Alkaline phosphatase bone isoenzyme and osteocalcin in the serum of hyperthyroid cats

F. Joy Archer, Susan M. Taylor

Abstract
The effect of hyperthyroidism on serum markers for increased bone metabolism and turnover was evaluated in 36 cats with elevated serum levels of thyroxine and alkaline phosphatase. Serum was analyzed for total and ionized calcium and phosphorus. Alkaline phosphatase isoenzymes were separated by agarose gel electrophoresis and osteocalcin was measured by radioimmunoassay. Values for hyperthyroid cats were compared with those for healthy cats. Alkaline phosphatase bone isoenzyme was markedly increased in all 36 hyperthyroid cats. Osteocalcin was increased in 44% of the cats. There was no correlation among the magnitude of increase in alkaline phosphatase bone isoenzyme, osteocalcin, and serum thyroxine concentrations. Increased serum phosphorus was found in 35% of the cats. Total calcium was within the reference range in all cats, while 50% of the cats had reduced levels of serum ionized calcium. We conclude that hyperthyroid cats do have altered bone metabolism, although it is usually clinically insignificant.

Introduction
Hyperthyroidism is the most common endocrine disease diagnosed in middle- to old-aged cats (1,2). Elevated serum alkaline phosphatase (ALP) and alanine aminotransferase (ALT) are the most common biochemical abnormalities reported in these cats, along with elevated thyroxine (T4) levels (1–5). The elevation in total ALP has been associated with increases in both its bone and liver isoenzymes in hyperthyroid cats (5,6). Similar isoenzyme increases have been reported in human hyperthyroidism (7). In humans with hyperthyroidism, increases in serum bone isoenzyme, osteocalcin (bone gamma carboxylglutamic acid (GLA)-protein), calcium, and phosphorus levels are common (8,9). These changes have been associated with increased bone metabolism attributed to the direct effects of T4 on bone cells (8,10–12). In contrast, no clinical signs attributable to altered bone metabolism associated with osteoporosis have been reported in hyperthyroid cats (1–5). Increases in serum phosphorus and ALP bone isoenzyme have been documented in a small number of hyperthyroid cats, but no evaluation of other markers of bone metabolism (osteocalcin) has been reported (2–6). The purpose of this study was to characterize the ALP isoenzyme patterns in cats with hyperthyroidism and to determine whether there was any relationship between the serum concentrations of T4 and total ALP or other markers of bone metabolism in these cats.

Materials and methods
Animals
Cats were selected from the hospital population at the Western College of Veterinary Medicine (WCVM)-Veterinary Teaching Hospital (VTH). Thirty-six cats with hyperthyroidism (44% ovariohysterectomized females, and 55% castrated males), between the ages of 6 and 19 y (average age 14 y), with clinical signs suggesting hyperthyroidism and a serum T4 of greater than 60 nm/L (reference range 19 to 59 nm/L) were evaluated. Criteria for inclusion in the study were hyperthyroidism and a serum total ALP greater than 80 U/L (reference range 10 to 35 U/L). Cats with signs referable to renal, bone, or liver disease (clinical signs, azotemia, palpable fractures, bone tumors, bone pain, icterus) were excluded from the study. Ten normal healthy cats between the ages of 14 y were included as a control group.

Can Vet J Volume 37, December 1996

735
of 3 and 18 y (average age 9 y) were also evaluated. Selection of these cats was based on lack of evidence of bone, liver, kidney, or thyroid disease (normal physical examination and serum calcium, phosphorus, ALP, ALT, gamma glutamyl transferase (GGT), creatinine, urea, and T4 within their reference ranges). Blood was collected aseptically and anaerobically from the jugular vein of each cat into a tube containing EDTA, a plain tube, and a tube containing cell separation material. Urine was obtained by cystocentesis. Two, 2-month-old, cats submitted to the Department of Pathology at the WCVM, after euthanasia at the SPCA, were used as a source of tissue for the preparation of extracts.

**Laboratory tests**

A complete blood count (CBC), chemistry panel, and urinalysis were performed for all cats. Levels of serum T4, ionized calcium, osteocalcin, and isoenzymes of ALP were also determined. A CBC was obtained on an automated hematology instrument (Coulter Electronics, Hileeah, Florida, USA), and differential counts were performed manually on smears stained with Wright’s Giemsa. The chemistry panel included 22 analytes and was performed using an automated chemistry analyzer (DACOS, Coulter Electronics). Urinalysis included measured specific gravity and microscopic examination of sediment. Concentrations of T4 in serum were determined by fluorescence polarized immunoassay (TDX, Abbott Laboratories, Abbott Park, Illinois, USA). The assay was validated for feline serum in the endocrinology laboratory at the WCVM. Levels of ionized calcium in venous blood were measured within 30 min of collection and separation of serum using a calcium sensitive electrode (ICA 2, Radiometer, Copenhagen, Denmark). Concentrations of osteocalcin (bone GLA-protein) in serum were determined by radioimmunoassay, utilizing 125I-bovine osteocalcin and rabbit anti-osteocalcin antiserum (Incastar, Stillwater, Minnesota, USA). For the measurement of alkaline phosphatase (ALP) isoenzymes in serum and tissue extracts, samples were pretreated with neurenamidase from Clostridium perfringens (ISOPAL, Beckman Instruments, Brea, California, USA); 20 uL of enzyme solution and 100 uL serum or tissue extract were incubated for 30 min at 37°C. A 5-uL sample of pretreated serum or extract was electrophoretically separated in agarose gel in barbital buffer pH 9.5 (ISOPAL, ALP isoenzyme electrophoresis kit, Beckman Instruments) at 150 volts for 40 min. The gels were stained for ALP activity by incubation for 60 min at 45°C in 5-bromo-4-chloro-iodoly-3-phosphate (BCIP) in 2-amino-2-methylpropanol buffer, pH 10.6. The gels were washed, air dried, and scanned at 600 nm for isoenzyme bands (Densitometer, Helena Laboratories, Beaumont, Texas, USA). Migration distances and band intensities were determined. Isoenzyme activity (U/L) was calculated by multiplying the peak area by the total ALP (tALP) activity (DACOS analysis).

Tissue extracts were prepared from fresh tissue of the 2 young cats by a modification of the method of Dorner (13) and stored at −20°C. Prior to use, aliquots of tissue extract were diluted in heat inactivated (3 h at 56°C) feline serum to provide a tALP activity of between 50 U/L and 80 U/L. Dilution and recovery studies were performed using these extracts. Separation of isoenzymes from each other and establishment of migration distances were done using the tissue extracts. Trial runs were performed using serum from young cats to confirm the migration distance of the bone isoenzyme in serum, and from cats with liver disease to confirm the migration distance of the liver isoenzyme in serum.

All sera from hyperthyroid and healthy cats in the study were subjected to electrophoresis, using gels and reagents with the same batch and lot number. Each gel contained and human serum ALP control (ISOPAL, Beckman Instruments), feline bone extract, and feline liver extract in heat inactivated feline serum as markers.

**Data analysis**

Descriptive statistics (median, mean, standard deviation linear regression analysis, single-tailed t-test, Pearson correlation, and Mann-Whitney U test) were performed using Statistics 4.1 (Analytical Software, Tallahassee, Florida, USA). Data sets with asymmetric distribution were log transformed before analysis. A significant difference was assumed to exist when the probability of making a type I error was less than 5% (P < 0.05).

**Results**

The majority of hyperthyroid cats had normal (within reference range) hemograms. Six cats (16%) had a mild neutrophilia, 2 (5.5%) lymphopenia, and 3 (8%) a mild erythrocytosis and increased hematocrit. These changes were consistent with stress and hemococoncentration.

### Table 1. Selected biochemical values of liver related analytes in 36 hyperthyroid cats

<table>
<thead>
<tr>
<th></th>
<th>ALP (U/L)</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
<th>GGT (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median</td>
<td>111.0</td>
<td>176.0</td>
<td>57.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Range</td>
<td>60–323</td>
<td>69–1360</td>
<td>30–376</td>
<td>0–5</td>
</tr>
<tr>
<td>Reference range</td>
<td>10–35</td>
<td>13–65</td>
<td>20–50</td>
<td>0–5</td>
</tr>
</tbody>
</table>

ALP = Alkaline phosphatase  
ALT = Alanine aminotransferase  
AST = Aspartate aminotransferase  
GGT = Gamma glutamyltransferase

### Table 2. Selected biochemical values of bone related analytes in 36 hyperthyroid cats

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>s</th>
<th>Range</th>
<th>Reference range</th>
</tr>
</thead>
<tbody>
<tr>
<td>T4 (nm/L)</td>
<td>192.9</td>
<td>71.5</td>
<td>72–300</td>
<td>19–59</td>
</tr>
<tr>
<td>Osteocalcin (ng/mL)</td>
<td>0.32</td>
<td>0.3</td>
<td>0–1.7</td>
<td>0–0.25*</td>
</tr>
<tr>
<td>Calcium total (mm/L)</td>
<td>2.42</td>
<td>0.15</td>
<td>2.25–2.95</td>
<td>2.23–2.80</td>
</tr>
<tr>
<td>Calcium ionized (mm/L)</td>
<td>1.23</td>
<td>0.04</td>
<td>1.16–1.31</td>
<td>1.27–1.47</td>
</tr>
<tr>
<td>Phosphorus (mm/L)</td>
<td>1.94</td>
<td>0.49</td>
<td>1.38–3.54</td>
<td>0.58–1.50</td>
</tr>
<tr>
<td>Calcium phosphorus ratio</td>
<td>0.82</td>
<td>0.22</td>
<td>0.58–1.50</td>
<td>1.50–2.00</td>
</tr>
</tbody>
</table>

*10 cats  
T4 = Thyroxine
Table 3. Levels of liver and bone isoenzymes of alkaline phosphatase (ALP) in 36 hyperthyroid and 10 normal cats

<table>
<thead>
<tr>
<th></th>
<th>ALP total (U/L)</th>
<th>ALP liver (U/L) (%)</th>
<th>ALP bone (U/L) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>125</td>
<td>23.8 (23.0)</td>
<td>99.3 (76.4)</td>
</tr>
<tr>
<td>s</td>
<td>61.7</td>
<td>13.4 (17.4)</td>
<td>59.7 (18.6)</td>
</tr>
<tr>
<td>Range</td>
<td>60–323</td>
<td>0–51.7 (0–48)</td>
<td>43–270 (52–100)</td>
</tr>
<tr>
<td>Normal range</td>
<td>10–35</td>
<td>8.6–24.2</td>
<td>2.3–40</td>
</tr>
</tbody>
</table>

Liver associated enzymes were increased in all hyperthyroid cats (Table 1). Total ALP levels were above the reference range in all 36 cats, because these cats had been selected for inclusion in this study based on a serum ALP level of >80 U/L (reference range 10 to 35 U/L). However, 53% of the cats had levels greater than 3 times the upper limits of normal. Similarly, ALT levels were above the reference range in all 36 cats; 33% of the cats had at least a 3-fold increase in levels. Aspartate aminotransferase (AST) levels were more variable. Twenty cats (66%) had increased levels of AST; 15 (75%) of these cats also had concomitant increases in the levels of serum creatine kinase (CK). Gamma glutamyl transferase (GGT) values were within the reference range in all cats.

Table 2 contains the values for analytes associated with the turnover of bone mineral. Levels of serum total calcium were within the reference range in all cats. Levels of ionized calcium were below the reference range in 18 (50%). The mean value for phosphorus was increased in hyperthyroid cats; 13 (36%) had serum phosphorus levels above the reference range. Osteocalcin values were variable. Sixteen (44%) of the hyperthyroid cats had osteocalcin concentrations greater than the 10 normal cats. These increases were not statistically significant (P = 0.05).

Electrophoretic separation of bone and liver isoenzymes of ALP in serum was possible after pretreatment with neuraminidase (Figure 1). Activities of these 2 isoenzymes were higher in the serum of hyperthyroid cats than in that of normal cats (Table 3). Bone isoenzyme activity was higher in all of the hyperthyroid cats than in the normal cats. Activity of the liver isoenzyme was higher than normal in 21 (58%) of the hyperthyroid cats. Five cats had approximately equal proportions (45% to 55%) of the bone and liver isoenzymes. In 17 cats, more than 80% of the tALP measured was the bone isoenzyme. There was a positive linear relationship between tALP and bone ALP r = 0.09 (P ≤ 0.001) and a negative linear relationship between the bone ALP and liver ALP isoenzyme, r = 0.79 (P ≤ 0.001) (linear regression analysis, Pearson correlation).

Although both serum T₄ and bone ALP were increased in all of the hyperthyroid cats, there was not statistically significant correlation between the serum T₄ concentrations and the activity of the bone ALP isoenzyme. There was a weak correlation between serum T₄ and serum phosphorus concentrations r = 0.45, (P = 0.006). There was no correlation between serum T₄ and the osteocalcin levels. An inverse linear relationship was seen between bone ALP and osteocalcin (r = -0.82), but this was not statistically significant (P = 0.05).

Discussion
Serum biochemical abnormalities are common in hyperthyroid cats, with elevated tALP being identified most frequently (2,3,5). Serum ALP comes from a number of tissue sources. The serum ALP of healthy mature cats originates primarily from the liver, while immature cats have a greater proportion of the bone isoenzyme in their serum (14,15). Isoenzymes from intestine, kidney, and placenta are generally not identified because of their very short half-life in circulation.

There is evidence that in cats, as in humans, both bone and liver isoenzymes of ALP are elevated in hyperthyroidism, resulting in the increase in measured tALP (4,6,7,16–18). Cats with serum tALP and T₄ values above the reference range were selected for this study to ensure adequate measurement of ALP isoenzymes by electrophoresis. Agarose gel electrophoresis of ALP isoenzymes allowed separation and quantification of the ALP isoenzymes in serum from these cats.

Quantification of ALP isoenzymes yielded similar results to those of a previously reported study (6). Increased levels of ALP bone isoenzyme were identified in all of the hyperthyroid cats in this study and in 9 of the 10 hyperthyroid cats previously studied. Levels of the liver isoenzyme of ALP were increased in 58% of the hyperthyroid cats in this study and in all of the cats in a previous study (6). Increases in the bone isoenzyme occur with greater frequency than increases in the liver isoenzyme in hyperthyroid humans (7,8,16–18).

Elevations in the levels of liver-related enzymes, including ALT, ALP, and AST, are common in hyperthyroid cats and humans (1–6,19–21). In this study,
ALT, a cytosolic enzyme and a sensitive indicator of hepatocyte damage, was elevated in all of the hyperthyroid cats. The elevations in hyperthyroidism are attributed to many factors, including malnutrition, hepatic hypoxia, congestive heart failure, infection, and a direct toxic effect of thyroid hormones on the liver (1–6,20). Induced production of the liver isoenzyme of ALP has also been suggested (6,16). Liver dysfunction in thyrotoxicosis is rare, and in both humans and cats, the histological changes seen in hepatic biopsies are mild, nonspecific, and are not related to the severity of the thyrotoxicosis (4,5,22,23).

Levels of the bone isoenzyme of ALP were increased in all of the cats in this study. Similarly, increases in this isoenzyme are common in human thyrotoxicosis (7–10,16–18,24). The thyroid hormones, T₄ and T₃, have been shown to directly stimulate osteclastic bone resorption, as well as stimulating osteoblastic activity in trabecular and cortical bone (7,11,12,25,26). Bone ALP is localized primarily within the plasma membrane of osteoblasts and is released during active bone formation (12). In addition, nuclear receptors for T₃ and T₄ in osteoblasts have been documented (12). Increased production and release of bone ALP and osteocalcin in response to increased T₃ and T₄ have been identified in osteoblast-like cell lines (12,27). In this study, as in most reports of human thyrotoxicosis, there was no detectable relationship between the magnitude of increase in the concentration of serum thyroid hormone and the observed increase in the activity of bone ALP (7,10,25).

Osteocalcin, or bone GLA-protein, is increased in many humans with hyperthyroidism (8,24). This protein is synthesized within the bone and is considered to be a specific marker of osteoblastic activity and bone remodelling (8,9,28,29). In most disease states, there is a high degree of correlation between the levels of osteocalcin in serum and the activity of bone ALP, although the 2 markers may not reflect exactly the same aspect of bone formation (29). Although 44% of the hyperthyroid cats in this study had higher serum levels of osteocalcin than those of the normal cats, there was no significant correlation between the levels of osteocalcin and the increases in bone ALP isoenzyme. In all of the hyperthyroid cats, the increase in bone ALP isoenzyme was more dramatic than the increase in osteocalcin. There was no detectable relationship between the serum T₃ concentration and the serum concentration of osteocalcin.

Alterations in serum calcium and phosphorus concentrations have been documented in human thyrotoxicosis. It is thought that the bone resorption caused by the elevated concentrations of thyroid hormones results in the release of calcium and phosphorus into the circulating pool. This has the effect of lowering serum parathyroid hormone (PTH) and subsequently decreasing the formation of calcitrol (1, 25-dihydroxy cholecalciferol) (24). Increased urinary and fecal loss of calcium results. Activation of osteoblasts with enhanced bone formation is inadequate to compensate for the severe bone resorption induced by thyroid hormones, and the net result is a loss of bone density.

All of the hyperthyroid cats evaluated in this study had normal concentrations of serum total calcium; 50% had lower levels if ionized calcium (Table 2). In humans with thyrotoxicosis, increased levels of total and ionized calcium in serum are commonly reported, as is a decreased concentration of PTH in serum, although these values may be normal in some patients (7,8,10,24,25,30). Hypercalcemia has not been associated with hyperthyroidism in cats. One study reported hypocalcemia in 18% of hyperthyroid cats (19); in most cases, levels of serum total calcium are normal (1–5). Levels of ionized calcium were decreased in 20% of hyperthyroid cats in another study (31). In the same study, 68% of the hyperthyroid cats also had increased levels of PTH in their serum (31). This is the reverse of findings reported in humans and may mask a negative calcium balance, similar to that in humans with thyrotoxicosis (25), in some hyperthyroid cats.

Increased concentrations of serum phosphorus are common in hyperthyroid cats and humans. Hyperphosphatemia may be the result of increased mobilization from bone, increased renal tubular resorption due to increased PTH, or release during muscle catabolism (1–6,31). Concurrent renal disease is another very common cause of hyperphosphatemia in hyperthyroid cats (2,4,5). Hyperphosphatemia has been reported to occur in 20% to 50% of hyperthyroid cats (1–5,19–21). In this study, 36% of the cats were hyperphosphatemic. None of these cats had any clinical or biochemical suggestions of renal dysfunction.

In humans, thyrotoxicosis is a risk factor for osteoporosis (24,25,32). Radiographic changes and occasional clinical symptoms associated with altered bone metabolism have been reported (10,11,16,17,24). Reduced mineral density in cortical and trabecular bone has also been documented (26). Affected patients are often asymptomatic, until the decrease in bone volume is sufficient to cause loss of bone strength, resulting in low energy fractures (25). There does not appear to be a link between the severity or duration of the thyrotoxicosis and the appearance of bone disease (10). Clinically detectable bone lesions are rare and are most often detected in postmenopausal women, in whom the contribution of the thyrotoxicosis to the osteoporosis is uncertain.

Clinically significant consequences of altered bone metabolism have not been identified in hyperthyroid cats. The findings of this study strongly suggest, however, that the sustained elevation of concentrations of thyroid hormones in hyperthyroid cats does have an effect on bone metabolism.

Acknowledgments
We thank S. Buczkowski and B. Trask for technical assistance.

References


Answers to Quiz Corner/Les réponses du Test Éclair

1. a  
2. b — If opiates are given, twitching does not quiet the horse.  
   b — Si des opiacés sont administrés, l’utilisation du tord-nez ne tranquillise pas le cheval.  
3. e  
4. b — Metestrual bleeding is observed in 5% to 10% of cycling cows on most farms.  
   b — Sur la plupart des fermes, le saignement vulvaire lors du métoestrus est observé chez de 5 à 10 % des vaches présentant un cycle oestral.  
5. d  
6. e  
7. c  
8. a — Intravenous xylazine causes a release of growth hormone in dogs.  
   a — La xylazine intraveineuse cause une libération de l’hormone de croissance chez les chiens.  
9. b — Terbutaline is an oral, beta-2 adrenergic agent used as a bronchodilator.  
   b — La terbutaline est un agent oral bêta-2 adrénergique utilisé comme bronchodilatateur.