ABSTRACT

Turnover of carnitine in the body is primarily the result of renal excretion, and high-fat (HF) diets have been shown to increase urine carnitine excretion in healthy people. Recently, increased renal excretion of carnitine was observed in dogs diagnosed with cystinuria and carnitine deficiency. Carnitine deficiency has been linked to dilated cardiomyopathy and lipid storage myopathies in dogs and humans, and low-fat (LF) diets have been beneficial in some human patients with carnitine deficiency. In addition, HF, protein-restricted diets are often recommended for management of cystinuria in dogs. However, whether HF diets increase renal carnitine excretion in dogs or whether dogs with carnitine deficiency would benefit from LF diets remains unknown. Therefore, the purpose of this study was to determine the influence of dietary fat and carnitine on renal carnitine excretion in healthy dogs. Results from this study revealed that an HF diet increased urine carnitine excretion in dogs; however, carnitine excretion with the HF diet was not significantly different from that in dogs consuming an LF diet. Nonetheless, these results raise the possibility that increased renal carnitine excretion associated with HF diets could be one risk factor for development of car-
Nineteen dogs with an underlying disorder in carnitine metabolism, and some dogs with carnitine deficiency may benefit from an LF diet. Another important observation in this study was that renal excretion of carnitine exceeded dietary intake in all diet groups, confirming previous reports that concluded that canine renal tubular cells reabsorb carnitine poorly when compared with those of humans.

**INTRODUCTION**

Carnitine (β-hydroxy-γ-N-trimethylamino-butyric acid) is a quaternary amine with a molecular weight of 161.2. It functions as a cofactor of several enzymes necessary for transport of long-chain fatty acids from the cytosol into the mitochondrial matrix. Once inside the mitochondria, fatty acids undergo β-oxidation to generate energy. This is especially important in tissues such as cardiac and skeletal muscles, which are heavily dependent on fatty acids as a source of energy. Another important function of carnitine is its buffering capacity, which modulates the intramitochondrial acyl–coenzyme A (CoA):coenzyme A ratio. This is important because the activated form of fatty acids used for both β-oxidation and lipid synthesis is acyl-CoA. However, build-up of acyl-CoA derivatives in the mitochondria results in decreased free CoA, which inhibits oxidative metabolism. Acyl-CoA derivatives also act as detergents at high concentrations.

Carnitine is obtained either from dietary protein or endogenous synthesis utilizing the essential amino acids lysine and methionine when dietary intake is low. Cardiac and skeletal muscles are significant storage sites, containing 95% to 98% of the carnitine in the body. Not surprisingly, carnitine deficiency has been linked to dilated cardiomyopathy and lipid storage myopathies in dogs and humans. Proposed causes for carnitine deficiency include defective carnitine biosynthesis, defective uptake or retention of carnitine by tissues, and excessive renal excretion.

Turnover of carnitine is primarily the result of renal excretion of either free carnitine or acyl-carnitine. Plasma carnitine is filtered by glomeruli, reabsorbed by proximal tubules, and released into the blood. Under normal circumstances, the kidneys preferentially reabsorb free carnitine over acylcarnitine, and renal excretion of acylcarnitine in humans is a normal physiologic response to elevated blood concentrations of this metabolite. Therefore, during extended periods of increased lipid consumption, carnitine is necessary not only for transporting long-chain fatty acids into and out of the mitochondria but also for binding with acyl groups and allowing their excretion in urine as acylcarnitine. In people with organic acidemias and diabetes mellitus, excessive accumulation of acyl-CoA and acylcarnitine metabolites leads to increased excretion of acylcarnitine in urine. This can increase carnitine loss in urine, and some patients require supplementation to prevent carnitine deficiency.

Dietary modification has been shown to be beneficial in managing humans with some disorders associated with carnitine deficiency. Carnitine supplementation and low-fat (LF), high-carbohydrate diets with medium-chain triglycerides have been used to successfully manage some human patients with lipid storage myopathies. Low-fat diets and carnitine supplementation have also been recommended in children with Reye’s-like syndromes associated with carnitine deficiency.

Diet has been shown to influence carnitine excretion in healthy humans as well. For example, high-fat (HF) diets appear to increase urinary carnitine excretion. In one study, two isocaloric diets having similar carnitine content but different carbohydrate and fat content were fed to healthy humans. Consumption of the HF, low-carbohydrate diet resulted in a 100%
increase in renal excretion of short-chain acyl-carnitine and a 30% increase in renal excretion of free carnitine compared with the LF, high-carbohydrate diet. Interestingly, the LF, high-carbohydrate diet did not significantly affect renal excretion of carnitine in comparison with diets usually consumed by the subjects. Therefore, even in healthy people, a normal physiologic response to consumption of HF diets is to increase renal excretion of carnitine esters.

The amount of carnitine excreted by the kidneys of healthy humans is also dependent, in part, on the amount of carnitine consumed in the diet. In one study, people eating high-carnitine diets excreted less carnitine in their urine than they consumed, whereas those eating low-carnitine diets excreted more than they consumed, indicating that in addition to dietary carnitine, endogenous synthesis influences urinary carnitine excretion. However, it has been reported that increased dietary intake of carnitine increased circulating concentrations but decreased efficiency (percentage of filtered load) of carnitine reabsorption by the kidney in humans, resulting in an increase in the rate of carnitine excretion. All studies of the effects of diet on carnitine excretion mentioned thus far were conducted in humans; however, human kidneys are able to synthesize carnitine, but canine kidneys have only negligible ability to do so. Also, it has been reported that canine renal tubular cells reabsorb carnitine poorly compared with those in humans. As a result, dogs may be at greater risk than humans for excess renal loss of carnitine, and their response to increased dietary fat may be different as well. In addition, a recent report described dogs diagnosed with cystinuria that had carnitine deficiency associated with increased renal excretion of carnitine, and some of these dogs developed dilated cardiomyopathy while consuming an HF, protein-restricted diet (Prescription Diet® Canine u/d®, Hill’s Pet Nu-

trition, Topeka, KS) to minimize cystine urolithiasis. It is not known at this time whether high dietary fat intake affected carnitine excretion in these cystinuric dogs. However, because dietary fat restriction is used for the management of some carnitine deficiency disorders in people, and short- and long-term intake of HF diets has been shown to increase urinary carnitine excretion in healthy people, the present study was designed to determine the long-term influence of dietary fat and carnitine on renal carnitine excretion in healthy dogs.

■ MATERIALS AND METHODS

Subjects

Eighteen beagles, including 15 spayed females (twelve 1 year of age and three 9 years of age) and three 2.5-year-old castrated males were selected for the study. One of the 9-year-old spayed females developed hypothyroidism during the study and had to be removed from the study. Dogs were determined to be healthy at baseline and at 6, 12, 18, 24, 30, and 36 months based on physical examination findings, complete blood count, serum chemistry profiles (specified below), complete urinalysis, and quantitative aerobic bacterial culture of urine samples collected by cystocentesis. In addition, baseline glomerular filtration rates were determined to be normal based on 24-hour endogenous creatinine clearance rates. Creatinine clearance was calculated using the following formula:

$$C\ (\text{ml/min}) = \frac{U_c\ (\text{mg/ml}) \times U_v\ (\text{ml/min})}{P_c\ (\text{mg/ml})}$$

where $C$ = clearance, $U_c$ = urine concentration of the analyte, $U_v$ = urine volume per unit time, and $P_c$ = plasma concentration of the analytes. All endogenous creatinine clearance rates were within previously established reference ranges for dogs.
Dogs were housed in individual cages with controlled lighting (12 hours of light and 12 hours of dark) and temperature, according to published guidelines. The study was approved by the University Animal Care and Use Committee.

Diet
At baseline, dogs were fed a canned canine maintenance diet (Science Diet® Canine Maintenance®, Hill’s Pet Nutrition), containing 27.5% protein (dry matter) and 19.4% fat (dry matter) (Table 1). After collecting baseline urine and blood samples, the dogs were randomly separated into three equally matched age and gender groups. Test diets, which were fed for 36 months, were randomly allocated to the groups thus formed. Test diet 1 was an LF diet (13.3% fat); test diet 2 was an HF diet supplemented with 344 mg/kg dry-matter basis of L-carnitine (HF + C) (24.1% fat); and test diet 3 was an HF diet (24.2% fat). The diets were essentially equal in all other nutrients. The protein content averaged 10% on a dry-matter basis and was intentionally restricted to minimize endogenous carnitine synthesis. The dog diagnosed with hypothyroidism had initially been assigned to the HF + C group prior to its exclusion, which resulted in only five dogs in that group completing the study.

Feeding Protocol
The baseline diet was fed once a day for a minimum of 2 weeks prior to 24-hour urine collections. The amount of food fed was based on energy requirements determined from ideal body weight using the following formula: Maintenance energy requirement = 2\[(30 \times \text{body weight \ [kg\]} + 70]. All dogs had a body condition score (BCS) of 3 (on a scale of 1 to 5) at entry into the study. Body weights and BCSs were evaluated monthly throughout the study, and the quantity of food fed to each dog was adjusted as necessary to maintain a BCS of 3. On the morning of 24-hour urine collections, dogs were fed at the beginning of the collection after the urinary bladder had been emptied. The same feeding protocol was used for all subsequent 24-hour urine collections while dogs were consuming the test diets. In addition, body weight and quantity of diet consumed by each dog were recorded throughout the study.

Urine Samples
Urine samples for complete urinalysis and quantitative aerobic bacterial culture were collected by cystocentesis at baseline and at 6, 12, 18, 24, 30, and 36 months after initiating the study. Twenty-four–hour urine collections were performed at baseline and 12, 24, and 36 months after diet assignments. Dogs were housed in individual metabolism cages to facilitate urine collection. To begin each collection, the urinary bladder was emptied by transurethral catheterization. The bladder was again catheterized at the end of 24 hours. All urine collected by voiding and transurethral catheterization was pooled, thoroughly mixed, and the volume measured at the end of the collection times. Aliquots (5 ml) of 24-hour pooled urine samples were stored at −70°C until analyzed for urine carnitine and creatinine concentrations.

To prevent catheter-induced bacterial urinary tract infections, cefadroxil (Cefa-Tabs®, Fort Dodge Laboratories Inc., Fort Dodge, IA) was administered orally (20 mg/kg of body weight every 12 hours) during the 24-hour urine collection period and for an additional 24 hours after collection. To minimize bacterial contamination of urine during collection, all voided urine was collected in a container surrounded by dry ice in an insulated box. Quantitative aerobic bacterial culture of pooled urine samples did not yield growth. At the end of each collection period, the urine samples from each dog were screened for blood con-
### TABLE 1. Proximate Analysis of Baseline and Three Protein-restricted Test Diets

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Diet</th>
<th>Baseline*</th>
<th>Low-fat</th>
<th>High-fat Plus Carnitine</th>
<th>High-fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture (% as fed)</td>
<td></td>
<td>71.7</td>
<td>7.6</td>
<td>6.4</td>
<td>7.6</td>
</tr>
<tr>
<td>Protein (% dry matter)</td>
<td></td>
<td>27.5</td>
<td>10.1</td>
<td>10.5</td>
<td>9.9</td>
</tr>
<tr>
<td>Fat (% dry matter)</td>
<td></td>
<td>19.4</td>
<td>13.3</td>
<td>24.1</td>
<td>24.2</td>
</tr>
<tr>
<td>Carbohydrate (NFE) (as dry matter)</td>
<td></td>
<td>45.6</td>
<td>70.5</td>
<td>59.0</td>
<td>59.4</td>
</tr>
<tr>
<td>Fiber (% dry matter)</td>
<td></td>
<td>1.4</td>
<td>2.3</td>
<td>2.4</td>
<td>2.5</td>
</tr>
<tr>
<td>Ash (% dry matter)</td>
<td></td>
<td>6.0</td>
<td>3.8</td>
<td>4.1</td>
<td>4.0</td>
</tr>
<tr>
<td>Lysine (% dry matter)</td>
<td></td>
<td>1.4</td>
<td>0.6</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>Methionine (% dry matter)</td>
<td></td>
<td>0.52</td>
<td>0.4</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>Methionine + cystine (% dry matter)</td>
<td></td>
<td>0.9</td>
<td>0.6</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>Carnitine (ppm or mg/kg of dry matter)</td>
<td></td>
<td>104</td>
<td>29</td>
<td>344</td>
<td>20</td>
</tr>
<tr>
<td>Metabolizable energy (kcal as fed)</td>
<td></td>
<td>36.8/oz</td>
<td>4.22/g</td>
<td>4.78/g</td>
<td>4.79/g</td>
</tr>
</tbody>
</table>

**Ingredient list**

- *Hill’s Science Diet® Canine Maintenance® Canned.
- Metabolizable energy calculated according to published guidelines.  
- NFE = nitrogen free extract.
tamination, using a reagent strip, and the collection was repeated within a week if blood contamination was detected.

**Serum Samples**

Blood samples were collected from the jugular vein of dogs approximately 8 hours after initiating 24-hour urine collections at baseline, 12, 24, and 36 months and approximately 8 hours after eating at 6, 18, and 30 months. Serum was obtained within 30 minutes of clot formation. Complete blood cell counts and serum chemistry profiles (albumin, alkaline phosphatase, alanine transaminase, amylase, aspartate transaminase, total bilirubin, cholesterol, phosphorus, total protein, triglyceride, blood urea nitrogen, glucose, creatinine, calcium, sodium, potassium, chloride, and carbon dioxide) were determined within 8 hours after blood was obtained.

**Carnitine Assay**

Carnitine in urine was measured as free carnitine, short-chain acylcarnitine, and total carnitine (nmol/ml). Assays of carnitine in urine extracts were conducted by modifications of the methods of McGarry and Foster and Parvin and Pande using L-carnitine as a standard. Duplicate aliquots of extract were added to a reaction mixture (0.55 mL) containing 50 mM HEPES-KOH buffer (pH 7.6); 0.25 mM N-ethyl-maleimide; and 0.01 µCi of [1-¹⁴C] acetyl CoA. The reaction was initiated by the addition of 0.5 U of carnitine acetyltransferase followed by frequent mixing at 25°C for 30 minutes. The reaction was terminated by the addition of charcoal in acidified alcohol followed by equilibration on ice for 30 minutes. After centrifugation, an aliquot of the supernatant was placed in scintillation fluid and radioactivity was counted. In this assay, sample results were compared with results for standard solutions of L-carnitine.

Using this modified method, percentage of recovery has been assessed for human plasma. Recovery of added L-carnitine (5.25 nmol/ml) to plasma was 96.2%. Recovery of added L-carnitine to extracted plasma was 99.3% (M.E. Pierpont, personal communication, February 1996).

**Calculations**

Twenty-four–hour urine carnitine excretion for free carnitine, short-chain acylcarnitine, and total carnitine were calculated (nmol/kg body weight/day). Twenty-four–hour urine short-chain acylcarnitine:free carnitine ratios were also calculated. Urine and serum creatinine concentrations were converted to nmol/ml.

**Statistical Analysis**

Statistical evaluations were performed using microcomputer software (SAS Institute Inc., Cary, NC) and a desktop computer. Response over time was assessed among the three treatment groups and within each treatment group. A combination of two-way analysis of variance (ANOVA) and least significant difference (LSD) test was used to analyze differences in urine carnitine excretion among the three test diets at each time period. Linear regression analysis, both as continuous variables and as discrete variables, was used to analyze differences in urine carnitine excretion within each treatment diet over time. The paired Student’s t-test was used to analyze beginning and ending body weights for each diet group. Significant differences were defined as \( P < .05 \).

**RESULTS**

**Body Weights and Caloric Intake**

Dogs on the LF diet weighed an average of 9.7 ± 1.2 kg at the start and 10.0 ± 1.3 kg at the end of the study (Table 2). The difference between these weights was not significant (\( P = \)
The dogs in this group consumed an average of 18.2 g of LF diet (76.8 kcal/kg body weight/day) and 0.49 mg of carnitine/kg body weight/day. Weight of the dogs in the HF + C group averaged 10.5 ± 0.8 kg at the start and 11.2 ± 1.0 kg at the end of the study ($P < .05$). The dogs in this group consumed 14.3 g of HF + C diet (68.4 kcal/kg body weight/day) and 4.61 mg of carnitine/kg body weight/day. The HF group weighed a mean of 10.7 ± 0.6 kg at the start and 11.0 ± 1.4 kg at the end of the study ($P = .42$). The dogs in this group consumed a mean of 12.2 g of the HF diet (58.4 kcal/kg body weight/day) and 0.23 mg of carnitine/kg body weight/day.

### Diet Comparisons

Significant differences were not detected in urinary excretion of free carnitine, short-chain acylcarnitine, total carnitine, or ester:free carnitine ratios. Least significant difference and least squared means (Table 3) tests revealed that free carnitine, short-chain acylcarnitine, and total carnitine were significantly higher in the HF + C group than in the LF and HF groups, and the ester:free carnitine ratio was significantly lower in the HF + C group than in the LF and HF groups. At 36 months, free carnitine and total carnitine were significantly higher ($P < .05$) in the HF + C group than in the LF and HF groups, and short-chain acylcarnitine was significantly higher ($P < .05$) in the HF + C group compared with the LF group (Table 3), although it was not significantly different from the HF group. Therefore, at 36 months, a significant difference in short-chain acylcarnitine excretion no longer existed between the HF + C and HF groups. Also of interest is that at all sampling periods (baseline, 12, 24, and 36 months), free carnitine, short-chain acylcarnitine, and total carnitine (Table

<table>
<thead>
<tr>
<th>Variable (mean ± SD)</th>
<th>Low-fat</th>
<th>High-fat Plus Carnitine</th>
<th>High-fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body weight (kg)</td>
<td>9.7 ± 1.2</td>
<td>10.5 ± 0.8*</td>
<td>10.7 ± 0.6</td>
</tr>
<tr>
<td>Final body weight (kg)</td>
<td>10.0 ± 1.3</td>
<td>11.2 ± 1.0*</td>
<td>11.0 ± 1.4</td>
</tr>
<tr>
<td>Caloric intake (g/kg BW/day)</td>
<td>18 ± 4.2</td>
<td>14 ± 2.2</td>
<td>12.2 ± 0.4</td>
</tr>
<tr>
<td>Dietary protein intake (g/kg BW/day)</td>
<td>1.7 ± 0.3</td>
<td>1.3 ± 0.2</td>
<td>1.1 ± 0.03</td>
</tr>
<tr>
<td>Dietary fat intake (g/kg BW/day)</td>
<td>2.2 ± 0.5</td>
<td>3.2 ± 0.5</td>
<td>2.8 ± 0.09</td>
</tr>
<tr>
<td>Dietary carnitine intake (mg/kg BW/day)</td>
<td>0.49 ± 0.01</td>
<td>4.61 ± 0.7</td>
<td>0.23 ± 0.008</td>
</tr>
<tr>
<td>(nmol/kg BW/day)</td>
<td>(3039.7 ± 710)</td>
<td>(28,598.0 ± 5,287)</td>
<td>(1426.8 ± 51.2)</td>
</tr>
</tbody>
</table>

*Significantly greater than initial body weight ($P < .05$).
2) were always highest in the HF + C group, followed by the HF and the LF groups.

Comparisons Within Diets Over Time
Evaluating the data as continuous variables by linear regression analysis revealed a significant difference (P < .05) in short-chain acylcarnitine excretion over time in the LF group. Excretion was increased from baseline at 12 months, peaked at 24 months, then decreased at 36 months to a level about twice that observed at baseline. A significant difference (P < .05) in free carnitine, short-chain acylcarnitine, total carnitine excretion, and ester:free carnitine ratio was also observed over time in the HF + C group. Free carnitine, short-chain

### TABLE 3. Twenty-Four-Hour Urine Carnitine Excretion in Healthy Dogs Fed Different Diets (Mean ± Standard Error of the Mean)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Diet</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low-fat</td>
<td>High-fat Plus Carnitine</td>
<td>High-fat</td>
<td></td>
</tr>
<tr>
<td>Free carnitine†</td>
<td>3475 ± 624</td>
<td>4402 ± 624</td>
<td>3855 ± 624</td>
<td></td>
</tr>
<tr>
<td>Short-chain acylcarnitine†</td>
<td>1061 ± 907</td>
<td>1571 ± 907</td>
<td>1318 ± 907</td>
<td></td>
</tr>
<tr>
<td>Total carnitine†</td>
<td>4599 ± 835</td>
<td>6013 ± 914</td>
<td>5192 ± 835</td>
<td></td>
</tr>
<tr>
<td>Ester:free carnitine ratio</td>
<td>0.30 ± 0.05</td>
<td>0.37 ± 0.04</td>
<td>0.33 ± 0.06</td>
<td></td>
</tr>
</tbody>
</table>

| 12 Months After Diet Assignment |                  |                |                |
| Free carnitine†                | 4202 ± 2856^b       | 41,486 ± 3498^a| 7574 ± 2856^b  |
| Short-chain acylcarnitine†     | 2166 ± 785^b        | 11,137 ± 962^a | 3802 ± 785^b   |
| Total carnitine†               | 6377 ± 3599^b       | 52,627 ± 4408^a| 11,383 ± 3599^b|
| Ester:free carnitine ratio     | 0.63 ± 0.16^b       | 0.25 ± 0.02^a  | 0.60 ± 0.09^b  |

| 24 Months After Diet Assignment |                  |                |                |
| Free carnitine†                | 5284 ± 2659^b       | 49,612 ± 2913^a| 8308 ± 2659^b  |
| Short-chain acylcarnitine†     | 3309 ± 986^b        | 12,122 ± 1080^a| 3851 ± 986^b   |
| Total carnitine†               | 8592 ± 3227^b       | 61,735 ± 3535^a| 12,160 ± 3227^b|
| Ester:free carnitine ratio     | 0.67 ± 0.13^b       | 0.25 ± 0.03^a  | 0.53 ± 0.05^b  |

| 36 Months After Diet Assignment |                  |                |                |
| Free carnitine†                | 5218 ± 2295^b       | 24,273 ± 2514^a| 8302 ± 2295^b  |
| Short-chain acylcarnitine†     | 2205 ± 540^b        | 5767 ± 592^a   | 3418 ± 540^a,b |
| Total carnitine†               | 7420 ± 2709^b       | 30,041 ± 2967^a| 11,720 ± 2709^b|
| Ester:free carnitine ratio     | 0.85 ± 0.38         | 0.25 ± 0.02    | 0.44 ± 0.06    |

*Values determined for each group of dogs after eating baseline diet (Science Diet® Canine Maintenance®, Hill’s Pet Nutrition, Topeka, KS) during 2-week period prior to feeding test diets.
†Nmol/kg body weight/24 hours.
^a,bMeans in the same row with different superscripts are significantly different (P < .05).
acylcarnitine, and total carnitine excretion were increased from baseline at 12 months, peaked at 24 months, and then had decreased to four to five times the baseline value at the 36-month evaluation. Conversely, the ester:free carnitine ratio was significantly decreased ($P < .05$) from baseline at 12 months and then remained stable for the duration of the study. The HF group also revealed a significant increase ($P < .05$) in short-chain acylcarnitine from baseline at 12 months and then remained stable from 12 to 36 months.

**Dietary Carnitine Intake Versus Renal Carnitine Excretion**

After test diet assignment, urine carnitine excretion increased from baseline levels in all diet groups. Mean dietary intake (Table 2) of carnitine in the LF group was 3039.7 nmol/kg body weight/day, and mean urine carnitine excretion ranged from 6377 to 8592 nmol/kg body weight/day (Table 3). Mean dietary intake of carnitine in the HF + C group was 28,598 nmol/kg body weight/day, and mean urine carnitine excretion ranged from 30,041 to 61,735 nmol/kg body weight/day. Mean dietary intake of carnitine in the HF group was 1426.8 nmol/kg body weight/day, and mean urine carnitine excretion ranged from 11,383 to 12,160 nmol/kg body weight/day.

**DISCUSSION**

Results from this study revealed that chronic consumption of an HF diet increased renal excretion of free, short-chain acylcarnitine and total carnitine in healthy dogs when compared with values for dogs consuming an LF diet, although this increase was not statistically significant. However, in healthy humans, consumption of an HF diet resulted in an increase in urine carnitine excretion that was primarily due to an increase in short-chain acylcarnitine (ester) excretion. In contrast, results from the present study revealed that dogs consuming an HF diet had an increase in urine carnitine excretion compared with dogs consuming an LF diet, and that the increase was primarily free carnitine, rather than short-chain acylcarnitine. The urine ester:free carnitine ratio decreased in the HF group as the study progressed but increased in the LF group. This indicates that excretion of free carnitine increased relative to excretion of short-chain acylcarnitine in dogs fed an HF diet, whereas the opposite was true for dogs fed an LF diet. This does raise the possibility that just as some people with disorders associated with carnitine deficiency benefit from carnitine supplementation and an LF diet, dogs with carnitine deficiency or those with a predisposing risk factor for developing carnitine deficiency (e.g., cystinuric dogs with increased renal excretion of carnitine) may also benefit from carnitine supplementation and an LF diet. However, studies in clinical patients are needed to confirm this.

Another noteworthy result was that the LF group excreted approximately two to 2.8 times more carnitine in their urine than they consumed in the diet, whereas the HF group excreted approximately eight to 8.5 times more carnitine than they consumed. Therefore, it appears that dogs have the ability to readily synthesize large amounts of carnitine from precursor amino acids when dietary intake of carnitine is low. This is similar to what was observed in humans, where those eating low-carnitine diets excreted more carnitine in their urine than they consumed. However, this same study also found that people eating high-carnitine diets excreted less carnitine in their urine than they consumed. This was not observed in the present canine study. Dogs consuming an HF diet supplemented with carnitine excreted more carnitine than they consumed, and as evident from the urine ester:free carnitine ratio, they excreted more free carnitine relative to short-chain acyl-
carnitine. The HF + C group did, however, excrete approximately one to two times more carnitine in their urine than it consumed; whereas the HF group, which did not receive carnitine supplementation, excreted eight to 8.5 times more carnitine than it consumed. It would have been ideal to have an LF diet group supplemented with carnitine to compare to the HF + C group, however, this was not logistically possible in the present study. Nonetheless, the observation that renal excretion of carnitine exceeded dietary intake in all three diet groups is consistent with previous reports concluding that canine renal tubular cells reabsorb carnitine poorly\textsuperscript{30,31} compared with findings in humans.

In humans, there is considerable variation in carnitine excretion among and within individuals tested.\textsuperscript{44–46} Similar variation in carnitine excretion was observed in the present canine study, and this may account in part for the large statistical variability and may explain the relatively wide normal range for urine carnitine excretion in healthy dogs.\textsuperscript{9} Therefore, establishing group trends in urine carnitine excretion over time decreased the likelihood that any one dog would significantly bias the results. The ability to document trends in levels of carnitine excretion from repeated sampling is one of the main reasons why dogs were evaluated over 36 months instead of just a few weeks. A larger number of dogs in the study would have been beneficial but was not possible due to a variety of constraints.

\section*{CONCLUSIONS}

Feeding an HF diet to dogs increased renal carnitine excretion but not significantly more than for dogs consuming an LF diet. However, it does raise the possibility that this increase in renal excretion of carnitine observed with the HF diet could be one risk factor for developing carnitine deficiency in some dogs with an underlying risk factor for developing carnitine deficiency, such as those with cystinuria and carnitininuria.\textsuperscript{8,9} In addition, as with humans,\textsuperscript{15,16,23,24} this study raises the possibility that dogs that develop carnitine deficiency may benefit from an LF diet, although further studies in clinical patients with carnitine deficiency are warranted to confirm this. Another important observation in this study is that dogs have the ability to increase endogenous synthesis of carnitine when dietary intake is low. Last of all, renal excretion of carnitine exceeded dietary intake in all three diet groups, confirming previous reports that concluded that canine renal tubular cells reabsorb carnitine poorly as compared with findings in humans.

\section*{REFERENCES}


