Skeletal muscular weakness, cramping, exercise intolerance, and pain or signs of pain have been reported as potential sequelae of hypothyroidism in humans and dogs.1,2 The pathophysiologic mechanisms that contribute to development of muscular weakness during hypothyroidism in humans and other animals may be multifactorial, but neuromuscular disease, manifesting as both peripheral neuropathy or myopathy, is frequently implicated.1–5 At the time of initial diagnosis of hypothyroidism, up to 80% of humans report problems that can be attributed to skeletal muscular dysfunction and nearly 40% have clinical evidence of skeletal muscle weakness.4 Although canine hypothyroid myopathy has been sporadically reported to occur as both a clinical and subclinical entity in spontaneous cases of canine hypothyroidism, the prevalence of this condition is unknown, its pathogenesis is poorly understood, and its pathologic phenotype is sparsely described in dogs.1,3 Carnitine is a carrier molecule that is essential for the transport of fatty acids into mitochondria for subsequent β-oxidation and plays an important role in the energy metabolism of skeletal muscle. Decreases in

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**Longitudinal study of the effects of chronic hypothyroidism on skeletal muscle in dogs**

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**Objective**—To study the effects of experimentally induced hypothyroidism on skeletal muscle and characterize any observed myopathic abnormalities in dogs.

**Animals**—9 female, adult mixed-breed dogs; 6 with hypothyroidism induced with irradiation with 131 iodine and 3 untreated control dogs.

**Procedures**—Clinical examinations were performed monthly. Electromyographic examinations; measurement of plasma creatine kinase, alanine aminotransferase, aspartate aminotransferase, lactate, and lactate dehydrogenase isoenzyme activities; and skeletal muscle morphologic-morphometric examinations were performed prior to and every 6 months for 18 months after induction of hypothyroidism. Baseline, 6-month, and 18-month assessments of plasma, urine, and skeletal muscle carnitine concentrations were also performed.

**Results**—Hypothyroid dogs developed electromyographic and morphologic evidence of myopathy by 6 months after treatment, which persisted throughout the study, although these changes were subclinical at all times. Hypothyroid myopathy was associated with significant increases in plasma creatine kinase, aspartate aminotransferase, and lactate dehydrogenase 5 isoenzyme activities and was characterized by nemaline rod inclusions, substantial and progressive predominance of type I myofibers, decrease in mean type II fiber area, subsarcolemmal accumulations of abnormal mitochondria, and myofiber degeneration. Chronic hypothyroidism was associated with substantial depletion in skeletal muscle free carnitine.

**Conclusions and Clinical Relevance**—Chronic, experimentally induced hypothyroidism resulted in substantial but subclinical phenotypic myopathic changes indicative of altered muscle energy metabolism and depletion of skeletal muscle carnitine. These abnormalities may contribute to nonspecific clinical signs, such as lethargy and exercise intolerance, often reported in hypothyroid dogs. (Am J Vet Res 2009;70:879–889)
skeletal muscle carnitine content, as well as increased urinary carnitine excretion, have been implicated in the pathogenesis of several hereditary and acquired metabolic myopathies of dogs and humans, including human hypothyroid myopathy.14,15 Considering the relatively ubiquitous role of thyroid hormones in regulation of lipid metabolism, thyroid hormonal imbalance has been determined to contribute to abnormalities of cellular energy generation in striated muscle through multiple carnitine-dependent mitochondrial biochemical pathways.6–13 To our knowledge, the effects of hypothyroidism on compartmental fractional carnitine concentrations have not been studied in dogs.

This study was undertaken as part of a collaboration examining the effects of chronic hypothyroidism on multiple canine organ systems.14 The specific objectives of this study were to evaluate the effects of experimentally induced hypothyroidism on skeletal muscle; to clinically, biochemically, electrophysiologically, and morphologically characterize any observed myopathic abnormalities; and to assess the effects of chronic hypothyroidism on carnitine metabolism. It was hypothesized that skeletal muscle carnitine deficiency occurs in dogs with chronic hypothyroidism and may contribute to the pathogenesis of hypothyroid myopathy.

Materials and Methods

Dogs—Nine healthy, mixed-breed, breeding bitches, aged 24 to 40 months and weighing from 7.5 to 11.5 kg, were obtained from a commercial source. Prior to the start of the study, each dog was determined to be healthy on the basis of lack of important abnormalities detected via clinical and neurologic examination, CBC, serum biochemical profile, urinalysis, zinc sulfate fecal flotation, and serum heartworm antigen test. Thyroid function tests (serum total T<sub>4</sub>, free T<sub>4</sub>, T<sub>3</sub> by equilibrium dialysis, total triiodothyronine, thyroid-stimulating hormone concentration, and determination of thyroglobulin, triiodothyronine, and T<sub>4</sub> autoantibody concentrations) performed by a commercial veterinary diagnostic laboratory service were within reference ranges in all dogs. Dogs were fed a nutritionally balanced commercially available ration throughout the duration of the study. The experimental protocol was approved by the Virginia Tech Institutional Animal Care and Use Committee.

Induction of hypothyroidism—Following 15 to 18 weeks of acclimation and preliminary data collection, hypothyroidism was induced in 6 randomly selected bitches via IV administration of 1 mCi of iodine 131<sup>131</sup>I/kg, a method determined to be successful for induction and long-term maintenance of hypothyroidism.14,15 Hypothyroidism was confirmed at 9, 40, and 75 weeks after treatment by finding serum T<sub>4</sub> concentrations < 5 nmol/L before and 4 hours after administration of 50 µg of recombinant human thyroid-stimulating hormone.6 The remaining 3 bitches served as untreated control dogs.

Clinical and diagnostic investigations and anesthetic protocol—Prior to induction of hypothyroidism and monthly thereafter, each dog underwent routine physical and neurologic (gait analysis; head, body posture, and cranial nerve evaluation; prioprioceptive and postural reaction testing; evaluation of segmental spinal reflexes and nociception; and palpation of muscle mass and tone) examinations performed by a board-certified neurologist (JHR). Diagnostic investigations were performed prior to (time 0) and at 6 (time 1), 12 (time 2), and 18 (time 3) months after the induction of hypothyroidism in all dogs.

The anesthetic protocol consisted of premedication with aropymazine maleate (0.02 mg/kg, IM) and morphine sulfate (0.25 mg/kg, IM), placement of a peripheral IV catheter, and induction with propofol (4 to 6 mg/kg, IV, to effect), with anesthesia maintained by use of isoflurane with supplemental 100% oxygen (50 mL/kg/min). During anesthesia, dogs received lactated Ringer’s solution (10 mL/kg/h, IV), the body temperature was monitored continuously with a rectal temperature probe, and normothermia was maintained with circulating warm water and air blankets. Dogs also received morphine sulfate (0.25 mg/kg, SC or IM) upon extubation and carprofen (2 mg/kg, PO, q 12 h) for 2 days following muscle biopsy.

Electrophysiologic examination—After anesthetic induction in each dog, a concentric needle EMG examination was performed with a commercial electrodagnostic system.6 The EMG examinations were performed with each dog lying in lateral recumbency to allow for complete examination of skeletal muscles of the head, neck, thoracic and pelvic limbs, and trunk of 1 side of the body only, while maintaining the muscles of the contralateral side of the body free of needle-induced artifact that might interfere with interpretation of muscle biopsy specimens. The body side subjected to EMG examination was determined via randomization. Three to 5 needle passes were routinely performed in all muscles examined. If present in a selected muscle, spontaneous EMG activity was semiquantitatively graded (1+ to 4+) on the basis of a published scale.16

Muscle biopsy procedure and processing—Following the EMG examination, biopsy specimens from the contralateral biceps femoris were harvested by use of a standard open muscle biopsy technique following aseptic preparation of the overlying skin. The biceps femoris was chosen as the biopsy site on the basis of the availability of normative morphometric data in dogs and our experience with its frequent involvement in cases of spontaneous hypothyroid-associated myopathy. Harvested muscle biopsy specimens were sharply separated into 3 approximately 0.5-cm<sup>3</sup> samples. Muscle biopsy specimens intended for fractional carnitine determinations were blotted dry and immediately frozen in liquid nitrogen at −80°C until assayed. The second muscle sample was further sharply divided into 3 smaller sections, each of which was immersed into 2.5% glutaraldehyde for ultrastructural examination. The final muscle biopsy specimen was processed for light microscopic and morphometric examinations.

Plasma, urine, and muscle biochemical analyses—At all sampling periods, heparinized jugular venous blood and urine samples were collected prior
to induction of general anesthesia for performance of EMG examinations and muscle biopsy procedures. Venous blood samples were immediately centrifuged for 15 minutes at 1,500 × g at 4°C. Plasma samples were then frozen at −80°C until analyzed for determination of activities of CK, AST, ALT, lactate, and total LDH and total, free, and esterified carnitine concentrations. Urine and muscle biopsy specimens were frozen at −80°C until assayed for total, free, and esterified carnitine concentrations. Plasma assays for activities of CK, AST, ALT, lactate, and total LDH were performed with a commercial biochemical analytical system, with canine reference ranges validated for our laboratory. Isoenzymatic fractions of LDH were determined via agarose gel electrophoresis at 100 V for 20 minutes as described, with individual fractions expressed as percentages of the total LDH activities. Plasma, urine, and muscle carnitine concentrations were determined by use of a described radioenzymatic method.

**Histopathologic and histochemical evaluation**—Fresh muscle biopsy specimens intended for light microscopic examination were flash frozen in isopentane precooled in liquid nitrogen and processed by use of a standard protocol of histologic and histochemical stains and reactions, including H&E, modified Gomori trichrome, periodic acid-Schiff, oil red O, myofibrillar ATPases (referred to as ATPases) at pH 9.8 and 4.3, acid phosphatase, alkaline phosphatase, esterase, NADH-dehydrogenase, and staphylococcal protein A–horseradish peroxidase.

**Morphometrics**—For quantitative morphometric analyses, 3 digital photomicrographs of representative sections of muscle oriented in the transverse plane and stained with H&E and ATPase (pH, 4.3) were obtained at 100X magnification from each dog at each sampling period. Morphometric analyses were performed with a personal computer with a custom macro written for public domain image analysis software. By use of the digital photomicrographs, the mean fiber type percentages of type I and type II myofibers were calculated by randomly counting 100 myofibers in each of the 3 photomicrographs from each dog and sampling period. Individual fiber type areas of type I and type II myofibers were determined in hand-drawn regions of interest from ATPase (pH, 4.3) stained images, and the mean fiber type areas subsequently calculated by obtaining the areas of 150 fibers of each fiber type (50 from each photomicrograph).

The mean number of internal (centralized) nuclei for type I and type II myofibers was determined by counting the total number of internal nuclei/100 myofibers on 3 H&E-stained sections from each dog and calculating the mean value from the 3 sections. By use of 3 sections stained with ATPase (pH, 4.3) from each dog, the mean number of internalized nuclei in type II myofibers was similarly calculated. The mean number of internal nuclei in type I myofibers was subsequently derived from the number in type II myofibers, the percentage of type I to type II myofibers (as described), and the total number of internal nuclei/100 myofibers.

**Ultrastructural examination**—Muscle biopsy specimens fixed in 2.5% glutaraldehyde were postfixed in 1% osmium and embedded in plastic by use of standard procedures. One-micron sections were stained with toluidine blue and examined via light microscopy to select appropriate fields for electron microscopy. Muscle biopsy specimens were then sectioned at 50 nm, treated with lead citrate and uranyl acetate, and examined with a transmission electron microscope.

**Statistical analysis**—Effects of group or treatment and time on each measured quantitative variable in the biochemical, morphologic, and carnitine categories were assessed by use of repeated-measures ANCOVA. The linear model included the baseline measurements as a covariate. Interactions between group and time were investigated by use of the slice option of a statistical software program followed by the Tukey procedure for multiple comparisons. Additionally, EMG results were compared between the 2 treatment groups at each of the time points by use of the Fisher exact test with a Bonferroni adjustment for multiple comparisons. Significance was set at α = 0.05. All analyses were performed with a statistical software package.

**Results**

**Clinical and EMG examinations**—No dog developed clinical signs of neuromuscular dysfunction at any time.

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### Figure 1

Mean (least square means of analyzed variables adjusted for time 0 values) ± SEM values for plasma biochemical analytes determined at various times (baseline [time 0], 6 months [time 1], 12 months [time 2], and 18 months [time 3]) in control dogs (solid line and diamonds) and dogs with hypothyroidism (dashed line and squares). A—Creatine kinase; reference range, 45 to 205 U/L. B—Total LDH; reference range, 53 to 590 U/L. C—Aspartate aminotransferase; reference range, 18 to 58 U/L. D—Alanine aminotransferase; reference range, 28 to 75 U/L. E—Lactate; reference range, 0.8 to 2.6 mmol/L. †Significant (P < 0.05) difference between control and hypothyroid groups. ▲, ▼, □, △Significant (P < 0.05) effect of time in the hypothyroid group at times labeled with matched symbols.
point in the study. However, by time 1, all hypothyroid dogs had at least 1 extraneural sign consistent with hypothyroidism, including weight gain (6/6 dogs), endocrine alopecia (3/6), seborrhea sicca (3/6), and superficial pyoderma or pododermatitis (2/6). At times 2 and 3, all hypothyroid dogs had at least 2 extraneural clinical signs compatible with hypothyroidism, which, apart from weight gain and obesity, were most often dermatologic in origin. One dog in the hypothyroid group died of necropsy-confirmed pulmonary thromboembolic disease 14 months after induction of hypothyroidism, which left 5 hypothyroid dogs for inclusion in the 18-month (time 3) sampling period.

At time 0, results of EMG examinations were normal in all dogs and remained normal in the 3 control dogs at all sampling points. At time 1, 5 of 6 hypothyroid dogs had abnormal results of EMG, consisting of increased insertional activity and variably severe (1+ to 3+) fibrillation potentials and positive sharp waves that were primarily detected in the proximal, appendicular extensor muscles (triceps, biceps femoris, semimembranosus, and semitendinosus). At times 2 and 3, variably severe (2+ to 4+) EMG abnormalities were detected in all hypothyroid dogs and had a diffuse distribution, being present in all muscles tested except for muscles of mastication. At times 1, 2, and 3, the proportion of dogs in the hypothyroid group with abnormal results of EMG was significantly ($P < 0.01$) higher than the control group.

Plasma biochemical assays—At time 0, no significant differences were detected between groups for any measured plasma biochemical analyte. No significant differences in activities of plasma CK, total LDH, AST, ALT, or lactate were detected between hypothyroid and control dogs at time 1 (Figure 1). At time 1, the only plasma biochemical analyte that exceeded the upper limit of the canine reference range was CK, which was detected in 2 of 6 hypothyroid dogs. At times 2 and 3, the plasma CK, total LDH, and AST activities were significantly higher in hypothyroid dogs than control dogs. Plasma CK and AST activities exceeded the upper end of their respective reference ranges in all hypothyroid dogs at times 2 and 3. At times 2 and 3, the total LDH concentration exceeded the upper limit of the reference range in 5 of 6 and 4 of 5 hypothyroid dogs, respectively. Among control dogs, plasma activities of CK, total LDH, and AST did not differ significantly throughout the duration of the study. In the hypothyroid group, activities of CK and total LDH were significantly ($P = 0.02$) higher at times 2 and 3, compared with time 1, and the plasma AST activity was significantly ($P = 0.02$) higher than the control group.

Figure 2—Mean (least square means of analyzed variables adjusted for time 0 values) ± SEM values for plasma LDH isoenzyme fractions, expressed as percentages of the total plasma LDH activity at various times in control dogs (white bars) and dogs with hypothyroidism (striped bars). A—LDH1. B—LDH2. C—LDH3. D—LDH4. E—LDH5. ○, △, ★, ▲Significant ($P < 0.05$) effect of time in the control group at times labeled with matched symbols. See Figure 1 for remainder of key.

Figure 3—Aggregates of nemaline rod-body inclusions in biceps femoris biopsy specimen from a hypothyroid dog. A—Photomicrograph. Gomori trichrome stain; bar = 50 µm. B—Electron photomicrograph. Notice nemaline rods with lattice-type appearance of expanded Z-line material. Bar = 500 nm.
higher at time 2, compared with time 1. No significant
group or time effects were detected for the plasma ALT
or lactate activities.

No significant group or time effects were detected
for the LDH1 or LDH3 isoenzymatic fractions (Figure 2).
Compared with controls at times 2 and 3, the LDH2
isoenzymatic fractional activities were significantly
(\( P = 0.01 \)) lower in hypothyroid dogs, whereas the LDH5
activities were significantly higher. Among hypothy-
roid dogs, the LDH2 activities were significantly lower
at times 2 and 3, compared with activity at time 1, and
the LDH5 activities at times 2 and 3 were significantly
(\( P = 0.03 \)) higher, compared with time 1. No differences
in LDH4 activities were detected between groups, al-
though in the control group, LDH4 isoenzymatic ac-
tivities were significantly (\( P = 0.02 \)) higher at time 1,
compared with activities at times 2 and 3.

Qualitative muscle histopathologic findings and
ultrastructure—At time 0, muscle biopsy specimens were
interpreted as histologically normal in all dogs in both
groups. Light and electron microscopic evaluations of
muscle biopsy specimens revealed normal findings at all
sampling points in all control dogs. When present in biop-
sy specimens from hypothyroid dogs, intramuscular nerve
fibers were reported as normal in appearance at all times.
At time 1, the predominant histopathologic abnormalities
observed in the hypothyroid group were multifocally dis-
tributed nemaline rod inclusions (Figure 3; Table 1) and
type II myofiber atrophy. Ultrastructurally, nemaline rods
had a classic lattice-type appearance of enlarged or repeat-
ing Z-band material and occurred in variable numbers
from single to large aggregates.

![Figure 4](https://placeHolder.com/4.png)

**Figure 4**—Photomicrographs of biceps femoris muscle biopsy
specimens from a control (A) and a hypothyroid dog (B) at time 2.
Notice the normal mosaic appearance of type I and II myofibers
in the specimen from the control dog and the type I myofiber
predominance and type II myofiber atrophy in the specimen from
the hypothyroid dog. ATPase stain (pH 4.3); bar = 50 \( \mu m \).

![Figure 5](https://placeHolder.com/5.png)

**Figure 5**—Photomicrograph (A) of subsarcolemmal accumula-
tions of NADH-dehydrogenase material in a muscle biopsy speci-
men from a hypothyroid dog. NADH-dehydrogenase stain; bar =
50 \( \mu m \). B—Electron photomicrograph of a muscle biopsy speci-
men from a hypothyroid dog. Notice foci of excessive numbers
of subsarcolemmal mitochondria and glycogen vacuoles (arrows).
Bar = 2 \( \mu m \).

<table>
<thead>
<tr>
<th>Histomorphologic feature</th>
<th>Time 0</th>
<th>Time 1</th>
<th>Time 2</th>
<th>Time 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I myofiber predominance</td>
<td>0/6</td>
<td>0/6</td>
<td>6/6</td>
<td>5/5</td>
</tr>
<tr>
<td>Type I myofiber atrophy</td>
<td>0/6</td>
<td>1/6</td>
<td>3/6</td>
<td>1/5</td>
</tr>
<tr>
<td>Type II myofiber atrophy</td>
<td>0/6</td>
<td>3/6</td>
<td>6/6</td>
<td>5/5</td>
</tr>
<tr>
<td>Nemaline rods</td>
<td>0/6</td>
<td>4/6</td>
<td>6/6</td>
<td>5/5</td>
</tr>
<tr>
<td>Subsarcolemmal accumulations of NADH-dehydrogenase-positive material</td>
<td>0/6</td>
<td>0/6</td>
<td>5/6</td>
<td>5/5</td>
</tr>
<tr>
<td>Perimysial lipid accumulation</td>
<td>0/6</td>
<td>0/6</td>
<td>3/6</td>
<td>2/5</td>
</tr>
<tr>
<td>Muscle fiber type grouping</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
<td>1/5</td>
</tr>
</tbody>
</table>

Table 1—Frequencies (number of affected dogs/total number of dogs) of observed qualitative microscopic changes in mus-
cle biopsy specimens from hypothyroid dogs at various times (baseline [time 0], 6 months [time 1], 12 months [time 2], and 18 months [time 3]).
Observed pathologic changes in all hypothyroid muscle biopsy specimens at time 2 included type I myofiber predominance, type II myofiber atrophy, and variably severe and multifocal nemaline rod inclusions in type I myofibers (Figure 4; Table 1). Less frequently observed abnormalities at time 2 included subsarcolemmal accumulations of NADH-dehydrogenase–positive material (Figure 5), perimysial lipid accumulations, and type I myofiber atrophy. Ultrastructurally, NADH-dehydrogenase–positive subsarcolemmal accumulations were composed of numerous mitochondria. Glycogen vacuoles were often found in close proximity to distorted or degenerating myofibers and subsarcolemmal mitochondrial accumulations. Mitochondria in subsarcolemmal foci had a heterogeneous mixture of normal and abnormal morphologies. Mitochondrial morphologic abnormalities included abnormal shapes (Figure 6), irregularly dilated cristae, single to multiple, variably sized and shaped, electron-dense inclusions within mitochondrial matrices, and scarce, intracytoplasmic, lamellated myelin-like inclusion bodies.

At time 3, nemaline rods, subsarcolemmal accumulations of excessive numbers of mitochondria, with similar ultrastructural features as those described at time 2, marked type I myofiber predominance, and severe type II myofiber atrophy were reported in all biopsy specimens from hypothyroid dogs (Table 1). Marked degeneration of actin and myosin filaments (Figure 7), regions of hypercontracted sarcomeres, and various phases of mitochondrial degeneration, often associated with hydropic matrices, within subsarcolemmal accumulations were noted on ultrastructural analysis of time 3 muscle biopsy specimens. Angular atrophy of type I and type II myofibers and foci of fiber type grouping were also observed in 1 hypothyroid dog at time 3.

Quantitative muscle morphometric analysis—At time 0, there were no significant differences between groups for any of the measured morphometric variables. No significant time effect for any measured morphometric variable was detected in the control group (Figure 8), and no significant group or time effects were detected for the percentage of internal nuclei in type I or type II myofibers. The percentage of type I and type II myofibers and the type I myofiber area were not significantly different between hypothyroid and control dogs at time 1. The type II myofiber area was the only variable in which there was a significant difference between groups at time 1, with hypothyroid dogs having a significantly ($P = 0.02$) smaller type II myofiber area, compared with that of control dogs. At times 2 and 3, the percentage of type I myofibers and the type I myofiber area were significantly ($P < 0.001$) higher and the percentage of type II myofibers and type II myofiber area significantly ($P < 0.001$) lower in hypothyroid
dogs, compared with values in control dogs. At each successive sampling point from time 1 on, within the hypothyroid group, the percentage of type I myofibers and type I myofiber area increased significantly (P = 0.002), whereas the percentage of type II myofibers and type II myofiber area decreased significantly (P = 0.002).

Quantitative carnitine fractional analysis—Plasma, urine, and muscle biopsy specimens dedicated to carnitine quantification from time 2 were excluded from the study because samples thawed prior to analysis because of a mechanical freezer failure. As a result, only samples from times 0, 1, and 3 were assayed. No differences in the time 0 fractional concentrations of plasma or muscle carnitine were detected between control and hypothyroid dogs, although urine total and free carnitine concentrations were significantly lower in the hypothyroid group than in the control group at time 0 (Table 2). No significant group or time effects were detected for any plasma fraction of carnitine, and no significant time effects for any urine or muscle fractions of carnitine were detected within the control group. Compared with control dogs, hypothyroid dogs had significantly higher urinary excretion of carnitine at times 1 and 3, characterized by an increased urinary concentration of the free carnitine fraction, and significant depletion of the muscle total (P = 0.02) and free (P = 0.03) carnitine concentrations at time 3. Among the hypothyroid group, significant decreases in muscle total (P = 0.01) and free (P < 0.02) carnitine concentrations were observed between times 1 and 3.

**Discussion**

This study revealed that experimentally induced hypothyroidism in dogs is a useful method for evaluation of skeletal myopathic changes induced by thyroid hormone deficiency. Although hypothyroid dogs did not have clinical neuromuscular dysfunction during the study, they developed important electrophysiologic, plasma biochemical, morphologic, and morphometric evidence of myopathy. The observed myopathic changes collectively mimicked those observed in both humans and dogs with spontaneous hypothyroid myopathy.

Figure 7—Electron photomicrographs of degenerative ultrastructural myopathic changes observed at time 3 in hypothyroid dogs. A—In the left section of the figure, notice myofiber degeneration resulting in complete distortion of the normal myofilamentous architecture. More normal appearing myofibers are present on the right, with nemaline rod inclusions in the myofiber in the center. Bar = 2 µm. B—Notice hypercontracted sacromeres adjacent to subsarcolemmal accumulations of mitochondria. Bar = 2 µm. C—Notice regions of subsarcolemmal mitochondria in various stages of degeneration; some have hydropic changes in their matrices. Bar = 500 nm.
skeletal muscle stores of free carnitine, with a concomitantly increased urinary excretion of the free carnitine fraction.

The observed qualitative histopathologic and ultrastructural features of nemaline rod inclusions, type I myofiber predominance, type II myofiber atrophy, and subsarcolemmal accumulations of morphologically abnormal mitochondria resembled similar changes reported in dogs and humans with naturally occurring hypothyroid myopathy.19–21 Ultrastructural abnormalities identified in this study, including abnormal mitochondrial shapes, dilated and irregular cristae, electron-dense matrix inclusions in mitochondria, myelin-like bodies, intracytoplasmic lipid inclusions, and mitochondrial and myofiber degenerative changes, have been reported as features of humans with hypothyroid myopathy.19 The ultrastructural mitochondrial changes noted in this and other studies19–22 suggest that the myopathic morphologic alterations observed with chronic hypothyroidism may be mediated through the intrinsic apoptotic pathway. Quantitative morphometric analyses also similarly revealed a significant and progressive loss and atrophy of type II myofibers and predominance of type I myofibers, which paralleled the temporal evolution of fiber type changes observed in humans with hypothyroid myopathy.19–21 The distribution of EMG changes initially observed in hypothyroid dogs also resembled what is commonly noted in humans in that hypothyroid myopathy initially develops in the large, proximal limb girdle and extensor muscles.19–21

Considering the severity of the morphologic and morphometric myopathic abnormalities identified in this study, it is surprising that clinical signs of neuromuscular dysfunction were not observed. This apparent clinical resistance to the myopathic effects of hypothyroidism in dogs may be partially explained in the context of the experimental design and the fiber-type–specific changes that are characteristic of hypothyroid myopathy. Type II myofibers are predominantly fast-contracting fibers that use anaerobic glycolytic metabolic pathways and are susceptible to fatigue.19

Table 2—Mean ± SEM (least square mean concentrations of specific carnitine fractions and adjusted for time 0 values) values (mmol/mL) for quantitative carnitine analysis in control and hypothyroid dogs at various times.

<table>
<thead>
<tr>
<th>Carnitine fraction</th>
<th>Control</th>
<th>Hypothyroid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time 0 (n = 3)</td>
<td>Time 1 (n = 3)</td>
</tr>
<tr>
<td><strong>Plasma</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>14.9 ± 3.2</td>
<td>14.6 ± 2.9</td>
</tr>
<tr>
<td>Free</td>
<td>13.0 ± 2.1</td>
<td>12.9 ± 1.9</td>
</tr>
<tr>
<td>Esterified</td>
<td>1.93 ± 1.3</td>
<td>1.85 ± 1.3</td>
</tr>
<tr>
<td>Ratio</td>
<td>0.14 ± 0.04</td>
<td>0.11 ± 0.06</td>
</tr>
<tr>
<td><strong>Urine</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>19.0 ± 9.8</td>
<td>14.0 ± 6.3</td>
</tr>
<tr>
<td>Free</td>
<td>8.56 ± 7.5</td>
<td>5.54 ± 5.1</td>
</tr>
<tr>
<td>Esterified</td>
<td>10.5 ± 4.4</td>
<td>6.25 ± 1.5</td>
</tr>
<tr>
<td>Ratio</td>
<td>1.79 ± 1.0</td>
<td>1.79 ± 0.29</td>
</tr>
<tr>
<td><strong>Muscle (mmol/mg)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>17.4 ± 6.6</td>
<td>18.4 ± 2.5</td>
</tr>
<tr>
<td>Free</td>
<td>15.0 ± 6.8</td>
<td>15.9 ± 3.5</td>
</tr>
<tr>
<td>Esterified</td>
<td>2.03 ± 1.5</td>
<td>2.28 ± 0.6</td>
</tr>
</tbody>
</table>

Ratio = Esterified/FREE.

*Muscle carnitine values reported in mmol of carnitine/mg of total muscle protein. †Significant (P < 0.05) difference in carnitine values between hypothyroid and control group at identical sampling times. ‡Significant (P < 0.05) difference in carnitine values in hypothyroid group between paired time points.
Although substantial and temporally progressive type II myofiber atrophy was a hallmark of hypothyroid myopathy in this and other human studies,16-20 clinical signs of muscle weakness or fatigue may have escaped clinical detection in this study because dogs were not subjected to vigorous exercise regimens that would more completely assess their anaerobic glycolytic muscle metabolic reserves. The consequent shift in fiber types to type I myofiber predominance that also accompanies hypothyroid myopathy likely preserves muscle mass, tempering the development of grossly visible or palpable muscle atrophy.20 Detection of subtle changes in muscle mass in hypothyroid dogs may also be complicated by the development of obesity, as commonly developed in the dogs of this study.

The present study also illustrated that plasma biochemical indices of muscle disease, such as CK, AST, total LDH, and LDH5 isoenzyme activities, are significantly but modestly increased in prolonged hypothyroidism. Similar findings have been reported for humans and dogs with spontaneous hypothyroid myopathy.4,5,16-20 However, results of the present study also suggested that clinical examination and analysis of plasma enzymes of muscle origin are insensitive for the early detection of hypothyroid myopathy in dogs. No significant differences in these biochemical markers were detected between control and hypothyroid groups at time 1, with only 2 of 6 hypothyroid dogs having CK activities exceeding the upper limit of the reference range, despite hypothyroid dogs having substantial electrophysiologic, morphometric, and histopathologic evidence of myopathic changes at this sampling point. The plasma LDH concentration also did not universally exceed the upper limits of the reference range in hypothyroid dogs and returned to reference ranges in some hypothyroid dogs at times 2 and 3, despite concurrent histopathologic and morphometric evidence of progressive myopathic change.

Thyroid hormones play a key role in energy and lipid metabolism and exert most of their known biological effects via stimulation of gene expression, although the target genes regulating tissue-specific energy metabolism and metabolic efficiency are largely unknown. Molecular investigations in animals with experimentally induced disease have identified several putative, thyroid-dependent targets that participate in skeletal muscle energy metabolism, including alterations in expression of carnitine palmitoyltransferase-1 and mitochondrial uncoupling protein genes, as well as disordered mitochondrial carnitine-acylcarnitine translocase activity that may contribute to the pathogenesis of hypothyroid myopathy.9-13 Although the phenotype of hypothyroid myopathy observed in the present study and other studies16-20 supports defects in skeletal muscle mitochondrial function, studies that conclusively determine thyroid-dependent alterations in mitochondrial function to be causative for the hypothyroid myopathic phenotype are needed. Because skeletal muscle is incapable of de novo carnitine synthesis, a shared common denominator for many of these putative targets is their dependence on carnitine for optimal mitochondrial fatty acid oxidation.

The carnitine analyses used in this study did not allow for kinetic assessment of the carnitine fractions among various tissues and the extracellular compartment. Thus, it remains unknown what mechanisms are responsible for the abnormalities in muscle and urine free carnitine fractions observed in hypothyroid dogs, although multiple thyroid-dependent mechanistic alterations could explain the observed increased renal excretion and depletion of skeletal muscle stores of free carnitine. Hypothyroidism diminishes the activity of γ-butyrobetaine hydroxylase, the cytosolic enzyme responsible for the final step in de novo synthesis of carnitine, which subsequently depletes the available pool of rapidly renewable extracellular carnitine, primarily originating from the liver in dogs.21,24 As a result, the rate and magnitude of turnover of carnitine in the skeletal muscle compartment would increase. A rapid efflux of skeletal muscle carnitine into the extracellular pool could result in increased renal excretion of carnitine if supraphysiologic plasma carnitine concentrations were attained. Proximal tubular resorption of carnitine into the cytoplasm of the myocyte are facilitated by organic cation transporter new 2, a transporter whose function may be thyroid hormone dependent.25

Intramuscular free carnitine fractions may also be depleted during conversion of long-chain acyl–coenzyme A esters to their carnitine derivatives to decrease mitochondrial toxicosis, another important physiologic function of carnitine.25 Depletion of nonesterified coenzyme A via sequestration as acetooctyl–coenzyme A and acetyl–coenzyme A results in inhibition of the citric acid cycle and has been incriminated as a mechanism of contractile failure in skeletal and cardiac muscle with impaired energy-generating capacity.12 However, this last possibility was not supported by our data that also revealed a temporally progressive decrease in the esterified fraction of skeletal muscle carnitine in hypothyroid dogs.

Our findings and those of others lead us to speculate that hypothyroid myopathy may be an underrecognized complication in dogs with spontaneous hypothyroidism because it can exist in the absence of specific clinical signs of neuromuscular disease or indirect screening-type biochemical markers of muscle damage commonly used in the clinical setting.1,6,8,9 Results of the present study were also supportive of the concept that overt external metabolic or dermatologic abnormalities are common in dogs with hypothyroidism and likely prompt timely diagnosis and treatment, thus precluding myopathy from evolving into a clinically important problem in most dogs.2,22 However, subclinical neuromuscular complications of hypothyroidism may contribute to the pathogenesis of frequently reported nonspecific signs of hypothyroidism, such as lethargy, exercise intolerance, and inactivity.1,20,21 Previ-
ous reports in humans with spontaneous hypothyroid myopathy indicate that clinical improvement of the myopathy often is evident within 1 to 2 weeks after the start of thyroid hormonal supplementation, despite morphologic evidence of hypothyroid myopathy that persists for several months. It is also common for owners of hypothyroid dogs to notice dramatic improvement in activity, energy, and exercise tolerance within the first 2 weeks of treatment, without having reported specific complaints related to neuromuscular dysfunction at the time of diagnosis. It is important to recognize the clinical spectrum of potential neuromuscular complications of spontaneous hypothyroidism because these may be the predominant or only clinical manifestation of disease in humans and dogs.

Although the results indicated that this experimental method was appropriate for the study of the effects of hypothyroidism on skeletal muscle, its design did not account for potential contributions from the immune-mediated effects that accompany nearly half of the cases of spontaneous hypothyroidism in dogs. Hypothyroidism has been documented as a coincident condition in dogs and humans with a variety of immune-mediated neuromuscular diseases, including myasthenia gravis and dermatomyositis.

The experimental hypothyroid method used in this study resulted in subclinical, biochemical, electrophysiologic, and morphologic myopathic changes that closely resembled those that develop in humans and dogs with spontaneous hypothyroidism. Chronic hypothyroidism was also associated with significant depletion in skeletal muscle free carnitine concentrations, as well as increased urinary excretion of nonesterified carnitine. Routine clinical examinations and serum biochemical assays of muscle enzymes are insensitive for early detection of hypothyroid myopathy in dogs. Additional studies are required to define and correlate the molecular mechanisms of thyroid hormone deficiency to the skeletal muscle phenotypic changes observed in dogs and humans with hypothyroid myopathy, elucidate the pathogenesis of muscle carnitine deficiency in hypothyroidism, and determine the prevalence of myopathic changes in dogs with spontaneous hypothyroidism.

References

