Nitrogen balance in clinically normal dogs receiving parenteral nutrition solutions

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Objective—To determine nitrogen balance in clinically normal dogs receiving parenteral nutrition solutions.

Animals—Eight clinically normal female Beagles.

Procedure—Dogs were randomly assigned to receive 4 treatments in random order. Treatment A consisted of IV administration of nonlactated Ringer’s solution. Treatments B, C, and D consisted of IV administration of isocaloric parenteral solutions containing 0, 1.36, and 2.04 g of amino acids/kg of body weight/d, respectively, for 7 consecutive days. Urine and feces were collected on days 5, 6, and 7 of each treatment period, and Kjeldahl analysis was used to determine nitrogen balance.

Results—Mean nitrogen balance was negative with treatments A and B but was not significantly different from 0 with treatments C and D. Dogs had the lowest nitrogen balance values and lost the most weight while receiving treatment A. Dogs were able to conserve protein and had higher nitrogen balance values when receiving treatment B, compared with treatment A. Dogs lost the least amount of weight while receiving treatment D. Regression analysis indicated that an IV amino acid intake of 2.32 g/kg/d (95% confidence interval, 2.00 to 2.81 g/kg/d), as supplied by the commercial product used in this study, would result in zero nitrogen balance in clinically normal dogs.

Conclusions and Clinical Relevance—Results suggest that IV amino acid requirement of clinically normal dogs is approximately 2.3 g/kg/d. (Am J Vet Res 2001;62:912–920)
of random numbers. Each dog was then randomly assigned to a different treatment for each of 3 additional trials. This ensured that every dog would receive all 4 treatments in random order. Each of the 4 trials lasted 7 days, with a 2-week period between trials. All experimental treatments and procedures were reviewed and approved by the Cornell University Institutional Animal Care and Use Committee.

The 4 treatments were designated A, B, C, and D. Treatment A consisted of IV administration of nonlactated Ringer's solution. Treatment B consisted of IV administration of an amino acid-free infusion that supplied 50% of estimated oral maintenance calories as 50% dextrose and 50% of maintenance calories as a 20% soybean oil emulsion. Treatment C consisted of IV administration of a crystalline amino acid solution with added electrolytes; a sufficient amount of this solution was administered to meet the estimated minimum daily oral requirement for the most limiting amino acid in the solution, leucine (Table 1). With the remaining daily caloric requirement supplied as equal caloric proportions of 50% dextrose and 20% lipid emulsion. Amino acid intake with this treatment was 1.36 g of amino acids/kg of body weight/d. Treatment D consisted of IV administration of 1.5 kcal ME/ml for 50% dextrose and the 20% lipid emulsion. Amino acid intake with this treatment was 2.04 (treatment D) g of amino acids/kg/d.

Estimated daily maintenance energy requirements (MER) in kilocalories of metabolizable energy (ME) were determined by use of the following equation:

$$\text{MER} = 144.4 + 62.2 \text{(BW}_{\text{kg}})$$

The caloric value of amino acids administered, if any, was subtracted from the total calculated energy requirement. Remaining calories were then divided equally between 50% dextrose and the 20% lipid emulsion. Caloric values of 1.7 kcal ME/ml for 50% dextrose, 2.0 kcal ME/ml for the 20% lipid emulsion, and 4.0 kcal ME/g for protein \(^{11,12}\) were used for all calculations.

Maintenance fluid requirements were estimated to be 60 ml/kg/d. For treatment A, therefore, this volume of Ringer's solution was infused. For treatments B, C, and D, supplemental Ringer's solution was administered as necessary to increase total fluid intake to 60 ml/kg/d. Ringer's solution was added directly to the PN solution for infusion. Parenteral nutrition solutions were compounded the day before administration; solutions were kept refrigerated until 2 to 3 hours prior to use. A 2,000-ml capacity all-in-one bag \(^{1}\) was used for compounding and delivery of PN solutions. The volumes of amino acid solution, Ringer's solution, 50% dextrose, and 20% lipid emulsion required by each dog during each 24-hour period were measured with 150-ml in-line burettes placed between each bulk container and the all-in-one bag. Solutions were mixed in the all-in-one bag for infusion as a single solution.

All dogs were weighed and received a complete physical examination each morning during feeding trials. The same scale was used throughout the study and had a sensitivity of ± 50 g. Parenteral nutrition solutions were delivered into a jugular vein or the caudal vena cava through percutaneously placed 16-gauge 9-in silicone elastomer infusion catheters \(^{1}\) or surgically implanted drug depot ports \(^{1}\) consisting of circular polysulfone depots attached to 16-gauge silicone elastomer catheters. \(^{1}\) No oral food or water intake of any kind was permitted during each 24-hour period. All 4 treatments were administered by gravity feed over a 12-hour period. One hundred fifty-milliliter in-line burettes were placed in the IV lines between the all-in-one bags containing the day's PN solution and a filterless IV administration set. The burettes were refilled from the all-in-one bags as necessary, but the access from the bags was kept closed at all other times; this prevented inadvertent overinfusion or loss of large volumes of PN solution directly from the bags. The PN solution was infused slowly initially; after 30 to 60 minutes, the rate was increased to the rate required to finish the infusion in 12 hours. This optimum infusion rate was calculated on a drops-per-minute basis and maintained as closely as possible throughout the feeding period by visually monitoring the flow rate through the drip chamber of each dog's administration set.

Dogs were closely monitored during infusion for development of problems, including adverse reactions to the infusion and mechanical difficulties such as tangled or disconnected IV lines. If the infusion was interrupted for mechanical reasons, the problem was addressed, and feeding was reinitiated as soon as possible at the original rate of infusion. When the required volume of solution had been infused, IV lines were disconnected, and catheters were flushed with heparinized saline solution and capped. Once all dogs had been disconnected, each received a physical examination and had its body temperature measured. Catheter bandages were changed every 48 hours; only sterile dressings were permitted to touch the catheter entry sites.

On days 5 through 7 of all 4 treatments, quantitative 24-hour urine and fecal samples were collected for determination of nitrogen balance. Serum biochemical analyses were also performed on days 5, 6, and 7; a CBC was repeated on day 6. All blood samples were collected in the morning prior to initiation of parenteral feeding. Collection of urine and fecal samples was begun on the morning of day 5 at the same time parenteral feeding was begun. The second collection was started with the initiation of feeding on day 6 and the third with the initiation of feeding on day 7. Urine and feces were collected, using funnel-shaped stainless steel metabolism pans that fit in the bottom of each metabolism cage. Urine passed through a central opening in each pan into a collection vessel underneath the cage and was retrieved and placed in a separate airtight storage container after every urination. Each of the three 24-hour urine samples from each dog was kept in a separate container. Fecal material was carefully removed from the surface of the pans after each defecation; each fecal sample was also kept in a separate airtight container. The third and final 24-hour urine and fecal collections were completed on the morning of day 8. Once day-
samples had been obtained, the metabolism pans were removed from the cages, the dogs were weighed, and all percutaneously placed catheters were removed.

A 2-week washout period was used between treatments. The dry dog food fed during the acclimatization period was fed ad libitum during these washout periods. At the end of each washout period, dogs were weighed, a complete physical examination was performed, and blood and urine samples were collected. Complete blood counts, serum biochemical analyses, and routine urinalyses were performed as before the first treatment, and results were compared with pretreatment values. In those dogs that were using drug depot ports, infusion catheters were inserted percutaneously immediately prior to the next treatment.

Nitrogen balance was defined as the difference between nitrogen intake and nitrogen output. For each dog, results for each of the three 24-hour fecal and urine collections during each of the treatments were considered separately; therefore, there were potentially 24 observations for each treatment. Nitrogen intake for each dog was calculated from the known volume of amino acid solution infused by dividing grams of amino acid administered by a factor of 6.25. Nitrogen output was calculated on the basis of total urinary and fecal nitrogen content, using standard Kjeldahl analysis. Analyses were conducted during each 2-week washout period. Briefly, 1 to 2 g of pooled lyophilized fecal material and 5 ml of pooled urine from each 24-hour collection period were assayed. Nitrogen in the sample was first oxidized to (NH₄)₂SO₄ through heat digestion with concentrated H₂SO₄. The digest was then alkalinized with concentrated NaOH, and the resultant NH₃ was distilled into a 4% solution of boracic acid. The ammonium borate produced was titrated with standard HCl. The amount of HCl required for titration was directly proportional to the amount of nitrogen in the sample; therefore, the volume of HCl could be used to calculate total nitrogen in the sample and total urinary and fecal nitrogen losses during each 24-hour period. Cutaneous nitrogen losses and nitrogen losses associated with blood loss during sampling were calculated from published values.

All statistical analyses were performed by use of a computer software package. Nitrogen balance and results for individual hematologic or biochemical tests were compared among treatments and among days within each treatment by use of 1-way repeated-measures ANOVA. When significant (ie, P ≤ 0.05) differences among groups were found, individual comparisons between treatments or days were performed, using the Newman-Keuls method. Any pairwise comparison for which P was ≤ 0.05 was considered significant. Nitrogen balance data were also examined by means of least squares regression analysis. A regression equation and coefficient of determination (r²) were obtained, using all individual data points for treatments B, C, and D. An estimate of the IV amino acid intake required for zero nitrogen balance in clinically normal dogs was calculated along with its 95% confidence interval (CI).

Results

Parenteral nutrition solutions—The nutrient content of the 4 treatments was calculated (Table 2). Minor changes in the volume of Ringer's solution administered were required on the basis of subject responses for all 4 treatments. It was not possible to administer 60 ml of Ringer's solution/kg during a 12-hour period to the dogs that received treatment A during the first trial without causing substantial overhydration. Therefore, dogs that received treatment A during subsequent trials received a fixed dose of 300 ml of Ringer's solution during the 12-hour infusion period. Additionally, during the first feeding trial, some dogs receiving treatment B, C, or D were thought to be marginally dehydrated on the basis of assessments of mucous membrane moistness. Therefore, an additional 100 ml of Ringer's solution was incorporated into the PN solution for dogs receiving treatment B, C, or D during the subsequent feeding trials.

Nitrogen balance—We did not detect any significant differences in nitrogen balance among days for each treatment; therefore, pooled data from the 3 days for each treatment were used for further analyses. Nitrogen balance could not be calculated for 1 dog while receiving treatment B and for 2 dogs while receiving treatment D because of catheter malfunction that necessitated discontinuation of PN. Infections complications also resulted in removal of data for 1 dog each for treatments A, C, and D prior to nitrogen balance calculations. Clinical signs of infection in these 3 animals included swelling and discomfort at the catheter entry site, pyrexia, and leukocytosis. One additional dog developed signs of early estrus (eg, a swollen vulva and scant serosanguineous vaginal discharge) on day 7 while receiving treatment D, but data from this dog were not removed from the study. No other dog was pregnant or exhibited signs of estrus during any portion of the study.

Mean nitrogen balance was significantly (P < 0.001) different among treatments (Table 3). Dogs had the most negative nitrogen balance values

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal/ml)</td>
<td>0</td>
<td>105.47± 7.58</td>
<td>107.87± 8.37</td>
<td>103.30± 5.38</td>
<td></td>
</tr>
<tr>
<td>Nitrogen (g/100 ml)</td>
<td>0</td>
<td>0.028± 0.0246</td>
<td>0.398± 0.0302</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lipid (g/100 ml)</td>
<td>0</td>
<td>0.28± 0.38</td>
<td>0.51± 0.37</td>
<td>4.62± 0.23</td>
<td></td>
</tr>
<tr>
<td>Carbohydrate (g/100 ml)</td>
<td>0</td>
<td>0.84± 1.12</td>
<td>13.86± 8.46</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium (mEq/L)</td>
<td>4.5</td>
<td>1.92± 0.19</td>
<td>1.04± 0.27</td>
<td>0.78± 0.21</td>
<td></td>
</tr>
<tr>
<td>Phosphorus (mmol/L)</td>
<td>0</td>
<td>0.39± 0.28</td>
<td>10.44± 0.83</td>
<td>13.16± 0.84</td>
<td></td>
</tr>
<tr>
<td>Potassium (mEq/L)</td>
<td>4.0</td>
<td>1.70± 0.16</td>
<td>14.29± 0.84</td>
<td>20.10± 1.14</td>
<td></td>
</tr>
<tr>
<td>Sodium (mEq/L)</td>
<td>147.5</td>
<td>62.86± 6.08</td>
<td>49.73± 7.62</td>
<td>48.36± 5.43</td>
<td></td>
</tr>
<tr>
<td>Osmolarity (mOsm/L)</td>
<td>310.0</td>
<td>981.91± 48.54</td>
<td>1,139.03± 62.25</td>
<td>1,173.21± 48.26</td>
<td></td>
</tr>
</tbody>
</table>

Values for treatments B, C, and D are given as mean ± SD.
Table 3—Nitrogen balance in dogs receiving parenteral nutrition with Ringer's solution (treatment A) or with isocaloric parenteral solutions containing 0, 1.36, and 2.04 g of amino acids/kg/d (treatments B, C, and D, respectively)

<table>
<thead>
<tr>
<th>Treatment (No. of observations)</th>
<th>Caloric intake (kcal/kg/d)</th>
<th>Nitrogen intake (g/kg/d)</th>
<th>Urine volume (mL/kg/d)</th>
<th>Urine nitrogen loss (g/kg/d)</th>
<th>Dry feces weight (g/kg/d)</th>
<th>Fecal nitrogen loss (g/kg/d)</th>
<th>Other nitrogen loss (g/kg/d)</th>
<th>Nitrogen balance (g/kg/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (21)</td>
<td>0</td>
<td>0</td>
<td>53.49 ± 9.59</td>
<td>0.2347 ± 0.0596</td>
<td>0.11 ± 0.28</td>
<td>0.0008 ± 0.0022</td>
<td>0.0181 ± 0.0156</td>
<td>-0.2536 ± 0.0500</td>
</tr>
<tr>
<td>B (21)</td>
<td>84.16 ± 1.93</td>
<td>0</td>
<td>53.90 ± 5.58</td>
<td>0.1641 ± 0.0434</td>
<td>0.19 ± 0.35</td>
<td>0.0117 ± 0.0030</td>
<td>0.0181 ± 0.0122</td>
<td>-0.1840 ± 0.0456</td>
</tr>
<tr>
<td>C (21)</td>
<td>82.54 ± 2.49</td>
<td>0.2315 ± 0.0860</td>
<td>42.34 ± 12.40</td>
<td>0.2633 ± 0.0544</td>
<td>0.26 ± 0.46</td>
<td>0.0304 ± 0.0100</td>
<td>0.0177 ± 0.0100</td>
<td>-0.0529 ± 0.0667</td>
</tr>
<tr>
<td>D (18)</td>
<td>79.97 ± 3.17</td>
<td>0.3400 ± 0.0098</td>
<td>48.31 ± 11.69</td>
<td>0.3511 ± 0.0771</td>
<td>0.38 ± 0.41</td>
<td>0.0003 ± 0.0036</td>
<td>0.0197 ± 0.0076</td>
<td>-0.0290 ± 0.0765</td>
</tr>
</tbody>
</table>

Data are given as mean ± SD. *Cutaneous nitrogen losses and losses associated with collection of blood samples. **Values with different letter superscripts are significantly (P < 0.05) different.

Figure 3—Nitrogen balance for clinically normal dogs treated IV with isocaloric parenteral solutions containing 0, 1.36, and 2.04 g of amino acids/kg/d (treatments B, C, and D, respectively) for 7 consecutive days. The solid and dotted lines represent the regression line and its 95% confidence interval, respectively.

Figure 2—Weight loss expressed as a percentage of initial body weight for dogs treated IV by means of administration of Ringer's solution (treatment A) or by administration of isocaloric parenteral solutions containing 0, 1.36, and 2.04 g of amino acids/kg/d (treatments B, C, and D, respectively) for 7 consecutive days. Error bars represent SD.

When receiving treatment A, mean nitrogen balance while dogs were receiving treatment B was significantly different from mean balance while receiving treatment A. Mean nitrogen balances while dogs were receiving treatment C or D were not significantly different from each other or from 0 but were significantly less negative than mean nitrogen balances when dogs were receiving treatment A or B.

Regression analysis indicated that there was a significant (P < 0.001; r² = 0.547) linear relationship between amino acid intake and nitrogen balance (Fig 1), according to the equation:

Nitrogen balance (g/kg/d) = -0.1793 + 0.4827 • nitrogen intake (g/kg/d)

Using this equation, it was estimated that an IV amino acid intake, as supplied by the commercial solution used, of 2.34 g of amino acid/kg/d (95% CI, 2.00 to 2.81 g/kg/d) would be necessary to result in zero nitrogen balance in clinically normal dogs.

**Body weight**—Dogs lost weight during feeding trials with all 4 treatments (Fig 2). Amount of weight lost, expressed as a percentage of the prestudy body weight, was significantly (P < 0.001) different among treatments. Mean weight loss during treatment A (mean ± SD, 12.30 ± 2.03%) was significantly (P < 0.001) greater than mean weight loss during any of the other treatments. Administration of calories alone prevented some of this weight loss, as dogs lost significantly less weight when receiving treatment B (6.11 ± 1.70%) than when receiving treatment A and did not lose significantly (P = 0.065) more weight than when receiving treatment C (4.18 ± 1.93%). Dogs lost significantly less weight when receiving treatment D (2.34 ± 1.62%) than when receiving treatment A or B (P = 0.002) but not when receiving treatment C (P = 0.103).

Examination of the progression of weight loss during the feeding trials also suggested that the higher dose of amino acids received with treatment D enabled dogs to maintain their body weight more effectively. By day 3 of the feeding trial, weight loss was significantly (P = 0.033) greater when dogs were receiving treatment B, compared with weight loss when dogs were receiving treatment D, and this difference persisted to the end of the feeding trial (P = 0.001). However, it was not until day 6 that a significant (P = 0.044) difference in weight loss was detected between treatments B and C. In addition, when dogs were receiving treatment D, mean percentage weight loss was not significantly different from 0 until day 4, and there was no significant difference in body weight for days 2 through 6. Regardless of the degree of weight loss, dogs were suc-
cessful in regaining prestudy body weight during the 2-week washout period between feeding trials.

Hematologic and serum biochemical analyses—Results of hematologic analyses performed on day 6 did not differ among treatments. We did not detect any significant differences in regard to results of serum biochemical analyses among days for each treatment; therefore, pooled data from the 3 days for each treatment were used for further analyses. Mean blood urea nitrogen concentration was decreased below the reference limits with all treatments, with dogs having significantly ($P < 0.001$) lower BUN concentrations when receiving treatment B than when receiving treatment A, C, or D (Table 4). A decrease in BUN concentration was associated with a decrease in serum creatinine concentration on a few individual occasions, but mean serum creatinine concentration remained within reference limits and did not differ significantly among treatments. Mean serum alkaline phosphatase (AP) activity was within reference limits with all 4 treatments, although dogs had significantly ($P < 0.001$) higher mean serum AP activity when receiving treatment B than when receiving treatment A, C, or D. Mean serum total protein concentrations were within reference limits and were not significantly different among treatments. Mean serum albumin concentrations were also within reference limits, but dogs had significantly ($P < 0.001$) lower mean serum albumin concentrations when receiving treatments A or B than when receiving treatment D. Finally, although mean blood glucose concentration measured prior to initiation of PN on days 3, 6, and 7 was within reference limits for all 4 treatments, it was significantly ($P < 0.001$) lower when dogs were receiving treatment A than when they were receiving any other treatment.

**Discussion**

Results of the present study indicate that an IV amino acid intake of 2.32 g/kg/d (95% CI, 2.00 to 2.81 g/kg/d), as supplied by the commercial preparation used and in combination with maintenance total caloric intake, will maintain zero nitrogen balance and stable body weight in clinically normal dogs receiving PN. This intake is lower than that recommended by several authors for clinical canine patients receiving PN and further investigation is required to characterize the IV amino acid requirements of healthy and diseased dogs. An important limitation of the present study was that only 3 levels of nitrogen intake were examined, and a statistically significant difference could not be demonstrated between 2 of these (treatments C and D). Additional work will be necessary to clarify the upper limit of the recommended amino acid intake, which appears to be well above the range investigated in the present study.

Nitrogen balance among the dogs in this study was found to vary with the IV intake of amino acid and nonprotein calories. Administration of nonprotein calories alone apparently had some protein-sparing effect; the carbohydrate and lipid administered with treatment B resulted in reduced nitrogen losses, compared with treatment A. Lean body mass was lost as a result of protein turnover, but the loss was not exacerbated to support gluconeogenesis. On the other hand, a combination of calories and amino acids was most effective in maintaining nitrogen balance. Dogs received enough amino acids with treatments C and D (1.36 and 2.04 g/kg/d, respectively) that nitrogen balance was not significantly different from zero, and catabolism of lean body mass was not required to support basal protein requirements.

Measurement of nitrogen balance represents a sensitive and accurate method for determining a subject's nutritional or metabolic response to nutritional manipulation. However, errors may be introduced if data are not collected and interpreted with care. For instance, urine nitrogen may be lost from open collection containers through evaporation of ammonia, resulting in a spurious increase in nitrogen balance values. Such nitrogen losses are minimized if urine is kept in containers that are closed or shaped to decrease the evaporative surface area; antibacterial preservatives may also be added to prevent bacterial conversion of urea to ammonia and further loss of nitrogen. Although closed containers were used in the present study, urine preservatives were not, so it is possible that some urine nitrogen was lost as ammonia prior to analysis. Factors that may result in spurious decreases in nitrogen balance values must also be considered. Fecal nitrogen losses were included in calculations of daily nitrogen balance for dogs in this study, as described. However, PN is well documented to cause small intestinal villus atrophy that is accompanied by decreases in gastrointestinal secretions and mucosal desquamation, and stools are passed much less frequently. Although the study protocol dictated that nitrogen losses in feces be incorporated with the nitrogen losses for the 24-hour period during which those feces were passed, the feces

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**Table 4—Results of biochemical analyses in dogs receiving parenteral nutrition with Ringer’s solution (treatment A) or with isocaloric parenteral solutions containing 0, 1.36, and 2.04 g of amino acids/kg/d (treatments B, C, and D, respectively)**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Reference range</th>
<th>Treatment A</th>
<th>Treatment B</th>
<th>Treatment C</th>
<th>Treatment D</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood urea nitrogen (mg/dl)</td>
<td>10–24</td>
<td>7.62 ± 1.86</td>
<td>5.50 ± 0.41</td>
<td>7.90 ± 0.68</td>
<td>8.87 ± 0.74</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td>0.8–1.5</td>
<td>0.60 ± 0.07</td>
<td>0.78 ± 0.11</td>
<td>0.62 ± 0.06</td>
<td>0.83 ± 0.38</td>
<td>NS</td>
</tr>
<tr>
<td>Serum alkaline phosphatase (U/L)</td>
<td>4–107</td>
<td>43.0 ± 10.7</td>
<td>94.48 ± 29.39</td>
<td>85.43 ± 31.04</td>
<td>78.07 ± 17.30</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Serum total protein (g/dl)</td>
<td>5.4–7.4</td>
<td>6.14 ± 0.49</td>
<td>5.97 ± 0.36</td>
<td>6.17 ± 0.41</td>
<td>6.13 ± 0.32</td>
<td>NS</td>
</tr>
<tr>
<td>Serum albumin (g/dl)</td>
<td>2.1–3.3</td>
<td>3.11 ± 0.19</td>
<td>3.05 ± 0.16</td>
<td>3.18 ± 0.14</td>
<td>3.29 ± 0.22</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Blood glucose (mg/dl)</td>
<td>58–120</td>
<td>69.76 ± 12.15</td>
<td>86.62 ± 10.55</td>
<td>83.90 ± 7.42</td>
<td>86.13 ± 16.19</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Data are given as mean ± SD.

NS = Not significant.
passed on days 5, 6, and 7 of each PN trial by dogs in this study probably contained nitrogen lost into the intestinal tract on preceding days. Thus, true nitrogen balance values may be slightly higher than those reported here.

Regression analysis of data obtained during this study suggested a linear relationship between IV amino acid intake and nitrogen balance. However, the r² value also indicated that a substantial proportion of the variation in nitrogen balance was attributable to factors other than amino acid intake. Nonprotein calorie intake appeared to be 1 of these factors. Individual variations in nitrogen metabolism and responses to the stress of confinement and IV feeding among dogs may also have been partly responsible for the variability observed. As with any nutrient, maintenance amino acid requirements for a population are best expressed as a range, whether healthy or diseased dogs are considered. A low r² value would also result if the relationship between amino acid or protein intake and nitrogen balance were not strictly linear. Some authors speculate that a linear relationship exists only at low levels of amino acid intake, and that incremental increases in protein intake result in relatively substantial improvements in nitrogen balance. However, as nitrogen balance approaches zero, expansion of lean body mass becomes more difficult, and there may be a plateau effect, resulting in a nonlinear relationship or a lower than expected r² value. Previous studies examining protein requirements in dogs support this hypothesis.34,35

A complex collection of factors determines the amounts of protein required by human patients receiving PN. The same factors are either known or likely to affect dogs as well. The most important of these is the primary illness itself. A 2- to 3-fold increase in the amount of amino acids required for synthetic and gluconeogenic functions is expected for human patients, with the increase proportional to the severity of the insult.20,30,37 Diseases characterized by substantial losses of protein into exudates, body cavities, the intestines, or the urinary tract will further increase requirements.46 Researchers studying dogs hospitalized in an intensive care unit for a variety of illnesses were recently able to demonstrate high rates of urinary nitrogen loss,64 a reflection of similarly accelerated protein catabolism in these patients. Critically ill dogs in this study had a mean total urinary nitrogen content of 0.81 g/kg0.75/d, compared with mean approximate losses of only 0.05 g/kg0.75/d when clinically normal dogs in the present study were receiving treatment D. Another factor known to affect protein requirements during PN in humans is the physiologic status of the patient during the period leading up to the illness necessitating PN.45 For instance, the protein requirements of a growing child with severe thermal burns may be as high as 4.8 g/kg/d.46 Conversely, extended malnutrition prior to the need for PN may increase the efficiency of protein utilization and decrease energy expenditure in human patients.39,41 Thus, a human patient who is hospitalized because of an acute complication of a chronic debilitating illness may well have protein requirements that are not as great as would initially be expected.44 It is also well accepted that physiologic stresses such as work, growth, old age, pregnancy, and lactation affect protein requirements in dogs.28,42-44 For instance, an increased protein intake of 39% on a dry matter basis was required by working sled dogs to prevent depletion of RBC mass.45 In addition, it appears that protein retention in aged dogs is less efficient than in their younger counterparts. An intake comparable to that predicted to be necessary for zero nitrogen balance among the clinically normal dogs in the present study (2.5 g/kg/d) was necessary for maximal filling of depleted protein mass among young adult dogs in a previous study.46 However, those authors found that an intake of 3.75 g/kg/d was required in 12- and 13-year-old animals. Nutrients such as the B vitamins and various trace minerals that function in metalloproteins are required for normal protein metabolism.28,30 and when intake is inadequate, the amount of protein necessary to meet requirements may be higher. Little is known regarding the specific micronutrient and vitamin requirements of critically ill canine patients, but partial PN is commonly administered by veterinary clinicians.9 Parenteral nutrition solutions lacking in these specific nutrients may increase patient protein requirements and could have falsely lowered the nitrogen balance of dogs in the present study. Finally, the amount and source of nonprotein calories provided will affect the total amount of protein required.46-48,49 There is conclusive evidence that addition of nonprotein calories as dextrose12-15 and, to a lesser degree, lipid emulsions16-19 to an amino acid infusion will improve ability to preserve lean body mass in humans receiving PN. A similar calorie-nitrogen effect has been demonstrated in dogs, in which an increase in enteral energy intake promotes more efficient utilization of ingested protein for tissue anabolism.13,31

Little is known about the specific protein requirements of sick dogs during PN, although the protein requirements of critically ill human patients have been explored in detail. Most investigators working with parenterally fed catabolic or malnourished human patients have found that protein requirements for nitrogen balance or maintenance of lean body mass are between 1.0 and 2.5 g/kg/d.30-32,38 Requirements appear to be highest in burn patients but will rarely be greater than 2.5 g/kg/d,34,41 even when incomplete utilization of infused amino acids is taken into account. The protein requirements of parenterally fed canine patients have not been systematically investigated. Dudrick et al6 originally estimated the required protein (as fibrin hydrolysate) of parenterally fed puppies to be 4 g/kg/d on the basis of National Research Council recommendations for growth in puppies fed oral diets, but this approach does not correct for digestive and other losses. The essential amino acid profiles of the commercial crystalline amino acid solutions in current use are also likely to be more optimal than that of fibrin hydrolysate. However, this value is still the basis for the amount of crystalline amino acids recommended for PN in a number of veterinary references.1,13 In the only previously published study7 investigating IV amino acid requirements in dogs, 24-hour nitrogen balance studies in 3 hospitalized canine patients receiving PN revealed positive nitrogen balance when 4 to 6 g of protein/100
kcal of energy (approx 2 to 3 g/kg) was administered. These results are comparable to those reported here. Other estimates of canine protein requirements during PN range from as low as 1 to 2 g/kg/d to as high as 7 to 8 g/kg/d for a moderately hypermetabolic patient. The latter dosage seems excessively high, compared with oral maintenance protein requirements for dogs, nitrogen balance measurements reported here and elsewhere, and human protein requirements during PN. Furthermore, clinical signs of hepatic encephalopathy associated with hyperammonemia have been reported when dogs were receiving PN at a dosage of 4 g of amino acid/kg/d.

Additional study in the area of parenteral protein requirements for canine patients is clearly required. However, until such data are available, results of the present study as well as reports from the human literature support a cautious approach. For instance, when PN first became widely available in human medicine during the mid-1970s, it was used aggressively in many patients. In a procedure called "hyperalimentation," protein and energy substrates were supplied in amounts greater than the calculated requirements in an attempt to force patients into positive nitrogen balance and promote tissue anabolism. However, renal, hepatic, or other metabolic dysfunction may prevent a critically ill individual from metabolizing high protein and energy loads. Serious complications such as cholestasis, hepatic lipidosis, respiratory failure, and hypermetabolism were repeatedly documented in association with hyperalimentation, and the practice is now considered dangerous and obsolete. With few exceptions, PN should only provide sufficient protein and calories to maintain lean body mass and stabilize body weight. Its purpose is simply to support patients and prevent further deterioration until they are able to support themselves. Large weight gains due to tissue accretion are neither expected nor likely, especially during the catabolic phase of injury. When adequate energy was supplied, increasing the amount of protein delivered from approximately 1.2 to 2.3 g/kg/d resulted in no further improvement in body composition, protein kinetics, or nitrogen balance in severely depleted or catabolic human burn patients. There is no evidence that a markedly positive nitrogen balance is any more beneficial than a moderately positive one, and administration of excessive amounts of amino acids may actually result in toxicoses. Critically ill patients may also have insufficient hepatic and renal reserve to process a large volume of nitrogenous waste products. Respiratory failure has been documented in human patients with compromised respiratory function. Additionally, the cost of PN may be substantially increased if large volumes of crystalline amino acid solutions are used. For all these reasons, conservative doses of protein are recommended for human PN patients and would seem to be indicated in canine patients as well. Finally, veterinarians must remember that the equations currently used to determine canine energy and nutrient requirements can only estimate an individual animal’s true requirements, and in critically ill canine patients with multiple organ dysfunction, the capacity of various metabolic pathways to process even modest amounts of nutrients may quickly be exceeded. The result may be hyperosmolality and dehydration, hyperglycemia, hyperlipidemia, hypophosphatemia, or azotemia. Constant reevaluation of the PN formula on the basis of results of blood biochemical analyses is required throughout the treatment period, with revision as necessary. Serum biochemical data gathered in the present study did not show evidence of excessive protein intake. Rather, the biochemical changes observed in dogs that were receiving treatment B were consistent with alterations previously reported for healthy dogs consuming a protein-deficient diet. Decreased serum urea nitrogen, albumin, and total protein concentrations as well as increased serum AP activity were evident among these dogs after they received a markedly protein-deficient enteral diet for 4 weeks. Cholestasis and high serum AP activity have also been reported for human patients receiving PN with insufficient amino acids in combination with an excessive nonprotein caloric intake. Dogs in this study were fed to meet maintenance energy requirements rather than the resting requirement that is conventional for critically ill hospitalized patients, because it was anticipated that resting requirements would be inadequate for these healthy active animals. Although this relatively increased caloric intake may have contributed to the biochemical differences observed when dogs were receiving treatment B, the fact that most dogs lost at least some body weight, regardless of treatment, suggests that restricting intake to resting requirements would have been insufficient to meet energy needs. In fact, maintenance energy intakes were apparently inadequate for some of the dogs in this study and may have falsely lowered nitrogen balance values as amino acids were used for energy in these animals. This interaction could have important consequences for clinical patients with inadequate parenteral caloric intake, in that protein requirements may be higher than expected in such situations.

References


41. Shaw SN, Elwyn DH, Askani J, et al. Effects of increasing nitrogen intake on nitrogen balance and enzyme expenditure in nutri-


