Selenium status of cats in four regions of the world and comparison with reported incidence of hyperthyroidism in cats in those regions

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Objective—To assess selenium (Se) status of cats in 4 regions of the world and to compare results for Se status with reported incidence of hyperthyroidism in cats in those regions.

Animals—50 cats (30 from 2 regions with an allegedly high incidence of hyperthyroidism and 20 from 2 regions in which the disease is less commonly reported).

Procedure—Hematologic samples (heparinized whole blood, plasma, and RBC fractions) were obtained from 43 healthy euthyroid cats and 7 hyperthyroid cats. Plasma concentration of Se and activity of glutathione peroxidase (GPX) in whole blood and plasma were determined.

Results—Plasma concentration of Se and GPX activity in whole blood or plasma did not differ significantly among cats from the 4 regions. However, cats had a plasma concentration of Se that was approximately 10 times the concentration reported in rats and humans. The GPX activity in whole blood or plasma in cats generally was higher than values reported in rats or humans.

Conclusions and Clinical Relevance—Cats have higher Se concentrations in plasma, compared with values for other species. However, Se status alone does not appear to affect the incidence of hyperthyroidism in cats. High Se concentrations may have implications for health of cats if such concentrations are influenced by the amount of that micronutrient included in diets. (Am J Vet Res 2001;62:934–937)

The thyroid gland contains more selenium (Se) per gram of weight than any other body tissue, suggesting an important role for this trace element in homeostasis of the gland. Selenium exerts its biological actions through the expression of selenoproteins. In the thyroid gland, these include glutathione peroxidases (GPX) and thioredoxin reductase, which catalyze detoxification of hydrogen peroxide and lipid hydroperoxides and, thus, protect thyrocytes from oxidative damage. Thioredoxin reductase also is involved in regulation of growth of healthy and tumorous cells. Deiodination of thyroid hormones is catalyzed in the thyroid gland by iodothyronine deiodinases; however, this family of selenoenzymes is not highly expressed in the thyroid gland of all species. Selenium status influences status of the thyroid gland and metabolism of thyroid hormones in rats and humans. In humans, thyrotoxicosis caused by Grave’s disease and multinodular goiter results in a decrease of serum Se concentrations and GPX activity in whole blood. Hyperthyroidism is the most common endocrine disease of cats and results almost exclusively from hyperfunctional adenomas. Little is known about the pathogenesis of the disease, but the reported incidence appears to vary among geographic locations. Selenium is a growth factor in many cell-culture systems, activating transcription factors that lead to cell division. Thus, it is possible that a dietary factor such as Se may play a role in the development of toxic nodular goiter in cats. To our knowledge, we are not aware of any reports of Se status of domestic cats. The seemingly apparent variation in the geographic incidence of hyperthyroidism in cats may reflect differences in environmental or dietary factors. Selenium status can influence status of the thyroid gland and expression of certain selenoenzymes such as iodothyronine deiodinases and growth-promoting selenoprotein thioredoxin reductase. The objective of the study reported here was to compare the Se status of cats from regions of the world; regions were selected on the basis of an apparent geographic variation in the incidence of hyperthyroidism in cats.

Material and Methods

Animals—Fifty cats from 4 regions were included in the study. Regions had an apparent geographic variation in incidence of hyperthyroidism in cats. These included cats from 2 regions of high incidence (United Kingdom [Edinburgh; n = 16] and eastern Australia [Sydney; 14]) and 2 regions in which the disease is less commonly observed and reported (Denmark [Greve; n = 10] and western Australia [Perth; 10]).
Collection of samples—Blood samples were obtained from cats being examined for reasons such as preanaesthetic evaluation and viral tests, and samples also were obtained from cats with suspected hyperthyroidism. Heparinized whole blood (2 ml) was immediately frozen at −40°C. Another 2 ml of heparinized blood was centrifuged immediately after collection, and plasma and RBC were harvested and stored separately at −40°C. Samples from Greve, Perth, and Sydney were stored at −40°C until they were transported on dry ice by air courier to our laboratory in Edinburgh.

**Plasma concentrations of t-thyroxine—Euthyroidism or hyperthyroidism was confirmed in each cat on the basis of compatible historical and physical features and concentrations of total t-thyroxine (TT4). Plasma TT4 concentration was determined by use of a double-antibody radioimmunooassay technique validated for use with feline plasma.**

**Plasma concentration of Se—Plasma concentrations of Se were measured, using reaction with 2,3-diaminonaphthalene followed by fluorimetry.** Briefly, 0.25 ml of plasma was digested overnight at room temperature (20 to 25°C) in 2 ml of concentrated nitric acid. Samples then were heated slowly to the boiling point and maintained boiling until dense brown fumes disappeared. Concentrated perchloric acid (2 ml) was added in a dropwise manner to each sample, and samples were refluxed for 30 minutes. Two milliliters of a 10% solution of HCl (vol:vol) then was added in a dropwise manner to drive off excess nitric acid and convert selenite to selenite.

After cooling, 5 ml of hydroxylamine-EDTA solution (25 g of hydroxylamine and 9.24 g of EDTA/L of distilled water) was added to each sample, followed by 2 drops of cresol red indicator. Ammonia solution (40% [vol:vol] in distilled water) was added to each sample until the sample turned green (approximate 3 ml), then a 10% solution of HCl (vol:vol) was added to each sample to turn the color of the solution orange (approximately 2 ml). After addition of those solutions, pH of each sample was 1.5 to 2.5, a pH that is optimal for the formation of the diamino naphthalene-Se complex. Samples then were diluted to 50 ml with distilled water prior to reaction with diamino naphthalene. The resulting fluorescent complex was extracted, using hexane.

**GPX activity in plasma and whole blood—Glutathione peroxidase activity in plasma and whole blood was determined, as described elsewhere.** Glutathione peroxidase activity in whole blood was adjusted on the basis of hemoglobin concentration. Hemoglobin concentration was determined by the use of Drabkin reagent and measured by an automated plate reader, using a standard solution of 18 g of Hb/dl for comparison.

**Statistical analysis—**The Kruskall-Wallis test was used to test for significant differences between groups from all 4 regions. The Mann-Whitney U test was used to test for significant differences between samples from 2 specific regions. Regression analysis was used to test for correlations between plasma concentration of Se and GPX activity in plasma and whole blood. Results of all tests were considered significant for values of $P < 0.05$.

**Results**

**Signalment and thyroid status—**Signalment of the cats in each region was summarized. Thyroid status of the cats also was determined.

Greve—Samples were obtained from 10 cats. Breeds represented were domestic shorthair (n = 5), Persian (3), Oriental shorthair (1), and British shorthair (1). Seven cats were castrated males, and 3 were ovariohysterectomized females. Cats ranged from 6 to 14 years old (median, 7.5 years). All cats were euthyroid.

Perth—Samples were obtained from 10 cats. Breeds represented were Persian (n = 7), Burmese (2), and Siamese (1). There were 3 sexually intact males, 3 castrated males, and 4 sexually intact females. Cats ranged from 1 to 7 years old (median, 3 years). All cats were euthyroid.

Sydney—Samples were obtained from 14 cats. Breeds represented were domestic shorthair (n = 13) and Siamese (1). There were 8 castrated males and 6 ovariohysterectomized females. Cats ranged from 2 to 18 years old (median, 8.5 years). Eleven cats were euthyroid, and 3 cats were hyperthyroid.

Edinburgh—Samples were obtained from 16 cats. Breeds represented were domestic shorthair (n = 15) and Siamese (1). There were 10 castrated males and 6 ovariohysterectomized females. Cats ranged from 7 to 16 years old (median, 10.5 years). Twelve cats were euthyroid, and 4 cats were hyperthyroid.

**Plasma concentrations of Se—**Data for plasma concentrations of Se for all cats were summarized (Table 1). Plasma concentrations of Se did not differ significantly between samples from cats in 4 regions with varying incidences of hyperthyroidism.

**Table 1—**Plasma concentrations of selenium (mmol/L) in cats from 4 regions with varying incidences of hyperthyroidism

<table>
<thead>
<tr>
<th>Region</th>
<th>Median</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Q1</th>
<th>Q3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Greve (n = 10)</td>
<td>8.64</td>
<td>4.20</td>
<td>7.88</td>
<td>5.51</td>
<td>7.41</td>
</tr>
<tr>
<td>Perth (n = 10)</td>
<td>5.19</td>
<td>3.95</td>
<td>7.24</td>
<td>4.38</td>
<td>6.56</td>
</tr>
<tr>
<td>Sydney (n = 14)</td>
<td>6.57</td>
<td>4.88</td>
<td>8.37</td>
<td>5.21</td>
<td>7.02</td>
</tr>
<tr>
<td>Edinburgh (n = 16)</td>
<td>5.36</td>
<td>3.93</td>
<td>8.70</td>
<td>4.76</td>
<td>6.40</td>
</tr>
<tr>
<td>All cats (n = 50)</td>
<td>5.76</td>
<td>3.95</td>
<td>8.70</td>
<td>5.13</td>
<td>6.87</td>
</tr>
</tbody>
</table>

*Q1 = Values for first quartile. Q3 = Values for third quartile.

**Table 2—Glutathione peroxidase activity* in whole blood obtained from cats in 4 regions with varying incidences of hyperthyroidism

<table>
<thead>
<tr>
<th>Region</th>
<th>Median</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Q1</th>
<th>Q3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Greve (n = 10)</td>
<td>404.9</td>
<td>230.7</td>
<td>587.0</td>
<td>312.0</td>
<td>493.1</td>
</tr>
<tr>
<td>Perth (n = 10)</td>
<td>409.0</td>
<td>236.0</td>
<td>492.5</td>
<td>347.1</td>
<td>404.4</td>
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<tr>
<td>Sydney (n = 14)</td>
<td>356.5</td>
<td>153.1</td>
<td>522.8</td>
<td>291.0</td>
<td>425.0</td>
</tr>
<tr>
<td>Edinburgh (n = 16)</td>
<td>320.9</td>
<td>200.9</td>
<td>427.0</td>
<td>286.7</td>
<td>377.0</td>
</tr>
<tr>
<td>All cats (n = 50)</td>
<td>354.3</td>
<td>153.1</td>
<td>587.0</td>
<td>304.1</td>
<td>449.0</td>
</tr>
</tbody>
</table>

*Values are micromoles of the reduced form of nicotinamide adenine dinucleotide phosphate oxidized per gram of hemoglobin.

**Table 3—Glutathione peroxidase activity* in plasma obtained from cats in 4 regions with varying incidences of hyperthyroidism

<table>
<thead>
<tr>
<th>Region</th>
<th>Median</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Q1</th>
<th>Q3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Greve (n = 10)</td>
<td>8.60</td>
<td>6.20</td>
<td>11.40</td>
<td>7.25</td>
<td>10.60</td>
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<tr>
<td>Perth (n = 10)</td>
<td>6.90</td>
<td>5.30</td>
<td>16.32</td>
<td>6.30</td>
<td>9.65</td>
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<tr>
<td>Sydney (n = 14)</td>
<td>7.60</td>
<td>4.50</td>
<td>11.70</td>
<td>6.33</td>
<td>9.38</td>
</tr>
<tr>
<td>Edinburgh (n = 16)</td>
<td>6.00</td>
<td>4.70</td>
<td>13.30</td>
<td>5.30</td>
<td>8.80</td>
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<tr>
<td>All cats (n = 50)</td>
<td>7.30</td>
<td>4.50</td>
<td>16.32</td>
<td>6.10</td>
<td>9.20</td>
</tr>
</tbody>
</table>

*See Table 2 for key.
significantly ($P = 0.17$) among the 4 groups of cats.

Data for GPX activity in whole blood were summarized (Table 2). The GPX activity in whole blood did not differ significantly ($P = 0.08$) among the 4 groups.

Data for GPX activity in plasma were summarized (Table 3). Although cats from the United Kingdom had lower values for GPX activity in plasma than other cats, these values did not differ significantly ($P = 0.051$) among the 4 groups.

We did not detect a correlation between plasma concentration of Se and GPX activity in whole blood ($P = 0.74, r = 0.010$) or plasma ($P = 0.08, r = 0.163$) in the 50 cats.

**Plasma Se status and hyperthyroidism**—Of the 50 cats in the study, 7 were hyperthyroid (3 from Sydney and 4 from Edinburgh). Plasma concentrations of Se for these 7 cats ranged from 0.31 to 0.60 mg/L (mean ± SD, 0.44 ± 0.10). We did not detect significant differences in plasma concentrations of Se ($P = 0.44$), GPX activity in whole blood ($P = 0.43$), or GPX activity in plasma ($P = 0.92$) between euthyroid and hyperthyroid cats. We did not detect significant differences in plasma concentration of Se ($P = 1.00$), GPX activity in whole blood ($P = 0.27$), or GPX activity in plasma ($P = 0.35$) between euthyroid and hyperthyroid cats from Edinburgh. We did not detect significant differences in plasma concentrations of Se ($P = 0.41$), GPX activity in whole blood ($P = 0.80$), or GPX activity in plasma ($P = 0.76$) between euthyroid and hyperthyroid cats from Sydney.

**Discussion**

Hyperthyroidism in cats is a relatively recently recognized disease and results from the formation of autonomous nodules in the thyroid gland. Although a specific factor has not been clearly identified as a cause of the disease, its incidence varies with geographic location, suggesting an environmental factor may play a role. In contrast, others have suggested that the disease may arise from the production of growth-promoting substances, possibly immunoglobulins that have been identified in the plasma of some affected cats.

A considerable body of evidence suggests that Se status may modify thyroid gland function and metabolism of thyroid hormones. The rate-limiting step in synthesis of thyroid hormones is believed to be the availability of hydrogen peroxide. The supply of hydrogen peroxide is believed to be regulated, in part, by the availability of selenoperoxidases and thioredoxin reductase. In cultured sheep thyrocytes, inhibition of selenoperoxidases by the addition of gold thioglucose leads to an increase in the de novo synthesis of triiodothyronine ($T_3$) and thyroxine ($T_4$). Thus, it is possible that Se deficiency in some species may give rise to increased thyroid gland synthesis of $T_3$ and $T_4$, although in Se-deficient animals, conversion of the prohormone $T_3$ to the bioactive $T_3$ in peripheral tissues would be impaired as a result of diminished expression of iodothyronine deiodinases. Alternatively, Se status is a potent regulator of thioredoxin reductase expression. Thioredoxin reductase is a selenoprotein that exerts many actions in addition to its antioxidant function.

Evidence suggests that thioredoxin reductase, acting in concert with thioredoxin, is involved in regulating cell growth and can activate transcription factors such as AP1 and NFkB. Thus, it is possible that overexpression of thioredoxin reductase as a result of high Se intake may give rise to autonomous nodules in the thyroid gland.

Because there are a number of theoretic mechanistic links by which low or high Se intake could give rise to hyperthyroidism, we tested the hypothesis that changes in Se status may be involved in the pathogenesis of hyperthyroidism in cats. Anecdotal evidence suggests that hyperthyroidism is a less common disease of cats in Denmark and western Australia than in the United Kingdom and eastern Australia. For that reason, we chose to compare the Se status of cats from locations within those 4 regions.

We did not find significant differences in plasma concentrations of Se or in the enzyme markers of Se status (GPX activity in whole blood or plasma) among cats of the 4 regions included in the study. Furthermore, although humans with hyperthyroidism have decreased plasma concentrations of Se and GPX activity, we did not find a similar relationship in hyperthyroid cats, compared with euthyroid cats. This may have been an effect of the population included in our study; because the euthyroid and hyperthyroid cats were not matched on the basis of age, or it may have been an effect of including only a small number of cats in the study.

Of particular interest was our observation that plasma concentrations of Se in cats were approximately 5-fold higher than concentrations reported in Se-replete rats and humans, using the same methods. In contrast, the GPX activity in whole blood or plasma of cats was similar to values for rats consuming Se in the diet at a rate of 0.1 mg/kg of body weight. Although we are not aware of any reports on the Se requirements of cats, 1 major pet food company uses National Research Council (NRC) requirements to specify the Se content of their diets that are formulated for cats. In those reports, it has been suggested that the Se content of their diets that are formulated for cats (range, 10 to 40 µg of Se/400 kcal of metabolizable energy). Because the stated caloric requirement of cats is 70 to 90 kcal/kg/d, this should lead to an average daily Se intake of approximately 7 to 36 µg of Se/cat/d. That amount of Se intake (approx 1.75 to 9 µg/kg/d) is considerably higher than the recommended intake for a typical human (55 and 70 µg/d in adult women and men, respectively, which is approx 1 µg/kg/d). Thus, the high concentrations of Se in the plasma of cats may result from increased dietary intake. A study of plasma concentrations of Se in cats fed noncommercial diets would be of interest, but analysis of results of the study reported here suggests that the calculation of the Se requirement for cats may be overestimated. The authors are aware of only 2 reports on bioavailability of Se in foods formulated for dogs and cats. In those reports, it has been suggested that Se appears to be poorly bioavailable in commercially formulated foods for dogs and cats. Analysis of our data would suggest that this assumption, at least for cats, is not reflected in low Se status.

Measurement of GPX in blood or plasma com-

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monly is used as an indicator of Se status. We did not find a correlation between plasma concentrations of Se and GPX activity in whole blood or plasma. Similar observations have been reported in other species when Se intake is high.21 Correlation between plasma concentrations of Se and GPX activity is apparent only when plasma concentrations of Se are below recommended amounts of intake. Indeed, many recommendations for Se intake are based on the amount of Se in the diet that will lead to maximal expression of GPX activity in blood. At higher intakes of Se, GPX activity in whole blood or plasma remains maximally expressed at plateau values, but plasma concentrations of Se continue to increase as Se intake increases.21 Lack of correlation between Se concentration and GPX activity in cats strongly suggests that most cats have adequate, if not high, intakes of Se.

Although high Se intake can be harmful, cats appear to be relatively resistant to such adverse effects. Selenosis results in conditions affecting the integument in animals (alopecia and hoof rot) and humans (loss of hair and fingernails).23 In addition, humans also have peripheral neuropathy.24 The increased values for measures of Se status in cats did not appear to cause any of the classic clinical signs of selenosis observed in other species. We were unable to document a link between Se status and the incidence of hyperthyroidism in cats. However, analysis of our results suggested that the Se content of cat food should be reassessed and, possibly, decreased.

We also believe that the role and adequacy of iodine in foods formulated for cats should be reassessed, because this trace element can have profound effects on function of the thyroid gland. In 1 study,22 it has been suggested that some foods formulated for cats can contain up to 10 times the recommended daily intake of iodine, possibly because of inclusion of thyroid glands from slaughtered animals. In another study,22 it has been reported that 2 brands of commercial food formulated for cats contained excessive amounts of iodine, whereas 9 others contained less than the recommended intake amount. Those authors speculated that a wide variation in iodine intake may be responsible for thyroid gland dysfunction in cats. One major pet food company now regulates iodine content in foods formulated for cats in an effort to provide adult cats with approximately 0.1 to 4.5 mg of iodine/cat/d.22 Additional research should address the possible interaction between Se and iodine intake on long-term effects of the thyroid gland in cats.

References