


ORIGINAL ARTICLE

Urinary excretion of advanced glycation end products in dogs and cats

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Funding information

This study was financially supported by a scholarship from the Faculty of Veterinary Medicine, Kasetsart University, Thailand.

Abstract

The present study was conducted with privately owned dogs and cats to investigate whether a relationship exists between the dietary AGEs and the urinary excretion of AGEs, as indication of possible effective absorption of those compounds in the intestinal tract of pet carnivores. For this purpose, data were collected from both raw fed and dry processed food (DPF) fed to dogs and cats, through spot urine sampling and questionnaires. Raw pet food (RF, low in AGE diets) was fed as a primary food source to 29 dogs and DPF to 28 dogs. Cats were categorized into 3 groups, which were RF ($n = 15$), DPF ($n = 14$) and dry and wet processed pet food (DWF, $n = 25$). Urinary-free carboxymethyllysine (CML), carboxyethyllysine (CEL) and lysinoalanine (LAL) were analysed using ultrahigh-performance liquid chromatography (UHPLC)—mass spectrometry, and were standardized for variable urine concentration by expressing the AGE concentrations as a ratio to urine creatinine (Ucr) concentration ($\mu\text{g}/\mu\text{mol}$ Ucr). Urinary excretion of CML, CEL and LAL in dogs fed with DPF was 2.03, 2.14 and 3 times higher compared to dogs fed with RF ($p < .005$). Similar to the dogs, a significant difference in CML:Ucr, CEL:Ucr and LAL:Ucr between the three diet groups was observed in cats (p -overall < 0.005 , ANOVA), in which the RF fed group excreted less AGEs than the other groups. Linear regression coefficients and SE of CML:Ucr, CEL:Ucr and LAL:Ucr showed that body weight and neuter status were significantly correlated with CML and CEL excretion, but not to LAL excretion. Our results revealed a significant correlation between dietary AGEs and urinary excretion of free CML, CEL and LAL, and also showed that endogenous formation of these AGEs occurs in both dogs and cats under physiological conditions.

KEYWORDS

advanced glycation end products, cats, diet, dogs, urinary excretion

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1 | INTRODUCTION

Globally, the lifespan of domestic pets is increasing, which is coincided with increased incidences of age-related diseases, like renal diseases and diabetes mellitus (Hoening, 2014; Rubin, 1997; Saunders, 2012). Interestingly, data from studies in humans, rats and dogs indicate a correlation between age-related diseases and the intake of advanced glycation end products (AGEs), but the cause and effect relationship is hitherto unclear (Ahmed, Frye, Degenhardt, Thorpe, & Baynes, 1997; Koschinsky et al., 1997; Uribarri et al., 2005). It is generally believed that specific AGEs, such as carboxymethyllysine (CML), carboxyethyllysine (CEL) and lysinoalanine (LAL), are possibly involved in pathogenesis of chronic diseases (Hamelin, Borot-Laloi, Friguet, & Bakala, 2003; Langhendries et al., 1992; Poulsen et al., 2013).

Advanced glycation end products are compounds consisting of sugar moieties and proteins and originate either from intermediates of glucose metabolism (i.e. endogenous AGEs, Rabbani & Thornalley, 2012) or from the diet (O'Brien & Morrissey, 1989). To improve nutritive values, hygiene and shelf life (Crane et al., 2010; Hullar, Fekete, & Szocs, 1998), processed pet foods (PF) are generally heat treated, which triggers a non-enzymatic glycation reaction known as the Maillard reaction thereby leading to the formation of dietary AGEs (Henle, 2005). Previously, van Rooijen et al. (2014) have shown that commercially available PF can contain high amounts of AGEs. However, it is currently not known whether dietary AGEs are effectively absorbed from the gastrointestinal tract of dogs and cats. This lack of knowledge prompted us to undertake the current observational study. The aim of this study was therefore to investigate whether a correlation exists between the dietary AGE intake and the urinary excretion of AGEs. For this purpose, urinary excretion of AGEs was compared between privately owned dogs and cats that were currently fed with either a PF (high in dietary AGEs) or a raw pet food (RF, low in dietary AGEs).

2 | MATERIALS AND METHODS

2.1 | Selection of animals and inclusion criteria

Data for this observational study were collected through spot urine sampling and questionnaires, from June to September 2015 (cat samples) and March to April 2016 (dog samples). Privately owned cats and dogs were recruited through veterinary clinics and Internet advertisement throughout the Netherlands. Participation in this study was voluntary, and informed consent was obtained from the owners. Only healthy dogs and cats over 1 year of age, and without history of renal diseases or diabetes mellitus were eligible for this study. The characteristics of the study population are summarized in Table 1. A completed feeding management questionnaire was obtained from the owners, which included questions on the general information and health of the animals, types and brands of pet food fed one week prior to enrolment in the study (van Rooijen et al.,

TABLE 1 Selected descriptive statistics of the study groups

Item	Dogs		Cats		
	Raw	Dry	Raw	Dry	Dry + Wet
Animals (numbers)					
Male					
Intact	7	6	6	1	2
Sterile	5	7	3	4	7
Female					
Intact	7	9	2	1	4
Sterile	10	6	4	8	12
Total	29	28	15	14	25
Age (year)					
Mean	5.1	6.6	7.5	8.4	6.7
Minimum	1	1	1	1	1
Maximum	11	15	14	19	18
SD	3.13	4.41	4.21	4.31	4.9
Body weight (kg)					
Mean	9.9	10.2	4.9	4.3	4.1
Minimum	4.8	1.1	3.4	2.2	1.9
Maximum	43.0	42.5	7.1	8.5	9.2
SD	3.19	4.39	1.21	1.84	1.64

Abbreviations: Dry + Wet: dry and wet processed pet food group; Dry: dry processed pet food group; Raw: raw pet food group; SD: standard deviation.

2016), feeding of snack and supplements, and possibility to feed outside the house. Types of pet food were categorized according to Crane et al. (2010), which were RF (home-made or commercially available), dry processed pet food (DPF, commercial dry form PF, contains 3%–11% water) and wet processed pet food (WPF, commercial moist or canned PF, contains 60 to more than 87% water).

A total of 57 dogs and 54 cats of different breeds, age and gender were included in this study. In the dog population, 25 dogs were male (13 intact and 12 neutered) and 32 were female (16 intact and 16 neutered). Age ranged from 1 to 15 years with a mean age of 5.8 years. The body weight ranged from 1.1 to 43 kg with a mean of 22.4 kg. The dogs belonged to different breeds and were categorized as small (<10 kg, $n = 8$), medium (≥ 10 kg to 25 kg, $n = 23$) and large breed dogs (≥ 25 kg, $n = 26$). RF was fed as a primary food source to 29 dogs and DPF to 28 dogs, whereas no dogs were fed WPF. Fifty dogs received additional snacks, and 13 dogs received dietary supplements (consisting of additional vitamins and minerals).

In the cat population, 23 cats were male (of which 14 neutered) and 31 were female (of which 24 neutered). The age ranged from 1 to 19 years with a mean age of 6.0 years. The body weight ranged from 1.9 to 9.2 kg with a mean of 4.37 kg. Fifteen cats primarily received RF, 14 primarily received DPF, and 25 received a combination of DPF and WPF (DWPF, ~50:50% volume basis). Of 3 cats, it was mentioned that eating outside the house occurred regularly, whereas 7 cats received table food and 18 cats received treats. None of the cats received dietary supplements.

2.2 | Urine sampling

Urine sample collection was conducted both during a visit to the participating veterinary clinics or by the owners at home. Urine collection in the participating veterinary clinics was conducted by manual compression of the urinary bladder or by cystocentesis during routine surgical procedures. Natural voided urine was sampled by the owners at home by direct sampling during spontaneous voiding in dogs or by using a litter box with non-absorbent litter in cats. Urine samples were stored at -18°C until further processing and analysis.

2.3 | Urine sample processing and analysis

Prior to the analysis of CML, CEL and LAL, aliquots of 2 ml urine samples were thawed and ultrafiltered using centrifugal filters (Ultracel 10K, Amicon Ultra 2 ml, Millipore) for 60 min at $4,000 \times g$ using a swing bucket rotor centrifuge (HERMLE z-383). The ultrafiltrates were stored at -20°C until analysis. Samples were analysed on an Accela Ultrahigh-performance Liquid Chromatography (UHPLC) System (Thermo Scientific, San Jose, CA, USA) using an Acquity UPLC BEH 300 Amide column (2.1×150 mm, $1.7\text{-}\mu\text{m}$ particle size) with an Acquity BEH Amide Vanguard precolumn (2.1×50 mm, $1.7\text{-}\mu\text{m}$ particle size). Eluent A was Millipore water, containing 0.1% (v/v) formic acid (FA), and eluent B was acetonitrile (ACN), containing 0.1% (v/v) FA. The supernatants of the dog/cat urine samples were diluted 50 times in eluent A containing 10^{-3} g/L labelled (^{13}C) lysine (Polypeptide laboratories, Strasbourg, France) as internal standard and centrifuged (5 min, $19,000 g$, 20°C). Supernatants (1 μl) were injected into the column thermostated at 35°C . The elution profile was as followed: 0–2 min isocratic on 90% B, 2–3 min linear gradient from 90% B to 65% B, 3–5 min isocratic on 65% B, 5–7 min linear gradient from 65% B to 40% B, 7–10 min isocratic on 40% B, 10–12 min linear gradient from 40% B to 90% B and 12–28 min isocratic on 90% B. The flow rate was 350 $\mu\text{l}/\text{min}$. Mass spectrometric data were obtained by analysing samples on a LTQ-VelosPro (Thermo Scientific) equipped with a heated ESI probe coupled to the UHPLC system. The capillary voltage was set to 3 kV with the source operation in positive ion mode and negative ion mode. The heater temperature was set at 225°C , and the capillary temperature was set at 300°C . The sheath gas flow rate was set at 30, and the auxiliary gas flow rate was set at 9 (arbitrary units). The compounds were analysed using selected reaction monitoring (SRM) method analysis in negative ion mode for LAL and in positive ion mode for CEL, CML and the labelled lysine. The normalized collision energy was set at 30 for LAL and at 35 for CML, CEL and labelled lysine. The m/z width on the fragment was set to 2. Compounds were quantified using an external standard calibration curve by plotting MS peak area of the SRM fragment divided by the MS peak area of the SRM fragment of $^{13}\text{C}_6^{15}\text{N}_2$ -lysine, used as internal standard. Data were acquired and analysed using Xcalibur 2.1 (Thermo Scientific). The quantification limit for the AGE compounds was 0.5, 0.35 and 0.4 $\mu\text{g}/\text{ml}$ sample for CML, CEL and LAL respectively.

Specific gravity of urine was analysed using a digital pocket refractometer (Pal-10S, Atago). Urine creatinine (Ucr) ($\mu\text{mol}/\text{l}$) was determined by the kinetic Jaffé reaction (Bartels & Böhmer, 1971), for the purpose of standardizing AGE excretion in the urine for variable urine concentration, by expressing the AGE concentrations as a ratio to Ucr concentration ($\mu\text{g}/\mu\text{mol}$ creatinine).

2.4 | Calculation and statistical analysis

To assess daily AGE intake, daily feed intake was estimated from the questionnaires. In case of ad libitum dry feeding (cats), a daily feed intake of 17.5 g/kg body weight/day was assumed (Gross et al., 2010). Dry matter (DM) of daily ingested commercial pet food was calculated assuming a DM content of 90% for DPF and 20% for WPF (Crane et al., 2010). The DM content of RF and other additional treats was calculated based on data from USDA National Nutrient Database for Standard Reference (US Department of Agriculture Agricultural Research Service, 2015), and DM content of 34 and 40% was estimated for home-prepared and commercial raw diets respectively. The latter was based on the average DM content of commercially available RF diets in the Netherlands.

Dogs were categorized to either RF or DPF fed group according to their primary food source. Categorizing cats to diet groups was more complicated, because of their food variety. Therefore, cats were categorized into 3 groups based on their primary food intake: RF, DPF and DWPF.

Statistical analysis was conducted in R (R Core Team, 2018), using package car (John & Sanford, 2009) and ggplot2 (Wickham, 2016). The 3 different AGEs (CML, CEL and LAL) were dependent variables in the analysis. The variables from the questionnaire were tested for univariable associations with the outcomes using t tests, ANOVA and Pearson's correlation coefficient. To correct for potential confounding, a multivariable linear regression analysis was used. Non-significant predictors ($p > .05$) were removed using a forward stepwise selection procedure. Confounding was deemed to be present when a change of at least 20% in the β -estimate was observed after inclusion of a variable to the model. The best model explaining the variance of the dependent variables was obtained. The assumptions on homoscedasticity, normality and linearity were checked by visual inspection of the plots of standardized residuals against standardized predicted values, quantile–quantile plots of standardized residuals and plots of standardized residuals against predictor variables.

3 | RESULTS

3.1 | Food intake

For dogs, the daily food intake of the RF group and the DPF group was 25.83 ± 6.716 and 28.05 ± 12.082 g DM/kg $^{0.75}$, which was not significantly different ($p = .6015$). However, in cats the food intake

of the DWPF group ($31.06 \pm 17.193 \text{ g DM/kg}^{0.75}$) was significantly different from the RF group ($19.53 \pm 5.871 \text{ g DM/kg}^{0.75}$) and the DPF group ($20.47 \pm 5.4 \text{ g DM/kg}^{0.75}$), $p = .018$ and $p = .037$ respectively. Noteworthy, the DWPF group contained a lactating cat with a significantly higher food intake compared with the rest of the cat population.

3.2 | Urinary AGE excretion

The specific types of AGEs present in dog and cat urine are reported in Table 2. The urine of dogs and cats that were fed RF contained significantly lower amounts of AGEs than urine of dogs and cats that were fed PF. Urinary excretions related to Ucr of CML, CEL and LAL in dogs fed with DPF versus RF were, respectively, 2.03, 2.14 and 3 times higher. Similar to dogs, the CML:Ucr and CEL:Ucr in cats fed with RF were 2.81 and 4.33 times lower than in cats fed with DPF, and also 2.72 and 6.33 lower than in the DWPF group. Urinary LAL excretion was significantly lower in the RF group compared with the DWPF group, but not compared with the DPF group. Interestingly, although dogs and cats in the RF groups had an estimated low intake of dietary AGEs, still significant excretion of CML, CEL and LAL via the urine was measured. This indicates endogenous formation of these AGEs in dogs and cats.

3.3 | Regression models of canine and feline CML:Ucr, CEL:Ucr and LAL:Ucr

Linear regression coefficients and SE of the significant explanatory variables on CML:Ucr, CEL:Ucr and LAL:Ucr of dogs and cats are reported in Table 3. In both dogs and cats, intake of heat-treated food was associated with an increased urinary excretion of specific AGEs compared with animals fed primarily with RF.

In the regression model, significant urinary excretion of CML, CEL and LAL was found in dogs fed with a RF diet. However, in cats fed with a raw diet, excretion of AGEs in urine was significant for CML and LAL, but not for CEL. In dogs, linear regression coefficients and SE of CML:Ucr, CEL:Ucr and LAL:Ucr showed that body weight and neuter status were significantly correlated with CML and CEL excretion, but not with LAL excretion. In cats, however, the body

weight was only significantly correlated with urinary CML excretion, whereas neuter status showed no significant correlation. Gender and age were not significant in the models. However, a negative but non-significant relationship between CML, CEL and LAL excretion in urine and age was observed in dogs and cats fed only RF. This indicates that endogenous formation and/or removal of AGEs might be slightly influenced by the age of the animal.

4 | DISCUSSION

4.1 | Relationship between type of pet food and urinary AGE excretion

In humans, consumption of heat-treated foods may lead to a significant AGE intake (Uribarri et al., 2010). In pet food production, thermal treatments are used to improve nutritive value, hygiene and shelf life, and a considerable amount of AGEs is formed during processing (van Rooijen et al., 2013). The presence of AGEs, such as LAL and CML, in commercial pet foods was assessed by van Rooijen et al. (2014). In this study, it was estimated that adult dogs and cats would, on average, consume CML 0.5 and 0.28 mg/metabolic body weight ($\text{kg}^{0.75}$)/day, when fed with a dry extruded diet. These values were reported to be even higher for canned food, since canned food contains higher CML concentration compared with extruded and pelleted foods (van Rooijen et al., 2014). However, a significant difference in urinary CML excretion between cats fed exclusively with DPF versus a combination of DWPF (=canned food) was not observed in the present study. This finding may be explained by the fact that most of the cats in the DWPF group were fed DWPF on a ~ 50:50% volume basis, which, when expressed on DM basis, shows that in this situation, more than 80% of energy intake comes from DPF. This may dilute the effect of higher AGE concentration in canned food. The higher CML:Ucr, CEL:Ucr and LAL:Ucr excretion when feeding PF may be explained by the higher AGE concentration available for intestinal absorption compared with RF. This finding indicates that dietary AGEs can indeed be absorbed by the gastrointestinal tract and excreted via the urine in dogs and cats, which is in line with findings in previous studies in other species (Friess et al., 2003; Hamelin et al., 2003). In the present study, urinary-free CML excretion showed a same trend as total urinary CML excretion in

TABLE 2 Urinary excretion of free carboxymethyllysine (CML), carboxyethyllysine (CEL) and lysinoalanine (LAL) ($\mu\text{g}; \mu\text{mol Ucr}$) in dogs and cats fed either raw, dry or dry and wet food

Parameters	Dog (group mean \pm SD)			Cat (group mean \pm SD)			<i>p</i> -overall
	RF (<i>n</i> = 29)	DPF (<i>n</i> = 28)	<i>p</i> -value	RF (<i>n</i> = 15)	DPF (<i>n</i> = 14)	DWPF (<i>n</i> = 25)	
CML	0.28 ± 0.140	0.57 ± 0.390	<.001	0.11 ± 0.069	0.31 ± 0.184^a	0.30 ± 0.118^a	<.001
CEL	0.21 ± 0.112	0.45 ± 0.214	<.001	0.06 ± 0.025	0.26 ± 0.133^a	0.38 ± 0.225^a	<.001
LAL	0.03 ± 0.069	0.09 ± 0.068	.003	0.02 ± 0.032	0.04 ± 0.025	0.06 ± 0.056^a	.005

Abbreviations: DPF: dry processed pet food group; DWPF: dry and wet processed pet food group; *n*: number; *p*-overall, ANOVA; RF: raw pet food group; SD: standard deviation.

^aMean is significantly different compared to RF group ($p < .05$).

TABLE 3 Estimate and SE of variables significantly ($p < .20$) contributing to urinary excretion of carboxymethyllysine (CML), carboxyethyllysine (CEL) and lysinoalanine (LAL) ($\mu\text{g}:\mu\text{mol Ucr}$) as estimated from spot urine samples of 57 dogs and 54 cats

Animal	Variable	CML ($R^2_{\text{adj}} = 0.35$)			CEL ($R^2_{\text{adj}} = 0.46$)			LAL ($R^2_{\text{adj}} = 0.13$)		
		Estimate	SEM	p	Estimate	SEM	p	Estimate	SEM	p
Dogs	Intercept	0.65	0.11	<.001	0.43	0.07	<.001	0.03	0.01	.01
	Food type									
	Dry	0.29	0.07	<.001	0.25	0.04	<.001	0.06	0.02	.003
	Raw	0			0			0		
	Metabolic body weight ($\text{kg}^{0.75}$)	-0.03	0.01	.003	-0.02	0.005	.002			NS
	Neutered status									
	Yes	-0.17	0.07	.02	-0.08	0.04	.043			NS
	No	0			0					
Cats	Intercept	0.31	0.08	<.001	0.06	0.04	.15	0.02	0.01	.09
	Food type									
	Dry	0.17	0.05	<.001	0.19	0.06	.003	0.02	0.02	.14
	Dry + Wet	0.15	0.04	<.001	0.31	0.06	<.005	0.05	0.01	.001
	Raw	0			0			0		
	Metabolic body weight ($\text{kg}^{0.75}$)	-0.06	0.02	.009			NS			NS

Abbreviations: Dry + Wet: dry and wet processed pet food group; Dry: dry processed pet food group; NS: not significant variable in regression model; p : p -value; R^2_{adj} : adjusted R square; Raw: raw pet food group; SEM: standard error of the means.

studies in adolescent human, infants and rats (Alamir et al., 2013; Delgado-Andrade, Tessier, Niquet-Leridon, Seiquer, & Navarro, 2012; Sebeková et al., 2008; Somoza et al., 2006), which all reported an increased excretion with higher dietary CML intake.

Interesting to note, CML, CEL and LAL were also present in the urine of dogs and cats fed with a RF. Considering the fact that these animals were assumed to have a negligible intake of AGEs via the diet, it seems likely that urinary-free CML, CEL and LAL in their urine were derived from endogenous formation. Ahmed (2005) has shown that in humans, endogenous glycation reactions also occur under physiological conditions. As a result, many AGEs that are present in the diet, such as CML and CEL, will also be formed in vivo (Ahmed, Argirov, Minhas, Cordeiro, & Thornalley, 2002). Therefore, the presence of significant urinary CML, CEL and LAL concentrations in dogs and cats fed with non-heated RF (except for CEL excretion in cats; Table 3) indicates existing endogenous AGE formation in dogs and cats.

4.2 | Metabolism and urinary excretion of dietary AGEs

In contrast to CML and CEL, the variation in urinary excretion of LAL was not explained by the dietary intake of LAL. This finding indicates a poor LAL absorption in the gastrointestinal tract, which might be explained by the fact that in cat food, LAL is present in the bound form (van Rooijen et al., 2016). Similar processing of dog and cat food suggests that LAL is likely also present in the bound form in dog food, and is thus also poorly absorbed in the dog gastrointestinal

tract. In a previous in vitro study (Hellwig, Matthes, Peto, Löbner, & Henle, 2014), in which gastrointestinal digestion of AGEs was simulated under in vitro conditions, LAL was mostly released into relatively long peptides (30–40 amino acids), which strongly impaired its availability for absorption. CML, however, appeared bound in small peptides (<1,000 Da), which were comparable in size to native amino acids. With regard to absorption kinetics, it was shown that dietary-free CML is absorbed by simple diffusion (Grunwald, Krause, Bruch, Henle, & Brandsch, 2006) and the absorption of CML and CEL in dipeptide is mainly carried out by peptide transporter 1 (PEPT1) (Hellwig et al., 2011). This is in correspondence with the results found by Uribarri et al. (2007), which showed that dietary CML strongly contributes to the concentrations of circulating CML in human subjects. Research in rats and humans showed that excretion of total urinary LAL is higher than the amount of urinary-free LAL (Finot, 2005). It was shown that approximated 6.2%–9.3% of LAL consumption in infants (Langhendries et al., 1992) and maximum of 5.6% in rats (Somoza et al., 2006) were excreted as total urinary LAL in the urine. In the present study, only free LAL was measured, which does not enable us to draw conclusions on the presence of bound LAL in the urine of dogs and cats.

A moderate correlation was found between CML and CEL excretion in urine, with a correlation coefficient of 0.77, $p < .001$ in dogs and a correlation coefficient of 0.73, $p < .001$ in cats. The correlation between CML and CEL was in line with a previous study in humans (Ahmed et al., 1997), in which CML and CEL deposition in human lens proteins showed significant resemblance. It was assumed that the found correlation was the result of the fact that CML and CEL share the common precursors.

Although a fair part of total variation is explained by the regression models for CML, CEL and LAL (35%, 46% and 13% in dogs and 38%, 39% and 15% in cats, respectively), there is still substantial residual variation. This may be explained by several factors. First, urinary excretion of total AGEs might be more representative of dietary AGE intake than free form excretion. It might also be that the bound form of AGEs is excreted in larger proportion than the free form, as is seen in excretion of LAL in rats (Finot, 2005). Second, digestion and absorption of dietary AGEs in dogs and cats may be less efficient and/or AGEs in feed may contain a higher amount of protein-bound forms, which are poorly available for absorption (Hellwig et al., 2014; Somoza et al., 2006). Third, dietary AGEs might significantly accumulate in canine and feline body tissue as reported in several rat studies. Intravenous injection of CML and CEL in rats showed temporary deposition in the liver (Bergmann et al., 2001). Ingestion of labelled-LAL in rat demonstrated a long retention time in the kidney, since it remained in the kidney until 9 days after dosing but not found in other organs such as spleen, lung, liver and gut (Struthers, Brielmaier, Raymond, Dahlgren, & Hopkins, 1980). Finally, fourth, other routes of excretion may also play a role in elimination of AGEs in dogs and cats, for example faecal excretion, gut microbiota degradation or biodegradation in the body. A study of Delgado-Andrade et al. (2012) showed that dietary CML is excreted mainly by faecal excretion, a route which was also shown in the excretion of LAL (Struthers et al., 1980).

4.3 | Possible animal-related factors associated with urinary AGE excretion

Animal-related factors were tested in this study by multivariable linear regression analysis (Table 3). The urinary excretion of CML and CEL in both dogs and cats showed a reduction with increasing metabolic body weight ($\text{kg}^{0.75}$), which may be explained by the fact that in this study only spot urine was used. Differences in urine production were corrected by expressing excretion as a ratio with creatinine. Creatinine infers to lean body mass or metabolic body weight (Forbes & Bruining, 1976). When a dog or cat has an increased body condition, the ratio of AGE excretion to creatinine is expected to be lower due to relatively more fat mass, versus lean body mass. Unfortunately, in this present study there was no information available of the body condition of the animals, and therefore, no assertion of lean body mass was possible.

Neuter status was found to be associated with urinary-free CML and CEL excretion in dogs; the respective levels were lower in sterile dogs than in intact dogs. This finding is in correspondence with results found in the previous study (Dammann, Sell, Begall, Strauch, & Monnier, 2012), in which non-breeder rats accumulated less CML in their skin than reproductively active rats. However, there was no clear explanation reported for this difference. On the other hand, in humans a reduction in testosterone in non-diabetic men was shown to be independently associated with elevation of serum AGEs, as a result of possible insulin resistance (Tahara, Imaizumi, Takeuchi, &

Yamagishi, 2010). This is not in agreement with the results found in this study. However, as testosterone levels in humans merely decrease due to a number of (subclinical) disease processes, while in dogs and cats, a decrease in the sex hormones is primarily due to castration, it might be that there are other confounding factors explaining the found correlation in humans.

Gender was not associated with differences in canine and feline urinary AGE excretion. However, a previous study in Ansell's mold-rats found higher CML and CEL accumulation in female skin collagen than in male (Dammann et al., 2012). As a large part of the population in our study was neutered (28 out of 57 dogs and 38 out of 54 cats), this might have influenced a possible relationship between sex hormones and AGEs, as seen in other species.

An association between age and urinary AGEs was not observed in the current study, which is in contrast with a study of Friess et al. (2003), in which a lower urinary excretion of free CML in younger human subjects was found. However, a negative but non-significant relationship between CML, CEL and LAL excretion in urine and age was observed in dogs and cats fed only RF. In another study, CEL deposition in human lens protein was also increased with age (Ahmed et al., 1997). The differences in significant results might be explained by a difference in longevity between companion animals and humans. Other than that, urinary excretion might not significantly reflect potential retention of AGEs in body tissue.

5 | CONCLUSIONS

The current data clearly show that type of pet food is related to urinary excretion of AGEs, which indicate that dietary AGEs can be absorbed in the canine and feline gastrointestinal tract and eliminated via the urinary system. Animals that were primarily fed with RF, with a low intake of dietary AGEs, showed significantly lower concentrations of free form AGEs in the urine compared to animals fed with PF. However, free form CML, CEL and LAL were still present in the urine of animals fed with RF, which may be the result of endogenous formation of AGEs. Further studies are necessary to elucidate urinary excretion of total CML, CEL and LAL, and the relative importance of endogenous versus exogenous AGEs as well as clinical relevance of AGEs on pet health. Assessment of AGEs in serum should be performed to confirm bioavailability of dietary AGEs and endogenous formation in experimental circumstances where the dietary intake can be fully controlled.

ACKNOWLEDGEMENTS

This study was financially supported by a scholarship from the faculty of Veterinary Medicine, Kasetsart University, Thailand.


CONFLICT OF INTEREST

The authors declared that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

ANIMAL WELFARE STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guideline page, have been adhered to and the appropriate ethical approval has been received. The authors confirm that they have followed EU standards for the protection of animals used for scientific purposes.

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How to cite this article: Palaseweenun P, Hagen-Plantinga EA, Schonewille JT, et al. Urinary excretion of advanced glycation end products in dogs and cats. *J Anim Physiol Anim Nutr*. 2020;00:1–8. <https://doi.org/10.1111/jpn.13347>