Correlation between Intravenously Supplied Energy Level and Zinc Metabolism in Laparotomized Rats

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Summary We investigated the relationship between intravenous energy loading and zinc status in laparotomized rats. One of three test solutions consisting of 3% amino acid, the same amount of electrolytes (excluding zinc) and different concentrations of glucose were infused through the jugular vein for 5 d. The total energy was 109, 191 and 273 kcal/kg/d, respectively. Significantly positive correlations were observed between infusion energy and rat body weight changes (% of initial value) and between infusion energy and cumulative nitrogen balance. Regarding the zinc status, a negative correlation was found between infusion energy and plasma zinc concentration, and a positive correlation was observed between infusion energy and urinary zinc excretion. There was no significant relationship between infusion energy and hepatic zinc content. These results indicate that the zinc requirement might be increased when infusion energy is elevated and the nutritional status is improved. Zinc supplementation in the post-operative period should be considered in light of not only catabolism but also anabolism. Anabolism may be more important than catabolism in regard to zinc metabolism under relatively mild stress.

Key Words zinc, energy, parenteral nutrition, laparotomy, rat

Zinc deficiency in patients receiving total parenteral nutrition (TPN) is well known. The earliest observation of this condition was reported in the 1970s (1-4). The topical symptom of zinc deficiency is skin lesions which are improved rapidly by the administration of zinc. The causes of zinc deficiency derived from the infusion solution administered as part of TPN are thought to be the amino acid (5), sugar-amine compounds (6, 7) and low zinc content in the TPN solution (8). Urinary zinc excretion is stimulated by the infusion of amino acid. The mechanism of the increase of urinary zinc excretion caused by the infusion of sugar-amine compounds has been suggested to be the chelating action of those compounds (6, 7). Other important factors are associated with a patient’s condition (e.g., catabolism). In
trauma patients and middle-to-severe surgical patients, the plasma zinc concentration was shown to be decreased (9-11) and the zinc excretion in their urine increased (11-13). However, a decrease in plasma zinc was also observed in the anabolic phase of patients undergoing TPN (1, 2). These findings suggest that the zinc requirement is influenced by several factors in post-operative patients.

Peripheral parenteral nutrition (PPN) has recently been recognized as a useful alternative to TPN because of its safety, simplicity and cost benefits. The use of PPN also avoids some of the disadvantages of TPN (e.g., the complexity of the technique and the risk of catheter-related sepsis) (14, 15). Although PPN is a beneficial technique, the infusion energy level provided by PPN is limited. If the osmotic pressure of the solution exceeds 800 to 1,000 mOsm/L, thrombophlebitis becomes a serious problem (16-18). For this reason, the infusion energy level in PPN is relatively lower than that in TPN. The zinc requirement of patients receiving TPN has been studied extensively, while the zinc requirement of patients receiving PPN has not yet been studied in detail. Furthermore, the relationship between infusion energy level and zinc metabolism under a parenteral nutritional regimen is not clear.

In this study, we examined the effects of the infusion energy level on body weight, plasma and hepatic zinc status, and urinary zinc excretion in laparotomized rats receiving parenteral nutrition.

**MATERIALS AND METHODS**

*Animals and test solutions.* Male Sprague-Dawley rats weighing 160–180 g were purchased from Charles River Japan (Shiga, Japan). The animals were maintained in a restricted access room with controlled temperature (23±2°C), humidity (55±15%) and lighting (light on 07:00–19:00 h), and fed a commercial diet (CRF-1; Oriental Yeast, Tokyo, Japan).

The three infusion test solutions were prepared with a commercial 50% glucose solution, branched chain amino acid-rich amino acid solution (Aminic; Roussel Morishita, Osaka, Japan), commercial vitamin mixture and injectable water. The electrolyte solution was prepared in our laboratory. The sodium dihydrogenphosphate solution was separated from the other solutions to avoid crystallization with calcium when all of these solutions were autoclaved. Trace elements were not included in this study. Each solution was mixed aseptically prior to use. The glucose concentrations were 7, 14.5 and 22% of the low, middle and high glucose solutions, respectively. The compositions of the test solutions are shown in Table 1.

*Experimental design.* We divided the rats into 4 groups: the Low (109 kcal/kg/d), Middle (191 kcal/kg/d) and High (273 kcal/kg/d) groups (n=5–6), and a control group (n=5). After overnight fasting, all of the rats in the three infusion groups underwent laparotomy (with a 3 cm abdominal incision and suture) and jugular vein cannulation under pentobarbital anesthesia. After the operation, the animals were placed individually in metabolic cages and infused with the solutions.
Intravenously Supplied Energy Level and Zinc Metabolism

Table 1. Compositions of test solutions.

<table>
<thead>
<tr>
<th>Composition</th>
<th>Low</th>
<th>Middle</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amino acid (g/L)</td>
<td>30</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Glucose (g/L)</td>
<td>70</td>
<td>145</td>
<td>220</td>
</tr>
<tr>
<td>Electrolytes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium (mEq/L)</td>
<td>40</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Potassium (mEq/L)</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Chloride (mEq/L)</td>
<td>40</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Calcium (mEq/L)</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Phosphate (mm)</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Magnesium (mEq/L)</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Total energy (kcal/L)</td>
<td>400</td>
<td>700</td>
<td>1,000</td>
</tr>
</tbody>
</table>

for 5 d. The daily dose of the solutions was 1.25 mL/h until post-operative day 1; thereafter, the dose was 2.50 mL/h. The control rats were supplied with CRF-1 and tap water ad libitum throughout the experimental period. In the infusion groups, urine was collected every day.

After the completion of 5-d infusion, all rats were anesthetized using pentobarbital, exsanguinated and their livers were removed.

This animal experiment was approved by the committee overseeing the care and use of animals in our institution.

Measurements. Plasma and urinary zinc were measured with a flame atomic absorption spectrophotometer (Model 180-80, Hitachi, Tokyo, Japan). Hepatic zinc was measured by the same method after slowly wet digesting with concentrated HNO₃ and 60% HClO₄.

The total protein, albumin and blood urea nitrogen (BUN) concentrations, alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) activity were analyzed with an automatic analyzer (Model 7150, Hitachi). Urinary nitrogen was measured with an analyzer (Yanako CN Corder; Yanagimoto Seisakusho, Kyoto, Japan).

Statistical analysis. Data are presented as means±SE. In the infusion groups, significance in the biochemical findings was determined by analysis of variance and Tukey’s multiple comparison test. Pearson’s correlation coefficient was used to examine the relationships between the infusion energy level and other parameters. The statistical analyses were performed by the software program Toukei Library ver. 4 (Yukumus, Tokyo, Japan). The level of significance was set at p < 0.05.

RESULTS

Significant positive correlations revealed between the infusion energy level and both body weight change and cumulative nitrogen balance over the 5 d are
Fig. 1. Correlations between the infusion energy level and body weight change, and between infusion energy level and nitrogen balance for 5d in laparotomized rats (n = 5–6).

Fig. 2. Correlations between the infusion energy level and plasma zinc concentration, hepatic zinc content, and urinary zinc excretion in laparotomized rats (n = 5–6). NS: not significant.

shown in Fig. 1. Although both the body weight and nitrogen balance were changed negatively in the Low group, they improved depending on the infusion energy level.

The correlation between infusion energy level and zinc status is shown in Fig. 2. The plasma zinc concentration decreased depending on the increase of infusion energy; significant negative correlation was observed. In contrast, urinary zinc excretion was significantly increased depending on the increase of infusion energy. The hepatic zinc content was not influenced by the infusion energy level (Fig. 2).

The plasma biochemical findings are shown in Table 2. The total protein and albumin concentrations were not changed in the infusion groups. The BUN concentration increased depending on the infusion energy level, and significant differences were observed among the groups. The BUN level in the Low group was higher than that in the control group. There were no differences among the infusion groups regarding ALP and LDH, which are zinc metallo-enzymes.
Table 2. Plasma biochemical findings after infusion of test solutions in laparotomized rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Total protein (g/100 mL)</th>
<th>Albumin (g/100 mL)</th>
<th>BUN (mg/100 mL)</th>
<th>ALP (IU/L)</th>
<th>LDH (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>5</td>
<td>4.8 ± 0.1</td>
<td>2.2 ± 0.1</td>
<td>16.2 ± 0.5</td>
<td>256 ± 24</td>
<td>118 ± 17</td>
</tr>
<tr>
<td>Middle</td>
<td>6</td>
<td>4.9 ± 0.0</td>
<td>2.2 ± 0.0</td>
<td>10.3 ± 0.4</td>
<td>288 ± 30</td>
<td>120 ± 15</td>
</tr>
<tr>
<td>High</td>
<td>5</td>
<td>4.8 ± 0.1</td>
<td>2.2 ± 0.1</td>
<td>7.3 ± 0.4</td>
<td>296 ± 14</td>
<td>129 ± 28</td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
<td>5.4 ± 0.1</td>
<td>2.5 ± 0.0</td>
<td>15.5 ± 0.9</td>
<td>774 ± 56</td>
<td>139 ± 29</td>
</tr>
</tbody>
</table>

Values are means ± SE. **p < 0.01.

DISCUSSION

In this experiment, the infusion energy level was divided into three categories (i.e., 109, 191 and 273 kcal/kg/d). Regarding the energy requirement for parenteral nutrition in rats, Martins et al (19) showed that the energy level of 270 kcal/kg/d was suboptimal for normal rats, and that rats receiving nutrition at the energy level of 350 kcal/kg/d gained body weight more rapidly than rats at the level of 270 kcal/kg/d. Chang and Silvis (20) reported that glucose infusion into rats in excess of 300 kcal/kg/d uniformly produced gross morphological fatty liver. From these observations, the 273 kcal/kg/d (High group) energy level used in this study was reasonable, and positive body weight changes and nitrogen balance were obtained in the High group. We therefore considered that the High group was in the anabolic phase. Conversely, negative body weight changes and an almost zero nitrogen balance were observed in the Low group (109 kcal/kg/d). In addition, the BUN level was increased when the infusion energy level was reduced. These results suggest that catabolism took place in the Low group; thus, a positive correlation was observed between infusion energy level and body weight change or nitrogen balance.

The plasma zinc concentration decreased and urinary zinc excretion increased depending on the energy intake in this study. It has been observed that the plasma zinc concentration decreased (9–11) and urinary zinc excretion increased (11–13) in surgical patients in the catabolic phase. However, we found that the plasma zinc concentration was relatively higher and the urinary zinc excretion relatively lower in the Low group (catabolic status) as compared to the High group (anabolic status). Although the zinc metabolism was observed to be influenced by amino acid infusion (5), the concentration in the solution used in that study was the same as that employed in our experiment. Kay et al (1, 2) suggested that zinc has an important role in the anabolic phase, and that its requirement increases and plasma zinc...
decreases during growth. Williams and Chesters (21) suggested that zinc is related to DNA and protein metabolisms. These metabolisms seem to be activated by growth, and the zinc requirement would thus be expected to increase during this period. Our present findings using laparotomized rats (3-cm abdominal incision and suture) suggest that the decrease in plasma zinc would be more influenced by anabolism than by catabolism in post-operative animals. In regards to the infusion energy level, the zinc requirement in PPN is thought to be relatively lower than that in TPN. The hepatic zinc content and plasma zinc metallo-enzyme activity were not significantly different among the infusion groups in this study. Van Rij et al (22) showed that a redistribution of body zinc occurred and the hepatic zinc content increased after trauma. The degree of trauma was severer (their model was 20% burned rats) and the infusion period was longer (7d) in their experiment than in our experiment. This may explain why we did not find any changes in hepatic zinc content and plasma zinc metallo-enzyme activity.

Based on our results and those of the above-mentioned investigations, we conclude that the zinc requirement under parenteral nutrition is affected by several factors. The requirement of zinc increases when the infused energy level is increased and the nutritional status is improved. Although the zinc requirement in PPN is relatively lower than that in TPN, zinc metabolism is probably affected by conflicting factors (i.e., those of anabolism and catabolism). In conclusion, zinc supplementation to post-operative patients should be considered not only catabolic but also anabolic. The zinc requirement seems to be influenced by the two aspects. Under relatively mild stress, anabolism would be more important than catabolism.

REFERENCES


