Use of Lipid Calories During Pneumococcal Sepsis in the Rhesus Monkey

ROBERT W. WANNEMACHER, JR., PH.D., MITCHELL V. KAMINSKI, JR., M.D.,* RICHARD E. DINTERMAN, B.S., AND THOMAS R. MCABEE, D.V.M.

From the United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland

ABSTRACT. A number of studies in patients and monkeys have suggested that intravenous infusion of lipids can be utilized as a major source of calories during sepsis and/or trauma. Others, however, have observed that lipid calories were not as effective as glucose calories in sparing body protein in severely septic and/or burned patients. In trying to resolve this controversy, a parenteral nutrition model was utilized during pneumococcal sepsis in the rhesus monkey. Earlier studies indicated that when monkeys were infused with 0.55 grams amino acid nitrogen and 13 kilocalories per kilogram per day, they lost approximately 13% of their body protein as a result of the clinical illness associated with pneumococcal sepsis. Addition of 85 kilocalories per kilogram per day of either dextrose or lipid emulsion (Intralipid) effectively prevented this infection-related loss of body protein. The present study was designed to determine whether the elevated plasma insulin concentrations associated with glucose infusions are necessary for protein sparing or would interfere with utilization of lipid calories. In this study, septic and control monkeys were infused with a hypocaloric amino acid-dextrose solution to which was added lipid emulsion. If septic monkeys were totally maintained on a hypocaloric infusion of amino acids and 32 kilocalories per kilogram per day from dextrose, they lost 6.8 ± 1.0% of their body protein over the 6-day experimental period. Addition of lipid calories (55 kilocalories per kilogram per day) to this mixture resulted in a gain of 1.1 ± 0.7% of body protein. In both experimental groups plasma insulin was approximately 100 to 200 microunits per milliliter, which resulted in a marked inhibition of ketogenesis and lipolysis. Despite elevated plasma insulin, the septic monkey appeared able to utilize the fatty acids from the infused lipid effectively as a calorie source to spare body protein. Since lipid emulsion contains approximately 13% glycerol calories, septic monkeys were infused with a hypocaloric amino acid-dextrose solution and an amount of glycerol calories equivalent to that found in Intralipid. Under these conditions, the loss of body protein was very similar to that seen when the hypocaloric amino acid-dextrose solution alone was infused in the septic monkey. Thus, the protein-sparing effects of the lipid emulsion are not related to its glycerol content.

As shown previously in a monkey model, an intravenous infusion of amino acids (AA) and calories will prevent protein wasting associated with pneumococcal sepsis. In this model, lipid calories were utilized at only slightly reduced efficiency when compared to an isocaloric infusion of dextrose. A number of investigators have observed that lipid calories in combination with AA will prevent weight loss and nitrogen (N) wasting in patients with combined sepsis and trauma. When traumatized patients were switched from a parenteral nutrition (PN) regimen that supplied the nonprotein calories effective in treating starvation but not catabolic response, the greater protein-sparing effects of dextrose as compared to fat when infused IV in severely catabolic patients is related to the insulin response excited by the former caloric substrate. In contrast, Long et al have observed increased protein-sparing effects of intravenous (IV) dextrose as compared to IV lipid in burned patients who were septic. IV infusion of dextrose calories was slightly better than lipid in traumatized patients who were highly catabolic. Several hypotheses have been advanced to explain the apparent controversial value of the use of IV lipid calories in critically ill patients; these include the following: (1) suggestion that glycerol content of the fat emulsion was the major caloric source utilized by burned patients; (2) that the greater protein-sparing effects of dextrose as compared to fat when infused IV in severely catabolic patients is related to the insulin response excited by the former caloric substrate; (3) that lipid calories are effective in treating starvation but not catabolic response to injury. To test these hypotheses, a PN model in Rhesus monkey was utilized to evaluate the efficiency of lipid calories during pneumococcal sepsis. In this model protein-wasting is associated with the catabolic effects of the infectious disease and not simple starvation. In the present study, a combined dextrose and lipid infusion was utilized to evaluate the effects on protein wasting in control and infected monkeys. This involved the infusion of a basal hypocaloric AA-dextrose solution with and without added lipid calories. To assess the contribution of the glycerol calories in the lipid emulsion, an equivalent amount of glycerol was added to the basal hypocaloric AA-dextrose solution and infused IV to control and infected monkeys.

METHODS

Thirty male Rhesus monkeys (Macaca mulatta), weighing 3.5 to 5.5 kg were adapted to chair-restraint and had indwelling catheters placed in the internal jugular
It was demonstrated that an automated procedure was needed to homogenize the samples. Fecal samples were frozen at -20°C for analysis of total glucose, protein, and ketones. Volumes were recorded; a specimen was analyzed for each monkey as described previously.

For sequential analysis within a group, data was analyzed by paired one-way analysis of variance. Comparison between group was analyzed by unpaired one-way analysis of variance. A p value less than 0.05 was considered significant under the null hypothesis.

**RESULTS**

Infusion with hypocaloric dextrose plus AA solution resulted in a marked increase in plasma insulin to approximately 200 μU/ml (Fig. 1). The addition of 55 kcal/kg/day of lipid emulsion to the hypocaloric AA-dextrose solution in control monkeys resulted in a markedly positive N balance during the 6-day experimental period (Fig. 2). In the septic monkeys, urinary N excretion was significantly increased when compared to controls. However, the monkeys tended to remain in N equilibrium or in slightly positive balance during pneumococcal sepsis. Plasma insulin concentrations were increased to approximately 200 μU/ml throughout the 6-day experimental period in both control and septic monkeys.

Since glycerol represents about 13% of the caloric value of the fat emulsion, an equivalent amount of this substrate was added to the hypocaloric AA-dextrose solution. When this mixture was infused into septic monkeys, urinary N excretion was not statistically different, by unpaired analysis of variance, from that observed when septic monkeys were infused with only the AA and 32 kcal/kg/day of dextrose (Fig. 3). Thus, the glycerol content of the fat emulsion did not appear to account for the protein-sparing action in the septic monkey.

Cumulative N balance over the 6-day period for the control and septic monkeys receiving these three different nutritional support infusions are summarized in Figure 4. These data emphasize the observations that this infection in the monkey stimulated an increased wasting of body protein. The addition of lipid calories to the hypocaloric AA-dextrose mixture reduced the wasting of body protein, but the monkeys accumulated less body protein that their noninfected controls.

By assuming that a monkey contains 15% protein which is 16% N, it was calculated that septic monkeys lost approximately 13% of their body protein if infused with 13 kcal/kg/day of AA alone (Table II). The addition of 32 kcal/kg/day dextrose significantly reduced this loss to 6.8% of their body protein. If 85 kcal/kg/day dextrose or lipid emulsion were infused together with 13 kcal/kg/day AA, protein-wasting was essentially prevented in septic monkeys. Further, the addition of lipid calories to the hypocaloric dextrose- AA mixture effectively spared body protein during pneumococcal sepsis, but the addition of only the equivalent amount of glycerol calories contained in the lipid source had no significant effect on the rate of loss of body protein (Table II).

When infused with 13 kcal/kg/day AA alone, control monkeys lost approximately 2% of their body protein.
over the 6-day experimental period (Table II). The addition of 32 kcal/kg dextrose to the AA mixture essentially maintained control monkeys in N equilibrium (Table II). The addition of more dextrose or lipid calories did not significantly improve N retention in the control monkeys. Thus, a chaired nonseptic monkey was maintained in N equilibrium with approximately 45 kcal/kg/day (32 from dextrose, 13 from AA), while the septic monkey, during febrile illness, was in slight negative balance when infused with approximately 100 kcal/kg/day.

The infusion of 32 kcal/kg/day dextrose resulted in only a slight increase in plasma glucose (Fig. 5). Plasma urea N was not significantly altered by the AA infusion with various caloric substrates (Fig. 5). The infusion of the hypocaloric AA-dextrose solution resulted in a significant decrease in plasma triglycerides, cholesterol, β-hydroxybutyrate, and free fatty acids (Fig. 5). The addition of lipid calories to this solution resulted in a significant increase in plasma triglycerides, cholesterol, and free fatty acids, but not in the β-hydroxybutyrate. Similar effects of dextrose or lipid calories were observed in both septic and control monkeys.

**DISCUSSION**

In the monkey model utilized in these studies, pneumococcal sepsis resulted in a marked catabolic response.
when compared to findings in noninfected controls. The protein wasting associated with this infectious disease could be prevented by adequate infusion of AA and calories from either dextrose or lipid emulsion. These results are in agreement with others that lipid calories can be utilized by the injured host to prevent wasting of body protein. In contrast, Long et al. observed that critically ill or severely burned patients were unable to utilize lipids for protein sparing. Similar reduced efficiency of utilization of lipid calories by critically ill patients has been suggested by others. McDougal et al. have postulated that the glycerol content of the fat emulsion was the only major calorie source utilized by burned patients. When septic monkeys were infused with a hypocaloric solution of dextrose and AA plus the amount of glycerol found in the lipid emulsion, protein wasting in septic monkeys was similar to that observed with AA-dextrose solution alone. Thus, in this model the protein-sparing effects of lipid emulsion were not related to its glycerol content.

It has also been argued that dextrose calories are more efficiently utilized than lipid calories because of the insulin response elucidated by infusing high concentrations of glucose. When septic or control monkeys were infused with the 32 kcal/kg/day dextrose and AA with or without lipid emulsion, plasma insulin concentrations were increased 10 to 20-fold over those observed in fasted monkeys. There appeared to be no significant difference in protein-sparing effects of an isocaloric mixture of AA plus dextrose and lipid emulsion or AA plus dextrose.

In an earlier study, infusion of AA plus lipid emulsion alone was slightly less than an isocaloric amount of dextrose plus AA in sparing body protein in septic monkey. While the difference between the two dietary regimes was not statistically significant, it did represent 2.9% body protein, which could be important from a physiologic point of view. Since the reduced protein-sparing effects of the lipid emulsion infusion was associated with a lower plasma insulin concentration, it could be argued that elevated plasma insulin concentrations...
Table II
Per cent change in body protein during 6 day experiment

<table>
<thead>
<tr>
<th>Diet</th>
<th>%</th>
</tr>
</thead>
</table>
| *1 Amino acids (0.55 g N and 13 cal/kg/day)                          | -12.8 ± 2.3
| *2 Amino acids + dextrose (85 cal/kg/day)                            | 1.1 ± 0.3
| 3 Amino acids + dextrose (32 cal/kg/day)                             | -6.8 ± 1.0
| *4 Amino acids + lipid (85 cal/kg/day)                               | -1.8 ± 0.5
| 5 Amino acids + dextrose (32 cal/kg/day) + lipid (85 cal/kg/day)     | 1.1 ± 0.7
| 6 Amino acids + dextrose (32 cal/kg/day) + dextrose (7.2 cal/kg/day)| -6.5 ± 0.5
| Heat-killed control                                                  | -2.3 ± 0.7
| *7 Amino acids                                                       | 2.5 ± 0.6
| *8 Amino acids + dextrose (85 cal/kg/day)                             | -0.2 ± 1.4
| *9 Amino acids + dextrose (32 cal/kg/day)                             | 1.1 ± 0.4
| *10 Amino acids + lipid (85 cal/kg/day)                              | 4.1 ± 0.7
| *11 Amino acids + lipid (55 cal/kg/day) + dextrose (32 cal/kg/day)   | 0.2 ± 0.3
| *12 Amino acids + dextrose (32 cal/kg/day) + dextrose (9.4 cal/kg/day)| 0.2 ± 0.3

* p < 0.05 compared to group 1.
# p < 0.05 compared to group 2.
" p < 0.05 compared to group 3.
" p < 0.05 compared to group 4.
| p < 0.05 compared to group 5.
| p < 0.05 compared to group 6.
| p < 0.05 compared to group 7.
| p < 0.05 compared to group 8.
| p < 0.05 compared to group 9.
| p < 0.05 compared to group 10.
| p < 0.05 compared to group 11.
| p < 0.05 compared to group 12.

The data for groups 1, 2, 4, 7, 8, and 10 from Wannemacher et al.¹

Fig. 5. Sequential changes in plasma glucose, urea N, triglycerides, cholesterol, β-hydroxybutyrate, or free fatty acids of septic monkeys infused with either basal AA solution (■) or basal solution plus lipid emulsion (□). Zero time values are from monkeys fasted for 24 hr. Each value is the mean for six monkeys and the vertical lines are SE of means that are significant (p < 0.05) different for fasting values.

Plasma cholesterol was progressively increased in the monkeys infused with the lipid, dextrose, and AA. Similar increases in plasma cholesterol have been observed in patients and monkeys infused with AA plus lipid or AA plus lipid and glucose solution.¹ The higher plasma

tend to reduce the wasting of body protein. Thus, during pneumococcal sepsis in monkeys, high plasma insulin concentration did not prevent the utilization of lipid calories and may have been beneficial in preventing wasting of body protein.
cholesterol value and elevated free fatty acids support the concept that the infused long-chain triglycerides are being utilized in metabolic processes by both septic and control monkeys. Further, this utilization of infused lipid appears to take place in the presence or absence of high plasma insulin concentrations. The high plasma insulin concentrations, however, blocked the ketone body production from the long-chain fatty acids. These observations are in agreement with the postulate that insulin blocks hepatic production of ketones.

REFERENCES