Acute-phase protein response to infection in severe malnutrition

JOHN F. MORLESE,1 TERRENCE FORRESTER,2 AND FAROOK JAHOOR1

1United States Department of Agriculture/ Agricultural Research Service, Children's Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine, Houston, Texas 77030; and 2Tropical Metabolism Research Unit, University of the West Indies, Mona, Kingston 7, Jamaica

Morlese, John F., Terrence Forrester, and Farook Jahoor. Acute-phase protein response to infection in severe malnutrition. Am. J. Physiol. 275 (Endocrinol. Metab. 38): E112–E117, 1998.—It is not known whether malnourished infants can mount a comprehensive acute-phase protein (APP) response and, if so, whether this is achieved by increasing APP synthesis rates. To address these issues, we measured 1) the plasma concentrations of five APPs (C-reactive protein, α1-acid glycoprotein, α1-antitrypsin, haptoglobin, and fibrinogen) and 2) the synthesis rates of three APPs (α1-antitrypsin, haptoglobin, and fibrinogen) using a constant intragastric infusion of [2H3]leucine in nine infected marasmic children at −2 days postadmission (study 1), −9 days postadmission when infections had cleared (study 2), and −59 days postadmission at recovery (study 3). Except for fibrinogen, the plasma concentrations of all APPs were higher in study 1 than in studies 2 and 3. Although the rate of synthesis of haptoglobin was significantly greater in study 1 than study 2, the rates of fibrinogen and α1-antitrypsin synthesis were similar in all three studies. These results show that 1) severely malnourished children can mount an APP response to infection which does not include fibrinogen and 2) the APP response is accomplished through different mechanisms.

α1-antitrypsin; C-reactive protein; haptoglobin; marasmus

The acute-phase response to the stress of injury or infection is characterized by higher plasma concentrations of the positive acute-phase proteins (APPs) (5, 6). These plasma proteins are of clinical relevance because they have important roles in combating infections, including modulating T-lymphocyte function and the complement system, scavenging hemoglobin, and protecting the integrity of healthy tissues against the effects of proteases produced by the pathogen or released from damaged cells (5, 15). Although the physicochemical properties of APPs have been studied in great detail, there is very little information on the in vivo metabolism of these proteins, particularly in pathological states such as severe protein-energy malnutrition.

Although it is well established that nearly every aspect of the body's defense system is damaged by severe malnutrition (2), it is not clear whether the capacity to mount an APP response is severely impaired. The finding that infected malnourished children have higher plasma concentrations of one family of APPs, the antiproteases (23, 24), suggests that these patients retain the capacity to mount an APP response involving that family of proteins. These findings do not necessarily prove, however, that the severely malnourished can elicit a comprehensive APP response to the stress of infection or injury. To find out whether the limited capacity of the severely malnourished to mount an APP response to infection includes a full spectrum of APPs, the present study aimed to determine the changes in plasma concentrations of a roster of five different APPs in infected malnourished children before and after treatment. The five APPs selected, C-reactive protein, α1-acid glycoprotein, α1-antitrypsin, haptoglobin, and fibrinogen, were chosen because they are representative of APPs with widely different functions (4, 5, 15, 20).

Another question concerns the mechanism by which malnourished individuals mount an APP response. Although there are few in vivo studies of the kinetic mechanisms responsible for the increased availability of APP in injured or infected well-nourished humans (19, 25), the evidence from these studies and from in vivo studies in infected animals (13, 14) suggests that a marked stimulation of the APP synthesis rate leads to increased availability of these proteins. In the malnourished state, however, the capacity to synthesize more protein may not be achievable because of a chronically poor dietary protein intake (29). It is therefore possible that, when stressed by infections, the severely malnourished may increase the availability of APPs through kinetic mechanisms other than stimulated synthesis rates.

The present study was therefore performed in marasmic children with infections to determine 1) the ability of malnourished children to mount a general APP response and 2) the kinetic mechanisms responsible for the increased plasma pool sizes of three APPs with widely different functions, i.e., α1-antitrypsin, haptoglobin, and fibrinogen. A newly developed stable isotope tracer technique (10) was used to directly measure the rates of synthesis of the three APPs in infected protein-energy malnourished children at three time points during hospitalization: when the children were both malnourished and infected (−2 days postadmission); after infections were under control, but the patients were still severely malnourished (−9 days postadmission); and after the patients were fully recovered and had achieved at least 90% of expected weight for height (−59 days postadmission).

METHODS

Subjects. This study was approved by the Medical Ethics Committee of the University Hospital of the West Indies and the Baylor Affiliates Review Board for Human Subject Research of Baylor College of Medicine. Nine Jamaican children (6 males, 3 females) were diagnosed as marasmic using the...
diarrhea and absence of chest crepitations). The clinical features of the infective episode (e.g., cessation of temperature, respiratory and pulse rates, and resolution of had cleared. The latter was determined by normalization of nance diet, their appetite had recovered, and their infections still severely malnourished, they were still on the mainte-
tance diet for 3 days before the isotope infusion. 2 h before the isotope infusion commenced. After a 2-ml venous blood sample was drawn, the [3H]leucine solution was infused nasogastrically at a rate of 26 µmol·kg⁻¹·h⁻¹ for 8 h. Additional 2-ml blood samples were drawn at 2-h intervals throughout the infusion. The same infusion and blood sampling protocol were repeated in studies 2 and 3.

Sample analysis. Blood was drawn in prechilled tubes (containing Na₂EDTA and a cocktail of sodium azide, thimer-
sal, and soybean trypsin inhibitor) and immediately centri-
fuged at 1,000 g for 15 min at 5°C. The plasma was removed and stored at −70°C for later analysis.

The plasma concentrations of five APPs, C-reactive protein, α₁-acid glycoprotein, haptoglobin, α₁-antitrypsin, and fibrino-
gen, were measured by radial immunodiffusion using Human RID kits (The Binding Site, San Diego, CA).

Two APPs, haptoglobin and α₁-antitrypsin, were isolated from plasma by sequential immunoprecipitation with anti-
human haptoglobin (Behring, Somerville, NJ) and anti-
human α₁-antitrypsin (Behring) as previously described (11). After being stained with Coomassie brilliant blue dye, the protein bands were cut out and washed several times. Fibrino-
gen was isolated as fibrin by thrombin precipitation, and very low density lipoprotein apolipoprotein B-100 (VLDL apoB-
100) was isolated by ultracentrifugation and isopropanol precipitation as previously described (11). The dried protein precipitates and gel bands were hydrolyzed in 6 M HCl at 110°C for 12 h. The amino acids released from the protein were purified by cation-exchange chromatography, and the tracer-to-tracee ratio of the protein-derived leucine was deter-
mined by negative-chemical-ionization gas chromatography-
mass spectrometry as previously described (11).

Calculations. The fractional synthesis rate (FSR) of each protein was calculated with the precursor-product equation

FSR (%/day) = \( \frac{E_{t_5} - E_{t_4}}{E_{pl}} \times \frac{2,400}{t_5 - t_4} \)

where \( E_{t_5} - E_{t_4} \) is the increase in enrichment of haptoglobin, α₁-antitrypsin, or fibrinogen-bound leucine over 4–8 h (t₄–t₅) of the infusion and \( E_{pl} \) is the plateau enrichment of apoB-100-bound leucine. In this calculation, the plateau enrichment of apoB-100-bound leucine in plasma is assumed to represent the enrichment of the intrahepatic leucine pool from which the three APPs are synthesized (11).

The intravascular absolute synthesis rate (ASR) of each APP was estimated as the product of FSR and the intravascu-
lar mass of a protein

intravascular ASR (mg·kg⁻¹·day⁻¹) = intravascular α₁-AT (or Hp or Fg) mass × FSR

where the intravascular mass of α₁-antitrypsin (α₁-AT) or haptoglobin (Hp) or fibrinogen (Fg) is the product of the plasma volume and the plasma concentration of the protein. The plasma volume of the acutely ill child with protein-energy malnutrition and that of the recovered child were based on previously published values (12, 27).

Statistical analysis. The data were analyzed by ANOVA with repeated measures by using the StatView II statistical package (Abacus Concepts, Berkeley, CA). When there were
significant differences over time, individual time points were compared with univariate post-ANOVA contrasts. Significance of difference was assumed at $P < 0.05$, and numerical data are expressed as means ± SE.

**RESULTS**

All nine subjects had evidence of infection at admission (Table 1). The mean age of the subjects was $10.1 ± 1.4$ mo. They were all severely malnourished at admission with a mean body weight of $5.0 ± 0.2$ kg and mean weight for age and height of only $55 ± 2.1$ and $75 ± 2.6\%$ of expected, respectively (Table 2). At study 2, after 9 days of antibiotic therapy, the patients' infections had cleared as determined by normalization of temperature, respiratory and pulse rates, and resolution of clinical features of the infective episode (e.g., cessation of diarrhea and absence of chest crepitations).

In the studies (Table 2), the mean body weight, weight for age, and height were $73 ± 2.5$ and $92 ± 2.3\%$, respectively (Table 2).

Plasma APP concentrations. With the exception of fibrinogen, the plasma concentrations of all five APPs in study 1 were significantly higher ($P < 0.05$) compared with the concentrations in study 2, when the infections had cleared, and with the concentrations in study 3, when the subjects were fully recovered (Tables 2 and 3). In study 2, the plasma concentrations of $\alpha_1$-antitrypsin and $\alpha_1$-acid glycoprotein were still significantly greater than the recovered values ($P < 0.05$); however, the concentrations of C-reactive protein, haptoglobin, and fibrinogen were not different from the values at recovery ($P > 0.05$; Tables 3 and 4). Unlike the other four APPs, the plasma concentration of fibrinogen was within the normal range in all three studies and did not differ in any of the studies (Table 4).

$\alpha_1$-Antitrypsin, haptoglobin, and fibrinogen synthesis rates. The tracer-to-tracee ratio of VLDL apoB-100-bound leucine reached a steady state after 4 h of the isotope infusion in all three studies (Fig. 1), and there was a linear increase in the amount of labeled leucine incorporated into plasma $\alpha_1$-antitrypsin, haptoglobin, and fibrinogen during this period of time (Fig. 1). Hence the FSRs of $\alpha_1$-antitrypsin, haptoglobin, and fibrinogen were calculated from the rate of incorporation of labeled leucine into each protein during the last 4 h of the isotope infusion.

The FSR of haptoglobin (proportion of haptoglobin pool synthesized per unit of time) was not different in any of the studies (Table 4). However, the 91% faster FSR in study 3 than in study 2 barely failed to reach statistical significance ($P = 0.08$). In study 1, the ASR of haptoglobin (i.e., amount of protein synthesized per unit of time) was significantly greater than in study 2. In study 2, when the infections had cleared, the ASR of haptoglobin was significantly slower than the study 3 value, although the haptoglobin pool sizes were similar in studies 2 and 3 (Table 4).

The $\alpha_1$-antitrypsin FSR was not different in any of the studies (Table 4). In study 1, the larger plasma pool of $\alpha_1$-antitrypsin was not associated with a higher ASR compared with the rates in studies 2 and 3. Similarly, although the $\alpha_1$-antitrypsin pool in study 2 was still significantly larger than in study 3, the ASR of $\alpha_1$-antitrypsin was not different in study 2 compared with the value in study 3 (Table 4).

There was no difference in the plasma fibrinogen concentration or the fractional or absolute synthesis rates among the three studies (Table 4).

---

Table 2. Physical characteristics of the infected children

<table>
<thead>
<tr>
<th>Physical Characteristic</th>
<th>Study 1</th>
<th>Study 2</th>
<th>Study 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mo</td>
<td>10.1 ± 1.4</td>
<td>10.2 ± 1.4</td>
<td>12.3 ± 1.4</td>
</tr>
<tr>
<td>Wt, kg</td>
<td>5.0 ± 0.2</td>
<td>4.9 ± 0.2</td>
<td>7.0 ± 0.2</td>
</tr>
<tr>
<td>Length, cm</td>
<td>64 ± 1.2</td>
<td>64 ± 1.2</td>
<td>67 ± 1.1</td>
</tr>
<tr>
<td>Wt for age, % of expected</td>
<td>55 ± 2.1</td>
<td>54 ± 2.2</td>
<td>73 ± 2.5</td>
</tr>
<tr>
<td>Wt for length, % of expected</td>
<td>75 ± 2.6</td>
<td>72 ± 2.2</td>
<td>92 ± 2.3</td>
</tr>
</tbody>
</table>

Values are means ± SE; $n = 9$. Studies 1–3, postadmission days 2, 9, and 59, respectively.
DISCUSSION

The aims of this study were to determine whether severely malnourished children can mount a general APP response to infection that includes different types of APPs and whether changes in the pool size of these proteins were achieved solely through changes in synthesis rates. The higher plasma concentrations of four of five APPs in the infected malnourished state confirm that severely malnourished children are capable of mounting an APP response, but the response does not include all of the APPs. The kinetic data demonstrate that the APP response in these children is accomplished through different mechanisms. Although the larger haptoglobin pool of the infected malnourished state was associated with an increased rate of synthesis of the protein, the larger \( \alpha_1 \)-antitrypsin pool was not associated with an increased rate of synthesis. Hence expansion of the \( \alpha_1 \)-antitrypsin pool may have occurred through a decrease in the rate of catabolism of this protein. These findings suggest that, in the severely malnourished, the APP response is not achieved solely through an increased rate of APP synthesis.

The advantage of any study of APP kinetics that uses an isotopic amino acid tracer and precursor-product analysis (as in present study) is that it permits the measurement of the rate of incorporation of the labeled amino acid precursor into the protein, hence a direct measurement of the fractional synthesis rate of the protein. A disadvantage of this method is that the kinetic information obtained is pertinent to APP within the intravascular pool only. Because the extravascular APP pool cannot be sampled, the isotopic enrichment and concentration cannot be measured in this pool. This precludes any calculation of rate of transfer of newly synthesized APP to the extravascular pool, hence calculation of the total ASR for an APP. As a consequence, studies of APP kinetics that use an isotopic amino acid tracer and precursor-product analysis are limited to calculations of intravascular APP synthesis rate as an index of the ASR.

In agreement with the earlier findings of others (3, 24), in the present study, the plasma concentrations of C-reactive protein, \( \alpha_1 \)-acid glycoprotein, haptoglobin, and \( \alpha_1 \)-antitrypsin in the infected marasmic children were markedly higher than the uninfected-malnourished and recovered values. The plasma fibrinogen concentration, however, was unaffected by the presence of infection, suggesting that marasmic children can mount an APP response to infection for most but not all APPs. At study 2, the plasma concentration of C-reactive protein had fallen to an almost undetectable level, suggesting that the concurrent infections had been cleared.

In the past, investigations of the kinetic mechanisms responsible for the APP response to injury or infection were limited to the study of fibrinogen synthesis because convenient tracer methods were not available to study the other APPs. The proposal that the increased availability of APPs was due to an increased rate of APP synthesis (5, 15) is primarily based on in vitro evidence of increased APP mRNA concentrations in rat hepatocytes after burn injury (21) or endotoxin administration (22), increased C-reactive protein production in perfused rabbit livers after injury (16), and in vivo measurements of fibrinogen synthesis rate in injured humans (25) and laboratory animals (14). In the malnourished state, however, there is no information to support the assertion that increased plasma APP concentrations in response to injury or infection are achieved through a stimulation of synthesis rate. Furthermore, whole body
protein synthesis is reduced in the infected malnourished child compared with both infected and uninfected well-nourished children (7, 26), suggesting that the malnourished child may be mounting an APP response through a mechanism other than a stimulation of synthesis rate. Our present results suggest that, in severe malnutrition, the increased plasma pool sizes of the APPs are achieved through mechanisms that involve changes in both the rate of synthesis and catabolism of these proteins.

For example, the higher plasma concentration of α1-antitrypsin in the infected malnourished state was not associated with an increased intravascular absolute synthesis rate of the protein. In fact, the amounts of α1-antitrypsin synthesized per unit of time in the infected malnourished state, the uninfected malnourished state, and the recovered state were almost identical (Table 4). This finding indicates that expansion and contraction of the α1-antitrypsin pool in response to the presence and absence of infection are achieved by a mechanism other than an increased synthesis rate. The pool size of a plasma protein is determined by the balance between its rates of synthesis and catabolism or its loss from the intravascular compartment. Hence an increase in the pool size of an APP can be achieved by one of two kinetic mechanisms: an increased rate of synthesis relative to catabolism-intravascular loss or a decreased rate of catabolism-loss relative to synthesis rate. Therefore, the most likely mechanism by which the α1-antitrypsin pool size is expanded in response to infection in the malnourished individual is through a reduction in the rate of catabolism relative to a normal rate of synthesis. Similarly, it can be argued that contraction of the pool size as the infection is cleared is due to normalization of rate of catabolism. In previous studies in infected malnourished children, we reported that the same mechanism, a decreased rate of catabolism, may be responsible for the initial expansion of the plasma pools of the negative APPs, albumin and transferrin, as the infection is cleared but the child is still malnourished (17, 18). It is possible that the ability of the severely malnourished individual to increase the availability of an APP by reducing its rate of catabolism may represent an adaptive mechanism which has the advantage of conserving the limited supply of amino acids.

On the other hand, in the infected malnourished state, when the plasma concentration of haptoglobin was higher, the rate of synthesis of haptoglobin was greater than in the uninfected malnourished state. In study 2, as the malnourished child’s infection cleared, both the plasma concentration and rate of synthesis of haptoglobin decreased by ~60%, suggesting that the haptoglobin pool of the malnourished child expanded (in response to infection) and contracted (as infection was cured) through changes in the rate of synthesis of the protein. A closer examination of the results, however, reveals that a concomitant reduction in the rate of catabolism of haptoglobin was also involved in mediating the expansion of the plasma pool. When the results of the infected malnourished state are compared with those of the recovered state, it can be seen that, despite the more than twofold expansion of the plasma haptoglobin pool in the infected malnourished state, the amount of haptoglobin synthesized is not different from the amount synthesized in the recovered state, suggesting that there had to be a concurrent reduction in the rate of catabolism of haptoglobin for its pool to expand.

Another interesting finding is that although the pools of haptoglobin are almost the same in the uninfected malnourished and recovered states, only half as much of the protein is synthesized by the malnourished child, and the pool turns over half as fast (indicated by 50% slower FSR). This finding suggests that, in the absence of infection, the severely malnourished child synthesizes far less haptoglobin but maintains a normal pool of protein by simultaneously suppressing the rate of catabolism. This is further evidence to support the argument that the malnourished are capable of increasing or maintaining normal or larger pools of APPs without stimulating the rate of synthesis.

Both the rate of synthesis and plasma concentration of fibrinogen were unaffected by the presence of an infection in the malnourished child. Typically, in response to an inflammatory episode, plasma fibrinogen concentration is markedly increased and remains elevated for several days (5). Furthermore, the rate of fibrinogen synthesis is increased 400% in rabbits inoculated with endotoxin (14), suggesting that infection does elicit a fibrinogen response. Hence the failure to find significantly higher plasma fibrinogen concentrations and rates of synthesis in the infected malnourished children was surprising and suggests that fibrinogen is not responding like a classical APP in severe malnutrition. The lack of a fibrinogen response to infection by the severely malnourished child may be related to the unique function of fibrinogen or to its relatively large pool size and fast synthesis rate. Although the lack of an increase in fibrinogen availability may not adversely affect the capacity to fight an infection, it will certainly have a detrimental effect on the ability of a malnourished individual to recover from surgery or injury because of the critical role of fibrinogen in wound healing (8). On the other hand, our finding does not rule out the possibility that malnourished patients may be able to mount a fibrinogen response to surgery.

Finally, it should be pointed out that the magnitude of the increase in plasma concentrations of the four APPs that had elevated concentrations when the malnourished children were infected was actually greater than that reported in the literature for healthy adults subjected to an infection (1) and similar to the response in infected well-nourished children (30). For example, Bostian et al. (1) reported that α1-acid glycoprotein, α1-antitrypsin, and haptoglobin increased by 150, 72, and 86%, respectively, in healthy men exposed to Salmonella typhi, and Wiedermann et al. (30) reported a 78% increase in the plasma α1-antitrypsin concentration in well-nourished children infected with Salmonella enteritidis. In comparison, in the present study, α1-acid glycoprotein, α1-antitrypsin, and haptoglobin increased by >600, 66, and 200%, respectively, in the infected malnourished children, suggesting that se-
verely malnourished individuals can mount an adequate response to infection for most APPs.

In conclusion, the findings of this study showed that marasmic children can mount an APP response to combat infection. The APP response in severely malnourished children is achieved by different mechanisms, either through a decrease in the rate of catabolism or a combination of increased APP synthesis and decreased catabolism.

We are grateful to the nursing staff of the Tropical Metabolism Research Unit, Kingston, Jamaica, for care of the children, Melanie Del Rosario and Margaret Frazer for technical support, and Leslie Loddke for editorial assistance.

This work was supported by National Institute of Child Health and Human Development Grant ROI-HD-34224-01A1, grants from the International Atomic Energy Agency and The Wellcome Trust, and federal funds from the US Dept. of Agriculture (USDA)/Agricultural Research Service under Cooperative Agreement 58-6250-1-003.

This work was presented in part at the Experimental Biology 1997 Meeting, New Orleans, LA, 6–9 April 1997, and was published in abstract form (Jahoor et al. Magnitude of the acute-phase protein response to infection in protein-energy malnourished children. FASEB J 11: 2105, 1997).

This is a publication of the USDA/Agricultural Research Service and Children’s Nutrition Research Center, Dept. of Pediatrics, Baylor College of Medicine and Texas Children’s Hospital, Houston, TX. The contents of this publication do not necessarily reflect the views or policies of the USDA nor does mention of tradenames, commercial products, or organizations imply endorsement by the US Government.

Address for reprint requests: F. Jahoor, Children’s Nutrition Research Center, Dept. of Pediatrics, Baylor College of Medicine, 1100 Bates St., Houston, TX 77030-2600.

Received 5 December 1997; accepted in final form 10 March 1998.

REFERENCES


