Urinary Isovalthine Excretion in Adult Cats Is Not Gender Dependent or Increased by Oral Leucine Supplementation

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EXPANDED ABSTRACT

Domestic cats and other Felidae species are known to excrete three unusual amino acids in their urine; felinine, isovalthine, and isobuteine. Felinine (2-amino-7-hydroxy-5,5-dimethyl-4-thiaheptanoic acid) was discovered in 1951 and is unique to certain Felidae species including the domestic cat (1). Isovalthine (2-amino-5-carboxy-6-methyl-4-thiaheptanoic acid) was found in the urine of healthy domestic cats (2), lions (3), and humans suffering from hypercholesterolemia (4), and can be induced in a number of animal species including guinea pigs, dogs, rats, and rabbits (5). Information on isobuteine (2-amino-6-carboxy-4-thiaheptanoic acid) is limited to the isolation of this amino acid from the urine of healthy humans and cats, and its characterization and structural confirmation by synthesis (4).

The biological roles of felinine, isovalthine, and isobuteine remain unknown. Felinine was hypothesized to be a precursor to a pheromone, and although much circumstantial evidence is available, this remains to be proven. The biological role of isovalthine and isobuteine, if indeed they have a biological function, remains a matter for speculation. The carbon backbone of the side chain of both felinine and isovalthine appears to be derived from isoprenoid units similar to that used to synthesize cholesterol. Consequently, both isovalthine as well as felinine have at various times been hypothesized to be involved in cholesterol regulation (1). However, because felinine excretion was shown to be gender dependent (6), the latter role seems unlikely.

There is little information available on normal excretion levels of isovalthine in domestic cats. One report (1) found that administration of leucine to cats resulted in increased urinary isovalthine excretion. The aim of this study was to determine normal daily urinary excretion levels of isovalthine in adult domestic cats of different genders, and the effect of oral leucine supplementation on daily urinary isovalthine excretion in male, female, and castrated cats.

MATERIALS AND METHODS

Diets and animals

The studies reported here were approved by and conformed to the requirements of the Massey University Animal Ethics Committee (7). Domestic short-haired cats (Felis catus), were obtained from the Centre for Feline Nutrition at Massey University (Palmerston North, New Zealand). The body weights of the cats at the start of the experiment ranged from 2.64 to 4.70 kg. Throughout the study, the cats were fed to appetite, a moist canned cat food that had passed an American Association of Feed Control Officials (AAFCO) minimum feeding protocol for proving an adult maintenance claim for a cat food (8). The diet provided the following nutrients (g/kg dry matter): crude protein 520, crude fat 290, leucine 48, cysteine 18, methionine 12, taurine 2, and calculated metabolizable energy of 4610 kcal/kg dry matter. During both studies, the cats were housed in metabolism cages (9) and urine was collected quantitatively. Fresh water was provided ad libitum. All cats had been vaccinated against feline rhinotracheitis, calicivirus, and panleukopenia using a modified live vaccine (Felocell CVR, Norden Laboratories, München, Germany). Feline leukemia and feline immunodeficiency virus have not been detected in the colony since its establishment in 1976.

Study 1

Sixteen adult cats (six intact males, four castrated males, three intact females, and three spayed females) were fed the diet for 5 d before urine was collected according to the previously described and validated procedures (6).
Significance, p

<table>
<thead>
<tr>
<th>Gender</th>
<th>n</th>
<th>Concentration (μmol/L)</th>
<th>24-h Excretion (μmol/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>6</td>
<td>32 ± 2.1</td>
<td>1.8 ± 0.11</td>
</tr>
<tr>
<td>Castrates</td>
<td>4</td>
<td>46 ± 8.1</td>
<td>1.9 ± 0.49</td>
</tr>
<tr>
<td>Female</td>
<td>3</td>
<td>39 ± 5.0</td>
<td>2.8 ± 0.50</td>
</tr>
<tr>
<td>Spays</td>
<td>3</td>
<td>37 ± 8.5</td>
<td>1.7 ± 0.10</td>
</tr>
<tr>
<td>Significance, p</td>
<td>&lt;</td>
<td>0.334</td>
<td>0.155</td>
</tr>
</tbody>
</table>

Values with different letters within column are significantly different.

1 Values are presented as mean ± SEM.

2 From reference 6.

Study 2

Twelve adult cats (four intact males, four castrated males, and four entire females) were fed the diet for 5 d before an 8-d quantitative urine collection period. On d 3, 4, and 5, each cat was given 2 gelatine capsules each containing 0.30 g of L-leucine: 1 at 0900 h and 1 at 1600 h.

Isolevithine analysis

Isolevithine was measured using a Waters ion-exchange HPLC system (Waters, Milford, MA), utilizing postcolumn O-phthalaldehyde derivatization and detection at 570 and 440 nm, after hydrolysis for 24 h at 110 ± 2°C in 6 M glass-distilled HCl containing 0.1% phenol in evacuated sealed tubes. An isolevithine standard was prepared using a previously published synthesis procedure (4) and purified by anion-exchange chromatography (DE 52, DEAE-cellulose).

Statistical analysis

No transformation of the data in study 1 or 2 was required as they were found to be normally distributed. A linear model with gender as the fixed effect was fitted to the data in study 1 using the General Linear Model (GLM) procedure in SAS 6.12 (10). The data from study 2 were subjected to repeated measures ANOVA with gender, and period as fixed effects. The urinary isolevithine concentration before leucine administration was used as a covariate. Effects were considered significant at a probability level of 5%.

RESULTS

All cats remained healthy throughout the two studies. The urinary concentration and 24-h urinary isolevithine excretions of the different groups of cats as well as previously published felinine excretion levels in adult cats are shown in Table 1. Daily urinary isolevithine excretion ranged from 1.2 to 3.8 μmol/kg body weight whereas the concentration of isolevithine in the urine of cats ranged from 24 to 66 μmol/L. Mean isolevithine excretion rates were highest in the female cats but there was no significant difference in the urinary concentration or 24-h excretion levels among the different groups of cats.

The daily excretion of isolevithine of the different groups of cats in study 2, before, during, and after leucine administration are shown in Figure 1. Urinary isolevithine concentrations ranged between 2.1 and 20.2 μmol/d. Statistical analysis revealed no significant difference among male, castrated, and female cats. There was also no significant effect of leucine addition on isolevithine excretion.

DISCUSSION

Daily urinary isolevithine excretion in adult cats was low and was not increased by oral leucine administration. This is in direct conflict with a previous finding where it was reported that cats administered with 0.21 g/d of L-leucine for 3 d by stomach tube resulted in an increase in isolevithine excretion in cats (2). However, in that study urinary isolevithine was detected by paper chromatography, an insensitive, nonquantitative method for measuring amino acids and this may have resulted in an erroneous conclusion. It is also possible that the diet fed in the latter study (2) was marginally deficient in leucine and the additional leucine merely restored isolevithine excretion levels to normal. In the current study, cats were fed a diet containing adequate levels of leucine and it is possible that the leucine addition may not have increased isolevithine synthesis.

Comparison of urinary isolevithine concentration with published felinine excretion levels shows that urinary isolevithine concentration in cats is ~1:25–1:30 times that of felinine, depending on gender (Table 1). Isolevithine concentrations in the urine ranged from 23 to 67 μmol/L in individual animals, compared to felinine, which ranges from 600 to 17,400 μmol/L.

![Figure 1](https://academic.oup.com/jn/article-abstract/134/8/2115S/4688891)
In addition, our results show that the excretion of isovalthine is not dependent on the gender of the animal. This is in contrast to felinine excretion, which was shown to be gender dependent with entire males excreting 3–6 times more felinine compared to cats of other genders (2).

Isovalthine was found to be a normal component of cat urine; in fact, research to date indicates that the domestic cat and the lion are the only two species that normally excrete isovalthine (2,3). Our group has recently provided further evidence for the latter observation, having tested the urine of healthy dogs, mice, rats, guinea pigs, pigs, rabbits, humans, and horses using amino acid analysis as the method of detection of isovalthine. However, many different species including the dog, guinea pig, rabbit, and rat have been reported to excrete isovalthine after the administration of a suitable induction agent such as isovaleric acid (5), although induction cannot always be reliably achieved (11).

The structural similarities of felinine and isovalthine are notable. Felinine synthesis in cats occurs via a conjugation reaction of glutathione and isopentenyl pyrophosphate and/or dimethylallyl pyrophosphate (12). It is likely that the resulting felinine-containing tripeptide (γ-glutamylfelinylglycine) is hydrolyzed in the kidney with the subsequent appearance of free felinine in the urine. This study provides indirect evidence that the biosynthesis of isovalthine does not appear to follow that of felinine, despite the structural similarities. If isovalthine synthesis occurred as a side reaction to felinine synthesis, it could be expected that isovalthine excretion levels would also be gender dependent, similar to felinine. In addition, isovalthine synthesis can be induced in other animals whereas felinine is a unique urinary amino acid of Felidae. Experiments to ascertain if leucine is a precursor to isovalthine, by orally administering 14C-leucine to rats (11), a species that does not normally excrete isovalthine, failed to show any incorporation of leucine into isovalthine. Efforts to show induction of isovalthinuria by leucine in guinea pigs also failed (13). Given these results and those presented here, the question of whether leucine is involved in isovalthine synthesis in the cat remains to be determined.

The biological role of isovalthine remains a matter for speculation. Although little scientific information is available, the fact that isovalthine excretion can be induced in other animals, and that adult cats excrete low concentrations of isovalthine in their urine and the concentrations are not dependent on gender, leads these authors to conclude that isovalthine biosynthesis may be the result of a side reaction in sulfur amino acid metabolism in many mammalian species, including cats.

ACKNOWLEDGEMENT

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LITERATURE CITED