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Urinary liver-type fatty acid-binding protein in clinically healthy elderly cats: Evaluation of its potential to detect IRIS stage 1 chronic kidney disease and borderline proteinuria

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Abstract

Background: Urinary liver-type fatty acid-binding protein (uL-FABP) is a promising biomarker to detect early chronic kidney disease (CKD) in cats. Few healthy cats show increased uL-FABP for unknown reasons.

Objectives: The objective of this study was to evaluate uL-FABP in a large healthy elderly cat population comparing cats with and without International Renal Interest Society (IRIS) stage 1 CKD and with and without borderline proteinuria.

Methods: This was a cross-sectional study. One hundred ninety-six clinically healthy client-owned cats of ≥7 years old were subdivided based on two criteria: (1) having either IRIS stage 1 CKD or no evidence of CKD and (2) having borderline proteinuria or no proteinuria. Urinary L-FABP was measured using a validated commercially available feline L-FABP ELISA.

Results: Overall, uL-FABP was detectable in 6/196 (3%) healthy elderly cats. For the first subdivision, nine (5%) cats had IRIS stage 1 CKD, 184 cats had no evidence CKD and three cats were excluded. All cats with IRIS stage 1 CKD had uL-FABP concentrations below the detection limit, whereas 6/184 (3%) cats without IRIS stage 1 CKD had detectable uL-FABP concentrations (median 1.79 ng/ml, range 0.79–3.66 ng/ml). For the second subdivision, 47 (24%) cats had borderline proteinuria, 147 cats had no proteinuria and two cats were excluded. One of the borderline proteinuric cats had a detectable uL-FABP concentration, whereas the other five cats with detectable uL-FABP concentrations were non-proteinuric.

Conclusion: With the current assay, the screening potential of uL-FABP as an early biomarker for feline CKD is limited as uL-FABP was rarely detected in clinically healthy elderly cats independently of the presence of either IRIS stage 1 CKD or borderline proteinuria.

KEYWORDS
renal biomarker, aged cats, screening, feline CKD, L-FABP

1 | INTRODUCTION

Chronic kidney disease (CKD) affects over one-third of the elderly cat population (Jepson et al., 2009; Marino et al., 2014). For better prognosis, early detection of CKD would be ideal to allow prompt therapeutic intervention before the disease progresses further towards azotemic stages (S. J. Ross et al., 2006). International Renal Interest Society (IRIS) stage 1 CKD is defined as early CKD in the absence of azotaemia and clinical signs but in the presence of a renal abnormality such as poorly concentrated urine, persistent proteinuria, abnormal renal imaging findings or persistently increased blood symmetric dimethylarginine (SDMA) concentration (International Renal Interest Society, 2021; Sargent et al., 2021). Unfortunately, the changes in these variables are not specific for CKD in cats (Paepe & Daminet, 2013). Glomerular filtration rate (GFR) estimation, the gold standard for detection of early CKD, is cumbersome for practitioners (Finch, 2014). Readily available in clinics, an increased serum creatinine (sCr) and/or SDMA concentration reflect a decreased GFR in cats with CKD, but these biomarkers have limitations for the detection of IRIS stage 1 CKD (Hokamp & Nabity, 2016). Additionally, at the start of renal damage, GFR is not yet declined in cats that will develop CKD (Finch, 2014). Therefore, using surrogate biomarkers of GFR may not be the most ideal option for early detection of feline CKD. Screening of at-risk populations, preferably healthy aged cats, is an important tool for the early diagnosis of CKD. Tubulointerstitial inflammation is the most common pathological change observed in cats with CKD (Chakrabarti et al., 2013). Hence, renal biomarkers reflecting tubular damage and/or dysfunction together with screening of healthy aged cats may be beneficial for the early detection of CKD.

Liver-type fatty acid-binding protein (L-FABP), physiologically synthesized by the liver and other organs, can pass the glomerular filtration barrier due to its low molecular weight and is then reabsorbed by proximal tubular cells (Xu et al., 2015). L-FABP plays a role in free fatty acid metabolism and is expressed by proximal tubular cells and excreted into urine when tubular cells are injured (Xu et al., 2015). Urinary L-FABP (uL-FABP) is promising as an early biomarker of tubular damage in people with acute kidney injury (AKI) as well as with CKD (Plesiński et al., 2019; Susantitaphong et al., 2013). According to recent findings, uL-FABP concentration increases in cats both with AKI and CKD (Katayama et al., 2019; Kongtasai et al., 2021). Furthermore, it has been shown that 7% of healthy adult cats have detectable uL-FABP concentrations (Kongtasai et al., 2021). The reason for this excretion is still unknown. Additionally, a significant correlation between uL-FABP and urinary protein: creatinine ratio (UPC) was found, both in cats with CKD and healthy cats (Kongtasai et al., 2021).

It is known from previous studies that borderline proteinuria is present in a significant number of healthy cats (Ghys et al., 2015; Williams & Archer, 2016), but its clinical importance and pathophysiology are not well understood (Jepson et al., 2009; Paepe, Verjans, et al., 2013). Tubular biomarkers such as uL-FABP might help to elucidate the relevance of borderline proteinuria in healthy cats.

Therefore, the major aim of this study was to describe uL-FABP in a large population of healthy elderly cats. Additional aims were to compare uL-FABP between cats with and without IRIS stage 1 CKD as well as between cats with borderline proteinuria and non-proteinuric cats. We hypothesized that uL-FABP might be useful for the early detection of early CKD in healthy elderly cats and would be increased in cats with either IRIS stage 1 CKD or borderline proteinuria.

2 | MATERIALS AND METHODS

2.1 | Animals

Frozen ($-80°$C) urine samples of healthy elderly ($\geq$7 years old) cats participating in another study were used. For the present study, urine taken at a single time point at the first visit was used from each cat enrolled in the original study.

To be included in the original study, cats needed to be clinically healthy based on the absence of significant abnormalities on history (e.g., no changes in general behaviour, stable body weight and absence of clinical signs), physical examination, complete blood count, serum biochemistry and urinalysis; and not receiving any medication within 2 months before enrolment, except for preventive medication. Based on history, physical, blood and urine examinations, cats were excluded from the original study if they had CKD IRIS stage 2 or higher (urine specific gravity [USG] persistently <1.035 in combination with sCr > 1.6 mg/dl) (International Renal Interest Society, 2021). Other exclusion criteria for the original study were urinary tract infection (based on a positive bacterial urine culture), diagnosis of a clinically relevant cardiovascular, inflammatory or systemic disease (e.g., hyperthyroidism) and being positive for feline immunodeficiency virus (FIV) antibodies or feline leukemia virus (FeLV) antigen. The Faculty of Veterinary Medicine, Ghent University, Belgium local ethical committee (EC2018-54) and the Belgian Federal Agency for the Safety of the Food Chain deontological committee approved the study protocol. Participating owners provided written informed consent.

For the present study, the group of included cats was subdivided as having either IRIS stage 1 CKD or no evidence of CKD. Initially, cats were diagnosed as having CKD if they had one of the following criteria: persistent poorly diluted urine (USG <1.035) without an identifiable non-renal cause, persistent renal proteinuria (UPC >0.4) or persistent increased SDMA (>14 µg/dl, RI of IDEXX Laboratories) (International Renal Interest Society, 2021). Diagnostic imaging was not available for IRIS staging in these cats because it was not part of the investigations of the original study. To confirm the persistence of USG <1.035, UPC >0.4 and SDMA >14 µg/dl, the values of these variables from the next visit (1–2 months after the inclusion for UPC and SDMA, and 1–6 months after the inclusion for USG) were assessed in cats with one of these abnormalities at the inclusion. Subsequently, cats with CKD with the aforementioned criteria were staged as having IRIS stage 1 CKD. Cats with CKD with persistent SDMA >17 µg/dl that were staged as having IRIS stage 2 or higher were excluded from this classification (International Renal Interest Society, 2021).

Also, the group of included cats was subdivided after eliminating pre- or post-renal cause into cats with borderline proteinuria

(UPC 0.2–0.4) or no proteinuria (UPC <0.2). Cats with overt proteinuria (UPC >0.4) were excluded from subclassification.

2.2 | Procedures

Complete and standardized history and physical examination including systolic blood pressure measurement using Doppler ultrasound technique following the American College of Veterinary Internal Medicine consensus were performed in all cats (Brown et al., 2007). Fasting blood samples and urine samples were collected by venipuncture and cystocentesis, respectively.

Laboratory parameters tested in all cats consisted of complete blood count; serum biochemistry profile including liver enzymes, total bilirubin, Cr, SDMA, electrolytes, total calcium, total thyroxine; FIV antibodies and FeLV antigen testing by SNAP FIV/FeLV Combo Test (IDEXX Laboratories); and complete urinalysis including dipstick, sediment analysis, USG, UPC and bacterial culture. Residual urine was centrifuged, and supernatant was immediately frozen at −80°C for 1–15 months until batch analysis of uL-FABP.

2.3 | Analyses

All laboratory variables except for uL-FABP concentration were measured at IDEXX Laboratories. Concentrations of uL-FABP were measured in batch with a commercial feline L-FABP ELISA (CMIC Holding, Tokyo, Japan) using an anti-human L-FABP antibody previously in-house validated for urine in cats by our group (Kongtasai et al., 2021). The assay was performed using procedures described in a previous study (Kongtasai et al., 2021). In brief, this ELISA presented acceptable intra- and inter-assay coefficient of variations (CVs) and adequate linearity without significant inaccuracy in serial dilution for uL-FABP (Kongtasai et al., 2021). In the current study, by assessing standard diluent in the L-FABP ELISA kit as blank ($n = 12$), the lower limits of detection (LOD) and of quantification (LOQ) were established based on the mean blank value + 4 × S.D. (0.10 ng/ml) and mean blank value + 10 × S.D. (1.47 ng/ml), respectively (Armbruster & Pry, 2008). Hence, the uL-FABP concentrations that were >LOD but <LOQ could be assigned the arbitrary value of 0.79 ng/ml. Mostly, 50 µl of centrifuged urine samples were applied undiluted to the ELISA plate. A 1:2 dilution was performed if measurements were higher than the highest calibration point of the standard curve.

2.4 | Statistical analysis

As most variables were not normally distributed according to the Shapiro–Wilks test, comparisons between the different groups based on IRIS stage and proteinuria level were based on the Wilcoxon rank sum test at a significance level of 5% using R Version 3.6.3 (2020, The R Foundation for Statistical Computing).

3 | RESULTS

Two hundred and five cats were included in the original study. For the present study, nine cats were excluded because of insufficient residual urine for uL-FABP measurement. The age of the included cats ranged from 7 to 18 years (median 9 years). Included cats were 69 male neutered, two male intact, 115 female spayed and 10 female intact. Represented breeds included 139 European shorthair or longhair cats and 57 purebred cats. The most commonly represented breeds were British shorthair or longhair cats ($n = 28$), Ragdolls ($n = 6$) and Maine Coon ($n = 4$). A diagram illustrating case selection and study overview is presented in Figure 1.

Ten cats (5%) were diagnosed with CKD of which nine were subsequently staged as having IRIS stage 1 CKD and one as IRIS stage 2 CKD (based on persistent SDMA > 17 µg/dl), and 184 cats did not have evidence of CKD on blood and urine examinations. For two cats, their CKD classification was unclear: in one cat with USG <1.035, follow-up data were not available and persistence of the poorly concentrated urine could not be confirmed, in another cat with persistent proteinuria (UPC >0.4), iatrogenic microscopic haematuria hampered the elimination of a post-renal cause for the increased UPC. These three cats with either IRIS stage 2 or unclear CKD classification were excluded for the comparison between cats with IRIS stage 1 CKD and no CKD. Two cats were excluded for the comparison between cats with borderline and no proteinuria, namely one cat with persistent renal proteinuria and the other with the persistent UPC >0.4 and iatrogenic microscopic haematuria in which a post-renal cause for the increased UPC could not be eliminated.

Of the nine cats with IRIS stage 1 CKD, seven cats had persistently inappropriately concentrated urine (USG <1.035) without an identifiable non-renal cause, one cat had persistently increased SDMA (15–17 µg/dl), and one cat had persistent renal proteinuria. For the proteinuric substaging, 47 cats had borderline proteinuria, and 147 cats were non-proteinuric.

From the 196 cats, only six (3%) had detectable uL-FABP concentrations. These six cats did not have evidence of IRIS stage 1 or stage 2 CKD based on blood and urine examinations, whereas only one cat was borderline proteinuric, the other five cats being non-proteinuric. Selected routine renal variables of the study population are presented as descriptive statistics in Table 1. For the six cats with detectable L-FABP, the median uL-FABP concentration was 1.79 ng/ml (range 0.79–3.66 ng/ml), and the median urinary L-FABP: creatinine ratio (uL-FABP/Cr) was 0.37 µg/g (range 0.24–1.06 µg/g). The concentrations of the six cats with detectable uL-FABP are presented in Figures 2 and 3 according to the subdivision based on the presence of IRIS stage 1 CKD or borderline proteinuria, respectively.

The 3-month follow-up urine samples from the six cats with detectable uL-FABP concentrations were analyzed for uL-FABP concentrations. The maximum duration of follow-up was 12 months for three cats (cats A, B and C), 18 months for two cats (cats D and E) and 24 months for one cat (cat F). No cats developed azotemic CKD during the follow-up period. Cat A had hyperthyroidism at 12 months after
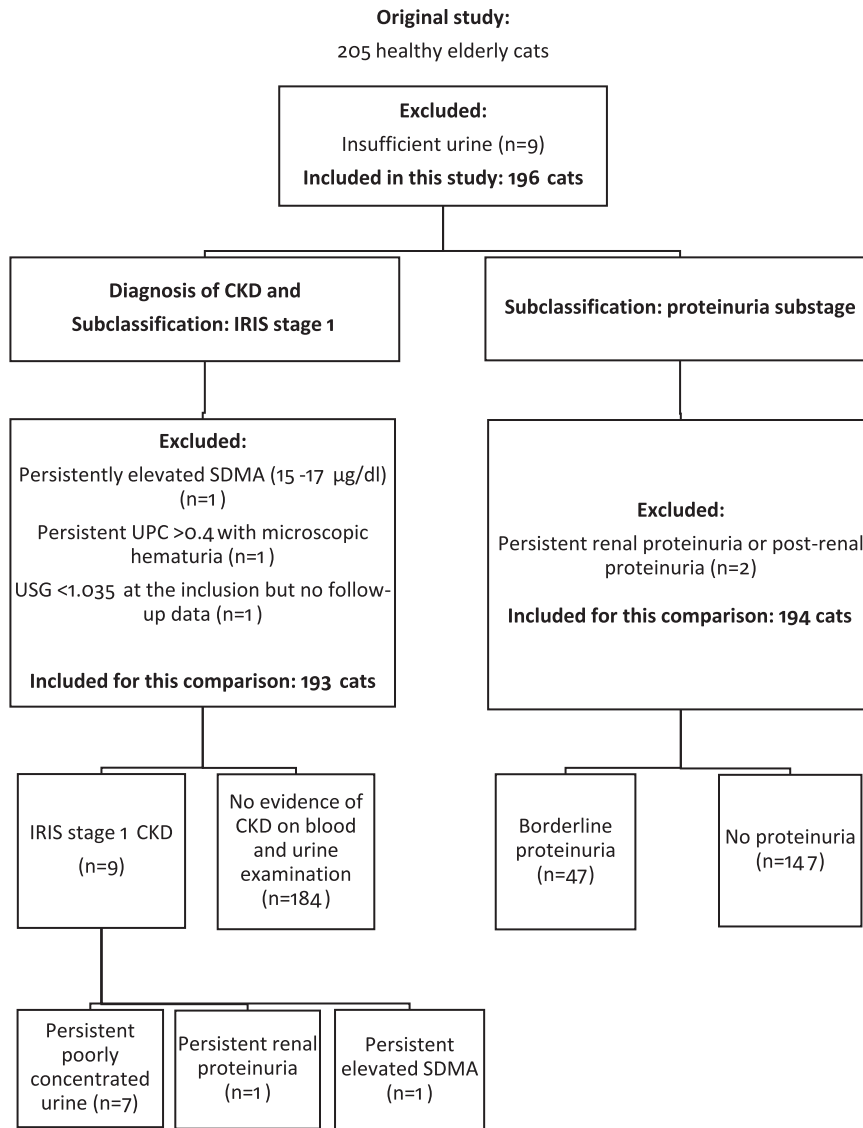
FIGURE 1 Flow chart on enrolled cats and the division in groups of this cohort. Abbreviations: IRIS: International Renal Interest Society; SDMA, symmetric dimethylarginine; UPC, urinary protein: creatinine ratio

baseline. Cat D had liver disease at 18 months after baseline and died 3 weeks later. Cat E had lymphoma at 18 months after baseline and died 4 months later. Cats B, C and F remained healthy. All six cats had uL-FABP concentration <LOD in all follow-up urine samples.

4 | DISCUSSION

The tubular marker urinary L-FABP was assessed in a large population of clinically healthy elderly cats including subpopulations of cats with IRIS stage 1 CKD and cats with borderline proteinuria. The main findings of this study are threefold: (1) few (3%) healthy elderly cats have detectable uL-FABP concentrations, (2) all cats matching at least one criterion of IRIS stage 1 CKD have uL-FABP <LOD and (3) borderline proteinuric cats rarely (2%) have detectable uL-FABP.

Because screening for CKD is strongly recommended in aged cats and because tubular damage might indicate early renal damage, uL-FABP concentrations were evaluated in 196 clinically healthy elderly

cats. Only six (3%) of them had detectable L-FABP concentrations which is comparable with a recent smaller cohort study on 44 healthy cats of which only three cats (7%) had uL-FABP concentrations >LOD (Kongtasai et al., 2021). These combined findings indicate that most healthy cats, independent of their age, produce uL-FABP <LOD as assessed by the only currently available commercial and validated immuno-assay. This corroborates findings in adult and elderly people with a normal kidney function, for which either undetectable or low levels of uL-FABP have been reported (Kamijo, Kimura, et al., 2004; Viswanathan et al., 2015). Moreover, the detectable uL-FABP concentrations in the elderly cats in the present study were even lower than those reported in healthy cats of different ages in a previous study (median 11.05 ng/ml; range 3.32–23.99 ng/ml) (Katayama et al., 2020).

Upregulation of uL-FABP expression reflects stress such as protein overload, ischemic injury or oxidative stress that causes tubulointerstitial damage in both cats and humans (Kamijo, Sugaya, et al., 2004; Katayama et al., 2020; Kongtasai et al., 2021). Increased uL-FABP

TABLE 1 Comparison of selected renal variables between cats with International Renal Interest Society (IRIS) stage 1 chronic kidney disease (CKD) versus cats with no evidence of CKD, and between cats with borderline proteinuria and cats without proteinuria

| Variables | All cats (n = 196) | IRIS staging | | | Proteinuria substaging | | |
		IRIS stage 1 CKD (n = 9)	No evidence of CKD (n = 184)	p	Borderline proteinuric (n = 47)	Non-proteinuric (n = 147)	p
Age (years)	9 [8, 11]	10 [8, 14.5]	9 [8, 11]	0.35	10 [9, 12]	9 [8, 11]	0.02
Body weight (kg)	4.45 [3.60, 5.25]	4.75 [3.85, 5.43]	4.40 [3.60, 5.23]	0.49	4.48 [3.43, 5.05]	4.40 [3.64, 5.40]	0.69
SBP (mmHg)	140 [125, 153]	150 [125, 162]	140 [125, 155]	0.24	148 [129, 160]	140 [120, 150]	0.05
sCr (mg/dl)	1.24 [1.14, 1.46]	1.23 [1.00, 1.50]	1.14 [0.70, 1.27]	0.75	1.20 [1.02, 1.33]	1.32 [1.16, 1.50]	<0.001
sSDMA (μg/dl)	10 [9, 13]	10.0 [11.0, 15.5]	10.0 [9.0, 12.0]	0.21	11.0 [9.0, 13.0]	10.0 [9.0, 12.0]	0.37
sTT4 (μg/dl)	24.5 [21.9, 29.6]	27.0 [20.6, 29.6]	24.5 [21.9, 29.0]	0.68	24.5 [21.9, 29.6]	24.5 [21.9, 28.3]	0.81
USG	>1.050 [>1.050, >1.050]	1.024 [1.02, 1.030]	>1.050 [1.049, >1.050]	<0.001	>1.050 [1.042, >1.050]	>1.050 [1.048, >1.050]	0.12
UPC	0.15 [0.12, 0.20]	0.18 [0.14, 0.28]	0.15 [0.12, 0.20]	0.20	0.25 [0.21, 0.30]	0.14 [0.11, 0.17]	<0.001

Note: Data are presented as median [25–75 percentiles].

Abbreviations: SBP, systolic blood pressure; sCr, serum creatinine; sSDMA, serum symmetric dimethylarginine; sTT4, serum total thyroxine; UPC, urinary protein: creatinine ratio; USG, urine specific gravity.
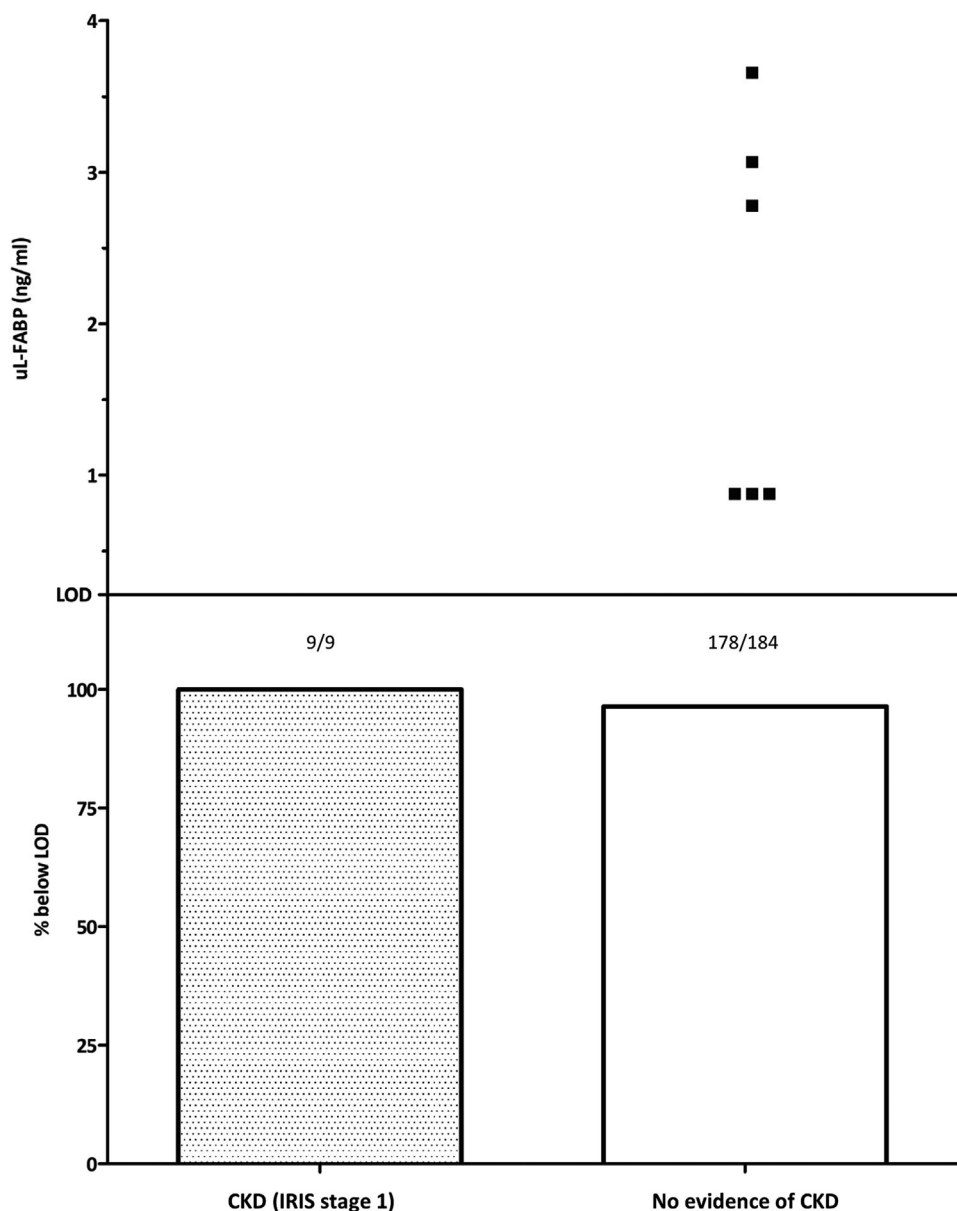
FIGURE 2 Urinary L-FABP concentration in relationship to the presence or absence of CKD. The bottom panel gives the percentage of cats that had uL-FABP concentrations below LOD. The top panel represents the uL-FABP concentrations that are above LOD for cats in both groups. Abbreviations: IRIS: International Renal Society; LOD, lower detection limit; uL-FABP, urinary liver-type fatty acid-binding protein

concentrations have been reported in cats with experimentally induced AKI, naturally occurring CKD and hyperthyroidism (Katayama et al., 2019; Kongtasai et al., 2021). In the present study, the few elderly cats with detectable uL-FABP concentrations seemed clinically healthy as azotemic CKD, hyperthyroidism and other systemic diseases were ruled out by blood and urine examinations. However, the detectable uL-FABP concentrations in these cats might be explained by early renal damage or by other unidentified diseases as diagnostic imaging was not performed in the present study. It is possible that tubular injury induces renal L-FABP expression and causes subsequent uL-FABP excretion. Nonetheless, after the standardization of uL-FABP concentrations to urinary Cr concentrations, the normalized uL-FABP/Cr ratios in most cats with detectable uL-FABP (median 0.37 μg/g) in the present study were lower than the cut-off (0.97 μg/g) to distinguish cats with CKD from healthy cats of the previous study which validated the same feline L-FABP ELISA for urine (Kongtasai et al., 2021). Furthermore, all cats with detectable uL-FABP in the present study had concentrated urine (USG $\geq$1.035) which implies that renal disease may not be the potential cause of detectable uL-FABP in these cats. Also, a low but detectable uL-FABP concentration in healthy elderly cats with concentrated urine may not have clinical significance. Indeed, in humans, increased uL-FABP expression predicts progressive renal disease in patients with either CKD or at risk for AKI (Kamijo et al., 2005; Manabe et al., 2012; Xu et al., 2015). In cats, the predictive value of uL-FABP for the development of CKD was recently evaluated in hyperthyroid cats but remains uncertain (Kongtasai et al., 2021).
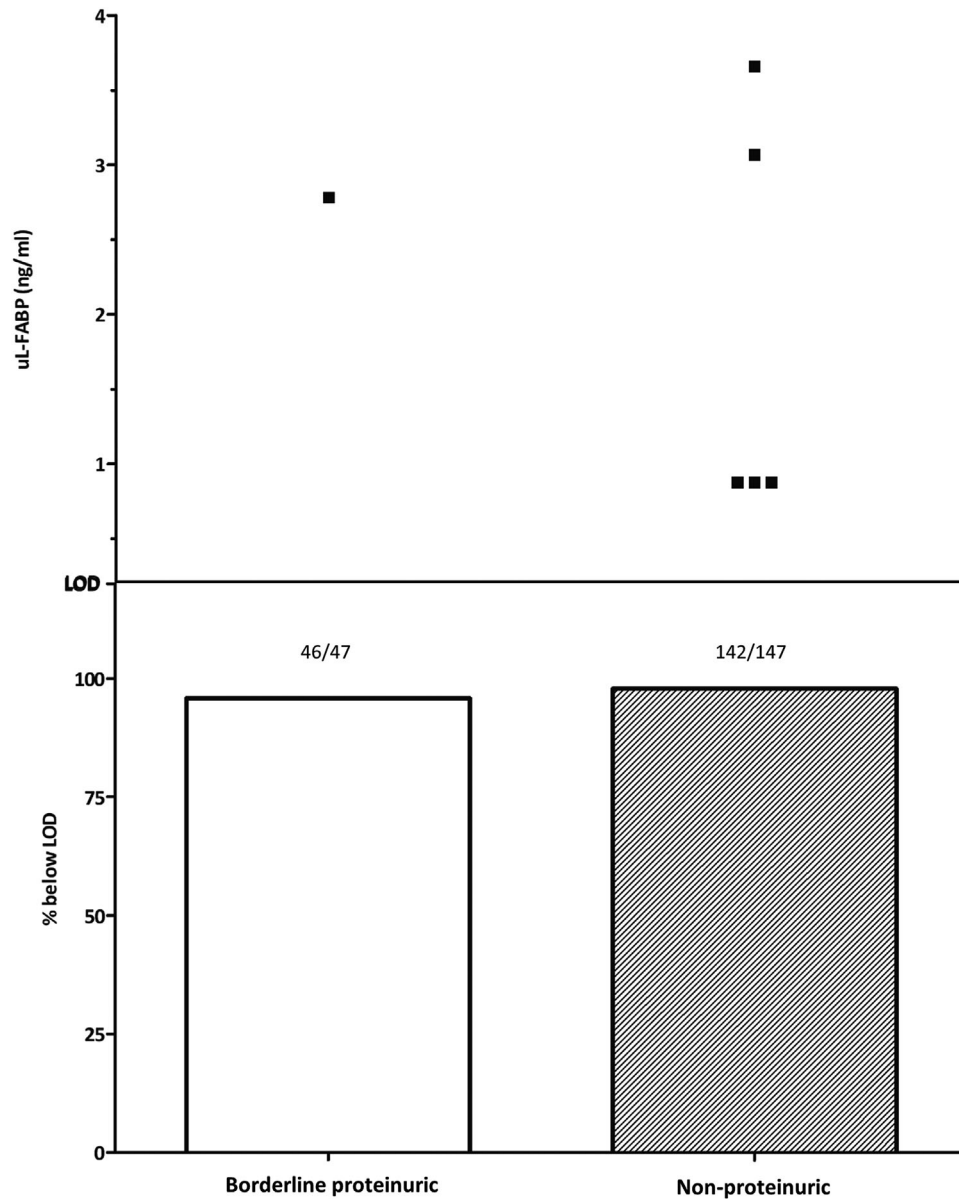
FIGURE 3 Urinary L-FABP concentration in relationship to the proteinuria substage. The bottom panel gives the percentage that had uL-FABP concentrations below LOD. The top panel represents the uL-FABP concentrations that are above LOD for cats in both groups. Abbreviations: LOD, lower detection limit; uL-FABP, urinary liver-type fatty acid-binding protein

Our findings in this population of healthy elderly cats question the potential value of uL-FABP as biomarker for early detection of CKD.

Studies that investigated tubular biomarkers in cats with IRIS stage 1 CKD remain scarce (Granick et al., 2020; Maeda et al., 2015). Urinary transferrin and albumin concentrations are higher in cats with IRIS stage 1 CKD than in healthy cats (Maeda et al., 2015). Urinary F2-isoprostanes, biomarkers of oxidative stress, are also significantly increased in cats with IRIS stage 1 CKD compared to healthy cats. These combined recent data imply that renal pathological events exist in IRIS stage 1 feline CKD (Granick et al., 2020). In the present study, we did not find a difference in uL-FABP concentrations between cats with and without criteria of IRIS stage 1 CKD. In contrast, all cats with IRIS stage 1 CKD even had uL-FABP concentrations <LOD. Hence, our

data strongly suggest that uL-FABP does not seem to be a reliable renal biomarker to detect IRIS stage 1 CKD in elderly cats. Also, in contrast to our early CKD biomarker hypothesis, all six healthy cats with detectable uL-FABP concentrations did not show evidence of IRIS stage 1 CKD on blood and urine examinations. In the present study, seven of the nine cats with IRIS stage 1 CKD had persistently poorly concentrated urine. Evaluating the persistence of poorly concentrated urine is important as healthy cats with initially low USG can have well-concentrated urine in a later evaluation (Ghys et al., 2015). Substantial tubular dysfunction is considered the cause of failing to produce concentrated urine (USG ≥1.035) in well-hydrated cats in the absence of non-renal causes for decreased USG such as diuretics and liver disease. On the other hand, some azotemic cats can also retain their urine

concentrating ability as reported both in an experimental model and naturally occurring CKD (DiBartola et al., 1987, L. A. Ross & Finco, 1981), and USG is not associated with the development of azotaemia in a prospective longitudinal study (Jepson et al., 2009).

In a retrospective longitudinal study, a single time point SDMA >14 µg/dl in non-azotemic cats corresponded to a >30% decrease in median GFR of healthy cats, demonstrating SDMA's potential to detect early CKD (Hall et al., 2014). Furthermore, a persistently increased SDMA concentration was added in 2019 as one of the criteria for the diagnosis of IRIS stage 1 CKD in cats with sCr <1.6 mg/dl (International Renal Interest Society, 2021). Because large prospective studies of SDMA in cats and studies on the specificity of feline SDMA are currently lacking, the IRIS guidelines stress the need for SDMA to be persistently increased in order to diagnose IRIS stage 1 CKD in cats (International Renal Interest Society, 2021). Unfortunately, the number of cats with persistently increased SDMA in the present study is very limited ($n = 2$) and both cats did not have detectable L-FABP in urine, thus limiting the interpretation of the relationship between persistently increased SDMA and detection of uL-FABP in cats.

Most cats with borderline proteinuria (98%) and also most non-proteinuric cats (97%) had undetectable uL-FABP concentration. In previous studies, UPC was moderately correlated with uL-FABP/Cr in cats with azotemic CKD, hyperthyroid cats and healthy cats (Kamijo, Sugaya, et al., 2004; Kongtasai et al., 2021). Theoretically, proteinuria increases the excretion of L-FABP in urine because proteinuria can damage proximal tubular cells via overloading of free-fatty acids that are bound to filtered albumin (Kamijo, Sugaya, et al., 2004) and because proteinuria overloads the protein resorptive ability, resulting in reduced L-FABP reabsorption in proximal tubules (Jepson et al., 2010). Although borderline proteinuria is associated with shorter survival in cats with CKD (Syme et al., 2006), the clinical importance and pathophysiology of borderline proteinuria in healthy cats is yet to be determined (Paepe, Bavegems, et al., 2013). Therefore, based on our findings, borderline proteinuria might either not be tubular in origin and/or not cause enough stress on proximal tubules by protein overload to the point that it induces tubular injury and/or increases uL-FABP excretion in healthy cats.

The main limitation of the current study is the low number (3%) within the large cohort of clinically healthy elderly cats with detectable uL-FABP. Because tubular injury, which contributes to the development of CKD (Chakrabarti et al., 2013), may be common in aged cats, we also expected a higher number of non-azotemic cats with a detectable uL-FABP concentration in our study population. Although only 5% of our cohort showed evidence of IRIS stage 1 CKD, which was different from the prevalence of IRIS stage 1 CKD in non-azotemic cats (22%) in a previous study (Marino et al., 2014), we still could indicate that the detectable uL-FABP in this cohort was not associated with IRIS stage 1 CKD. Nevertheless, the second limitation of our study is the small number of cats with IRIS stage 1 CKD. Also, diagnostic imaging for detection of renal abnormalities, one of the criteria to diagnose IRIS stage 1 CKD in cats, is lacking in this study. Consequently, we cannot unequivocally conclude that the cats that we classified as 'no evidence of CKD' did not have IRIS stage 1 CKD. The median age of

healthy elderly cats in the current study was about 9 years. Older cats have a high risk for renal tubulointerstitial lesions (Chakrabarti et al., 2013; Jepson et al., 2009). It is possible that if more older cats had have been included in this study, we might have found more cats that had detectable uL-FABP concentration. Hence, this might be another limitation of our study. However, aging seems not associated with higher uL-FABP concentrations in humans and dogs (Kamijo, Kimura, et al., 2004; Takashima et al., 2021; Viswanathan et al., 2015). In a previous study, we suggested that liver disease may be one of the causes for increased uL-FABP concentration in cats as serum L-FABP was reported to be increased in human patients with liver diseases (Kamijo, Sugaya, et al., 2004; Kongtasai et al., 2021). Although this concern has not been proven yet, we attempted to minimize this limitation by measuring total bilirubin and liver enzymes including alanine aminotransferase, alkaline phosphatase and gamma glutamyl transferase. Nonetheless, without diagnostic imaging, we cannot completely rule out liver disease in the included cats. The final limitation is the effect of long-term storage of feline L-FABP in frozen urine might have impacted the uL-FABP concentrations in the current study. However, the oldest urine samples were frozen for 16 months before uL-FABP was evaluated in this study. Human uL-FABP is reported to be stable at $-70°C$ for at least 18 months (Liu et al., 2016), whereas the stability of uL-FABP in feline frozen urine remains unknown.

5 | CONCLUSION

In conclusion, we demonstrated that L-FABP is undetectable in the urine of the large majority of clinically healthy elderly cats. Still, a limited number of these healthy cats show detectable uL-FABP concentrations, not related to either the presence of IRIS stage 1 CKD on blood and urine evaluation or borderline proteinuria. Our combined findings question that uL-FABP is a promising biomarker to detect early CKD in healthy aged cats.

AUTHOR CONTRIBUTIONS
Conceptualization, data curation, investigation, methodology, project administration, writing—original draft, and writing—review and editing: Thirawut Kongtasai. Dominique Paepe: *Conceptualization, methodology, and writing—review and editing*. Femke Mortier: *Conceptualization, data curation, investigation, methodology, and writing—review and editing*. Sofie Marynissen: *Methodology and writing—review and editing*. Evelyne Meyer: *Conceptualization, validation, and writing—review and editing*. Luc Duchateau: *Formal analysis and writing—review and editing. Conceptualization, methodology, supervision, and writing—review and editing*: Sylvie Daminet.

CONFLICT OF INTEREST
Authors declare no conflict of interest

ETHICS STATEMENT
This study was approved by the local ethical committee of the Faculty of Veterinary Medicine, Ghent University, Belgium and the deontological committee of the Belgian Federal Agency for the Safety of the Food Chain (EC 2018/54).

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author upon reasonable request.

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PEER REVIEW
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