Dietary Copper Influences Reproduction in Cats1,2

ABSTRACT The objective of this study was to determine the copper requirement of female cats (queens) for gestation. Cuproenzyme activities were evaluated to identify a noninvasive indicator of copper status. This study used a depletion-repletion model. Specific pathogen–free queens (n = 28) were adapted to a purified diet; after consuming a copper-depletion diet (0.8 mg Cu/kg diet) for 4 mo, they were randomly allocated to three dietary treatment groups receiving copper sulfate at 4.0, 5.8 or 10.8 mg Cu/kg diet. Four queens underwent liver biopsies at two time points during the study. Plasma samples were analyzed for copper concentrations, extracellular superoxide dismutase, ceruloplasmin and diamine oxidase activities. Only liver copper concentrations were responsive to dietary copper intake. The dietary concentration of copper had a significant effect on the time taken for queens to conceive (P = 0.04). There was a negative linear relationship between dietary copper (x = Cu mg/kg diet) and the mean time (y = days) for queens to conceive (y = 43.38 – 2.87x; R² = 0.97). The current NRC recommendation of 5 mg/kg diet copper for cats appears marginal for optimal reproduction. J. Nutr. 130: 1287–1290, 2000.

KEY WORDS: • copper • feline • reproduction
• ceruloplasmin • diamine oxidase

Dietary copper is an essential trace element for all species and is required for normal fetal and neonatal development (Keen et al. 1998). Characterization of enzootic ataxia (or swayback disease) in lambs provided the initial evidence that copper was essential for normal fetal development (Bennetts et al. 1948). The authors showed that the frequency and severity of the disease was reduced when supplemental copper was provided to the ewes either before or early in gestation. Similar neurologic and skeletal abnormalities have been reported in newborn copper-deficient goats, swine, guinea pigs, rats, dogs and cattle (Keen et al. 1998).

Other clinical signs in the offspring of copper-deficient reproducing animals include connective tissue abnormalities, depigmentation of hair and wool, impaired keratinization of fur, hair and wool, and a hypochromic, microcytic anemia (Rucker et al. 1998, Turnlund 1994). Copper deficiency in the dam also results in poor overall reproductive performance, including low fertility, fetal death, resorptions and abortions (Turnlund 1994).

Copper functions as a component of metalloenzymes that catalyze electron transfer reactions involving molecular species. Cuproproteins also have nonenzymatic functions including copper transport and storage, and coagulation (Linder 1996). Suboptimal dietary copper intake can result in decreased activities of cuproenzymes (Prohaska 1991). In some cases, changes in enzyme activities have been associated with the clinical and pathologic changes observed in copper deficiency.

Before the initiation of this project, there was only one publication on the copper requirements for kittens (Doong et al. 1983). That study determined the copper requirements for kittens on the basis of changes in growth in response to differing levels of dietary copper. The authors also determined that liver copper concentrations were responsive to dietary copper intake. Although gross pathologic defects were not reported, histologic evidence of connective tissue lesions of the aorta were assumed to be secondary to low dietary copper concentrations. In contrast to other species, anemia was not found in copper-deficient cats or kittens. On the basis of that study, and the requirements of rats, the NRC (1986) proposed a copper requirement of 5 mg Cu/kg diet for kittens for growth.

During the testing of three diets using protocols of the Association of American Feed Control Officials (AAFCO), kittens with clinical signs compatible with copper deficiency were born to female cats (queens) consuming different commercial diets. Observed clinical signs included neonatal death, premature kittens, hypochromatricia and collagen abnormalities (frequently manifested as twisted limbs and curly tails). The common factor in all of the diets was the presence of copper oxide as the supplementary dietary copper source. Copper oxide has been demonstrated to be an unavailable form of dietary copper in chickens and pigs (Baker et al. 1991, Cronwell et al. 1989). The addition of copper sulfate to one of the commercial diets resolved the abnormalities in the kittens.

The objectives of this study were to determine the copper requirement of queens for gestation and to examine the response of several cuproenzymes to dietary copper intake as a noninvasive alternative to liver biopsy that would serve as an indicator of copper stores in the cat. The enzymes analyzed were extracellular superoxide dismutase (EC SOD; EC


2 Supported in part by grants from the George and Phyllis Miller Feline Health Fund, Center for Companion Animal Health, School of Veterinary Medicine, University of California, Davis, The UC Davis Clinical Nutrition Research Unit, NIDDK 35747 and The Winn Feline Foundation, Manasquan, NJ.

3 To whom correspondence should be addressed.

0222-3166/00 $3.00 © 2000 American Society for Nutritional Sciences.
Materials and Methods

The experimental protocols adhered to the NIH guidelines (NRC 1985) and were approved by the Animal Use and Care Administrative Advisory Committee of the University of California at Davis.

Animals and their management. Twenty-eight 2- to 3-y-old specific pathogen–free domestic short-hair proven queens from the Feline Nutrition and Pet Care Center of the University of California at Davis were used. Queens were group housed in large wire cages (2.5 × 2.5 × 2.5 m), in temperature-controlled rooms (21 ± 2°C) with a light-dark cycle of 14 h:10 h. Queens were transferred to queen cages (0.8 × 0.8 × 0.8 m) before giving birth and remained there until the kittens were weaned. Queens had free access to experimental diets and tap water.

Diets. Experimental diets were prepared by adding varied amounts of copper sulfate (Fisher Scientific, Fair Lawn, NJ) to a casein/lactalbumin-based purified diet. The purified diet exceeded NRC recommended levels of all ingredients other than copper and has been demonstrated to support adequate growth and reproduction in cats at the Feline Nutrition and Pet Care Center. Copper concentrations were confirmed by atomic absorption spectrophotometry (model 3030B, Perkin-Elmer, Clay Adams, NJ). Analyses revealed the following copper concentrations (mean ± SEM, n = 3) in the dietary treatments: 0.84 ± 0.2 mg Cu/kg diet for the copper depletion diet; and 4.0 ± 0.7, 5.8 ± 0.4 and 10.8 ± 2 mg Cu/kg diet for the 3, 6 and 10 mg Cu/kg diets containing copper sulfate. The differences between the actual and analytical copper concentrations in the experimental diets were most likely due to variations between and within each 40 kg (dry weight) batch of diet. The copper concentration of the standard colony commercial dry diet was 32.8 mg Cu/kg. Diets were stored at 4°C before preparation and feeding.

Design. A depletion-repletion study design was used. Queens were weighed and examined weekly throughout the study. The queens were adapted to a purified diet containing 10.8 mg Cu/kg diet; when weight maintenance was achieved, they were given the copper-depletion purified diet (0.8 mg Cu/kg diet) for 4 mo. This time period was selected in part on the basis of results from the study of Doong et al. (1983). In that study, a queen consuming a copper-deficient diet (0.58 mg Cu/kg diet) for ~60 d had a liver copper concentration similar to the predemotion values from this study (0.83 μmol/g fresh tissue). Dietary treatments were not started until postpartum liver copper analyses were evaluated. Further depletion was rejected to avoid the potentially negative consequences of severe dietary copper depletion reported in other species. After copper depletion, the queens were randomly allocated to one of three dietary treatments with copper supplied as copper sulfate, at concentrations of 4.0, 5.8 and 10.8 mg Cu/kg diet. These amounts were selected to cover a range of copper concentrations higher and lower than the current NRC recommendations for growth. Queens were given their respective dietary treatments for ~1 y.

Blood samples were collected by routine venipuncture every other week throughout the study. Samples were analyzed for copperoxygenase activities at the following four time points in the study: (1) the start of the experiment (just before copper depletion); (2) after 4 mo of copper depletion; (3) after 2 mo of copper repletion; and (4) the end of the study (~1 y). Four of the 28 queens underwent exploratory laparotomies for liver biopsies before starting the copper-depletion diet and after the depletion diet for 4 mo.

Plasma copper concentration did not reflect the dietary copper intake of the queens. The mean plasma copper concentration for all 28 queens before entering the study was 10.7 ± 0.5 μmol/L; after 4 mo of consuming the copper-depletion diet, it was 9.4 ± 0.4 μmol/L. There was no difference in plasma copper concentration among the groups after queens consumed their respective dietary treatments for 1 y. After 1 y, the mean plasma copper concentrations were 10.9 ± 2.8, 12.9 ± 2.3, and 12.3 ± 1.1 μmol/L for the queens given the 4.0, 5.8 and 10.8 mg Cu/kg diets, respectively. The mean liver copper concentration in the four queens that underwent exploratory laparotomies for liver biopsy be-

RESULTS

Plasma copper concentration was determined using a colorimetric assay by Takagi et al. (1994). Two modifications to that assay were the use of 200 μL of plasma per sample (DiSilvestro et al. 1997) and the incubation of samples for 2 h before and after the addition of the chromagen. The DAO activity in each sample was determined in units per liter (U/L) on the basis of a standard curve using porcine kidney DAO (Sigma Chemical, St. Louis, MO). One unit of DAO will oxidize 1 μmol/min of substrate using a molar extinction coefficient of 9600 (mol/L)−1 cm−1.

Copper oxidase (CP) activity was measured using a modification of the colorimetric assay by Takagi et al. (1994). Two modifications to that assay were the use of 200 μL of plasma per sample (DiSilvestro et al. 1997) and the incubation of samples for 2 h before and after the addition of the chromagen. The DAO activity in each sample was determined in units per liter (U/L) on the basis of a standard curve using porcine kidney DAO (Sigma Chemical, St. Louis, MO). One unit of DAO will oxidize 1 μmol/min of substrate at pH 7.2 at 37°C.

Statistical analysis. Statistical analyses were performed using PC-SAS (version 6.08, SAS Institute, Cary, NC). One-way ANOVA was used to test for differences in means, and a Bonferroni post-hoc test was used for comparisons. One-factor repeated-measures ANOVA was used to compare copperoxygenase activity within a group over time. Chi-square contingency table analysis was used to evaluate the relationship between conception frequency and dietary treatment. Analysis of dietary copper concentration and the mean time until the onset of gestation was computed using least-squares regression analysis. Data are shown as mean ± SEM, unless otherwise specified. Differences were considered significant at P ≤ 0.05.

1 Each diet was composed of the following constant ingredients (g/kg diet): casein (New Zealand Milk Products, Petaluma, CA); 220; lactalbumin (see casein), 220; rendered animal tallow (Florin Tallow, Dixon, CA); 270; sucrose, 75.5; starch (cornstarch, Melijoe), Bridgewater, NJ), 144; taurine (Taisho Pharmaceutical, Torrance, CA), 1.5; choline chloride (International Mineral and Chemical, Terre Haute, IN), 3; vitamin mixture (Williams et al. 1987), 10; l-methionine (Ajinomoto USA, Raleigh, NC), 3; l-arginine (see l-methionine), 3; mineral mixture, 50; mineral mixture (containing copper supplied as copper sulfate, at concentrations of 4.0, 5.8 and 10.8 mg Cu/kg. Diets were stored at 4°C between preparation and feeding.

SEMs, unless otherwise specified.
fore the start of the copper-depletion diet was 0.82 ± 0.03 μmol Cu/g fresh tissue. The mean decreased to 0.32 ± 0.08 μmol Cu/g fresh tissue (P = 0.001) after the copper depletion diet was consumed for 4 mo. Mean plasma copper concentrations did not change with dietary copper depletion or repletion (9.8 ± 0.2 μmol/L predepletion and 10.8 ± 0.6 μmol/L postdepletion). Packed cell volumes did not change with dietary copper depletion (36% predepletion and 33% postdepletion).

There were no differences in kitten liver copper concentrations among the dietary treatment groups, even when concentrations were compared with kittens born to queens consuming the commercial food. Liver copper concentrations were 0.26 ± 0.13, 0.37 ± 0.22, 0.43 ± 0.13 and 0.39 ± 0.22 μmol/g fresh tissue for the 4.0, 5.8 and 10.8 mg Cu/kg diets and commercial diet, respectively (n = 5/group). Ceruloplasmin concentrations were different among the three dietary treatment groups at all four time points in the study. The CP activities at the start of the study were 25.2 ± 4.6 and 25.5 ± 2.3 U/L for the 4 and 5.8 mg Cu/kg dietary treatments and 43.8 ± 4.4 U/L for the queens consuming the 10.8 mg Cu/kg diet (P = 0.03). Significant differences remained between queens consuming the 4 and 5.8 mg Cu/kg diets (22.7 ± 1.5 and 30.2 ± 3.6 U/L) and the 10.8 mg Cu/kg dietary treatment group by the end of the study (43.8 ± 4.4 U/L; P = 0.001). Regardless of the significant differences among the treatment groups, dietary copper did not affect CP activity between or within queens in the same experimental group with time. There were no significant differences in DAO or EC SOD activity among the treatment groups.

The concentration of dietary copper affected (P = 0.04) the time interval for queens to conceive after exposure to the tom (defined as the number of days from the introduction of a proven tom until conception) (Table 1). There was a significant difference between the queens consuming diets containing 4.0 and 10.8 mg Cu/kg diet (P = 0.05). There was a negative linear relationship between dietary copper concentration (x = Cu mg/kg diet) and the mean time (y = days) necessary for queens to conceive (y = 43.38 – 2.87x; R² = 0.97). No resorptions were observed and one litter from each dietary treatment group was aborted. No significant differences among dietary treatment groups in the number of kittens born per litter, birth defects, kitten mortality or birth weights were observed.

DISCUSSION

Among the variables measured, liver copper concentration was the most sensitive to dietary copper intake and the best reflection of copper stores in the queen. This finding is similar to reports in other species (Klevay and Medeiros 1996) and supports the conclusions by Doong et al. (1983). In that study, liver copper concentration in kittens reflected dietary copper intake over the range of 0.58–9.64 mg Cu/kg diet.

Plasma copper concentration was not a sensitive indicator of dietary copper intake in this study. This conclusion is similar to findings in other species (Milne 1998). The decline in liver copper concentrations was not accompanied by a decline in plasma copper concentrations. Moreover, there was no response in plasma copper concentrations to dietary copper repletion. It was hypothesized initially that plasma copper concentrations may change only with extensive depletion. However, in an ongoing study in our laboratory, plasma copper concentrations have not changed in queens consuming a copper depletion diet for 2 y, despite severe declines in liver copper concentrations. Plasma copper concentrations may be maintained at the expense of other storage sites, or alternatively, conserved efficiently by cats in times of copper depletion.

There was an overall lack of response to dietary copper deficiency and copper repletion in the cuproenzymes analyzed. All of the enzymes evaluated have been demonstrated previously to respond to dietary copper deficiency in rodents (Keen et al. 1998). There were no differences in EC SOD or DAO activities. The response of EC SOD to dietary copper depletion and supplementation has been variable in the literature (Milne 1998). Several studies have demonstrated a decrease and increase in DAO concentrations secondary to dietary copper depletion and supplementation, respectively (DiSilvestro et al. 1997, Jones et al. 1997). The extent of copper depletion and repletion in this study may not have depleted storage sites (primarily liver) sufficiently to induce a change in the activity of these enzymes.

There were significant differences in the CP concentrations between the dietary treatment groups at all time points throughout the study. These differences were not likely to be the result of dietary treatment because they were present at the beginning and persisted throughout the study. The differences observed in CP activities were probably the result of hormonal changes in the cycling queens. Ceruloplasmin is sensitive to estrogen and increases in states of pregnancy (Milne 1998). We have documented elevations in CP concentrations in queens during gestation, followed by a gradual decline to prepartum values after parturition (unpublished data). Queens were cycling and breeding throughout the study and this would explain the fluctuations in CP activity.

Compared with reports in other species (Brewer 1987), the hematopoetic system of the queen is more resistant to changes in dietary copper intake. Packed cell volumes were not affected by dietary copper depletion in queens that underwent biopsy. The relative resistance of cats to anemia from copper deficiency was also demonstrated by Doong et al. (1983). Cats may possess alternative methods of iron transport that make them resistant to anemia from copper deficiency.

This is the first report of dietary copper deficiency depressing reproductive efficiency in cats. The findings from this study suggest that the copper requirement for the queen for gestation should be at least 3.8 mg Cu/kg diet. The optimal amount of copper may even be higher on the basis of the strong correlation between the amount of copper sulfate, in the range of 4–10.8 mg Cu/kg diet, and the mean time it took queens to become pregnant (R² = 0.97).

The lack of clinical signs compatible with copper deficiency, such as angular limb deformities and hypochromatemia, in the kittens born to the queens receiving low concentrations of dietary copper were not anticipated. Queens

### TABLE 1

<table>
<thead>
<tr>
<th>Dietary Cu, mg/kg</th>
<th>4.0</th>
<th>5.8</th>
<th>10.8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Queens, n</td>
<td>10</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Pregnancy rate, %</td>
<td>83</td>
<td>82</td>
<td>87</td>
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<tr>
<td>Mean days until onset of gestation²</td>
<td>36 ± 26a</td>
<td>25 ± 18ab</td>
<td>16 ± 12b</td>
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</tbody>
</table>

¹ There were no significant differences among the treatment groups. ² Significant effect of treatment P = 0.04. Values are means ± SEM. Values in a row not sharing a common superscript are significantly different (P = 0.05).
consumed their aborted fetuses so it is unknown how many of these kittens were abnormal. The consumption of abnormal kittens has been documented previously in our colony and the literature (Hart and Hart 1980).

There were no differences in the liver copper concentrations among the kittens still-born or that died after birth. A plausible explanation may be that regardless of the queen’s copper stores, copper transport to the fetus is a priority.

The results of this study indicate that liver copper levels, but not plasma copper concentrations, CP, DAO or EC SOD activities were responsive to dietary intake of copper in cats. The current NRC recommendation of 5 mg Cu/kg diet is probably marginal for optimal reproduction in the queen, especially if the copper is a source with an availability lower than that of copper sulfate.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the technical assistance of Debbie Bee and Jennifer Larsen in caring for the cats in this study and Roche Products (Nutley, NJ) for providing the vitamin mixture.

LITERATURE CITED


