Effect of Excess Dietary Methionine on Weight Gain and Plasma Amino Acids in Kittens\textsuperscript{1,2}

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\textbf{ABSTRACT} Four groups of five kittens each were individually fed for 6 wk either a purified control diet containing 0.5\% methionine (Met) (based on 18\% casein supplemented with arginine, cystine and threonine) or one of three experimental diets containing an additional 2, 3 or 4\% L-Met. The diets with added Met caused a reduction in food intake (FI) on the second day and a negative body weight gain (BWG), in proportion to the level of added Met. After 10 d, kittens fed the 2 and 3\% Met diets increased their FI and had positive BWG. During the last 10 d of the experiment, the control, 2\% Met and 3\% Met groups had BWG of 28, 15 and 0 g/d, respectively. Kittens given the 4\% Met diet showed no adaptation and continued to lose weight. In these kittens plasma concentration of Met was 50–70 times and cystathionine about three times greater than in control kittens. Four male kittens were fed the same 4\% Met diet for 6 wk and then switched to a diet containing 4\% L-Met plus 4\% glycine (Gly) for 12 d. Average daily FI was 21.4 ± 1.3 g with 4\% Met and 48.5 ± 2.5 g after the addition of Gly, and BWG went from negative to positive. These results indicate that growing kittens are more sensitive than rats to excess Met and have a limited adaptive capacity. Kittens did not grow normally when the diet contained 2\% or more dietary Met, which was equivalent to 0.6 g Met/(kg body wt·d). \textit{J. Nutr.} 117: 1838–1843, 1987.

\textbf{INDEXING KEY WORDS:}
\begin{itemize}
  \item cat nutrition
  \item methionine excess
  \item glycine
  \item plasma amino acids
\end{itemize}

Methionine (Met) has been shown to be one of the most toxic amino acids when given in amounts that substantially exceed the requirement for growth in rats (1, 2), chicks (3), guinea pigs (4) or pigs (5). Previous experiments (6) have shown that growing rats fed a diet containing 2\% excess Met needed 8 d to adapt to the new dietary conditions and stabilize their metabolism and weight gain.

The toxicity of dietary Met has not been studied in cats. Daily doses of 800 mg or greater Met per cat are often used as a urinary acidifier in the treatment of feline urologic syndrome (7). The highest dietary Met content reportedly used in experimental diets was 1.5\% total Met (8), which caused a slight depression in weight gain.

The object of this study was to determine the minimal concentration of dietary Met that would cause an adverse effect on kittens and to evaluate their capacity to adapt to excess Met. We also wanted to determine whether a glycine (Gly) supplement, which has been shown to ameliorate the adverse effects of excess dietary Met in rats (9), was effective in kittens.

\textbf{MATERIALS AND METHODS}

\textit{Animals}

Twenty-four male specific-pathogen-free kittens, vaccinated against panleukopenia and accustomed to the purified basal diet, were used as experimental animals. Their initial body weight ranged between 1100 and 1600 g, and they were individually housed in stainless steel cages.

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\end{itemize}
TABLE 1

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Control</th>
<th>2% Met</th>
<th>3% Met</th>
<th>4% Met</th>
<th>4% Met</th>
<th>4% Met + 4% Gly</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food intake, g/d</td>
<td>77.6 ± 1.5</td>
<td>42.3 ± 1.9</td>
<td>30.1 ± 1.9</td>
<td>15.6 ± 1.0</td>
<td>21.4 ± 1.3</td>
<td>48.5 ± 2.5</td>
</tr>
<tr>
<td>Casein Met&lt;sup&gt;1&lt;/sup&gt; ingested, g/d</td>
<td>0.334 ± 0.006</td>
<td>0.182 ± 0.008</td>
<td>0.129 ± 0.008</td>
<td>0.067 ± 0.004</td>
<td>0.092 ± 0.006</td>
<td>0.209 ± 0.011</td>
</tr>
<tr>
<td>Extra L-Met&lt;sup&gt;2&lt;/sup&gt; ingested, g/d</td>
<td>—</td>
<td>0.84 ± 0.038</td>
<td>0.90 ± 0.057</td>
<td>0.62 ± 0.040</td>
<td>0.86 ± 0.052</td>
<td>1.94 ± 0.100</td>
</tr>
<tr>
<td>Total Met ingested, g/d</td>
<td>0.33 ± 0.006</td>
<td>1.02 ± 0.046</td>
<td>1.03 ± 0.065</td>
<td>0.69 ± 0.044</td>
<td>0.95 ± 0.058</td>
<td>2.15 ± 0.11</td>
</tr>
<tr>
<td>Total Met (last 10 d)</td>
<td>0.14</td>
<td>0.62</td>
<td>0.72</td>
<td>0.64</td>
<td>0.55</td>
<td>1.16</td>
</tr>
</tbody>
</table>

<sup>1</sup>Average intake in g/d (±SEM) during the last 10 d of the experiment.
<sup>2</sup>Average intake in g/d (±SEM) for 12 d before and after the change of diet.
<sup>3</sup>The casein diet contained 0.43% Met.
<sup>4</sup>Additional free L-Met ingested.

**Diets**

A basal (control) purified diet was prepared containing 18% casein supplemented with 1.46% amino acids (L-arginine·HCl, 0.88%; L-cystine, 0.35%; and L-threonine, 0.23%), 20% sucrose, 26.57% starch (Melojel, National Starch and Chemical, Bridgewater, NJ), 25% animal tallow (Florin Tallow, Dixon, CA), 2% safflower oil, 4% mineral mixture<sup>4</sup>[10], 1% vitamin mixture<sup>4</sup>[8], 0.63% choline chloride, 1% cellulose (Solka-Floc) and 0.34% sodium acetate (to balance the hydrochloride of arginine).

Four experimental diets were prepared by addition of either 2, 3 or 4% Met or 4% Met + 4% Gly to the basal diet. These substitutions were made at the expense of starch with the exception of the 4% Met + 4% Gly diet, which was accommodated by a 6% reduction in starch and a 2% reduction in sucrose.

**Design**

**Experiment 1: effects of increasing levels of dietary Met.** Twenty male kittens were divided into four groups of five animals each; the mean body weight (±SEM) was 1425 ± 69 g. Each group of kittens received either the control diet or one of the experimental diets containing an additional 2, 3 or 4% Met for 6 wk. Food intake was recorded daily and body weight was recorded every 2 d. At d 10 and 42 after initiation of the experimental diets, 3-ml samples of blood were taken in heparinized syringes from the jugular vein of the unanesthetized kittens at approximately the same time (1000–1200 h) on each sampling day. Plasma was immediately treated with sulfosalicylic acid and then prepared and analyzed for free amino acids as previously described[11], using an amino acid analyzer (Model 121 MB, Beckman Instruments, Palo Alto, CA).

**Experiment 2: effects of dietary Gly on response of kittens to excess Met.** Four male kittens weighing 1870 ± 77 g were fed a diet containing 4% Met for 6 wk (same protocol as in experiment 1) and then switched to a diet containing 4% L-Met plus 4% Gly for a further 12 d. Food intake and body weight were recorded daily.

**Statistics**

Analysis of variance[12] was used to determine differences between groups and P ≤ 0.05 was taken as significant.

**RESULTS**

**Experiment 1.** Effects of dietary Met concentration on mean daily food intake and weight gain are shown in Table 1 and Fig. 1, respectively. The control animals exhibited a rectilinear weight gain of 28.0 ± 2.2 g/d during the 6 wk of the experiment, and had a mean food intake of 77.6 ± 1.5 g/d during the last 10 d of the experiment (food efficiency was 36 g body weight

<sup>4</sup>Mineral mix (g/kg diet): CaHPO<sub>4</sub>·2.25, K<sub>2</sub>HPO<sub>4</sub>·5.50, CaCO<sub>3</sub>·5.50, MgSO<sub>4</sub>·7.50, KCl·5.50, KHCO<sub>3</sub>·5.50, NaHCO<sub>3</sub>·7.50, MnSO<sub>4</sub>·H<sub>2</sub>O 0.19, ZnSO<sub>4</sub>·H<sub>2</sub>O 0.2235, CuSO<sub>4</sub>·H<sub>2</sub>O 0.040, FeC<sub>4</sub>H<sub>5</sub>·3H<sub>2</sub>O 0.500, C<sub>6</sub>(H<sub>10</sub>N<sub>7</sub>O<sub>6</sub>)·0.0075, SnCl<sub>2</sub>·H<sub>2</sub>O 0.0050, Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>·0.0015, [NH<sub>4</sub>]<sub>2</sub>(MoO<sub>4</sub>·4H<sub>2</sub>O) 0.002, CrCl<sub>3</sub>·H<sub>2</sub>O 0.013, NaCl·6H<sub>2</sub>O 0.015, NaF 0.007, NH<sub>4</sub>V<sub>2</sub>O<sub>7</sub>·4H<sub>2</sub>O 0.001, NaCl 0.2435.

<sup>5</sup>Contribution of vitamin mix (mg/kg diet): retinyl palmitate 11, cholecalciferol 0.05, dl-α-tocopherol acetate 160, menadione sodium bisulfite 14, thiamin mononitritre 25, riboflavin 10, pyridoxine hydrochloride 10, nicotinic acid 100, calcium pantothenate 20, myo-inositol 200, pteroylmethonoglutamic acid 10, cobalamin 0.05, D-biotin 1, ascobic acid (as a preservative).
gain per 100 g food ingested). By the second day, the kittens fed the high Met diets decreased their food intake and lost weight in proportion to the Met level fed. After 10 d, the kittens fed the 2 and 3% Met diets showed some enhancement of their food intake and body weight gain. Kittens fed the 2% excess Met diet had an average weight gain of 15.3 ± 1.8 g/d during the last 10 d of the experiment; during this time food intake was stabilized at 42.3 ± 1.9 g/d [food efficiency was 36 g body weight gain per 100 g food]. In the group fed 3% Met, the kittens just maintained their body weight (body weight gain of 2.1 ± 4.2 g/d) and mean food intake was 30.1 ± 1.9 g/d during the last 10 d of the experiment [food efficiency was 7 g body weight gain per 100 g food ingested]. The 4% Met group showed no adaptation and continued to lose weight (−6.0 ± 1.6 g/d from d 32 to 42), while the food intake remained low (15.6 ± 1.0 g/d).

On d 10, the plasma free amino acid pattern [Fig. 2] was greatly modified by the excess Met intake. Plasma Met was 50- to 70-fold higher in kittens fed the 2–4% excess Met diets than in the controls. Plasma cystathionine was 3- to 4-fold higher, plasma proline and asparagine 0.5- to 2-fold higher and plasma alanine threonine and serine 2- to 3-fold higher than in controls. The high concentration of free Met [2–3 mM] prevented an accurate determination of cystine, since the peaks elute too close to each other for separate determination when the Met level is more than 1 mM. There were no changes in plasma concentrations of other dispensable amino acids including taurine, glutamine, glycine, glutamic acid and citrulline. At the end of the experimental period (d 42), the plasma free amino acid pattern was similar to that on d 10, except that Met was about 40% higher (>4 mM) than on d 10 and cystathionine had returned to normal. The largest changes in plasma free amino acid concentrations were found in the plasma of animals fed the 3% excess Met, which also had the highest daily Met intake (Table 1). The 4% Met group had a severely reduced food intake and a lower Met intake than the 3% Met group.

**Experiment 2.** Four male kittens were fed the 4% Met diet for 6 wk. One of them died on the second day after transfer to the 4% Met + 4% Gly diet and was excluded from the results [necropsy results to be reported elsewhere]. The other three kittens had an initial body weight of 1870 ± 77 g [mean ± SEM]. Their body weight decreased to 1717 ± 73 g by d 42, at which time they were fed the 4% Gly-supplemented diet (Fig. 3). The kittens responded on the first day that supplemental Gly was added to the diet by increasing food intake and gaining body weight. After 12 d of Gly supplementation, body weight was 1989 ± 110 g. The mean food intake was 21.4 ± 1.3 g/d during the last 12 d of
feeding the 4% Met diet, increased significantly \([P \leq 0.01]\) to 48.5 ± 2.5 g/d after addition of Gly and remained at this level until the end of the experiment.

**DISCUSSION**

From the results of these experiments, kittens appear to be somewhat more sensitive than rats to Met excess. An 18% casein diet supplemented with arginine, threonine, taurine and cystine was sufficient to support rectilinear weight gains and apparently meet the kittens' requirements for essential amino acids [13]. This casein diet contained a concentration of 4.3 g Met/kg (by analysis). A supplement of 2% L-Met caused a severe growth depression in kittens, similar to that reported in young rats fed a 10% casein diet containing 3% excess DL-Met [1].

The high requirement of kittens for protein [14] has been related to the regulation of enzymes involved in the catabolism of amino acids [15] (urea cycle enzymes, alanine aminotransferase, aspartate aminotransferase). These enzymes of general nitrogen catabolism in cats have relatively high activities but are nonadaptive to increases or decreases in protein intake. It therefore might be expected that an excess amount of a specific amino acid would be handled with less adverse effect in cats than in rats. Harper, Becker and Stucki [16] suggested that the more protein an animal (rat) eats, the easier the adaptation to an amino acid excess, a direct result of higher activity of the catabolic pathways. This may not be true for the kitten, since the hepatic enzymes involved in the catabolism of Met showed activities much lower than those of rats [6, 17], and the adaptation was much slower in our experiment with kittens fed an 18% casein diet than for rats fed a 12% casein diet under similar conditions. Young rats fed a 2% Met diet needed 8 d to adapt and gain weight linearly (1.8 g/d versus 3.3 g for control rats), while the same phenomenon took place in 14–21 d in the growing kittens (15 g/d compared with 28 g in control kittens). Since ingestion of a diet containing 2% excess Met changed the body composition of rats, any comparisons of weight gain of kittens could be misleading.

Excess dietary Met has been shown to increase the requirement of rats for energy [18] and to decrease body fat content of rats [19] and pigs [5]. This response may be related to the energy concentration of the diet. A higher level of fat was used for the kitten diets (25% animal tallow + 2% safflower oil) than for the rat diets (8% peanut oil). Because of the higher fat content of the kitten diet, it might be expected that kittens would more readily adapt than rats or pigs, but this was not observed.

Schaeffer, Rogers and Morris [8] showed that the requirement of growing kittens for Met in the absence of cystine was about 0.75%. In their experiments, 1.5% total dietary Met slightly reduced the daily weight gain.
also applies to kittens. In the second experiment, a 4% Gly supplement alleviated part of the toxic effects of Met excess and kittens increased their intake up to 2.15 g Met/d (Table 1). Nevertheless, this concentration of Met remained somewhat aversive, since after 12 d the three kittens gained only about 22 g/d. Accumulation of a highly toxic product of Met catabolism such as methanethiol or hydrogen disulfide (20) could be responsible for the adverse effect.

The marked elevation of plasma Met appeared to depend on the daily Met intake and not on the percentage of excess Met in the diet. As food intake and weight gain increased, Met concentration in the plasma increased rather than decreased. This is consistent with the finding of low enzyme activities for Met catabolic enzymes in cat liver. Plasma taurine was unchanged or lower after feeding the high Met diets. Taurine synthesis, which was shown to be low in cats (21, 22), does not seem to be increased under these conditions (the diets contained 0.075% taurine). A consistent accumulation of cystathionine was found on d 10 in the plasma of kittens fed the excess Met diet. The subsequent return of cystathionine concentration to normal levels may indicate that there was a delay in the induction of cystathionase. An increase in activity occurred faster (8 d) in rats receiving the same level of dietary Met (6). An increase in plasma neutral amino acids, alanine, proline, threonine, serine and asparagine, was found in blood samples taken on d 10 and 42. The high concentrations of plasma Met (3–4 mM) could be responsible for impaired (or inhibited) uptake of these amino acids by the liver.

These observations indicate limited adaptation of kittens to dietary Met excess. A supplement of 2% Met was sufficient to have a severe adverse effect on kittens, whereas glutamic acid had an adverse effect only at dietary concentrations at or above 9% (23). Leucine at 10% did not depress body weight gain and food intake of growing kittens unless isoleucine and valine were already limiting (24). Kittens given the 2% excess Met diet were able to adapt to the excess Met by stabilizing their food intake and having rectilinear growth, but this adaptation occurred more slowly than in rats fed a similar diet. The pattern of plasma free amino acids of kittens was greatly modified by excess Met. Concentrations of Met as high as 3–4 mM were found in the plasma of kittens; these are much greater than the 1–2 mM reported in rats fed excess Met in the diet (1). A Gly supplement improved food intake and weight gain of kittens fed a diet with excess Met but did not completely restore them to normal.

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LITERATURE CITED


