Chapter 4
The Biological Function of Cauxin, a Major Urinary Protein of the Domestic Cat (*Felis catus*)

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Abstract A major protein component of domestic cat urine is the carboxylesterase family member termed cauxin. Cauxin is secreted into the urine from the proximal straight tubular cells of the kidney, and the level of cauxin excretion is species-, sex-, and age-dependent. Cauxin is excreted in large amounts in the closely related members of the Felidae lineage, the cat (*Felis catus*), bobcat (*Lynx rufus*), and lynx (*Lynx lynx*). Male and female immature cats begin excreting cauxin about 2.5 months after birth, and excretion levels increase with age. In mature cats, cauxin excretion is significantly higher in intact males than in castrated males or female cats. The physiological function of cauxin is to provide species-, sex-, and age-dependent regulation of 2-amino-7-hydroxy-5,5-dimethyl-4-thiaheptanoic acid (felinine) production. Cauxin hydrolyzes the peptide bond of the felinine precursor, 3-methylbutanol-cysteinylglycine, to produce felinine and glycine. The sulfur-containing volatile compounds, 3-mercapto-3-methyl-1-butanol, 3-mercapto-3-methylbutyl formate, 3-methyl-3-methylthio-1-butanol, and 3-methyl-3-(2-methyl-disulfanyl)-1-butanol, are identified as species-specific odorants and candidates of felinine derivatives from the headspace gas of cat urine. These cat-specific volatile compounds may represent pheromones used as territorial markers for conspecific recognition or reproductive purposes by mature cats. The elucidation of cauxin-dependent felinine production provides new evidence for the existence of species-specific odorants and pheromones produced by species-specific biosynthetic mechanisms in mammalian species.

4.1 Introduction

Proteinuria, the excretion of large amounts of proteins into the urine, is often considered to be an indication of pathological event of the kidney because mammals only excrete small amounts of proteins into the urine under physiological conditions

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to prevent proteins loss from the body (D'Amico and Bazzi 2003). However, it is known that some mammalian species, including mice (*Mus musculus*) and rats (*Rattus norvegicus*), exhibit proteinuria under physiological conditions and this type of proteinuria is involved in chemical communication. Mouse urine contains a high concentration (10–15 mg/ml) of the 19-kDa lipocalin family proteins termed major urinary proteins (MUPs) that remain stable in urine marks over many weeks without degradation (Cavaggioni and Mucignat-Caretta 2000; Beynon and Hurst 2004). Many polymorphic variants of MUPs are expressed in the liver and secreted into the blood. The secreted MUPs pass through glomerular barriers of kidneys and are excreted into the urine. In male mice, MUPs bind male-specific volatile pheromones such as 2-*sec*-butyl-4,5-dihydrothiazole and 3,4-dehydro-*exo*-brevicomin (Bacchini, Gaetani and Cavaggioni 1992; Robertson, Beynon and Evershed 1993) and release them from deposited urine over several hours (Hurst, Robertson, Tolladay and Beynon 1998). In addition, polymorphic variants of MUPs are known to play a direct role in individual recognition during male mice (Hurst, Payne, Nevison, Marie, Humphries, Robertson, Cavaggioni and Beynon 2001; Nevison, Armstrong, Beynon, Humphries and Hurst 2003).

In this chapter, we review a new type of physiological proteinuria found in the domestic cat (*Felis catus*) and illustrate the biological relevance of this process. Chronic renal diseases represent the leading cause of illness and death among aging cat populations. Therefore, we carried out a precise analysis of cat urine to diagnose early-stage renal diseases. In this process, we determined that male cats exhibit proteinuria under physiological conditions. Since previously identified MUPs were not present in cat urine, we investigated the protein contents of cat urine and found that the major protein component of cat urine was the carboxylesterase family member termed cauxin involved in the production of felinine, a putative precursor of cat pheromones.

4.2 Cauxin Discovery and Biochemical Characterization

Urinary proteins were analyzed by SDS–polyacrylamide gel electrophoresis (PAGE), and a 70-kDa protein was identified as the major component of cat urine (Fig. 4.1A). Comparative analysis of urinary proteins in several other mammals such as humans, mice, dogs, and cattle did not detect a 70-kDa protein. Therefore, the 70-kDa protein was purified from cat urine and characterized by biochemical methods (Miyazaki, Kamiie, Soeta, Taira and Yamashita 2003). Analysis of tissue distribution indicated that the 70-kDa protein is expressed in the kidney in a tissue-specific manner and secreted from the proximal straight tubular cells of the kidney into the urine (Fig. 4.1B). A full-length cDNA for a 70-kDa protein was cloned from a cat kidney cDNA library. The cDNA clone encoded a polypeptide of 545 amino acid residues. The deduced amino acid sequence shared 47% identity with cat carboxylesterase (CES, EC 3.1.1.1), and contained both the CES family protein motif (EDCLY) and a conserved active site motif (GESAG) associated with

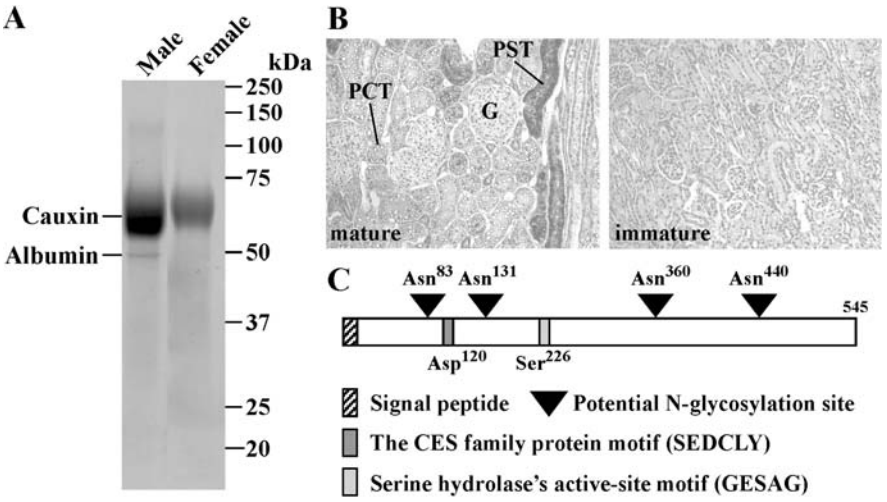

Fig. 4.1 (A) Cat urine (10 µl) was analyzed by SDS-PAGE followed by Coomassie blue staining. A single heavily stained band of cauxin was detected at 70 kDa in the urine of mature intact male and female cats. (B) Immunohistochemical images of cauxin in paraffin-embedded renal sections of 2-year-old mature and 1-month-old immature male cats. Cauxin-positive proximal straight tubular cells (PST) are observed between the inner cortex and outer medulla regions in the mature cat, but not in the immature cat. PCT, proximal converted tubules; G, the renal glomerulus. (C) A schematic image of the primary structure of cauxin

serine hydrolase family members (Fig. 4.1C). *In vitro* enzyme assays confirmed that the 70-kDa protein has esterase activity and can hydrolyze the artificial substrates *p*-nitrophenylacetate and 1-naphthylacetate. Therefore, we concluded that the 70-kDa protein is a novel member of the mammalian CES family and named the protein cauxin (carboxylesterase-like urinary excreted protein).

Cauxin is markedly different from previously reported mammalian CESs in term of urinary excretion. Other mammalian CESs comprise multigene families, and CES isozymes are highly and ubiquitously expressed in tissues such as the brain, liver, kidney, lung, and small intestine (Satoh and Hosokawa 1998). Our work on cauxin was the first description of a carboxylesterase excreted in urine.

4.3 Sex and Age Dependence of Cauxin Excretion

Although cauxin is excreted in the urine of both male and female cats, the average urinary concentration of cauxin was found to be significantly higher in intact males (0.87 ± 0.19 g/L) than in females (0.23 ± 0.12 g/L) (Miyazaki, Yamashita, Hosokawa, Taira and Suzuki 2006a). RT–PCR indicated that the expression of cauxin mRNA in the kidney was about fivefold higher in intact males than females (Miyazaki, Yamashita, Suzuki, Soeta, Taira and Suzuki 2006b). In addition, we found that cauxin expression decreased in the kidney proximal straight tubular cells

immediately after castration of intact males, and that the average urinary concentration of cauxin in castrated males (0.13 ± 0.10 g/L) was significantly lower than in intact males. These results suggest that sex hormones such as testosterone are important regulatory factors of cauxin production in the proximal straight tubular cells.

Additionally, we found that cauxin excretion levels are dependent on the age of the cat (Miyazaki et al. 2006a). Cauxin was not detected in the urine of cats of less than 3 months of age by SDS–PAGE followed by Coomassie blue staining. Consistent with this finding, the expression of cauxin in the proximal straight tubular cells was not detected with an anti-cauxin antibody using immunohistochemistry (Fig. 4.1C). Temporal analysis of cauxin excretion by Western blotting with the anti-cauxin antibody indicated that immature male and female cat begin excreting cauxin about 2.5 months after birth, and the excretion level increases with age.

4.4 Species-Specific Excretion of Cauxin

The cauxin gene is conserved in several mammals, including humans, mice, rats, dogs, and cattle, although only the cat excretes cauxin. Cauxin mRNA expresses in the liver and kidney of humans and mice (M. Miyazaki, T. Yamashita, H. Taira and A. Suzuki, unpublished data). Ecroyd et al. reported that cauxin is present in ram (*Ovis aries*) reproductive fluids (Ecroyd, Belghazi, Dacheux, Miyazaki, Yamashita and Gatti 2006). Ram cauxin was secreted from the caudal epididymis and associated with epididymal soluble prion protein (Ecroyd, Belghazi, Dacheux and Gatti 2005). Based on these findings, we investigated whether additional Felidae species excrete cauxin into the urine. Urinary proteins were analyzed by SDS–PAGE followed by Coomassie blue staining and Western blotting with an antibody raised against cauxin purified from cat urine. Our analysis indicated that cauxin is a major component in bobcat (*Lynx rufus*) and Siberian lynx (*Lynx lynx*) urine, as well as in domestic cat urine (Miyazaki et al. 2006a). We could not detect cauxin in the urine of the puma (*Puma concolor*), leopard (*Panthera pardus*), tiger (*P. tigris*), jaguar (*P. onca*), snow leopard (*P. uncia*), or lion (*P. leo*). However, McLean and colleagues detected cauxin in the urine of the Asiatic lion (*P. leo persica*), Amur tiger (*P. tigris altaica*), Persian leopard (*P. pardus saxicolor*), clouded leopard (*Neofelis nebulosa*), and jaguar by MALDI–ToF mass spectrometry (L. McLean, J. Lewis, J. Hurst and R. Beynon, personal communication). Interestingly, in these Felidae animals, cauxin is excreted in part as a disulfide linked multimer. At present, Felidae animals are classified into eight lineages including *Panthera*, *bay cat*, *caracal*, *ocelot*, *Lynx*, *puma*, *leopard cat*, and *domestic cat* (Johnson, Eizirik, Pecon-Slattery, Murphy, Antunes, Teeling and O'Brien 2006). The bobcat and Siberian lynx are same members of the *Lynx* lineage and near to the cat in the phylogenetic tree of Felidae animals. These results suggest that over time, each Felidae animal developed unique machinery for the kidney-specific expression and the urinary excretion of cauxin, and the closely related members, the domestic cat, bobcat, and lynx,

posses the most highly developed machinery. Cauxin would play species-specific physiological roles in each mammalian species.

4.5 Physiological Function of Cauxin

The CES family of proteins is characterized by the ability to hydrolyze a wide variety of aromatic and aliphatic substrates containing ester, thioester, and amide bonds (Heymann 1980, 1982). Cauxin is a member of the CES family, and is secreted from the proximal straight tubular cells into the urine in a species-, sex-, and age-dependent manner. Therefore, we postulated that cauxin was involved in an enzymatic reaction in cat urine and the products made by the reaction should vary with species, sex, and age. Based on this hypothesis, we searched for physiological substrates and products of cauxin in cat urine and identified 2-amino-7-hydroxy-5,5-dimethyl-4-thiaheptanoic acid, also known as felinine.

Felinine is excreted in a sex- and age-dependent manner in the urine of the cat, ocelot (*Felis pardalis*), and bobcat (Hendriks, Tarttelin and Moughan 1995c). Felinine is suggested to be a putative pheromone precursor in the cat (MacDonald, Rogers and Morris 1984; Hendriks, Moughan, Tarttelin and Woolhouse 1995b) because chemically synthesized felinine is odorless but develops a characteristic catty odor during storage (Hendriks, Woolhouse, Tarttelin and Moughan 1995a). Previously, it had been suggested that felinine is synthesized in the kidney because felinine is not present in blood or other cat tissues. However, Rutherfurd et al. found that 3-methylbutanol-glutathione (3-MBG) identified from cat blood contains the chemical structure of felinine (Rutherfurd, Rutherfurd, Moughan and Hendriks 2002). They suggested that the synthesis of the felinine precursor is formed via a glutathione S-conjugation reaction between glutathione and isopentenylpyrophosphate, an intermediate of cholesterol biosynthesis. Hendriks et al. found that cat urine contains 3-MBG, 3-methylbutanol-cysteinylglycine (3-MBCG), and *N*-acetyl felinine, in addition to felinine, and suggested that the breakdown of 3-MBG occurs in a similar manner to other glutathione S-conjugates (Hendriks, Harding and Rutherfurd-Markwick 2004). It is well known that glutathione S-conjugates are hydrolyzed to cysteinylglycine S-conjugates by γ-glutamyl transferase (EC 2.3.2.2) in the kidney, and the cysteinylglycine S-conjugates are hydrolyzed by renal dipeptidase (EC 3.4.13.11) (Lohr, Willsky and Acara 1998; Wang and Ballatori 1998). In this degradation pathway, the cysteine S-conjugates like felinine are reabsorbed into the renal tubular cells, *N*-acetylated by *N*-acetyltransferase (EC 2.3.1.5), and ultimately excreted as *N*-acetyl cysteine S-conjugates in the urine. However, the excretion level of *N*-acetyl felinine is much lower than that of felinine in cat urine (Hendriks et al. 2004; Rutherfurd-Markwick, McGrath, Weidgraaf and Hendriks 2006). Therefore, we hypothesized that the metabolic pathway from 3-MBG to felinine is cat-specific and cauxin is involved in this pathway. Thus, we tested whether cauxin hydrolyzed the felinine precursors, 3-MBG and 3-MBCG *in vitro*. Enzymatic assays determined that cauxin hydrolyzes the peptide bond of 3-MBCG

to felinine and glycine (Fig. 4.2) (Miyazaki et al. 2006b). This finding suggested that cauxin regulates the production of felinine in a species-, sex, and age-dependent manner *in vivo* and this suggestion was consistent with *in vivo* data correlating cauxin and felinine levels in the urine with the sex and age of the cat.

Figure 4.2 shows the proposed metabolic pathways for the conversion of 3-MBCG to felinine in the cat kidney. The 3-MBG is filtered through the glomerulus of kidneys and converted to 3-MBCG by γ-glutamyl transferase localized at the brush border of proximal tubular cells. A small portion of the 3-MBCG is hydrolyzed to felinine and glycine by renal dipeptidase localized in the proximal tubular cells. Felinine thus formed is absorbed by the proximal tubular cells, where it is converted to *N*-acetyl felinine by *N*-acetyltransferase. Most of the remaining

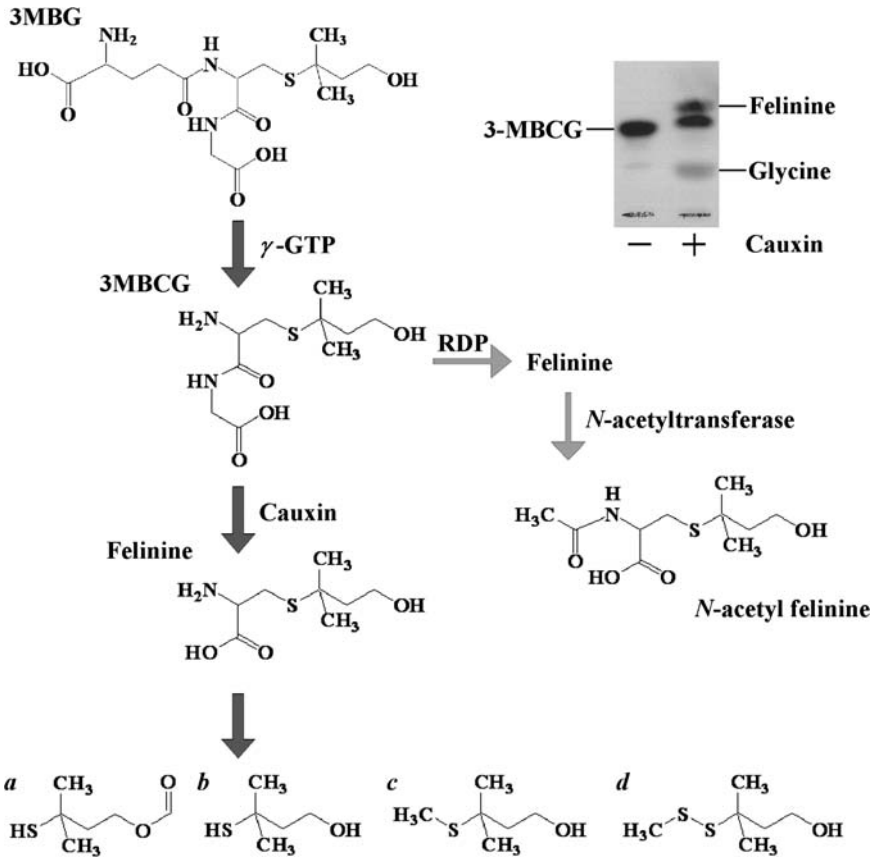

Fig. 4.2 Hydrolytic activity of cauxin on 3-MBCG and proposed metabolic pathways for the conversion of 3-MBG to felinine in the cat kidney. The 3-MBCG (20 mM) was incubated with or without cauxin (1.5 mg/ml) at 38°C for 6 h, and the reaction mixtures were analyzed by thin layer chromatography with ninhydrin staining. γ-GTP, γ-glutamyl transferase; RDP, renal dipeptidase; *a*, 3-mercapto-3-methylbutyl formate; *b*, 3-mercapto-3-methyl-1-butanol; *c*, 3-methyl-3-methylthio-1-butanol; and *d*, 3-methyl-3-(2-methyldisulfanyl)-1-butanol

3-MBCG is hydrolyzed to felinine and glycine by cauxin in the renal tubules and the bladder and/or *ex vivo*. The felinine produced in this manner is excreted into the urine.

4.6 Biological Relevance of Cauxin-Dependent Felinine Production

To elucidate the biological relevance of cauxin-dependent felinine production, it was necessary to determine the bioactivity of felinine and/or felinine derivatives. Felinine was postulated to be biologically important as a territorial marker for intraspecies communication (MacDonald et al. 1984; Hendriks et al. 1995b), or, although the direct evidence remains to be demonstrated, a putative precursor pheromone involved in attracting females (Tarttelin, Hendriks and Moughan 1998). Hendriks et al. (1995b) hypothesized that felinine degradated to 3-mercapto-3-methyl-1-butanol contributed to cat-specific urinary odor. Joulain and Laurent (1989) suggested that 3-mercapto-3-methyl-l-butanol, 3-methyl-3-methylthio-l-butanol, and 3-methyl-3-(2-methyldisul-fanyl)-1-butanol were present

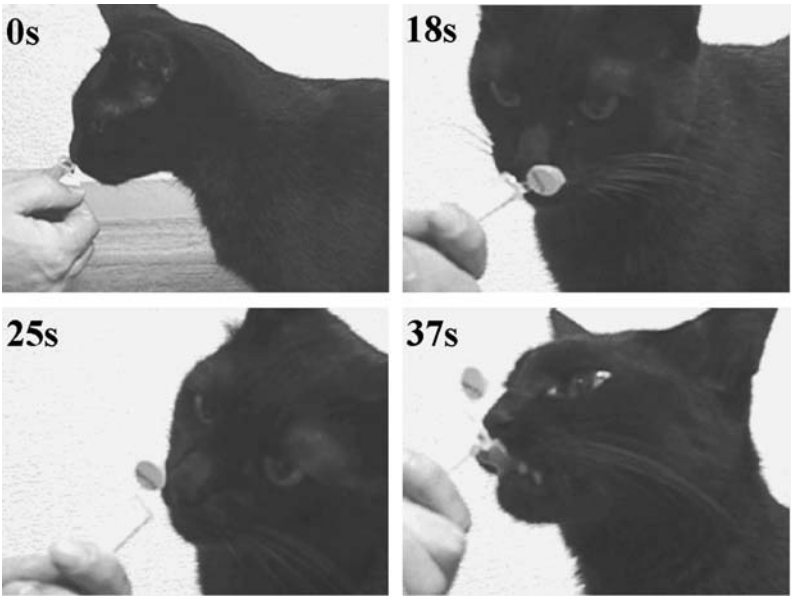

Fig. 4.3 Behavioral bioassay by using a felinine derivative. Felinine purified from cat urine by HPLC was dissolved in water at a concentration of 10 mg/ml, and 200 µl of the solution was stored in a 1.5-ml eppendorf tube at room temperature for 5 days. GC–MS analysis detected 3-mercapto-3-methyl-1-butanol in the headspace gas of the tube. The cat (6-year-old castrated male) was able to sniff the opening of the tube, but not contact the felinine solution. The cat sniffed 3-mercapto-3-methyl-1-butanol with considerable interest (18s and 25s) and then licked his lips five times (37s)

in cat urine. These compounds were identified in bobcat urine (Mattina, Pignatello and Swiharat 1991), but Rutherfurd et al. (2004) could not detect these compounds in cat urine. To identify the volatile compound responsible for cat-specific urinary odor, we analyzed the components of volatile compounds in the headspace gas of cat urine using a gas chromatograph mass spectrometry (GC–MS). Our analysis detected four sulfur-containing volatile compounds, 3-mercapto-3-methyl-1-butanol, 3-mercapto-3-methylbutyl formate, 3-methyl-3-methylthio-1-butanol, and 3-methyl-3-(2-methyl-disulfanyl)-1-butanol, as candidates of felinine derivatives (Miyazaki et al. 2006b). The levels of these compounds were found to be sex- and age-dependent. Based on these results, we are now designing studies to determine the bioactivity of felinine derivatives with cats. Since sulfur-containing volatile compounds would give species-, sex-, and age-specific odor to cat urine, it is possible that these compounds are putative pheromones used for conspecific recognition or reproductive purposes in the cat. Our preliminary behavioral bioassays indicated that cats sniffed 3-mercapto-3-methyl-1-butanol with considerable interest and sometimes licked their lips (Fig. 4.3), but we could not identify a functional behavioral response. Further studies using behavioral bioassays are needed to clarify the biological relevance of cauxin-dependent felinine excretion.

4.7 Conclusion

Although proteinuria is often considered to be a pathological event, we demonstrated that this is not the case for the domestic cat. Male cat urine contains a large amount of the mammalian carboxylesterase family member termed cauxin. Cauxin is excreted in a species-, sex-, and age-dependent manner and regulates the production of felinine, a putative pheromone precursor. This finding provides an example of a previously unknown type of proteinuria involved in chemical communication.

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References

Bacchini, A., Gaetani, E. and Cavaggioni, A. (1992) Pheromone binding proteins of the mouse, *Mus musculus*. Experientia 48, 419–421.

Beynon, R.J. and Hurst, J.L. (2004) Urinary proteins and the modulation of chemical scents in mice and rats. Peptides 25, 1553–1563.

Cavaggioni, A. and Mucignat-Caretta, C. (2000) Major urinary proteins, α_{2U}-globulins and aphrodisin. Biochim. Biophys. Acta 1482, 218–228.

D'Amico, G. and Bazzi, C. (2003) Pathophysiology of proteinuria. Kidney Int. 63, 809–825.
Ecroyd, H., Belghazi, M., Dacheux, J.L. and Gatti, J.L. (2005) The epididymal soluble prion protein forms a high-molecular-mass complex in association with hydrophobic proteins. Biochem. J. 392, 211–219.
Ecroyd, H., Belghazi, M., Dacheux, J.L., Miyazaki, M., Yamashita, T. and Gatti, J.L. (2006) An epididymal form of cauxin, a carboxylesterase-like enzyme, is present and active in mammalian male reproductive fluids. Biol. Reprod. 74, 439–447.
Hendriks, W.H., Woolhouse, A.D., Tarttelin, M.F. and Moughan, P.J. (1995a) Synthesis of felinine, 2-amino-7-hydroxy-5,5-dimethyl-4-thiahep-tanoic acid. Bioorg. Chem. 23, 89–100.
Hendriks, W.H., Harding, D.R. and Rutherfurd-Markwick, K.J. (2004) Isolation and characterisation of renal metabolites of γ-glutamylfelinylglycine in the urine of the domestic cat (*Felis catus*). Comp. Biochem. Physiol. B Biochem. Mol. Biol. 139, 245–251.
Hendriks, W.H., Moughan, P.J., Tarttelin, M.F. and Woolhouse, A.D. (1995b) Felinine: a urinary amino acid of Felidae. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 112, 581–588.
Hendriks, W.H., Tarttelin, M.F. and Moughan, P.J. (1995c) Twenty-four hour felinine excretion patterns in entire and castrated cats. Physiol. Behav. 58, 467–469.
Heymann, E. (1980) Carboxylesterases and amidases. In: W.B. Jakoby (Ed.), *Enzymatic Basis Detoxification, Vol. II*, Academic Press, New York, pp. 291–323.
Heymann, E. (1982) Hydrolysis of carboxylic esters and amides. In: W.B. Jakoby, J.R. Bend and J. Caldwell (Eds.), *Metabolic Basis Detoxification*, Academic Press, New York, pp. 229–245.
Hurst, J.L., Payne, C.E., Nevison, C.M., Marie, A.D., Humphries, R.E., Robertson, D.H., Cavaggioni, A. and Beynon, R.J. (2001) Individual recognition in mice mediated by major urinary proteins. Nature 414, 631–634.
Hurst, J.L., Robertson, D.H.L., Tolladay, U. and Beynon, R.J. (1998) Proteins in urine scent marks of male house mice extend the longevity of olfactory signals. Anim. Behav. 55, 1289–1297.
Johnson, W.E., Eizirik, E., Pecon-Slattery, J., Murphy, W.J., Antunes, A., Teeling, E. and O'Brien, S.J. (2006) The late Miocene radiation of modern Felidae: a genetic assessment. Science 311, 73–77.
Joulain, D. and Laurent, R. (1989). The catty odour in blackcurrant extracts versus the blackcurrant odour in the cat's urine? In: (S.C. Bhattacharyya, N. Sen and K.L. Sethi (Eds.), *11th International Congress of Essential Oils, Fragrances, and Flavours*, Oxford IBH Publishing. New Delhi, India, pp. 89.
Lohr, J.W., Willsky, G.R. and Acara, M.A. (1998) Renal drug metabolism. Pharmacol. Rev. 50, 107–141.
MacDonald, M.L., Rogers, Q.R. and Morris, J.G. (1984) Nutrition of the domestic cat, a mammalian carnivore. Annu. Rev. Nutr. 4, 521–562.
Mattina, M.J.I., Pignatello, J.J. and Swiharat, R.K. (1991) Identification of volatile components of bobcat (*Lynx rufus*) urine. J. Chem. Ecol. 17, 451–462.
Miyazaki, M., Kamiie, K., Soeta, S., Taira, H. and Yamashita, T. (2003) Molecular cloning and characterization of a novel carboxylesterase-like protein that is physiologically present at high concentrations in the urine of domestic cats (*Felis catus*). Biochem. J. 370, 101–110.
Miyazaki, M., Yamashita, T., Hosokawa, M., Taira, H. and Suzuki, A. (2006a) Species-, sex-, and age-dependent urinary excretion of cauxin, a mammalian carboxylesterase family. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 145, 270–277.
Miyazaki, M., Yamashita, T., Suzuki, Y., Soeta, S., Taira, H. and Suzuki, A. (2006b) A major urinary protein of the domestic cat regulates the production of felinine, a putative pheromone precursor. Chem. Biol. 13, 1071–1079.
Nevison, C.M., Armstrong, S., Beynon, R.J., Humphries, R.E. and Hurst, J.L. (2003) The ownership signature in mouse scent marks is involatile. Proc. Biol. Sci. 270, 1957–1963.
Robertson, D.H.L., Beynon, R.J. and Evershed, R.P. (1993) Extraction, characterization and binding analysis of two pheromonally active ligands associated with major urinary protein of house mouse (*Mus musculus*). J. Chem. Ecol. 19, 1405–1416.
Rutherfurd, K.J., Rutherfurd, S.M., Moughan, P.J. and Hendriks, W.H. (2002) Isolation and characterization of a felinine-containing peptide from the blood of the domestic cat (*Felis catus*).

J. Biol. Chem. 277, 114–119.
Rutherfurd, S.M., Zhang, F., Harding, D.R., Woolhouse, A.D. and Hendriks, W.H. (2004) Use of capillary (zone) electrophoresis for determining felinine and it's application to investigate the stability of felinine. Amino Acids 27, 49–55.
Rutherfurd-Markwick, K.J., McGrath, M.C., Weidgraaf, K., and Hendriks, W.H. (2006) γ-glutamylfelinylglycine metabolite excretion in the urine of the domestic cat (*Felis catus*). J. Nutr. 136, 2075S–2077S.
Satoh, T. and Hosokawa, M. (1998) The mammalian carboxylesterases: from molecules to functions. Annu. Rev. Pharmacol. Toxicol. 38, 257–288.
Tarttelin, M.F., Hendriks, W.H. and Moughan, P.J. (1998) Relationship between plasma testosterone and urinary felinine in the growing kitten. Physiol. Behav. 65, 83–87.
Wang, W. and Ballatori, N. (1998) Endogenous glutathione conjugates: occurrence and biological functions. Pharmacol. Rev. 50, 335–356.