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NOTE

Physiology

The profile of urinary lipid metabolites in cats

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ABSTRACT. Polyunsaturated fatty acids including arachidonic acid (AA), docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), are converted to lipid mediators by oxidation. Unlike other mammals, cats cannot synthesize AA. Since their lipid metabolic features remain unknown, we qualitatively analyzed 118 types of urinary lipid metabolites in healthy neutered cats. Using LC-MS, we found 26 lipid metabolites in urines of all individuals. In detail, 20 AA-, 5 EPA- and 1 DHA-derived lipid mediators were detected. Focusing on oxidative pathway, 17 cyclooxygenase-metabolites and 5 metabolites produced by non-enzymatic pathway were detected. Of interest, few lipoxygenase- or cytochrome P450-metabolites were excreted. Thus, AA-derived cyclooxygenase-metabolites mainly composed the urinary lipid metabolites in cats.

KEY WORDS: cat, lipid metabolite, urine

Polyunsaturated fatty acids (PUFAs) are fundamental components of cell membrane as well as the precursor of lipid mediators. PUFAs are categorized into n-6 and n-3 fatty acids by the position of double bond. Arachidonic acid (AA) belongs to n-6, while eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) doses to n-3 fatty acid [18]. AA, EPA and DHA are converted to many types of bioactive lipid called lipid mediators. Prostaglandins (PGs) and leukotrienes (LTs) are well-known lipid mediators which play a pivotal role in the regulation of several pathophysiological functions [1].

Since mammals can synthesize AA but not enough EPA and DHA from their parent fatty acid [7], we need to obtain n-3 PUFAs from dietary food. Uniquely, cats cannot synthesized AA as well as EPA and DHA, because of the lack of the D6-desaturase activity [2]. They need to gain both n-6 and n-3 PUFAs from dietary food. Thus, cats presumably repressent unique PUFA metabolic profile. However, it remains unknown.

Many PUFAs-derived lipid mediators are excreted into urine. There are several reports showing that the urine lipid profile reflects host physiological and/or inflammatory condition. In human, urinary metabolite level of a major proinflammatory mediator PGE_2 are elevated upon cigarette smoking [10]. In human and mice, urinary level of PGD_2 metabolite reflects the severity of the food allergic symptoms [6, 9]. Cats often suffer from urinary disorders such as chronic kidney disease, idiopathic cystitis, and urinary calculus. Urinary lipid metabolites can be a beneficial index which reflect these diseases. On basis of these backgrounds, we first performed a LC-MS-based comprehensive analysis of lipid metabolites in the urines of healthy cats to investigate the metabolic features of PUFAs in cats.

As shown in Table 1, we recruited neutered 5 cats (4 castrated male; 3 Japanese domestic cat and 1 Munchkin, and 1 spayed female; Japanese domestic cat, 7.2 ± 3.2 years old), and collected their urines by cystocentesis from June to December 2019. All cats recruited in this study were diagnosed as healthy based on the results from biochemistry blood test, complete blood count test and physical condition at Pigeon Animal Hospital, Tokyo. These cats had no underlying diseases or medication at urine collection. As shown in Table 2, each cat ate different kinds of commercially available food which contained larger amount of n-6 fatty acid than n-3 fatty acid. Urine samples were stored until used at −28°C for 3–7 months. Informed written consent was obtained from all the owners at admission. The collected urines were centrifuged (20,000 × *g*, 5 min) and the supernatant were mixed with internal standards (Table 3). After the solid phase extraction (Oasis HLB, Waters, MA, USA), the sample solutions containing lipids fractions were eluted with methanol. The sample solution (5 μl) was injected to liquid chromatography (Nexera 2, Shimadzu, Kyoto, Japan) equipped with mass spectrometer (LCMS-8060, Shimadzu).

Total 118 types of metabolites (Substrate classification: 86 types; n-6 (AA)-derived, 32 types: n-3 PUFA (EPA and DHA)-derived metabolite. Enzyme classification: 35 types: Cyclooxygenase (COX), 50 types: lipoxygenase (LOX), 19 types: cytochrome P450 (CYP) and 14 types: non-enzymatic metabolites) (Fig. 1) were measured and analyzed by using LC/MS/MS Method Package for Lipid Mediators Ver. 2 with LabSolutions software (Shimadzu) as manufactures instruction. Each metabolite was identified by retention time and selected reaction monitoring ion transition. The metabolites were defined as “Detected” only in the case that the

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Table 1. Gender and the results of blood test

Number	Gender	TP (g/d*l*)	ALT (GPT) (U/*l*)	Cre (mg/d*l*)	WBC (10^2/*μl*)	HCT (%)
1	M	6.7	63	1.53	85	46.5
2	M	6.7	59	0.99	176	38.2
3	M	7.4	39	1.36	77	45.7
4	M	7.1	52	1.24	52	41.5
5	F	7.1	37	1.52	73	42.0

M, castrated male; F, spayed female. TP, total protein; ALT, alanine transaminase; GPT, glutamic pyruvic transaminase; Cre, serum creatinin; WBC, white blood cell; HCT, hematocrit.

Table 2. The amount of food contained polyunsaturated fatty acids (PUFAs)

Number	Food types	Contained PUFAs (g/100 g)	
		AA	EPA+DHA
1	Brand A, type A	3.33	0.21
2	Unknown	-	-
3	Brand A, type B	Unknown	0.87
4	Brand A, type C	Unknown	1.21
5	Brand A, type C	Unknown	1.21

AA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

Table 3. The list of internal standards (IS)

	Name	Concentration (*n*g/m*l*)
1	Tetranor-PGEM-d6	25.0
2	6-keto-Prostaglandin F1α-d4	25.0
3	Thromboxane B2-d4 (TXB2-d4)	25.0
4	Prostaglandin F2α-d4 (PGF2α-d4)	25.0
5	Prostaglandin E2-d4 (PGE2-d4)	25.0
6	Prostaglandin D2-d4 (PGD2-d4)	25.0
7	Leukotriene C4-d5 (LTC4-d4)	25.0
8	Leukotriene B4-d4 (LTB4-d4)	25.0
9	5(S) HETE-d8	25.0
10	12(S) HETE-d8	25.0
11	15(S) HETE-d8	25.0
12	Oleoyl Ethanolamide-d4 (OEA-d4)	0.5
13	Eicosapentaenoic Acid-d5 (EPA-d5)	500.0
14	Docosahexaenoic Acid-d5 (DHA-d5)	50.0
15	Arachidonic Acid-d8 (AA-d8)	500.0

metabolites were constantly detected in all urine samples.

Figure 1 shows metabolite maps based on substrate and enzymatic pathway. In all urine samples, we found that 26 types of the lipid metabolites were detected in the analyzed metabolites. Among these, 20 types were AA-derived (Fig. 1A), 5 types were EPA-derived (Fig. 1B) and 1 type was DHA-derived metabolite (Fig. 1C). The detection rate of n-6 (AA) or n-3 PUFA (EPA and DHA) derived metabolite were 23.3% (20/86) and 18.8% (6/32), respectively.

The profiles of urinary lipid metabolites can also be influenced by consumed PUFAs. As shown in Table 2, their foods were different. We could not reveal the amount of AA, DHA or EPA in their foods in the present study. Even in this situation, the detection rate of n-6 or n-3 PUFA derived metabolites was almost the same in all the cat urines (23.3% vs 18.8% in measurement items).

COX, LOX, CYP mediated oxidation or non-enzymatic oxidation convert PUFAs (AA, DHA, and EPA) to lipid mediators [20]. We next compared the detected metabolites by oxidative pathways. In the 26 types of metabolites, the number of COX-, LOX- or CYP-metabolites was 17, 2 and 1, respectively. Non-enzymatic metabolites were 6 types (Fig. 1). The detection rate of COX-, LOX-, CYP- or non-enzymatic oxidized metabolites was 48.6% (17/35), 4.0% (2/50), 5.3% (1/19) and 42.9% (6/14), respectively. Thus, COX-metabolites accounted for the largest proportion of the urinary lipid metabolites in healthy cats.

It is reported that COX was localized in many renal structures of cats and COX inhibition decreased urinary PGE_2 excretion of healthy cats [17]. Satoh H *et al.* reported that COX is expressed on duodenal mucosa and plays a tissue protective role in cats [19]. These reports suggest the importance of COX metabolites in tissue homeostasis of cats, and they also support our findings that COX metabolites are mainly consisted of the urinary lipid profile of cat.

There are accumulation evidences showing pathophysiological role of major PGs. PGE_2 is well-known to play and extensive role in inflammatory responses [13]. PGI_2 maintain circulation healthy [11]. These PGs and their metabolites were constantly detected in cat urine (Fig. 1), suggesting their crucial role in tissue homeostasis of cat.

Previous study showed that a major LOX-derived metabolite, LTE_4 was detected in urine of healthy human and dogs [5, 8]. On the other hand, few LOX metabolites were detected in the cat urines. Previous *in vitro* study showed that cultured feline tracheal epithelial cells produced 12-LOX metabolite 12-hydroxyeicosatetraenoic acid (HETE) and 15-LOX metabolite 15-HETE [22]. Oral treatment of a 5-LOX inhibitor decreased the concentration of leukotriene B_4 (LTB_4) in pyloric mucosal biopsy specimens of healthy cats [4]. Although these reports suggest the production of LOX metabolites in feline tissue, we did not detect many. Further

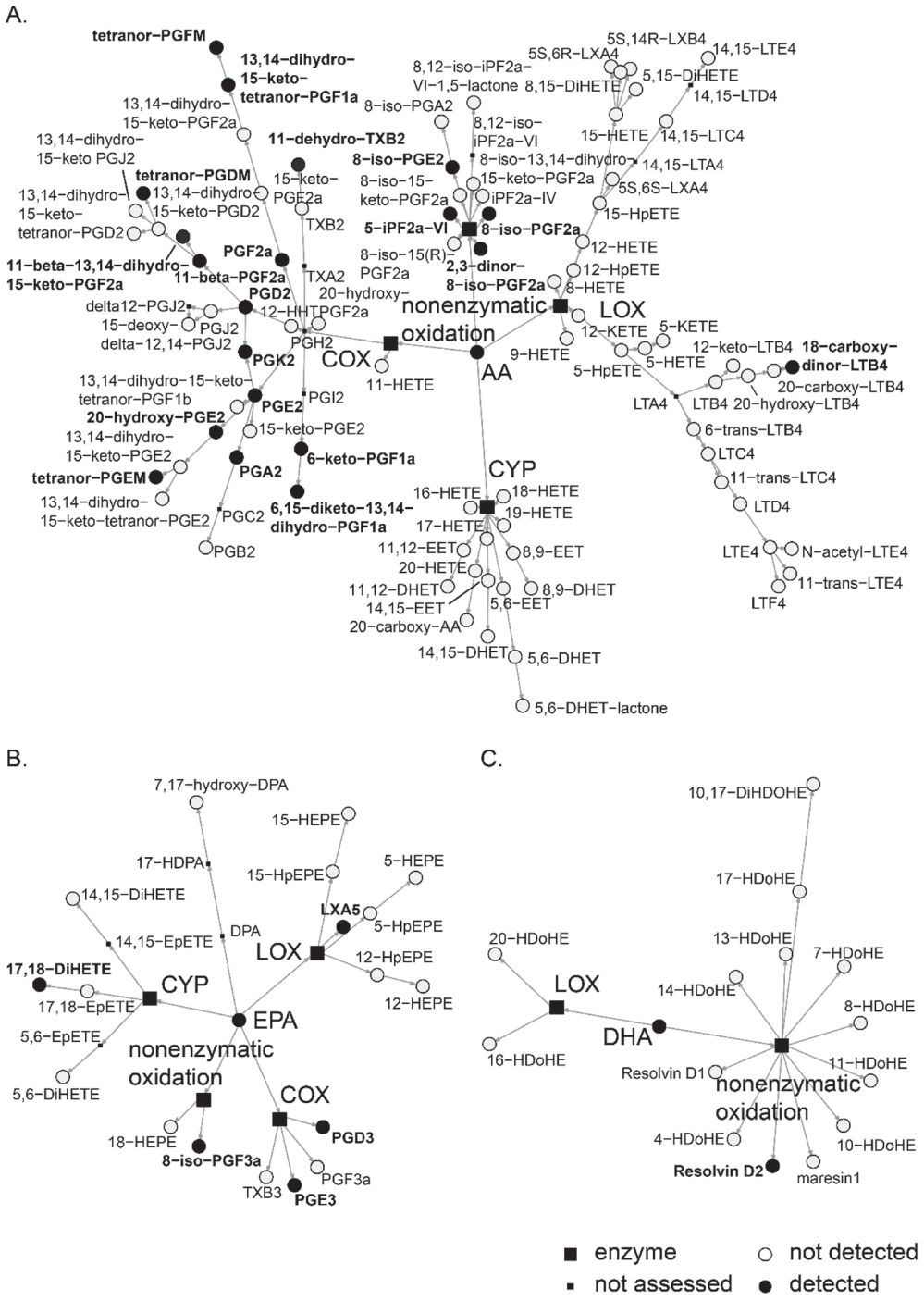

Fig. 1. Metabolic pathway of arachidonic acid (AA), icosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). AA (A), EPA (B) and DHA (C) metabolic pathway classified by cyclooxygenase (COX), lipoxygenase (LOX), cytochrome P450 (CYP) or non-enzymatic oxidation (large black square). A black circle indicates analyzed lipid metabolite in urine of healthy cats. A black filled circle indicates detected metabolite. A black open circle indicates non-detected metabolite. A small black square indicates non-assessed metabolite.

investigation is needs to reveal the urinary excretion of LOX-metabolites in cats.

CYP-derived metabolites, 5,6-EET, 11,12-EET, and 14,15-EET were detected in urine from healthy human [15], while they were not detected in cat urine. There have been several reports showing that cats possess unique expression pattern of CYP enzymes. Cat liver expresses less mRNA of most major CYP isoform CYP2E1 than dog and human livers [14]. Ono. Y *et al.*, also reported the low activation of another major CYP isoform CYP2C in liver of cat [16]. These features may be responsible for the low detection number of CYP metabolites in healthy cat urine.

We detected several non-enzymatic oxidation products of AA called isoprostanes, such as 8-iso-$PGF_{2\alpha}$, 8-iso- PGE_2 and

2,3-dinor-8-iso-$PGF_{2\alpha}$ (Fig. 1). Isoprostanes are reported to be an index of lipid peroxidation in both animal and human models of oxidative stress [3, 12]. This lipid metabolic pathway may play roles in cat homeostasis.

There are some limitations of the present study. First, we could not assess the breed and sex, neutering difference because of small sample size. Second, pro-inflammatory lipid mediators including PGE_2 are released by surgical trauma [21]. Indeed we detected PGE_2 or its metabolites, 20-hydroxy-PGE_2, tetranor-PGEM and PGA_2 in cat urines. Cystocentesis might stimulate the production of these lipids. Third, preservation of urine also possibly influences the detection rate of lipids. Inagaki. S. *et al.* reported that PGD_2 metabolite tetranor-PGDM in urine was stable in −20°C for 7 days [6]. However, we did not assess the stability of each lipid metabolite detected in cat urines. Further investigations are needed to clarify these points.

In summary, we revealed urinary lipid profiles in healthy neutered cats, and they were uniquely characterized by AA-COX-metabolites. Several non-enzymatic oxidation products of AA were also detected. These findings will help to understand lipid metabolisms and to analyze pathophysiological features of cats in future study.

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