Folate Requirement of Growing Kittens to Prevent Elevated Formiminoglutamic Acid Excretion Following Histidine Loading1,2

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EXPANDED ABSTRACT

KEY WORDS: • folate • kittens • requirement • formiminoglutamic acid • growth

Folate is a generic descriptor referring to a group of related compounds of which folic acid is the simplest form. Folic acid is readily assimilated and converted to the active cofactors necessary for single-carbon metabolism and DNA synthesis. Folate has been shown to be an essential dietary component for growing kittens (Thenen and Rasmussen 1978). Because the folate requirement of growing kittens had not been experimentally determined, the National Research Council (1986) suggested a tentative requirement of 0.8 mg/kg diet based on data from swine. In this study, we determined the minimal folate requirement of growing kittens based on hematological parameters and formiminoglutamic acid excretion after a histidine load.

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

Animals and housing. Specific-pathogen–free domestic short-haired kittens (initial mean body weight 1.33 ± 0.03 kg) from the Feline Nutrition and Pet Care Center of the University of California, Davis were used. Kittens were housed individually in stainless steel metabolism cages (60 × 60 × 60 cm) in rooms with a controlled temperature (21 ± 2°C) and light cycle (lights on: 0600–2000 h). They had free access to the experimental diets and tap water.

Diets. A gelatin-based purified diet supplemented with amino acids was used as a basal diet1, the diet contained all nutrients in sufficient amounts to meet the requirements of growing kittens except for folate. Experimental diets were prepared by adding various amounts of folic acid (pteroylglutamic acid, Sigma Chemical, St. Louis, MO) to the basal diet at the expense of cornstarch. The folic acid concentrations of the experimental diets were 0.1, 0.15, 0.2, 0.3, 0.6, 0.9 and 1.2 mg/kg diet, which were confirmed by a Lactobacillus casei microbiological assay (Martin et al. 1990).

Experimental design. Kittens were weaned at 8 wk of age and given the basal diet and tap water until 10 wk of age (wk 0 of the experiment) when they were randomly allocated into seven groups of eight kittens (4 males and 4 females) in each group. Each group was randomized to one of the seven experimental diets and maintained on the diet for 20 wk. Food intake was measured daily and body weight weekly. Blood samples were taken at wk 0 and 20 from the jugular vein of unanesthetized kittens with potassium EDTA as an anticoagulant. Plasma was prepared from the blood samples by centrifugation at 11000 × g for 20 min. For the measurement of formiminoglutamic acid (Figlu),3 urine was collected for a period of 48 h from kittens given a histidine load (0.22 g l-histidine/kg body weight) administered by gastric tube at wk 0 and 20. Urine was collected into a plastic bottle containing 1 mL of 18 mol/L sulfuric acid (Fisher Scientific, Fair Lawn, NJ). Food was withheld overnight before the oral histidine load. A broken-line method (Robbins 1986, Robbins et al. 1979) was employed to estimate the minimum folate requirement of growing kittens.

Sample analysis. Hematological parameters were measured using a blood cell counter (Mascot, CDC Technologies, Oxford, CT). A microbiological assay described by Tamura (1990) with L. casei (7469, American Type Culture Collection, Rockville, MD) and the Bio-Rad Model 2550 EIA

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4 Composition of the basal diet (g/kg): gelatin (150 Bloom Type A, Grayslake Gelatin, Grayslake, IL), 380; amino acid mixture, 40; sucrose, 100; animal fat (Fornin Tallow, Dixon, CA), 300; starch 93 (Melojel, Bridgewater, NJ); cellulose, 20; mineral mixture (Williams et al. 1987), 50; vitamin mixture, 10; choline chloride, 4.7 and taurine, 2.5. The amino acid mixture (Ajinomoto U.S.A., Raleigh, NC) was composed of (g/kg diet): l-isoleucine, 4.64; l-leucine, 7.32; l-methionine, 7.04; l-phenylalanine, 3.12; l-threonine, 3.72; l-tryptophan, 2.32; l-valine, 4.24; l-histidine, 3.20 and l-lysine • HCl, 4.40. The vitamin mixture contained (mg/kg diet) retinyl palmitate, 40; cholecalciferol, 5; dl-a-tocopheryl acetate, 320; menadione, 15; thiamin mononitrate, 25; riboflavin, 10; pyridoxine • HCl, 10; nicotinic acid, 100; calcium pantothenate, 20; myo-inositol, 200; cyanocobalamin in mannitol, 0.06; b-biotin, 1; ascorbic acid, 400 (as a preservative).

5 Abbreviations used: Figlu, formiminoglutamic acid; MCH, mean corpuscular hemoglobin; MCV, mean corpuscular volume.
Reader (Bio-Rad, Richmond, CA) was used to determine folate concentrations in whole blood and plasma. Urinary Figlu was measured according to an enzymatic method (Tabor and Wyngarden 1958).

Statistical analysis. All statistical analyses were performed using SPSS/PC+* Version 2.0 (SPSS Institute, Chicago, IL) and PC-SAS (for broken-line analysis), Version 6.03 (SAS Institute, Cary, NC). Two-way ANOVA, with diet and sex as main effects, was used for all parameters except plasma folate concentration (wk 20), which was analyzed by the Kruskal-Wallis test because of unequal variances among groups (Bartlett's test). Mean corpuscular volume (MCV, wk 20), plasma folate concentration (wk 0), whole-blood folate concentration (wk 20) and urinary Figlu excretion were logarithmically transformed before ANOVA tests because of unequal variances among dietary groups (Bartlett's test). Break points of selected variables (wk 20) were calculated using a nonlinear least-square method (Robbins 1986) if diet effect was significant (ANOVA). $P < 0.05$ was considered significant for all tests; there were no significant interactions.

**FIGURE 1** Folate concentrations in (A) whole blood, (B) erythrocytes and (C) plasma of kittens given a purified diet with various levels of folic acid. Each point represents a mean for 8 kittens (4 males and 4 females) with a vertical bar as SEM. At wk 20, there was a significant diet effect on plasma (Kruskal-Wallis test, $P < 0.01$), erythrocytes (two-way ANOVA, $P < 0.01$) and whole blood (two-way ANOVA, $P < 0.01$) folate concentrations.

**FIGURE 2** (A) Mean corpuscular volume (MCV) (B) mean corpuscular hemoglobin (MCH) and (C) urinary formiminoglutamic acid (Figlu) excretion of kittens given a purified diet with various levels of folic acid. Each point represents a mean for 8 kittens (4 males and 4 females) with a vertical bar as SEM. Two-way ANOVA showed a significant diet effect ($P < 0.01$) on MCV, MCH and urinary Figlu excretion. Break points for MCV, MCH and urinary Figlu excretion as functions of dietary folate concentrations were 0.32, 0.31 and 0.46 mg folic acid/kg diet, respectively.
**Results and discussion.** Folate levels in the experimental diets had no significant effect on food intake, body weight and body weight gain (mean 13.0 g/d). However, kittens receiving the diet containing 0.1 mg folate/kg diet tended to have a lower food intake at wk 20, (43 g/d vs. 55 g/d for the other treatments) and a lower body weight gain throughout the experiment. These measurements indicated that the kittens were exhibiting incipient clinical signs of folate deficiency. Male kittens had a significantly higher food intake and body weight gain at wk 20 than female kittens. There were no significant diet × sex interactions.

Folate concentrations in whole blood, erythrocytes and plasma blood increased with the increase of dietary folate intake (Fig. 1). Whole-blood folate concentration (Fig. 1, A) attained a maximum when dietary folate reached 0.9 mg folate/kg. In contrast, plasma folate concentration (Fig. 1, C) was positively correlated with dietary folate concentrations over the range of 0.1–1.2 mg folate/kg diet. Erythrocyte folate concentration followed a relationship similar to that of whole blood to dietary folate concentration (Fig. 1, B). Plasma folate appeared to be a better indicator of current folate intake than status, and may be a useful parameter for measuring the bioavailability of folate in food.

The hematologic parameters, white blood cell count, hemoglobin concentration, hematocrit, mean corpuscular hemoglobin (MCH) concentration and platelet count, were not affected by the experimental diets. However, MCV (Fig. 2, A) and MCH (Fig. 2, B) were significantly elevated at wk 20 when dietary folate was < 0.3 mg/kg diet. These observations are in agreement with those of Thenen and Rasmussen (1978) who reported megaloblastic erythropoiesis in kittens fed a purified diet without added folate.

Urinary Figlu excretion of kittens at 20 wk was significantly enhanced by diets containing <0.6 mg folate/kg (Fig. 2, C). Folate is a cofactor for the removal of the formimino group from formiminoglutamic acid in the histidine catabolic pathway. The histidine load test has been used for the diagnosis of folate deficiency in humans and results in an elevated urinary Figlu excretion in deficient individuals (Brody 1994). The fact that urinary Figlu excretion in folate-deficient kittens increased after a histidine load indicates that the catabolic pathway of histidine in cats is similar to that of other animals, and the load test is useful in the diagnosis of folate deficiency in kittens.

Because plasma, whole blood, and erythrocyte concentrations of folate were directly related to dietary concentration of folate, they were not regarded as useful indicators of the folate requirement. However, they may be used as potential diagnostic aids for adequacy. In contrast, urinary Figlu, MCV and HCH were sensitive indices of folate status of kittens, and exhibited similar response relationships to dietary concentrations of folate. These measurements were used along with dietary folate concentrations to construct broken-line relationships by a least-square analysis method (Robbins 1986) to determine the folate requirement. Estimates of the requirement as milligrams of folic acid per kilogram diet (energy density of 20 kJ/g diet) were as follows: Figlu, 0.46, MCV, 0.32 and HCH, 0.31. When asymptotic relationships were fitted, the 95% confidence interval estimates were 0.43–0.48, 0.32–0.32 and 0.28–0.34 mg folic acid/kg diet, respectively. These estimates are all < 0.5 mg folic acid/kg diet. However, because we did not test a dietary concentration between 0.3 and 0.6 mg/kg diet, on the basis of actual measured Figlu excretion, we recommend 0.6 mg folic acid/kg diet as a minimal folate requirement for growing kittens. This value provides a margin of safety and is less than the estimated requirement of the National Research Council (1986) of 0.8 mg folic acid/kg diet. For a dietary concentration of 0.6 mg folic acid/kg diet, the mean (± SEM) concentration of folate in whole blood, erythrocyte and the plasma of kittens was 5.9 ± 1.1, 17.8 ± 2.3 and 3.6 ± 0.3 μg/L, respectively. The Figlu excretion for kittens given the diet containing 0.6 mg/kg folic acid and a histidine load was 129 ± 13 mmol/d.

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**LITERATURE CITED**


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