: I hope so.

Dr. Gershwin: I like the thesis and I do not think we should be overly concerned about re-exploration of studies that were done in the 1950s and 1960s. But for the data to be reconciled, we are going to have to consider the nature of the flora and what the specific organisms are, because obviously there are going to be differences.

Dr. Marini: Do you feel that the reaction of the body to acute infection is independent of the agent—gram-positive or gram-negative bacteria, fungi, and so on—or does it depend on the agent to some extent? In neonates, differences are seen according to the agent causing the sepsis.

Dr. Fjeld: My intuition tells me that the responses have to differ markedly, but that is certainly not in my area of expertise.

Dr. Marini: I asked the question because in the baby with gram-negative sepsis is found hyperglycemia and hyperinsulinemia, in other words insulin resistance. When we manage these babies with total parenteral nutrition, they do better when we add medium chain triglycerides.

Dr. Griffin: In your balance experiments with muscle protein and acute phase proteins, did you take into account the negative synthesis of albumin during the acute phase response, which would be contributing to the amino acid pool for the acute phase proteins?

Dr. Fjeld: No, we looked at overall muscle composition and overall acute phase protein composition. Those are described in our 1994 paper in the *Journal of Nutrition* (3).

Dr. Griffin: During the acute phase response albumin synthesis falls considerably.

Dr. Fjeld: That is right, the nutrient transport proteins in general fall precipitously. An extension of this hypothesis is that a driving force may exist: if the host is trying to synthesize the acute phase proteins, that may be a factor in reducing the synthesis rate of the nutrient transport proteins.

Dr. Griffin: That is transcriptionally controlled. The lack of amino acids going into those pools will contribute to your acute phase pool as well.

Dr. Fjeld: But the idea that phenylalanine and other amino acids of that group are limiting would still hold true.

Dr. Suskind: Some years ago, when we first started looking at the endocrine changes in malnutrition, we found that these children had very high growth hormone levels and very low IGF-1 levels. We began looking at recovery of IGF-1 during renutrition and it seemed to follow the other visceral proteins. So it appeared that IGF-1 was a very sensitive visceral protein for assessing recovery from malnutrition and also from infection, which was obviously a major factor in the low IGF-1 levels. I was wondering whether you might comment on the relative roles of infection and growth. Perhaps what is happening is that the increase in production of cytokines is traded against the production of visceral proteins and that one of those visceral proteins is IGF-1; when the infectious insult is over, this can return to normal to allow a normal endocrine profile and, hence, normal growth to be re-established.

The second point I would like you to comment on is the question of whether it could be the products of muscle catabolism that promote the loss of zinc, water-soluble vitamins, vitamin A, and other nutrients. I wonder if you might comment on that to try to put the whole picture together in terms of the catabolic response.
Dr. Fjeld: I think your explanation about the downregulation of the IGF-1 as a visceral protein is a lovely idea; it certainly fits with this whole hypothesis. With regard to your second point, I have not made a quantitative assessment, but one can appreciate the concept that when skeletal muscle is catabolized, not only is there a loss of nitrogen but also of the other constitutive elements of lean body mass, including potassium and the micronutrients. Also, there is downregulation of the rate of synthesis of the nutrient transport proteins such as retinol-binding protein and transferrin, and that is partly why we see, for instance, more vitamin A loss from the body during times of infection.

Dr. Wasantwisut: I would like to hear your comments on the implications in terms of protein quality for a population at high risk of infection. In the past, only the absolute amount of protein in the diet has been considered and this is usually derived mainly from plant protein in developing countries. You imply that it may be important to have animal protein.

Dr. Fjeld: I think that is the key question. We do not have enough data yet; however, as I see it, this brings into question some of our fundamental concepts about protein requirements, given that metabolic demands may be placed on protein that determine what levels of individual amino acids are best suited to the individual under particular conditions. Most data on amino acid requirements are based on nitrogen balance studies, which are less sensitive to flux of single amino acids than the tracer studies that can now be carried out internationally. The International Dietary Energy Consultancy Group met a few years ago in London and examined the question of whether protein requirements needed to be reconsidered in infected children. It was considered that the data were too sparse to support such a re-evaluation. However, this notion can now be explored with newer tracer methodology that allows us to follow the flux of individual amino acids under defined conditions.

Dr. Coovadia: I would like to comment on an issue that was raised earlier: although it is important to look at the host, it may be very important to look at the agent too. Your elegant methods may help to unravel some important clinical problems. I want to illustrate this by experiences that go back over many years. Not all infections are associated with weight loss at high risk of infection. In the past, only the absolute amount of protein in the diet has been considered and this is usually derived mainly from plant protein in developing countries. You imply that it may be important to have animal protein.

Dr. Fjeld: I think that is the key question. We do not have enough data yet; however, as I see it, this brings into question some of our fundamental concepts about protein requirements, given that metabolic demands may be placed on protein that determine what levels of individual amino acids are best suited to the individual under particular conditions. Most data on amino acid requirements are based on nitrogen balance studies, which are less sensitive to flux of single amino acids than the tracer studies that can now be carried out internationally. The International Dietary Energy Consultancy Group met a few years ago in London and examined the question of whether protein requirements needed to be reconsidered in infected children. It was considered that the data were too sparse to support such a re-evaluation. However, this notion can now be explored with newer tracer methodology that allows us to follow the flux of individual amino acids under defined conditions.

Dr. Coovadia: I would like to comment on an issue that was raised earlier: although it is important to look at the host, it may be very important to look at the agent too. Your elegant methods may help to unravel some important clinical problems. I want to illustrate this by experiences that go back over many years. Not all infections are associated with weight loss at high risk of infection. In the past, only the absolute amount of protein in the diet has been considered and this is usually derived mainly from plant protein in developing countries. You imply that it may be important to have animal protein.

Dr. Fjeld: I think that is the key question. We do not have enough data yet; however, as I see it, this brings into question some of our fundamental concepts about protein requirements, given that metabolic demands may be placed on protein that determine what levels of individual amino acids are best suited to the individual under particular conditions. Most data on amino acid requirements are based on nitrogen balance studies, which are less sensitive to flux of single amino acids than the tracer studies that can now be carried out internationally. The International Dietary Energy Consultancy Group met a few years ago in London and examined the question of whether protein requirements needed to be reconsidered in infected children. It was considered that the data were too sparse to support such a re-evaluation. However, this notion can now be explored with newer tracer methodology that allows us to follow the flux of individual amino acids under defined conditions.

Dr. Coovadia: I would like to comment on an issue that was raised earlier: although it is important to look at the host, it may be very important to look at the agent too. Your elegant methods may help to unravel some important clinical problems. I want to illustrate this by experiences that go back over many years. Not all infections are associated with weight loss at high risk of infection. In the past, only the absolute amount of protein in the diet has been considered and this is usually derived mainly from plant protein in developing countries. You imply that it may be important to have animal protein.

Dr. Fjeld: I think that is the key question. We do not have enough data yet; however, as I see it, this brings into question some of our fundamental concepts about protein requirements, given that metabolic demands may be placed on protein that determine what levels of individual amino acids are best suited to the individual under particular conditions. Most data on amino acid requirements are based on nitrogen balance studies, which are less sensitive to flux of single amino acids than the tracer studies that can now be carried out internationally. The International Dietary Energy Consultancy Group met a few years ago in London and examined the question of whether protein requirements needed to be reconsidered in infected children. It was considered that the data were too sparse to support such a re-evaluation. However, this notion can now be explored with newer tracer methodology that allows us to follow the flux of individual amino acids under defined conditions.

Dr. Coovadia: I would like to comment on an issue that was raised earlier: although it is important to look at the host, it may be very important to look at the agent too. Your elegant methods may help to unravel some important clinical problems. I want to illustrate this by experiences that go back over many years. Not all infections are associated with weight loss at high risk of infection. In the past, only the absolute amount of protein in the diet has been considered and this is usually derived mainly from plant protein in developing countries. You imply that it may be important to have animal protein.

Dr. Fjeld: I think that is the key question. We do not have enough data yet; however, as I see it, this brings into question some of our fundamental concepts about protein requirements, given that metabolic demands may be placed on protein that determine what levels of individual amino acids are best suited to the individual under particular conditions. Most data on amino acid requirements are based on nitrogen balance studies, which are less sensitive to flux of single amino acids than the tracer studies that can now be carried out internationally. The International Dietary Energy Consultancy Group met a few years ago in London and examined the question of whether protein requirements needed to be reconsidered in infected children. It was considered that the data were too sparse to support such a re-evaluation. However, this notion can now be explored with newer tracer methodology that allows us to follow the flux of individual amino acids under defined conditions.

Dr. Coovadia: I would like to comment on an issue that was raised earlier: although it is important to look at the host, it may be very important to look at the agent too. Your elegant methods may help to unravel some important clinical problems. I want to illustrate this by experiences that go back over many years. Not all infections are associated with weight loss at high risk of infection. In the past, only the absolute amount of protein in the diet has been considered and this is usually derived mainly from plant protein in developing countries. You imply that it may be important to have animal protein.

Dr. Fjeld: I think that is the key question. We do not have enough data yet; however, as I see it, this brings into question some of our fundamental concepts about protein requirements, given that metabolic demands may be placed on protein that determine what levels of individual amino acids are best suited to the individual under particular conditions. Most data on amino acid requirements are based on nitrogen balance studies, which are less sensitive to flux of single amino acids than the tracer studies that can now be carried out internationally. The International Dietary Energy Consultancy Group met a few years ago in London and examined the question of whether protein requirements needed to be reconsidered in infected children. It was considered that the data were too sparse to support such a re-evaluation. However, this notion can now be explored with newer tracer methodology that allows us to follow the flux of individual amino acids under defined conditions.

Dr. Coovadia: I would like to comment on an issue that was raised earlier: although it is important to look at the host, it may be very important to look at the agent too. Your elegant methods may help to unravel some important clinical problems. I want to illustrate this by experiences that go back over many years. Not all infections are associated with weight loss at high risk of infection. In the past, only the absolute amount of protein in the diet has been considered and this is usually derived mainly from plant protein in developing countries. You imply that it may be important to have animal protein.

Dr. Fjeld: I think that is the key question. We do not have enough data yet; however, as I see it, this brings into question some of our fundamental concepts about protein requirements, given that metabolic demands may be placed on protein that determine what levels of individual amino acids are best suited to the individual under particular conditions. Most data on amino acid requirements are based on nitrogen balance studies, which are less sensitive to flux of single amino acids than the tracer studies that can now be carried out internationally. The International Dietary Energy Consultancy Group met a few years ago in London and examined the question of whether protein requirements needed to be reconsidered in infected children. It was considered that the data were too sparse to support such a re-evaluation. However, this notion can now be explored with newer tracer methodology that allows us to follow the flux of individual amino acids under defined conditions.

Dr. Coovadia: I would like to comment on an issue that was raised earlier: although it is important to look at the host, it may be very important to look at the agent too. Your elegant methods may help to unravel some important clinical problems. I want to illustrate this by experiences that go back over many years. Not all infections are associated with weight loss at high risk of infection. In the past, only the absolute amount of protein in the diet has been considered and this is usually derived mainly from plant protein in developing countries. You imply that it may be important to have animal protein.

Dr. Fjeld: I think that is the key question. We do not have enough data yet; however, as I see it, this brings into question some of our fundamental concepts about protein requirements, given that metabolic demands may be placed on protein that determine what levels of individual amino acids are best suited to the individual under particular conditions. Most data on amino acid requirements are based on nitrogen balance studies, which are less sensitive to flux of single amino acids than the tracer studies that can now be carried out internationally. The International Dietary Energy Consultancy Group met a few years ago in London and examined the question of whether protein requirements needed to be reconsidered in infected children. It was considered that the data were too sparse to support such a re-evaluation. However, this notion can now be explored with newer tracer methodology that allows us to follow the flux of individual amino acids under defined conditions.

Dr. Coovadia: I would like to comment on an issue that was raised earlier: although it is important to look at the host, it may be very important to look at the agent too. Your elegant methods may help to unravel some important clinical problems. I want to illustrate this by experiences that go back over many years. Not all infections are associated with weight loss at high risk of infection. In the past, only the absolute amount of protein in the diet has been considered and this is usually derived mainly from plant protein in developing countries. You imply that it may be important to have animal protein.

Dr. Fjeld: I think that is the key question. We do not have enough data yet; however, as I see it, this brings into question some of our fundamental concepts about protein requirements, given that metabolic demands may be placed on protein that determine what levels of individual amino acids are best suited to the individual under particular conditions. Most data on amino acid requirements are based on nitrogen balance studies, which are less sensitive to flux of single amino acids than the tracer studies that can now be carried out internationally. The International Dietary Energy Consultancy Group met a few years ago in London and examined the question of whether protein requirements needed to be reconsidered in infected children. It was considered that the data were too sparse to support such a re-evaluation. However, this notion can now be explored with newer tracer methodology that allows us to follow the flux of individual amino acids under defined conditions.
sponse or is the body overreacting to a stimulus? My question is, do you think that there would ever be a time when one might consider modulating the acute phase response to limit the amount of somatic damage? Are there any clever ways whereby we might at least modulate it? Particularly, as we know that nutritional restoration in the acute phase of the illness is never going to entirely restore the negative nitrogen balance and somatic mass?

Dr. Fjeld: I could think about blunting the nutritional effect of the acute phase response, but I do not think I can comment on whether it is an exaggerated response relative to the biological need.

Dr. Farthing: But I think it is an interesting research question.

Dr. Woodward: It seems to me that we can let the body give us the answer, or at least initiate an answer. By which I mean that we can now say the body for some reason places a high metabolic priority on maintaining acute phase protein concentrations in the blood, regardless of metabolic status; this is done even in the marasmic child, although interestingly by a different mechanism from what is accepted for you and me (4). We seem to increase synthesis, whereas the marasmic child accomplishes the same endpoint by decreasing catabolism. We need to find out the reason for this high priority, but in the meanwhile I would suggest that as acute phase proteins are maintained at a high level even under extreme circumstances, there must be an important protective reason. Of course, antioxidant defense is among them.

Dr. Griffin: If you look at animal models of infection and you remove some of the cytokine effect, for example by blocking TNF in salmonella models in rodents, the immune response is almost completely ablated and mortality increases greatly. In Listeria models, you see the same effect if you block TNF or IL-6. In humans, in the intensive care situation, given high dose methyl prednisolone, mortality from secondary infections is greatly increased, and that treatment is now contraindicated, of course, in the ICU setting. If we start to manipulate the acute phase response, we must be very careful not to remove crucial elements of the response. I do not think we are in a position yet to know what they are. Tuberculosis is a prime example. TNF is crucial in the formation of granulomas and the sequestration of that organism; if you ablate that response, you get much more profound metabolic change and death in animal models. So, I would advise caution.

Dr. Meydani: I can offer two examples from our own work where a nutritional manipulation was effective in reducing some of the effects associated with the acute phase response. One example is a study looking at IL-1 induced weight loss in an animal model, where we used fish oil to reduce the production of prostaglandin E\textsubscript{2}, and, therefore, obviate the weight loss that you normally see (5,6). In that case, the effect of limiting the acute phase response was beneficial. Also, in our vitamin E studies in influenza infection, we were able to reduce the weight loss associated with influenza without really affecting several other mechanisms, for example TNF production or IL-6 production or some of the inflammatory mediators that probably will be needed for defense against the infection. So, I think the key question is how to manipulate the response without, as Dr. Griffin said, affecting crucial functions. In that regard nutritional manipulation might be somewhat different from using antibodies against specific cytokines, where you would totally reduce or inactivate the function of the cytokine.

Dr. Roberton: Do you have any specific suggestions for the immune outcome in your immunization model? The purpose of the protein catabolism and of nutritional restitution, it is hoped, is to mount a more efficient inflammatory response. The acute phase response has a lot of nonspecific elements to it that create an immediate inflammatory response, but also some longer term components. Do you have any suggestions how you might measure the efficiency of the immune response in your immunization model as a long-term effect resulting from the transient nutritional supplementation? What are the outcome indicators that would be useful?
Dr. Fjeld: One of the indicators we would be interested in is turnover—how much futile cycling of amino acids there is. We would want to get the most synthesis out of the least amount of breakdown. We may be also interested in the rate at which nutrient transport proteins are synthesized, and the relative compromise between the positive acute phase proteins and the decrement in nutrient transport proteins. For instance, we know that children who are asymptomatic in the field but who have a raised leukocyte count also have disturbances in micronutrient nutrition. We think these are caused, at least partially, by the unavailability of transport proteins, so the lack of those transport proteins seems to be compromising the child’s micronutrient status, with all its attendant consequences. So, we want to look at the balance between the synthesis of the acute phase proteins and the maintenance of adequate nutrient transport proteins, for instance. And both of those things could be looked at using a tracer methodology.

Dr. Suskind: If we were to provide the limiting amino acids for the acute phase reaction, such as phenylalanine, tyrosine, and tryptophan, during the infectious state, do you think the loss of nitrogen would be mitigated, thereby providing us an approach to treatment in the acutely infected patient?

Dr. Fjeld: It is tempting to say yes, but I am sure many other nutrient and dietary factors need to be considered along with that. It would not simply be a matter of including those amino acids.

REFERENCES