The requirements of protein & amino acid during acute & chronic infections

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Received July 15, 2005

Nutrition and infection interact with each other in a synergistic vicious cycle, leading to an adverse nutritional status and increased susceptibility to infection. Infectious episodes result in hypermetabolism and a negative nitrogen balance which is modulated by hormones, cytokines and other pro-inflammatory mediators, and is compounded by a reduced food intake. The extent of the negative nitrogen balance varies with the type of infection and its duration; however, it is reasonable to suggest that the loss of body protein could be minimized by the provision of dietary nitrogen, although anorexia will limit this. Further, distinctions need to be made about the provision of nutrients or protein during the catabolic and anabolic or recovery phase of the infection, since the capacity of the body to retain protein is enhanced in the anabolic recovery phase. Meeting the increased requirement for protein (and other nutrients) in infection does not imply a complete therapeutic strategy. Infections need to be treated appropriately, with nutrition as an adjunct to the treatment. Prior undernutrition could also impair the body’s response to infection, although the weight of the evidence would suggest that this happens more particularly in oedematous undernutrition. In general, the amount of extra protein that would appear to be needed is of the order of 20-25 per cent of the recommended intake, for most infections. In acute infections, this is particularly relevant during the convalescence period. Community trials have suggested that lysine supplementation to the level required for normal daily nutriture, in predominantly wheat eating or potentially lysine deficient communities, improves immune function among other functional nutritional parameters; however, there is as yet insufficient evidence to suggest a specific requirement for amino acids in infections over and above the normal daily requirement as based on recent evidence. Some clinical studies that have showed benefits with specific amino acids through selected clinical outcomes, however, these do not provide enough evidence for a firm recommendation.

Key words Amino acid supplementation - infection - protein requirement - undernutrition

Nutrition and infection interact with each other synergistically. Recurrent infections lead to a loss of body nitrogen and worsen nutritional status; the resulting malnutrition, in its turn, produces a greater susceptibility to infection. In children, linear growth and weight gain, which are important indices of child
health, are lower in underprivileged communities\(^2\)\(^-\)\(^5\). This reduced growth rate, which leads to stunting in later life, is associated with long-term effects, including decreased productivity, and functional deficits\(^6\). The cause of this reduction in growth rate is likely to be multifactorial, reflecting the interactions of a poor diet and a poor environment\(^7\), and particularly the consequences of systemic and parasitic infections\(^8\).

Infections usually cause a set pattern of metabolic and clinical changes in individuals. The metabolic pattern, which includes hypermetabolism, a negative nitrogen balance, increased gluconeogenesis and an increased fat oxidation is modulated by hormones, cytokines and other pro-inflammatory mediators\(^9\), and is always compounded by a reduced food intake. The clinical features include fever, and depending on the site of infection, include symptoms like cough or diarrhoea, compounded by nutritional changes. In terms of endogenous protein availability for the metabolic processes, the major reservoir in the lean body mass is represented by muscle, as visceral mass is usually preserved in infection\(^10\). The total body skeletal muscle mass varies in different populations, based on their ethnicity, activity level and prior nutritional status\(^11\)\(^,\)\(^12\), and the negative nitrogen balance after injury tends to be higher in muscular well nourished individuals than in malnourished individuals\(^13\)\(^-\)\(^15\). Significant reductions in lean tissue or by extension, the muscle mass will attenuate the immunological response, as well as reduce physical activity. The provision of protein in the diet could meet some of the requirements of amino acids for the immune and restorative response. In this review the influence of infection on daily protein and amino acid requirements will be covered. The influence of infection on protein nutritional status has been extensively reviewed earlier for different pathogens and nutrients\(^16\).

### Pattern of protein and amino acid response to infection

Injuries or infections lead to an increased nitrogen loss from the body. The specific response to bacterial or viral infections, in terms of the N balance has been reviewed\(^17\), and the catabolic response of adults to infections with different organisms like bacteria, rickettsiae and viruses was prospectively evaluated by metabolic balance studies, during exposure as well as during overt infection. The infections were treated by antibiotics in the more severe cases. There was a loss of nitrogen in all cases, that was proportional to the number of days the subjects had fever\(^17\) (Table I), and was most serious with bacterial infections like typhoid fever. Although the intake of the subjects fell with the illness, pair-fed healthy controls showed that only about a third of the infection induced nitrogen loss could be attributed to dietary restriction\(^18\), and therefore suggests that dietary supplementation could directly reduce part of the negative nitrogen balance observed. However, given the anorexia that exists during the acute phase of the infection it is unlikely that efforts to increase protein intake would be successful. The striking feature of these data is the dependence of the negative nitrogen balance on the duration of fever, and negative nitrogen balance was not observed until the febrile response began, although the catabolic response can be observed in the absence of fever or other clinical symptoms as well\(^18\).

<table>
<thead>
<tr>
<th>Infection</th>
<th>Nitrogen loss (g)</th>
<th>Average fever duration (day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severe typhoid</td>
<td>186</td>
<td>25</td>
</tr>
<tr>
<td>Moderate typhoid</td>
<td>87</td>
<td>15</td>
</tr>
<tr>
<td>Tularaemia</td>
<td>52</td>
<td>6</td>
</tr>
<tr>
<td>Q fever</td>
<td>40</td>
<td>5</td>
</tr>
<tr>
<td>Sandfly fever</td>
<td>16</td>
<td>3</td>
</tr>
</tbody>
</table>

*Source: Ref. 17*
negative nitrogen balance persists for days or even weeks (for example, in children after a bout of pertussis) after the fever subsides. Even in mild viral fever lasting for 3 days (for example, sandfly fever), subjects took 10-11 days to recover from their negative nitrogen balance, when offered normal amounts of dietary protein and energy.

In chronically undernourished Indian adults exposed to vaccination, oxidative responses measured 24 h later by leucine oxidation increased by about 15 per cent. In children aged 3-6 yr, similar findings were found at the Instituto de Nutricion de Centro America y Panama, Guatemala (INCAP), where the effects of bacterial infections of the upper and lower respiratory tract, as well as gut showed a decrease in nitrogen retention by 15 to 45 per cent. Further, in children, a negative nitrogen balance was observed with infections even in the absence of symptoms or a febrile response. This finding was also observed in terms of leucine oxidation in Indian children below 5 yr of age, where vaccination with DPT vaccine increased the fraction of tracer leucine oxidized by about 17 per cent, one day after the vaccination. In general, there is a catabolic response with increased nitrogen losses from the body, and Wilmore has summarized the pattern of the catabolic response to infection with the following observations: (i) The increased nitrogen loss occurs via the urine, mainly as urea, although it is possible that with fever and sweating (significant losses could occur through sweat); (ii) there is a dose response in that the greater the infection (in terms of the degree and duration of the fever), the more extensive the nitrogen loss; (iii) more nitrogen is lost from a well nourished individual than a depleted patient following a comparable insult; and (iv) the response is not constant, and follows a time course, increasing to a peak and then gradually returning to equilibrium.

Other responses also occur in the infected individual. The catabolic response is characterized by nitrogen loss and the flux of amino acid nitrogen from muscle. The principal amino acids released from muscle are alanine and glutamine, although they do not constitute as much in muscle protein. Therefore, there is de novo synthesis of these amino acids in muscle, with an increased loss of skeletal muscle protein. This is evident in severe or prolonged infection as wasting of muscle. The export of amino acid nitrogen from muscle meets the needs of gluconeogenesis in the liver; the conversion of alanine to glucose results in nitrogen residues which are then excreted. Glutamine is a preferred fuel for enterocytes and immune cells such as lymphocytes and macrophages. In the kidney, glutamine supplies ammonia which combines with filtered hydrogen ions to form ammonium ions which are then excreted.

There is also a reduction in amino acids in the plasma, and in skeletal muscle. In general, the majority of plasma amino acids shows a decline early in the infection, and precedes the onset of fever. A decline in plasma glutamine is also seen. In critical illness, plasma levels of leucine, lysine, threonine, histidine and glutamine have been observed to fall. In contrast, plasma phenylalanine and tryptophan levels have been found to rise. Acute phase proteins, which are produced by the liver in response to infection, have a high phenylalanine and tryptophan concentration, and it may be that the increased plasma phenylalanine and hepatic uptake will fuel this process. These changes in plasma amino acid levels tend to persist until the infection subsides, and are associated with an anabolic response through cytokine-stimulated increase in the synthesis of positive acute-phase proteins by the liver. Changes are also evident in muscle concentrations of proteins and amino acids. Total free amino acids and glutamine concentrations have been observed to fall by more than half during critical illness associated with bacteraemia. On the other hand, branched chain amino acid and aromatic amino acid concentrations increased with critical illness.
These alterations in nitrogen flow, and in muscle catabolism and liver anabolism, are associated with changes in protein kinetics which vary with the tissue involved, as infusions of stress hormones have shown early and late effects on different cells such as muscle or lymphocytes. In general, as opposed to a simple reduction in food intake, infection, although accompanied by anorexia, is associated with an increased whole body protein turnover rate, including an increased protein synthesis rate. In response to vaccination, a 37 per cent increase in protein synthesis and a 55 per cent increase in protein breakdown was observed. Similarly, in chronically undernourished Indian adults, the response to vaccination (1 day after) was an increase in protein turnover by about 15 per cent, associated with an increase of about 13 and 20 per cent in protein synthesis breakdown. In these subjects, who were adapted to a constant diet prior to the vaccination, the 6 h N excretion (corrected for urea pool size) increased by 6 per cent after vaccination. A similar vaccination in well nourished adults induced a 25 per cent increase in protein synthesis and a 15 per cent increase in breakdown 2 days after the event. The increase in protein synthesis correlates with the clinical indices of the severity of infection, and the fractional synthesis rate (FSR) was increased for liver albumin synthesis (12.8%, normal value 6.7%) as well as lymphocyte protein synthesis (11.1%, normal value 7.2%), but slightly decreased in muscle (1.5%, normal value 1.7%) in critically ill adult patients. In specific terms of albumin synthesis, this was found to be sensitive to endotoxin challenge in humans, where the FSR increased by about 50 per cent above basal levels, one hour after administration of the endotoxin. At a cellular level, stress hormones have been shown to decrease protein synthesis in T lymphocytes and mononuclear cells.

Tuberculosis is now a major cause of infection worldwide, particularly in underprivileged populations. It is estimated that about 2 million or more people die of tuberculosis every year. The protein kinetic response in tuberculosis mimics, in general, the response to other infective insults, however, in a study on patients with tuberculosis (just diagnosed, without treatment), it was also found that there was a reduced rate of muscle synthesis in response to feeding in the patients when compared to body mass index (BMI) matched controls. This anabolic block in response to feeding may be one of the reasons for the accelerated weight loss seen in these patients. The excessive production of sputum and expectoration may also be an additional source of protein loss. In melioidosis, another chronic disease associated with wasting, protein turnover was increased by nearly 40 per cent when compared to uninfected subjects, however, in contrast to tuberculosis, the net catabolic rate was not significantly changed, nor was an anabolic block in response to feeding observed. An intriguing finding about tuberculosis is the apparent dependence of the immune response on protein. Mice fed a 2 per cent protein diet rapidly succumbed to infection with \textit{Mycobacterium tuberculosis}, when compared to mice fed a 20 per cent protein diet. What was noteworthy was that feeding a 20 per cent protein diet to previously malnourished mice (fed a 2% protein diet) reversed the fatal course of the infection. The mechanism by which murine phagocytes kill \textit{M. tuberculosis} is by the arginine dependent production of NO and reactive nitrogen intermediates as well as interferon gamma (INF-\(\gamma\)) and tumour necrosis factor alpha (TNF-\(\alpha\)). Malnourished mice showed a decrease in the inducible form of NO in the lungs, as well as a lung specific decrease in INF-\(\gamma\) and TNF-\(\alpha\) production. This study suggests that malnutrition induced susceptibility to infection and associated mortality can be ameliorated by nutritional intervention; however, a 2 per cent protein diet is quite abnormally low in protein and it would be interesting to assess the effects of a more realistic (or less severe) low protein intake.
The response of tissues to specific illness varies depending on the severity and chronicity of the infection, as well as the pathogen. The increase in protein synthesis is probably reflective of the anabolic response in the liver in the synthesis of acute phase proteins, which help in combating infection. In a study on protein kinetics in children with marasmus and kwashiorkor, it was found that children with marasmus had an increased protein turnover and synthesis, while those with kwashiorkor did not. Although the authors did not measure the fractional synthesis rate of any particular acute phase protein, they did find that the marasmic children had a higher plasma total globulin concentration, and speculated that the accelerated protein kinetics allowed for a better immunological response in marasmic children, to infection, probably with a better outcome. Reeds et al. have suggested that the increased protein oxidation (measured by leucine oxidation or N excretion) seen with infections, is due to a mismatch in the composition of acute phase proteins and muscle protein; the process of converting the latter into the former is associated with the release of excess amino acids that are oxidized. If this is so, feeding infected subjects with a dietary protein that more closely resembles the amino acid composition of acute phase proteins, should lead to a lower rate of protein breakdown. In a study on children who were fed isoenergetic, isonitrogenous meals containing either egg white-tryptophan or milk as a protein source, those who received the former protein had lower rates of plasma urea appearance, although the authors could find no differences in whole body protein kinetics, nor could they demonstrate any urea recycling during the study.

**A framework to explore the effect of infection on protein and amino acid requirements**

Why do infections cause an increase in nitrogen loss and therefore increase the dietary requirement for nitrogen? From a mechanistic viewpoint, there are several reasons for the increased nitrogen loss. The loss of body protein following the stress of injury or infection is largely from skeletal muscle. Although skeletal muscle water increases due to an increase in its extracellular water, total free amino acids and the glutamine content decrease by 59 and 72 per cent respectively, during critical illness. This breakdown of muscle protein resulting in the export of amino acid nitrogen, principally in the form of alanine and glutamine, fuels the gluconeogenic, oxidative and ureagenic processes in the liver. Thus, the response to infection includes an increased rate of release of amino acids from muscle and an increased splanchnic uptake of amino acids. The increased splanchnic uptake of amino acids, particularly glutamine is stimulated by the increased levels of stress hormones and results in lowered plasma glutamine (and amino acid) concentrations, a feature that has been observed in many studies dealing with stress and infection. This decrease in plasma glutamine concentration is in spite of a maintained rate of appearance of plasma glutamine and an increase in protein turnover indicated by a 50 per cent higher leucine flux. In this process, the carbon skeleton of the amino acid is utilized, leaving a nitrogen residue which is excreted. The requirement for glucose is increased with infection because white cells at the site of infection would use glucose for glycolysis rather than oxidation. Injured tissues also show this response; using arteriovenous studies in burned patients, Wilmore et al. showed that leg areas with large burns consumed more glucose than small burns, with almost all the glucose consumed being recovered as lactate.

The net release of amino acids from muscle is associated with a decline in intracellular amino acid concentrations. Although the intracellular concentration of most individual amino acids is quite low, the sum of all amino acids significantly contributes to cellular osmolarity in cells exposed to isotonic extracellular fluid. Since amino acids are
osmolytes, this decline in intracellular amino acid concentration is likely to be associated with a decrease in muscle cell volume. The reduced cell volume is associated with increased catabolism and proteolysis within the cell, mediated in part by alterations of lysosomal pH, which modifies the activity of acidic lysosomal proteases such that intracellular proteolysis increases\textsuperscript{56,57}. Hormones also exploit the influence of cell volume on metabolism to exert their effects\textsuperscript{58}. The effect of infection \textit{per se} on cell volume is not known with much certainty, since reports on the interplay of cell volume and infection are scarce. It is known that cholera toxin activates both Cl\textsuperscript{-} and K\textsuperscript{+} channels via cAMP\textsuperscript{59}, an effect expected to result in cell shrinkage. The heat-stable toxin from \textit{Escherichia coli} has been shown to activate Cl\textsuperscript{-} channels via cGMP\textsuperscript{60}, and erythrocytes infected with malaria display new ion permeation pathways\textsuperscript{61}. However, the situation is not as clear in infections with other agents such as viruses, and overall, the role of cell volume in the interaction of host and pathogen is far from understood. From the clinical viewpoint in humans, it has been shown that there is a progressive decline in intracellular water in critically ill septic patients, and this intracellular water loss is associated with protein and potassium losses from the body\textsuperscript{62}.

The support of the immune response comes from the synthesis of acute phase proteins in the liver, and this increased liver protein synthesis has to be supported either by amino acids derived from the diet, or by breakdown of body protein. The plasma levels of acute phase proteins rise during infection, and these proteins have important roles in combating the infection through the modulation of T-lymphocyte function and the complement system, as well as through scavenging haemoglobin and protecting tissues against the effects of proteases released from damaged cells\textsuperscript{63,64}. However, it is clear that there is a cost to the synthetic process, as, even when there is an apparently adequate supply of dietary protein, there is still a net loss of nitrogen from the body during active infection\textsuperscript{48}. It has been hypothesized that the reason why negative nitrogen balance occurs during an acute-phase response is that the amino acid composition of the positive acute phase proteins differs from that of muscle protein\textsuperscript{65,66}, in that it has high concentrations of aromatic amino acids. This leads to an internal amino acid imbalance, and as a result, approximately 2 g of mixed muscle protein must be broken down to support the synthesis of approximately 1 g of the typical mixture of positive acute phase proteins. In a study on patients with melioidosis, there was a close relationship between the plasma C-reactive protein level and the rate of protein synthesis, suggesting that protein synthesis is weighted towards liver anabolism and the synthesis of acute phase proteins\textsuperscript{42}. The excess amino acids liberated from muscle are then oxidized, leading to an increased N loss from the body. This process may be operative at a subclinical level; it is possible that the occurrence of inapparent infections in children (no clinical findings, but abnormal erythrocyte sedimentation and white cell count) is a factor that increases the demand for nutrients, which is particularly significant in underprivileged children from developing countries\textsuperscript{67}, since, during chronic immunostimulation there is a partitioning of nutrients towards the support of the immune defenses and an effective reduction in the availability of nutrients for growth.

A diminished absorption of nutrients could also act synergistically to the above mentioned catabolic effects and anorexia in inducing negative nitrogen balance. This has been shown, in terms of mucosal injury, to be a factor that influences catch up growth in children\textsuperscript{68}. In this study on mucosal permeability to sugars, it was found that up to 43 per cent of growth faltering could be attributed to long term intestinal lesions in children aged 2-15 months. The effect was present when invasive pathogens were present as opposed to those, such as \textit{Vibrio cholerae}, that caused
a secretory diarrhoea. Similar findings were found in a later study in the same area, where Gambian children between 0.5 to 3 yr of age, had evidence of chronic cell-mediated enteropathy with crypt hyperplasia, villous stunting, regardless of their nutritional status. This type of tropical enteropathy predates the onset of marasmus and could lead to decreased oral tolerance. In rodents, bacterial translocation from the intestine to the mesenteric lymph nodes has also been demonstrated with systemic infections, suggesting a defect in mucosal integrity. In children < 1 yr in age, there is a transfer of immunogenic macromolecules from the gut lumen into the body across the mucosal barrier, leading to growth impairment. There is also an extensive literature on the occurrence of enteropathy in tropical populations, and structure and function (measured by sugar absorption and permeability) were modestly correlated. The cause of the enteropathy is likely to be environmental, since the changes that occur in tropical enteropathy are acquired and reversible to some extent. A more contemporary survey of small intestinal biopsies from Zambian AIDS patients showed that there was decreased villous height and crypt depth compared to controls; these findings were related to the nutritional status of the subjects.

Measurements of the lysine requirement in chronically undernourished adult Indian men, from poor socio-economic backgrounds, with low habitual protein and lysine intakes, showed their daily lysine requirement to be about 50 per cent higher, when expressed on a per kg body weight basis, than the requirements in similar, but healthy, well nourished Indian men. It was likely that subjects from urban slums would have intestinal parasites, since other studies in south India, have shown a high prevalence of nematodinths, hookworm and pinworm infestations, ranging from 30 to 60 per cent for different parasites and areas.

It is possible that this environmental influence could, through direct (diversion of nutrients) or indirect (hyperplastic intestinal epithelial response, or malabsorption) means, increase these requirements. Following treatment for intestinal parasites, studies on similar chronically undernourished men who had mixed intestinal parasitic infestation (having one or more of the following: Ascaris lumbricoides, Giardia lamblia, Ankylostoma duodenale, Trichuris trichura and Entamoeba histolytica/dispar), showed that most of the increased lysine requirement could be explained by the presence of these parasites. A hyperplastic response of the intestinal epithelium to the presence of parasites could also explain the higher lysine requirement, since studies in neonatal pigs have shown that intestinal lysine oxidation is significant and accounts for the utilization of a disproportionately large amount of dietary supply when protein intake is restricted. Thus, the lower efficiency of lysine utilization, or higher lysine requirement, may be a consequence of the anatomic changes in the intestinal wall that accompany chronic intestinal parasitic infestations and possibly subclinical enteric bacterial and viral infections. Specifically, morphological changes such as a reversible villous flattening and lowering of the villus to crypt ratio are associated with Giardia infection, while tapeworm infestations are associated with villous damage at the point of attachment of the worm to the intestinal wall. Beside the effect of parasites, tropical enteropathy, related to environmental factors such as subclinical enteric infections and undernutrition, also leads to similar intestinal epithelial changes. These factors could lead to impaired nutrient absorption and increased losses from the gastrointestinal tract, which are also causal factors in the nutritional disturbance due to these intestinal events.

Finally, infection is associated with anorexia and low physical activity. Anorexia leads to a diminution
of food and hence protein intake; this in turn is capable of inducing a negative nitrogen balance. As noted earlier, in infective illness, it has been found that about one third of the negative nitrogen balance can be explained by a lowered protein intake; however, this also means that anorexia is not the major determinant of protein loss during infection. In addition, a lowering of physical activity would also contribute to net proteolysis, as prolonged bed rest has been shown to reduce skeletal muscle synthesis, although higher dietary protein intakes tend to modulate this process.

The regulation of these metabolic responses is due to the neuroendocrine response and the release of counterregulatory hormones during infective stress. The hormones, including cortisol, glucagons and the catecholamines are responsible for the persistent nitrogen loss and hyperglycaemia observed, but they are not solely responsible for the extent of these metabolic alterations. In addition to these hormonal mediators are the release of cytokines like the interleukins (IL-1 and IL-6), along with TNF. These factors are important in infection related changes in metabolism, particularly in septicaemia.

**Effect of prior undernutrition on the response of protein metabolism to infection**

It is well established that infection and malnutrition act synergistically, and while it is known that nutrition affects every aspect of the body’s defense mechanism, the nutritional status does not affect all infections equally. Tuberculosis, pneumonia, bacterial and viral diarrhoea and measles are known to be influenced by the nutritional status. The effect of infection on nitrogen loss is variable depending on the prior nutritional status of the individual. However, there may be urea salvage taking place in malnourished and infected individuals, such that the oxidative process, measured in terms of leucine oxidation may be more than what is seen in urea nitrogen loss. In terms of the acute phase proteins (C-reactive protein and serum amyloid A), severely malnourished children show an impaired response to DPT vaccination, and this has been observed in children with kwashiorkor as well. Similarly, undernourished patients were found to have an attenuated C-reactive protein response to surgery. The influence of protein intake on the magnitude of the acute phase response was demonstrated in rats exposed to turpentine; increasing protein deficiency tended to reduce total protein, albumin and α2 macroglobulin responses. On the other hand, it has been shown that the acute phase response of malnourished patients with Crohn’s disease was essentially normal. A detailed kinetic investigation into the acute phase response in marasmic children found that severely malnourished children were able to mount an adequate acute phase response for most acute phase proteins, except for fibrinogen. Interestingly, this study showed that the plasma concentration of some acute phase proteins (APP) could increase as a result of a diminished clearance from the plasma, showing that the APP response can be mounted through different mechanisms of an increased synthesis rate or a diminished clearance. Similar results were found in a well controlled animal model of malnutrition (by feeding a 3% protein diet) and inflammation (by turpentine injection) in piglets. Malnutrition and inflammation caused a similar decline in muscle protein fractional synthesis rate (FSR), but inflammation, in contrast to malnutrition, caused an increased fractional synthesis rate of albumin and fibrinogen. However, the plasma level of albumin fell with inflammation, showing that plasma concentrations are dependent on the synthesis rate as well as catabolism or loss from the vascular space. Similarly, Malawian children with marasmus also showed an increased total body protein turnover, and an increased globulin fraction of the plasma proteins, in contrast to those children who had kwashiorkor. Similar findings were observed in protein-energy...
Epidemiological confirmation that the acute phase and immune response may be normal when malnutrition is present comes from a survey of 200 Kenyan children, in whom a large battery of immune assays was performed\textsuperscript{102}. In this study, nearly all the children had elevated titres of antibody to a variety of pathogens, despite a high prevalence of stunting, anaemia and apparent biochemical micronutrient deficiencies. One group of children was supplemented with an isocaloric meat and milk containing diet, and in these children, the only detectable effect was a high titre of IgM (short-lived) \textit{Helicobacter pylori} antibodies. It is not clear why this occurred; it may have been due to a beneficial response form the diet, although the possibility that the meat was a carrier for \textit{H. pylori} could not be excluded\textsuperscript{102}. In adults as well, an enhanced acute phase response has been observed in a study on otherwise clinically normal, undernourished Indian slum dwellers, whose plasma levels of interleukin-6 and TNF-\(\alpha\) were higher than those in urban middle class subjects\textsuperscript{103}.

Overall, the evidence suggests that in undernutrition uncomplicated by oedema, the ability to mount an acute phase response is retained, and that changes in plasma levels of these proteins are due to an interaction between their synthesis and catabolic rate. In children with oedematous malnutrition and infection, it appears that they are not able to effectively increase protein turnover and mount a catabolic response\textsuperscript{101}.

**Protein requirement in infection**

Meeting the increased requirement for nutrients and protein in infection does not imply a complete therapeutic strategy. In general, infections need to be treated appropriately, with nutrition as an adjunct to the treatment. Severe infections need appropriate antibiotic treatment, and the accompanying fever, which increases the demands for nutrients, has to be treated. In general, the aim of the increased allowance in acute infection is to cover the losses that were sustained, and extends into the entire convalescence. The anorexia that accompanies illness prevents additional protein intake, and aggressive feeding of protein during the illness is counterproductive. In chronic infections, the aim is to replace the additional daily loss, during, for instance, tuberculosis, in which the course of the illness and its treatment are prolonged.

However, a greater concern at the population level is the situation of individuals in environments in which persistent immune activation, along with a decline in intestinal absorptive capacity from tropical enteropathy, may be present, which is not manifested as an overt clinical syndrome, but which will still increase the demand for protein\textsuperscript{104}. In addition to this concern, one needs to consider the allowance in chronic infections such as HIV and tuberculosis, which are widely prevalent in the world today, and are likely to be associated with some amount of tropical enteropathy.

From the viewpoint of chronic infections, an analysis of the nitrogen loss in these conditions based on kinetic data is presented in Table II. Two chronic infections, tuberculosis and melioidosis\textsuperscript{41,42}, in different countries, studied by the same technique using leucine kinetics as the marker for protein kinetics, have been compared. Since one of the studies presented oxidation data normalized to the lean body mass, these data were recalculated for body weight, so that all data were comparable. Although these were short term studies, measured over 4 h in the fasted and fed state, leucine balance values for the fast and fed state could be calculated as the difference between intake and oxidation for the fasted and fed phase, and extrapolated to 12 h in each case. The 12 h fasted and fed balance was added to get a 24 h value; the 24 h leucine balance was converted into an N value (based
on a leucine content of diet and body protein of approximately 8%), and the N balance so obtained represented the increased requirement. Based on this recalculation, patients with chronic bacterial disease did have an increased nitrogen loss, while in the tuberculosis patients, the balance was about 45 mg N/kg/day more negative than the controls for that study. To use these data effectively in coming to a recommendation, the assumption was made that the same systematic error in extrapolation was made in both control and infected groups. Then, the difference in N balance between the control and infected groups represents the extra N loss in each disease, and this could be expressed as a percentage of the N intake in each study, to arrive at an allowance for meeting the increased requirement level for nitrogen. With this method, the N requirement in tuberculosis would be about 25 per cent higher than in the controls, when the N loss was assessed against the N intake (Table II).

The tuberculosis group were all clinically ill, many with fever, and cough, at the time of the study. Additionally, most of the tuberculosis patients had not yet begun treatment, as they had just been diagnosed. In the other chronic bacterial disease of melioidosis, there was an increase in N loss of about 15 per cent by the same method of calculation as detailed above. The lower loss in this group could be due to their relative heterogeneity in pathology, as well as their having received antibiotics for up to 2 wk before the study, although they were mildly febrile on the day of the study. They also had a higher energy intake than in the tuberculosis patients (Table II). These data would suggest that in chronic bacterial infections, the allowance for protein is of the order of about 25 per cent. It is most important to recognize that the replacement of protein in wasting conditions will be most effective when adequate amounts of energy are provided and when physical activity is encouraged.

### Table II. Comparison of short term fed and fasted state leucine kinetics and balance in patients with pulmonary tuberculosis, melioidosis, and asymptomatic subjects with mixed intestinal parasites

<table>
<thead>
<tr>
<th>Group studied</th>
<th>Leucine parameters</th>
<th>24 h balance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fasted</td>
<td>Fed</td>
</tr>
<tr>
<td></td>
<td>(µmol/kg/h)</td>
<td>(µmol/kg/h)</td>
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<tr>
<td>Tuberculosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
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<td>60  37  23</td>
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<tr>
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<td>2  25  -23</td>
<td>60  47  13</td>
</tr>
<tr>
<td>Melioidosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>2  16  -14</td>
<td>72  48  20</td>
</tr>
<tr>
<td>Infected + treated</td>
<td>2  17  -15</td>
<td>72  53  13</td>
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<tr>
<td>Intestinal parasites</td>
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<td></td>
</tr>
<tr>
<td>Untreated</td>
<td>3  15  -12</td>
<td>29  18  11</td>
</tr>
<tr>
<td>Treated</td>
<td>3  13  -10</td>
<td>29  17  12</td>
</tr>
</tbody>
</table>

Oxid, leucine oxidation; Bal, leucine balance; Delta N loss, difference in N balance between infected and control subjects

Superscript numerals denote reference numbers

Ref. 41: India study. Protein source: Wheat/soy protein = 40/60. Diet: Energy = 138 kJ/kg/day, protein: fat: carbohydrate = 15: 30: 55; N intake = 175 mg/kg/day

Ref. 42: Thailand study. Protein source: Soy protein. Diet: Energy = 161 kJ/kg/day, protein: fat: carbohydrate = 14: 32: 54; N intake = 170 mg/kg/day

Ref. 82: India study. Protein source: Amino acid mix based on egg protein except for leucine (5%). Diet: Energy = Basal metabolic rate (BMR) x 1.3 Physical activity level (PAL), based on actual measurements, protein: fat: carbohydrate = 14: 32: 54; N intake = 160 mg/kg/day
There is also an increased requirement with mixed intestinal parasite infestations\textsuperscript{82}. In this study, 24 h leucine balance data have been used to compare treated and untreated subjects. In these data, there were no extrapolations involved, as the data were collected for 12 h fast and 12 h fed periods, with careful energy balance conditions observed, and a week of adaptive feeding to a diet that provided amino acids at or above requirement levels and all micronutrients. On the day of the experiment, energy was provided at requirement for a recumbent person. It is worth noting that converting leucine balance to N balance depends on the leucine content of protein oxidized; in this study, the diet contained 5 per cent leucine, and converting leucine balance to N balance should use this value. The difference in N balance calculated in this manner between the treated and untreated groups was about 10 per cent of the N intake. It is possible that intestinal absorption may be impaired in this condition. Carbohydrate malabsorption occurs with intestinal giardiasis\textsuperscript{105}, which disappeared after treatment. In neonatal rats, Cryptosporidium parvum infection resulted in 47 and 34 per cent reductions in leucine and glutamate fluxes across the ileal mucosa\textsuperscript{106}, while the absorption of a labeled dose of EDTA has been shown to be reduced by half in patients with intestinal strongyloides stercoralis\textsuperscript{107}.

During the course of a mild febrile episode, for instance, that induced by vaccination, the increase in amino acid (and hence protein) oxidation was of the order of 15 per cent\textsuperscript{19}. While this may be taken to be indicative of the acute protein loss, another approach to approximating the extra protein allowance is to measure, by N balance, the N loss during the course of the infection. This approach has been used in relatively acute and limited bacterial conditions\textsuperscript{16,104}. If the mean additional loss of protein is 0.6 g/kg/day, and if it takes 2-3 times the duration of depletion to replete this protein in an individual, then assuming the additional protein in the diet was available during convalescence, it would require 0.2 to 0.3 g protein/kg/day to meet the increased demand for protein. This is about 20-30 per cent of the normal requirement, depending on the age of the individual, since protein requirements vary with childhood and growth, as well as in the elderly. If the protein losses are higher, as they are in diarrhoea and dysentery, or typhoid fever, it would take even more protein to cover the losses. Thus if the losses are assumed to be 0.9 g protein/kg/day in diarrhoea or dysentery, then the allowance, assuming a 2-3 wk convalescence, would be 0.2 - 0.3 g protein/kg/day\textsuperscript{16,104}. However, in chronic disease, where the course of the illness is in months or longer, it is important to provide for the N loss completely on a daily basis. There will continue to be an increased requirement in convalescence as well due to the repletion process, and it is quite possible that alterations in the efficiency of utilization will persist in the recovery period. These allowances for an extra protein requirement during infection are summarized in Table III.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Allowance (as a percentage of protein requirements)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated tuberculosis\textsuperscript{1}</td>
<td>25</td>
</tr>
<tr>
<td>Partly treated melioidosis\textsuperscript{1}</td>
<td>15</td>
</tr>
<tr>
<td>Mixed intestinal parasites\textsuperscript{1}</td>
<td>10</td>
</tr>
<tr>
<td>Acute bacterial infection\textsuperscript{2} (including convalescence)</td>
<td>20</td>
</tr>
<tr>
<td>Acute diarrhoea\textsuperscript{2} (including convalescence)</td>
<td>30</td>
</tr>
<tr>
<td>Mild febrile illness, as induced by vaccination\textsuperscript{3}</td>
<td>15</td>
</tr>
<tr>
<td>Sepsis\textsuperscript{4}</td>
<td>30</td>
</tr>
</tbody>
</table>

1: Based on calculations in Table II and text.
2: Data from Ref 16. The requirement is calculated based on replacing the extra losses throughout convalescence.
3: Data from Ref 19.
4: Data from Ref. 108. Note that the protein loss is approximately 2 g/kg/day in sepsis, however, the maximum that can effectively be utilized is a 30 per cent increase in protein intake over the safe value of 1 g/kg/day.
The protein loss experienced in more severe critical illness such as sepsis and trauma can be more dramatic, associated as it is with the altered metabolic profile of these patients. In a careful study on critically ill patients who were haemodynamically stable, and which included detailed and precise evaluations of body protein stores by neutron activation analysis, it was found that the protein loss was about 1.8 kg over a 10 day period\textsuperscript{108}. When expressed per kg corrected body weight (body weight was corrected for overhydration), this works out to a loss, or balance, of -2.2 g protein per kg/day. The protein intake of the patients with this negative protein balance was 0.9 g protein/kg/day with an energy intake of 114 kJ/kg fat free mass/day. Replacing this negative protein balance will represent an enormous increase of the protein intake, and is a wasted exercise when considering the metabolic state of the patient, including the insulin resistance that would accompany severe infection. Indeed, these investigators found that when the protein intake of the patients was raised by 30 per cent to 1.2 g/kg/day, with the same energy intake, the protein loss or negative protein balance reduced by half to about 1 g/kg/day. However, the protein loss did not decline any further, with further increments in protein intake. At an intake of 1.5 g protein/kg/day, even when the energy intake was increased to 131 kJ/kg FFM/day, the protein loss was not significantly reduced further. The 30 per cent increase in protein intake was therefore a metabolically effective increment. Studies on intestinal absorption in sepsis also suggest that the absorptive capacity of the intestine is reduced by half\textsuperscript{109}, suggesting overall, that the efficiency of utilization will be reduced by about 50 per cent in critical illness and sepsis. In this severe illness, the ability of the body to respond to increased protein intake will be reduced due to increased insulin resistance, limiting the usefulness of an enhanced protein intake, and increments of protein and energy beyond a certain point do not result in lower body protein losses. However, when the body is in an anabolic phase during recovery, the ability to retain protein will be greater.

The specific amino acids requirement in infection depends on what these are used for. There are several lines of evidence to believe that there is a specific pattern of amino acid requirement in infection. Since there is evidence of increased hepatic acute phase protein synthesis, along with efflux of amino acids from muscle, due to muscle protein breakdown\textsuperscript{110}, and since there is a mismatch between the composition of muscle protein and acute phase protein\textsuperscript{48}, it is possible that the provision of an amino acid mixture that more or less mimics the composition of the acute phase proteins will diminish the demand for endogenous amino acids. Reeds\textsuperscript{111} has suggested that the amino acids that are required to maintain the immune system in a state of optimal condition are glutamine, arginine and aspartate for lymphocyte proliferation and function, and cysteine, glutamine and glycine for glutathione synthesis. All these are dispensable amino acids, and their synthesis in the body will depend on the availability of its amino acid precursor.

The indispensable amino acids are also important; the provision of lysine fortified wheat flour to study communities in Pakistan\textsuperscript{112} and China\textsuperscript{113} has been shown to result in an improvement of immune parameters. In China\textsuperscript{113}, where the major proportion of protein intake came from wheat, the provision of wheat flour fortified with lysine to the level of 0.45 mg/g protein, for a period of 3 months, resulted in an improvement of CD3 T cells, C3 complement and IgA in women and children, while in men, CD8 T cells, C3 complement and IgG increased significantly. This short term study however could not bring out differences in observed morbidity. In a similar study carried out in northern Pakistan\textsuperscript{112}, the 3 month provision of lysine fortified wheat flour (resulting in a similar level of fortification as the
Chinese study\(^{113}\) to a community that derived its protein intake predominantly from wheat, resulted in an increase in CD3, CD8 and C3 complement levels in men, women and children. Importantly, the positive effect on immune function was independent of the socio-economic status of the families. These studies highlight the importance of lysine limiting diets in the developing world, where environmental risks for infection are greater, due to poverty, overcrowding and poor sanitation. The daily requirement of lysine in a predominantly cereal eating population is also relevant. If the protein quality were to be assessed using the 1985 FAO/WHO/UNU requirement pattern\(^{114}\) (16 mg/g protein), then wheat protein alone would provide this requirement, with an amino acid score of greater than 100. However, recent evidence based on more accurate stable isotope technique suggests that the requirement for lysine is much higher\(^{12,74,79}\), resulting in a requirement pattern of 45 mg/g protein. This would mean that the amino acid score of wheat protein is less than 50, and subjects consuming predominantly wheat based diets, and getting most of their protein from wheat, would be at risk of lysine deficiency. Indeed, such populations are usually poor, with low food diversity, and it is likely that even if their energy and protein requirements were met, their micronutrient intake would be suboptimal. This makes it difficult to define a pure lysine deficiency in terms of morbidity. Nevertheless, the children from the Pakistan supplementation study grew better after lysine fortification\(^{112}\), and other nutritional parameters in adults as well as children, related to serum proteins such as albumin, pre-albumin and transferrin, also improved after lysine fortification. Muscle function has also been shown to improve after adequate intake of lysine in well nourished young men over the short term\(^{79}\).

With reference to Indian dietary intakes recorded by the National Nutrition Monitoring Bureau (NNMB) surveys\(^{115-117}\), it would appear that mixed Indian diet tends toward being lysine deficient. If lysine is taken to be the limiting amino acid in these diets, then the protein digestibility corrected amino acid score (PDCAAS) of diets from tribals, or subjects from urban slums (Bhuvaneshwar/Cuttack) or rural areas (mean of 8 States) ranges from 73 to 80, assuming a digestibility of 80 per cent. In addition, the bioavailability of protein is likely to be low with tropical enteropathy. In terms of common foods eaten, only legumes and protein from animal sources have an amino acid score greater than 100\(^{118}\), with cereals, millets, vegetables and nuts/seed having amino acid scores ranging from 50 to 96 (Table IV).

There are several studies available, relating to laboratory or clinical outcomes, based on specific amino acid supplementation. These studies are essentially qualitative, as they identify a need, but do not quantify it through dose response studies. For example, the specific requirements for glutathione, and its dependence on the availability of cysteine\(^{120}\), will raise the requirement for that

<table>
<thead>
<tr>
<th>Protein source</th>
<th>Lysine content** mg/g protein</th>
<th>FAO/WHO/UNU 1985** requirement pattern (16 mg/g protein)</th>
<th>New requirement value (45 mg/g protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat</td>
<td>27</td>
<td>&gt;100</td>
<td>60</td>
</tr>
<tr>
<td>Rice</td>
<td>35</td>
<td>&gt;100</td>
<td>78</td>
</tr>
<tr>
<td>Sorghum</td>
<td>24</td>
<td>&gt;100</td>
<td>53</td>
</tr>
<tr>
<td>Millet</td>
<td>22</td>
<td>&gt;100</td>
<td>50</td>
</tr>
<tr>
<td>Nuts / seeds</td>
<td>35</td>
<td>&gt;100</td>
<td>77</td>
</tr>
<tr>
<td>Vegetables</td>
<td>43</td>
<td>&gt;100</td>
<td>96</td>
</tr>
<tr>
<td>Legumes</td>
<td>73</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Animal protein</td>
<td>82</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
</tbody>
</table>

*not corrected for digestibility  
**rounded off values from Ref. 119; average values of common foods used for nuts/seeds, vegetables, legumes and animal protein  
\(^{119}\) Refs 78, 79  
\(^{110}\) Ref 78
amino acid and its precursor methionine. Cysteine supplementation has been shown to improve the erythrocyte glutathione synthesis rate in malnourished children\textsuperscript{121}. There is also evidence that the plasma amino acid concentrations fall during infection\textsuperscript{24,122}, and specifically, those amino acid levels that do not rise during feeding have been identified as those that are possibly limiting during infection. The amino acids that have been identified as limiting by this process are threonine and methionine\textsuperscript{117}. The reduction in the intracellular volume leading to cell shrinkage can be attributed to the efflux of glutamine and other osmolytes from the cell\textsuperscript{56}. This reduction in intracellular volume is also seen in clinical studies of sepsis and trauma\textsuperscript{62}. A strategy to reduce intracellular shrinkage is to supplement glutamine in the diet, so as to increase the intracellular glutamine content.

Glutamine also has other effects, notably on epithelial and immune function. With respect to the latter, glutamine enriched diets have been shown to improve the HLA-DR expression on blood monocytes in trauma patients\textsuperscript{123}; this function is important for T lymphocyte recognition of bacterial antigens. Other clinical outcome based research has also shown that glutamine has beneficial effects with respect to length of stay in hospital, however, the results are variable, this variability in the benefit of specific nutrient supplementation may be due to different study conditions as well as due to the genetic makeup of the individual\textsuperscript{124,125}. There are also data to show that arginine supplementation in endotoxaemic pigs, improves muscle protein turnover and reduces liver protein turnover, as well as whole body protein turnover\textsuperscript{126}. There has only been one set of studies that specifically set out to identify the lysine requirement in undernourished men living in slums, who had intestinal parasites\textsuperscript{12,74,79,82}, in whom the lysine requirement was found to be about 50 per cent higher. It is important to recognize that the oversupplementation of amino acids (with or without whole protein supplementation) also has its problems. In rats, the oversupplementation of amino acids (particularly threonine, methionine and branched chain amino acids) leads to an increased severity of malaria infection\textsuperscript{127}, and certainly the supplementation of methionine in the diet will increase the need for glycine, as dietary supplementation studies with methionine in normal women receiving low protein diets have shown an increased 5-L-oxoproline excretion\textsuperscript{128}.

**Conclusion**

Protein loss occurs from the body due to the catabolism induced by infection, and this loss should be made good through the provision of additional protein intake particularly during recovery. Prior undernutrition attenuates the response of the body to infection and this is particularly affected in oedematous undernutrition. The needs for protein are reflected in the negative N balance that occurs during acute and chronic infections, leading to a loss of body weight. This can be made good during recovery, however, the recovery period can last for much longer than the original acute illness. The capacity to retain protein is enhanced in the recovery phase of infection and the need for protein should particularly be met during this time. In chronic infections, it would appear that an increase in protein intake of up to 25 per cent is warranted, however, the consideration to feed protein should include an adequate energy and micronutrient intake, as well as a consideration of a decreased absorptive capacity that may occur with co-existing tropical enteropathy in poor or undernourished populations. In all these situations, the protein requirement is an adjunct to the primary medical treatment, and should be delivered in a varied and wholesome diet. In terms of specific amino acids, there are insufficient data to propose specific quantitative allowances for amino acids during infection. The supplementation of some amino acids
such as methionine will also increase the requirement for other dispensable amino acids. Short term studies of clinical nutrition in acutely ill patients suggest that the supplementation of specific amino acids may be relevant. Studies with lysine supplementation in the community have shown that immune capability is enhanced in men, women and children. However, there is no morbidity based evidence on the effect of specific amino acid supplementation on chronic infection or in prevention of infection in the community, nor are there adequate data to suggest that amino acids need to be supplied beyond the daily recommended requirement based on recent evaluations of the indispensable amino acid requirement.

References


15. Scrimshaw NS. Historical concepts of interactions, synergism and antagonism between nutrition and infection. J Nutr 2003; 133 : 316S-21S.


17. Powanda MC, Beisel WR. Metabolic effects of infection on protein and energy status. J Nutr 2003; 133 : 322S-7S.


21. Gandra YR, Scrimshaw NS. Infection and nutritional status. II. Effect of mild virus infection induced by 17-D
yellow fever vaccine on nitrogen metabolism in children. 

22. Elia M. The inter organ flux of substrates in fed and 
fasted man, as indicated by arteriovenous balance studies. 

23. Muhlbacker F, Kapadia CR, Colpys MF, Smith RJ, Wilmore 
DW. Effects of glucocorticoids on glutamine metabolism 

1975; 26 : 9-20.

25. Wannemacher RW Jr. Key role of various individual 
aminos acids in host response to infection. Am J Clin Nutr 

26. Wannemacher RW Jr, Pekarek RW, Bartelloni PJ, 
Vollmer RT, Beisel WR. Changes in individual plasma 
aminos acids following experimentally induced and fly fever 
virus infection. Metabolism 1972; 21 : 67-76.

27. Souba WW, Klimberg VS, Plumley DA. The role of 
glutamine in maintaining a healthy gut and supporting 
the metabolic response to injury and infection. J Surg Res 

28. Jackson NC, Carroll PV, Russell-Jones DL, Sonksen PH, 
Treacher DF, Umpleby AM. The metabolic consequences 
of critical illness: acute effects on glutamine and protein 

29. Roth E, Funcovics J, Muhlbacker F, Schemper M, 
Mauritz W, Sporn P, et al. Metabolic disorders in severe 
abdominal sepsis: glutamine deficiency in skeletal muscle. 

A descriptive study of skeletal muscle metabolism in 
critically ill patients: free amino acids, energy-rich 
phosphates, protein, nucleic acids, fat, water, and 

phase protein response is attenuated by protein deficiency 

32. Keusch GT. Immune function in the malnourished host. 

muscle, lymphocytes, and albumin with stress hormone 

34. Long CL, Jeevanandam M, Kim BM, Kinney JM. 
Whole body protein synthesis and catabolism in septic man. 

35. Garlick PJ, McNurlan MA, Fern EB, Tomkins AM, 
Waterlow JC. Stimulation of protein synthesis and 

36. Cayol M, Taueron I, Rambourdin F, Prugnaud J, 
and hepatic protein synthesis are increased by vaccination 

37. Essen P, McNurlan MA, Gamrin L, Hunter K, Calder G, 

MA, Garlick PJ, et al. Albumin synthesis in humans 
increases immediately following the administration of 

Garlick PJ, Wernerman J. A combined stress hormone 
infusion decreases in vivo protein synthesis in human T 
lymphocytes in healthy volunteers. Metabolism 2001; 50 : 
1308-14.

40. The global plan to stop TB 2006-2015. Towards a world 

41. Macallan DC, McNurlan MA, Kurpad AV, de Souza G, 
Shetty PS, Calder AG, et al. Whole body protein metabolism 
in human pulmonary tuberculosis and undernutrition: 
evidence for anabolic block in tuberculosis. Clin Sci Lond 

42. Paton NI, Angus B, Chaowagul W, Simpson AJ, 
Suputtamongkol Y, Elia M, et al. Protein and energy 
metabolism in chronic bacterial infection: studies in 

43. Chan J, Tian Y, Tanaka KE, Tsang MS, Yu K, Salgame P. 
Effects of protein calorie malnutrition on tuberculosis in 

44. Flynn JL, Chan J, Triebold KJ, Dalton DK, Stewart TA, 
Bloom BR. An essential role for interferon gamma 
in resistance to Mycobacterium tuberculosis infection. 

45. Flynn JL, Goldstein MM, Chan J, Triebold KJ, Pfeffer 
K, Lowenstein CJ, et al. Tumor necrosis factor-alpha is 
required in the protective immune response against 
Mycobacterium tuberculosis in mice. Immunity 1995; 
2 : 561-72.


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