NOS inhibitors may not alter systemic NO homeostasis and inflammatory response but may facilitate the production of arginine-associated amino acids and nitrogen excretion in cases of subacute peritonitis.

Key words  
\( N^\text{G}-\text{nitro-}l\text{-arginine methyl ester; arginine; subacute peritonitis; parenteral nutrition; nitric oxide} \)

Nitrergic nerve fibers and their functional responses are associated with inflammation. In stressful conditions, especially in inflammation, nitric oxide (NO) production is increased, and NO-releasing agents reduce blood pressure in rats with peritonitis. These agents may reduce NO overproduction that occurs during peritonitis, a condition that is accompanied by arginine deficiency. However, the variations in the disease severity and the dosage, route, and period of NOS inhibitor administration are debatable. Therefore, we investigated the dose effects of chronically infused NOS inhibitor, \( N^\text{G}-\text{nitro-}l\text{-arginine methyl ester (L-NAME)} \), on the anabolism, inflammatory responses, and arginine metabolism in parenterally fed rats with cecal puncture-induced subacute peritonitis. Male Wistar rats were divided into 4 groups and were administered total parenteral nutrition solutions with 0, 5 (low dose), 25 (medium dose), or 50 (high dose) mg \( \text{kg}^{-1}\cdot\text{d}^{-1} \) of L-NAME for 7 d. Sham-operated rats administered total parenteral nutrition solution and normal healthy rats fed chow diet were also included. Our results showed that parenteral infusion significantly decreased body weight gain and plasma citrulline concentrations. In rats with subacute peritonitis, the parenteral infusion-induced increases in circulating white blood cells and NO were significantly decreased, whereas the decrease in serum albumin levels was significantly increased. Rats with subacute peritonitis that were administered chronic infusion of L-NAME had a significantly reduced nitrogen balance. In addition, rats administered the medium dose of L-NAME had significantly increased plasma arginine, ornithine, glutamate, and proline. In conclusion, chronic infusion of NOS inhibitors may not alter systemic NO homeostasis and inflammatory response but may facilitate the production of arginine-associated amino acids and nitrogen excretion in cases of subacute peritonitis.

Patients with peritonitis develop arginine deficiency due to decreased consumption, impaired absorption, and increased utilization of arginine. Arginine, a precursor for the synthesis of nitric oxide (NO), urea, creatinine, glutamate, proline, and polyamines, is a conditionally essential amino acid in stressed conditions, especially in inflammation. Green et al. was the first research group to show that nitrate/nitrite concentrations and the severity of infection are closely correlated. Subsequent studies showed that patients with peritonitis have uncontrolled activation of inducible nitric oxide synthase (NOS), which results in NO overproduction and the following sepsis. Therefore, inhibition of NOS might be a useful strategy to treat arginine deficiency and inhibit excess NO production.

In animal and human studies, the results of NOS inhibition in inflammation have been ambiguous. For example, \( N^\text{G}-\text{nitro-}l\text{-arginine methyl ester (L-NAME)} \), a nonselective NOS inhibitor which inhibits both constitutive and inducible NOS, effectively ameliorated the inflammatory lesions in the skin of zinc-deficient rats, improved the myocardial function in sepsis, and attenuated the lipopolysaccharide-induced peritoneal permeability and NO release in mice. On the contrary, some studies have shown that L-NAME may cause hypertension, augment the expressions of interferon (IFN)-\( \gamma \) and interleukin (IL)-2, and result in markedly severe disease in rats with T cell-dependent autoimmune interstitial nephritis. The adverse effects of NOS inhibitors have also been observed in subjects with hemodynamic instability and sepsis. Evidence suggests that the therapeutic effects of NOS inhibitors may depend on the dose, timing, and the severity and category of diseases.

In clinical practice, a number of in hospital patients with subacute peritonitis developed chronic malnutrition and inflammation. Administration of total parenteral nutrition solution is an efficient method to supply all daily nutrition requirements; however, it is associated with occurrence of increased complicated infections. Zheng et al. indicated that long-term parenteral nutrition-induced liver steatosis may be attenuated by L-NAME administration. Up to now, only few reports have described the long-term application of L-NAME for subacute peritonitis. Additionally, no study has ever addressed the effects of L-NAME on the inflammatory response and amino acid metabolism in subjects administered parenteral nutrition. In our study, we used a rat model with subacute peritonitis induced by cecal puncture and administered total parenteral nutrition solution to provide adequate nutrition, and we investigated the dose effects of parenterally supplemented L-NAME on the anabolism, inflammatory response, and arginine-derived metabolites such as the intermediates of the urea cycle, circulating profile of amino acids, NO, and cytokines.
room maintained at 22 °C on a 12 : 12-h light–dark cycle. The animals were acclimatized to the animal facility for 7 d before surgery. The animal facilities and protocols were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Changhua Christian Hospital, Changhua, Taiwan.

After fasting overnight, the animals were anesthetized by intramuscular injection with ketamine (100 mg) and xylazine (10 mg per kg of body weight). Subsequently, the animals underwent 2 surgeries (day 0): (1) the placement of catheters in the superior vena cava through the external jugular vein for infusing the total parenteral nutrition solution and (2) a modified cecal puncture procedure for inducing subacute peritonitis. In brief, following the midline incision of the abdomen, the cecum was exposed and punctured through both sides with an 18G needle without causing blood vessel damage. A small amount of fecal material, which was approximately the size of a rice grain, was extruded from each puncture site, and the cecum was placed back into the peritoneum. The abdomen was closed with a 3-0 chromic gut suture for the muscle layer and surgical staples for the skin. The study design was as follows:

R group: Healthy rats without surgical operation and with free access to water and chow.

TPN group: Rats received sham abdominal operations and total parenteral nutrition solution.

CPP group: Rats with subacute peritonitis and received 0 mg · kg⁻¹ · d⁻¹ of L-NAME in the total parenteral nutrition solutions for 7 d.

LNA group: Rats with subacute peritonitis and received 5 mg · kg⁻¹ · d⁻¹ of L-NAME (low dose) in the total parenteral nutrition solutions for 7 d.

MNA group: Rats with subacute peritonitis and received 25 mg · kg⁻¹ · d⁻¹ of L-NAME (medium dose) in the total parenteral nutrition solutions for 7 d.

HNA group: Rats with subacute peritonitis and received 50 mg · kg⁻¹ · d⁻¹ of L-NAME (high dose) in the total parenteral nutrition solutions for 7 d.

After surgery, the infusion of total parenteral nutrition solution was initiated and water was provided ad libitum. During the experimental period, total parenteral nutrition solution provided the sole source of nutrition, and the infusion rate of the solution was gradually increased from 25 kcal on day 0 to about 65 kcal (270 kcal · kg⁻¹ · d⁻¹) on day 6, thereby providing adequate parenteral energy and nutrients for healthy rats of this size. The final sample size was 11 to 12 rats per parenterally fed group with 1 animal lost in the TPN, CPP, and MNA groups because of the misplacement of intravenous infusion line mostly found on day 0 to day 2.

**Composition of Total Parenteral Nutrition Solutions**

Total parenteral nutrition solutions were prepared aseptically with crystalline amino acids, dextrose, lipid emulsion, vitamins, trace elements, and electrolytes. Each liter of total parenteral nutrition solution had 42 g of amino acids (Aminosyn 10%), 160 g of dextrose (Parent-aid 50%, Taita No. V, Taiwan), and 34.6 g of lipid emulsion (Fresenius AG, D-6380 Bad Homburg v.d.H. Fed. Rep. of Germany). The amounts of arginine, proline, glutamate, ornithine, and citrulline were 3.97, 3.48, 0.0, and 0 g in per liter of solutions, respectively. The freshly prepared L-NAME solution was filtered through a 0.22 μm disposable sterile filtration apparatus and added into the dextrose-amino acid admixture before mixing with the lipid emulsion daily.

**Analytical Measurements**

During the experimental period, the body weights were recorded and daily urine samples were collected into urine cups containing HCl to maintain the pH at less than 2 and then stored at 4 °C before determination of nitrogen concentration by micro-Kjeldahl analysis. The amount of total parenteral nutrition infusion and nitrogen intake were observed daily. Nitrogen balance was calculated from the nitrogen intake and urinary nitrogen excretion.

Seven days after the surgery, the animals were euthanized after being anesthetized with ketamine (150 mg · kg⁻¹) and xylazine (15 mg · kg⁻¹). Blood samples were collected by cardiac puncture and separated into whole blood, serum, and plasma for further analysis. The liver, heart, lungs, kidneys, spleen, small intestine, and gastrocnemius muscles of each rat were dissected, weighed, and put into liquid nitrogen immediately. The carcasses were stored at −20 °C until analysis of carcass composition, including water, protein, and fat.

Complete blood counts, including numbers of circulating white blood cells (WBC), red blood cells (RBC), and platelets, concentrations of hemoglobin, and percentages of hematocrit were determined using a hematology analyzer (GEN, Coulter Inc., FL, U.S.A.). Serum concentrations of albumin, glucose, triglyceride, glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), blood urea nitrogen (BUN), and creatinine were measured using an automatic analyzer (Hitachi 747, Tokyo, Japan). Commercially available enzyme-linked immunosorbant assay (ELISA) kits were used to measure serum concentrations of insulin (Mercoda AB, Uppsala, Sweden) and tumor necrosis factor (TNF)-α (DuoSet, R&D System, Minneapolis, MN, U.S.A.). Serum concentrations of nitric oxide (NO, represented by nitrite and nitrate) were measured using a colorimetric assay kit (lactate dehydrogenase method, Cayman Chemical, MI, U.S.A.). The plasma amino acid profiles, including glutamate, arginine, proline, ornithine, and citrulline, were analyzed using reversed-phase HPLC. All samples for the ELISA and colorimetric assays were analyzed in one assay batch in duplicate. The inter-assay coefficients of variance were 5—10%.

**Statistical Analysis**

The data were analyzed using one-way analysis of variance (ANOVA) by using the SAS general linear models program. The values were expressed as mean ± S.E.M. Group means were considered to be significantly different at p values less than 0.05, as determined by the protective least-significant difference (LSD) technique when the ANOVA analysis indicated an overall significant treatment effect (p<0.05).

**RESULTS**

The daily body weight and body weight gain (day 0 to day 7) are shown in Fig. 1. Animals with parenteral nutrition, i.e., the TPN, CPP, LNA, MNA, and HNA groups, weighed significantly less on day 6 and day 7 and gained lesser weight in 7 d (insert of Fig. 1) than animals that were fed orally, i.e., the R group. The body weight, weight gain, or carcass weight (data not shown) were not significantly different among the groups with subacute peritonitis. The absolute amount and percentage of water in the carcass of parenterally fed rats...
were significantly lower than those of the rats in the R group (Table 1). The MNA group had significantly lower percentages of carcass water, and the CPP, MNA, and HNA groups had significantly lower amount and percentage of carcass protein than the TPN group. In addition, the administration of L-NAME significantly decreased the higher fat percentages induced by subacute peritonitis.

The daily nitrogen intakes were not significantly different among the parenterally fed groups because an approximately equal amount, that is, 60—63 ml per day, of total parenteral nutrition solution was infused. However, urinary nitrogen excretion in the MNA group was significantly higher than that of the TPN and CPP groups (Fig. 2A). After subtracting urinary nitrogen excretion from intake, the nitrogen balance in the LNA, MNA, and HNA groups was significantly lower than that in the TPN and CPP groups (Fig. 2B).

The relative weights of the liver, heart, kidneys, spleen, gastrocnemius muscle, and small intestine are shown in Table 2. Parenterally fed rats, that is, the TPN, CPP, LNA, MNA, and HNA groups had significantly higher spleen weights and lower small intestine weights than the orally fed rats, i.e., the R group. In rats with subacute peritonitis, the relative weights of the liver and spleen were significantly high in the parenterally fed rats. In addition, rats administered high doses of L-NAME had lower liver weights than the rats of the

<table>
<thead>
<tr>
<th>Group</th>
<th>Water g</th>
<th>%</th>
<th>Protein g</th>
<th>%</th>
<th>Fat g</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>R</td>
<td>134.3±7.2</td>
<td>62.6±0.4</td>
<td>39.8±3.5</td>
<td>18.0±1.0</td>
<td>44.5±4.2</td>
<td>17.4±1.3</td>
</tr>
<tr>
<td>TPN</td>
<td>122.9±2.9*</td>
<td>61.0±0.4*</td>
<td>41.6±1.0</td>
<td>20.4±0.4*</td>
<td>41.0±1.8</td>
<td>20.3±0.7*</td>
</tr>
<tr>
<td>CPP</td>
<td>120.4±2.8*</td>
<td>61.2±0.5*</td>
<td>35.2±1.0†</td>
<td>17.9±0.4†</td>
<td>45.9±1.6</td>
<td>23.9±0.8*†</td>
</tr>
<tr>
<td>LNA</td>
<td>119.7±3.1†</td>
<td>61.3±0.6*</td>
<td>39.3±2.6</td>
<td>19.3±0.4</td>
<td>39.9±1.4</td>
<td>20.4±0.5*†</td>
</tr>
<tr>
<td>MNA</td>
<td>113.9±2.2†</td>
<td>59.2±0.4*</td>
<td>33.6±2.6†</td>
<td>17.4±1.3†</td>
<td>43.0±2.0</td>
<td>21.7±0.6*†</td>
</tr>
<tr>
<td>HNA</td>
<td>118.0±2.3†</td>
<td>60.7±0.3*</td>
<td>33.8±2.1†</td>
<td>17.6±0.8†</td>
<td>42.2±1.8</td>
<td>21.7±0.8*†</td>
</tr>
</tbody>
</table>

\[1\] Values are represented as mean±S.E.M., n=11—12 for each group. * Indicates significant differences from the R and TPN groups, respectively (one-way ANOVA with least significant difference, \(p<0.05\)). a, b Significant differences among the CPP, LNA, MNA, and HNA groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Liver g/kg</th>
<th>Heart g/kg</th>
<th>Kidneys g/kg</th>
<th>Spleen g/kg</th>
<th>Muscle g/kg</th>
<th>Small intestine g/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>R</td>
<td>5.41±0.11</td>
<td>0.39±0.01</td>
<td>1.06±0.03</td>
<td>0.31±0.01</td>
<td>1.46±0.10</td>
<td>3.53±0.10</td>
</tr>
<tr>
<td>TPN</td>
<td>5.43±0.12</td>
<td>0.40±0.01</td>
<td>1.09±0.02</td>
<td>0.71±0.03*</td>
<td>1.50±0.04</td>
<td>2.54±0.13*</td>
</tr>
<tr>
<td>CPP</td>
<td>6.54±0.27*</td>
<td>0.47±0.03*</td>
<td>1.13±0.02</td>
<td>0.87±0.04*</td>
<td>1.45±0.04</td>
<td>2.55±0.05*</td>
</tr>
<tr>
<td>LNA</td>
<td>6.04±0.20*</td>
<td>0.43±0.01</td>
<td>1.13±0.03</td>
<td>0.82±0.04*</td>
<td>1.48±0.05</td>
<td>2.51±0.10*</td>
</tr>
<tr>
<td>MNA</td>
<td>5.97±0.12*</td>
<td>0.44±0.02*</td>
<td>1.14±0.03</td>
<td>0.81±0.04*</td>
<td>1.52±0.04</td>
<td>2.53±0.06*</td>
</tr>
<tr>
<td>HNA</td>
<td>5.59±0.09*</td>
<td>0.44±0.02*</td>
<td>1.12±0.03</td>
<td>0.75±0.03*</td>
<td>1.49±0.03</td>
<td>2.49±0.06*</td>
</tr>
</tbody>
</table>

\[1\] Values are represented as mean±S.E.M., n=11—12 for each group. * Significant difference compared with the R group (one-way ANOVA with least significant difference, \(p<0.05\)). a, b Significant differences among the CPP, LNA, MNA, and HNA groups. Gastrocnemius muscle was used to represent muscle.
were administered a medium dose of L-NAME (i.e., the MNA group) had significantly increased concentrations of plasma glutamate, arginine, proline, and ornithine.

Table 3. White Blood Cells and Serum Biochemical Data in Normal Rats and in Peritonitic Rats Parenterally Supplemented with or without Different Doses of \(N\)-nitro-L-arginine methyl ester (L-NAME)

<table>
<thead>
<tr>
<th>Group</th>
<th>WBC (10^3/\mu l)</th>
<th>Albumin g/l</th>
<th>Glucose g/l</th>
<th>Triglycerides mg/l</th>
<th>GOT U/l</th>
<th>GPT U/l</th>
<th>BUN mg/l</th>
<th>Creatinine mg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>R</td>
<td>6.84 ± 0.81</td>
<td>40.3 ± 0.7</td>
<td>3.19 ± 0.27</td>
<td>1171 ± 71</td>
<td>96.4 ± 6.0</td>
<td>48.6 ± 2.2</td>
<td>254.0 ± 8.3</td>
<td>5.13 ± 0.12</td>
</tr>
<tr>
<td>TPN</td>
<td>11.29 ± 0.77*</td>
<td>34.4 ± 0.7*</td>
<td>2.35 ± 0.2*</td>
<td>1043 ± 31*</td>
<td>398.6 ± 36.1*</td>
<td>25.6 ± 2.1*</td>
<td>123.1 ± 4.1*</td>
<td>4.00 ± 0.19*</td>
</tr>
<tr>
<td>CPP</td>
<td>9.23 ± 0.66**</td>
<td>30.8 ± 0.7**</td>
<td>1.49 ± 0.18</td>
<td>506 ± 32*</td>
<td>325 ± 41.4*</td>
<td>20.8 ± 2.6*</td>
<td>127.8 ± 7.3*</td>
<td>4.18 ± 0.12*</td>
</tr>
<tr>
<td>LNA</td>
<td>8.74 ± 0.62†</td>
<td>31.0 ± 0.4†</td>
<td>1.54 ± 0.17†</td>
<td>467 ± 44†</td>
<td>295.4 ± 19.9†</td>
<td>20.0 ± 1.5†</td>
<td>127.6 ± 3.3†</td>
<td>4.17 ± 0.11†</td>
</tr>
<tr>
<td>MNA</td>
<td>7.86 ± 0.64†</td>
<td>31.8 ± 0.6†</td>
<td>1.49 ± 0.91†</td>
<td>566 ± 67†</td>
<td>325.6 ± 30.7†</td>
<td>23.6 ± 1.5†</td>
<td>136.5 ± 5.1†</td>
<td>4.27 ± 0.14†</td>
</tr>
<tr>
<td>HNA</td>
<td>9.73 ± 0.51**†</td>
<td>32.1 ± 0.7**†</td>
<td>1.68 ± 0.18†</td>
<td>455 ± 27†</td>
<td>343.9 ± 33.6†</td>
<td>24.7 ± 2.2†</td>
<td>140.9 ± 1.1†</td>
<td>4.42 ± 0.19†</td>
</tr>
</tbody>
</table>

1 Values are represented as mean ± S.E.M., \(n = 11—12\) for each group. \(*\) and \(†\) indicated values significantly different from the R and TPN groups, respectively (one-way ANOVA with least significant differences).

Table 4. Plasma Concentrations of Arginine Metabolism-Associated Amino Acids in Normal Rats and in Peritonitic Rats Parenterally Supplemented with or without Different Doses of \(N\)-nitro-L-arginine methyl ester (L-NAME)

<table>
<thead>
<tr>
<th>Group</th>
<th>Glutamate mol/l</th>
<th>Arginine mol/l</th>
<th>Proline mol/l</th>
<th>Ornithine mol/l</th>
<th>Citrulline mol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>R</td>
<td>1079 ± 97</td>
<td>299 ± 14</td>
<td>258 ± 22</td>
<td>107 ± 4</td>
<td>84.1 ± 3.7</td>
</tr>
<tr>
<td>TPN</td>
<td>329 ± 36*</td>
<td>255 ± 13</td>
<td>172 ± 15</td>
<td>115 ± 4</td>
<td>47.1 ± 3.4*</td>
</tr>
<tr>
<td>CPP</td>
<td>332 ± 30*,b</td>
<td>254 ± 20*</td>
<td>151 ± 11*,c</td>
<td>116 ± 7*,b</td>
<td>45.9 ± 2.6*</td>
</tr>
<tr>
<td>LNA</td>
<td>350 ± 36*,b,†</td>
<td>251 ± 12*</td>
<td>307 ± 40*,a,b</td>
<td>132 ± 8*,b</td>
<td>46.8 ± 2.2*</td>
</tr>
<tr>
<td>MNA</td>
<td>759 ± 106*,a,b</td>
<td>359 ± 20*,a,b</td>
<td>364 ± 43*,a,b</td>
<td>173 ± 14*,a,b</td>
<td>53.7 ± 1.6*</td>
</tr>
<tr>
<td>HNA</td>
<td>439 ± 41*,a,b</td>
<td>306 ± 11*,a,b</td>
<td>223 ± 20*,a,b</td>
<td>169 ± 11*,a,b</td>
<td>48.3 ± 1.5*</td>
</tr>
</tbody>
</table>

1 Values are represented as mean ± S.E.M., \(n = 11—12\) for each group. \(\times\) and \(†\) indicated significantly different from the R and TPN groups, respectively (one-way ANOVA with least significant differences, \(p<0.05\)). \(a, b, c\) Significant differences among the CPP, LNA, MNA, and HNA groups.

CPE, LNA, and MNA groups.

The results of the WBC and serum biochemical assays are shown in Table 3. The TPN group had significantly higher levels of circulating WBC and serum concentrations of GOT, as well as significantly lower serum concentrations of albumin, glucose, triglycerides, GPT, BUN, and creatinine than the R group. There were no significant differences in the circulating levels of RBC and platelets, concentrations of hemoglobin, or percentages of hematocrit among groups. However, circulating WBC and serum albumin levels were significantly lower in the CPP group than the TPN group. In addition, \(N\)-NAME administration had no significant impact on the serum biochemical indices that we measured.

The serum concentration of insulin was significantly higher in the CPP group than in the R and TPN groups (Fig. 3A). In addition, rats with parenteral feeding had significantly higher serum NO levels, and the increase in the serum NO level in the subacute peritonitis groups (Fig. 3B) was low. Parenteral feeding, subacute peritonitis, and \(N\)-NAME administration did not have a significant impact on the serum concentrations of TNF-\(\alpha\) (Fig. 3C).

To investigate the effects of parenteral nutrition, subacute peritonitis, and different doses of \(N\)-NAME supplementation on arginine metabolism, the plasma concentrations of glutamate, arginine, proline, ornithine, and citrulline were measured using reversed-phase HPLC (Table 4). Parenterally fed rats had significantly low plasma glutamate and citrulline concentrations, but subacute peritonitis did not have a significant impact on these plasma amino acid concentrations. However, parenterally fed rats with subacute peritonitis that were administered a medium dose of \(N\)-NAME (i.e., the MNA group) had significantly increased concentrations of plasma glutamate, arginine, proline, and ornithine.

Fig. 3. Serum Concentrations of Insulin (A), Nitric Oxide (B), and TNF-\(\alpha\) in Normal Rats and in Parenterally Fed Rats with Subacute Peritonitis with or without Different Doses of \(N\)-NAME. Values are represented as mean ± S.E.M., \(n = 11—12\) for each group. Symbols * and † represent significant differences from the R and TPN groups, respectively (one-way ANOVA and least significant differences).
DISCUSSION

Inflammation is a condition associated with uncontrolled excessive production of NO and relative arginine deficiency.\textsuperscript{1,2,21} Partial inhibition of NOS activity may have beneficial effects on alleviating arginine deficiency and inhibiting excess NO-induced complications in inflammation.\textsuperscript{5,7,14} However, both animal and human studies have shown inconsistent results on the therapeutic effects of NOS inhibitors. To date, only few reports have described the dose effects of NOS inhibitors in subjects receiving parenteral nutrition, especially in peritonitis. Using parenterally fed rats with subacute peritonitis, we showed that L-NAME significantly increased urinary nitrogen excretion and plasma concentrations of arginine, ornithine, proline, and glutamine but did not alter circulating levels of albumin, NO, or citrulline.

In this parenterally fed model, subacute peritonitic rats gained less body weight (Fig. 1) and had less carcass protein content (Table 1) and serum albumin concentrations (Table 3). They also had increased liver and spleen weights (Table 2) accompanied by sacs composed of muscular and connective tissue filled with intra-abdominal abscesses.\textsuperscript{16} All these symptoms indicate that these peritonitic animals were under catabolic and inflammatory stress, even though their circulating levels of TNF-\(\alpha\) and NO were not significantly increased (Fig. 3). In addition, we found that the circulating numbers of WBC were significantly increased in rats receiving parenteral nutrition, and the increases were attenuated by subacute peritonitis. These results indicated that these parenterally fed rats were under inflammatory stress. Recently, it has been demonstrated that parenteral nutrition may result in abnormal T-lymphocyte function.\textsuperscript{22} Therefore, we speculated that these parenterally fed, peritonitic rats might be adapted to the inflammatory stress. Recently, it has been demonstrated that activated arginase downregulates NO production in activated macrophages.\textsuperscript{29} This contradictory result on circulating NO may be at least partially attributed to the differences between continuous infusion and bolus injection. Meanwhile, the urinary NO excretion should be considered, especially since urinary nitrogen excretion was significantly increased in peritonitic rats that received L-NAME.

L-Arginine can be catalyzed by NOS to produce NO and L-citrulline, and it can be catalyzed by arginase to produce urea and L-ornithine.\textsuperscript{26} Usually, these 2 pathways compete with the same substrate arginine,\textsuperscript{27,28} and it has been suggested that activation of arginase downregulates NO production in preventing NO-mediated apoptosis in activated macrophages.\textsuperscript{29} In this study, we found that a medium dose of L-NAME may significantly elevate plasma ornithine but not citrulline (Table 4). Even though we did not measure the protein expression and activities of arginase and NOS, the results of this study suggest that chronic infusion of a medium dose of L-NAME may preserve arginine for arginase and the urea cycle in subacute peritonitis.

Metabolism of glutamate, proline, ornithine, and arginine are completely interchangeable in living animals,\textsuperscript{29} and their metabolic pathways function in the liver, small intestine, and kidneys.\textsuperscript{30} The parenteral feeding-induced decreases in the plasma glutamate and citrulline concentrations were confirmed in this study, and the decreases may be attributed to an effect on the production of these 2 amino acids in the small intestine.\textsuperscript{30} We did not find a significant impact of peritonitis on plasma arginine levels, even though it has been shown that the production of \textit{de novo} arginine and citrulline decreases to about 30\% of the normal levels in septic patients.\textsuperscript{31} However, rats administered a medium dose of L-NAME had significantly increased plasma glutamate, arginine, proline, and ornithine concentrations. Therefore, all our findings indicate that a medium dose of L-NAME may inhibit NOS activity and facilitate the arginine-derived amino acids, that is, ornithine, glutamate, and proline.

In summary, the results of our study revealed that chronic, intravenous administration of a medium dose of L-NAME may facilitate the production of arginine-derived amino acids but results in catabolism in parenterally fed rats with peritonitis. For instance, a medium dose of L-NAME treatment significantly increased plasma arginine, ornithine, glutamate, and proline concentrations and significantly decreased the nitrogen balance. However, circulating levels of WBC, NO, and albumin were mainly affected by parenteral feeding and subacute peritonitis but not by L-NAME treatment. These findings imply that long-term inhibition of NOS within the dose range that we used may not alter systemic NO homeostasis or inflammatory response but may facilitate the production of arginine-associated amino acids and urinary nitrogen excretion in subacute peritonitis. Therefore, NOS inhibitor L-NAME may have a potential therapeutic use in correcting arginine deficiency status in peritonitic patients with parenteral nutrition. The use of NOS inhibitors in various
diseases needs to be further investigated.

Acknowledgements We thank Su-Chen Lin, Fu-Ann Tsai, and Ya-Chi Lai for their technical support. This work was supported by the National Science Council of the Republic of China under the grant number NSC-90-2320-B-371-001 and by Changhua Christian Hospital under the grant number CCH-4604.

REFERENCES