

## ORIGINAL ARTICLE

# Tear glucose, creatinine, and urea nitrogen concentrations in cats with normal or increased plasma concentrations

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## Abstract

**Objective:** The aim of the study was to determine the lacrimal fluid (LF) contents of glucose, urea nitrogen, and creatinine in cats.

**Animal studied:** A total of 96 cats were included in the study.

**Procedure:** Venous blood and LF samples were collected. For LF sampling, three small polyurethane sponges were placed in the ventral fornix of both eyes. Both LF and plasma concentrations of glucose, urea nitrogen, and creatinine were quantitatively analyzed and compared.

**Result:** Glucose (n = 40) and urea nitrogen concentrations (n = 42) measured in LF from both eyes highly correlated. While there was a very strong correlation ( $\rho = 0.97$ ) between urea nitrogen concentrations in blood plasma and the corresponding tear levels (with the median LF urea nitrogen being 109% of that measured in plasma), the LF glucose concentrations were significantly lower than the corresponding plasma concentrations (with only 13% of the blood glucose concentration detected in the LF). The creatinine concentrations in tears were much lower than those in plasma, and LF creatinine was detectable in only 12/48 cats (25%). Hence, a comparison of the LF creatinine concentrations between both eyes or with the corresponding plasma creatinine concentration was not possible.

**Conclusion:** Measurement of LF urea nitrogen concentrations in cats appears to be reliable and might have potential clinical utility. Measurement of LF glucose concentrations is less reliable but may still be useful in some cats. Creatinine is not reliably detected in the LF in cats. Further studies determining clinical utility of LF metabolites in cats and other companion animals are warranted.

## KEYWORDS

azotemia, biochemical analysis, comparison tears and blood, hyperglycemia, lacrimal fluid sampling, tear levels

## 1 | INTRODUCTION

The functional unit of the “ocular surface” comprises the tear production, tear film, corneal and limbal epithelium, conjunctival epithelium and goblet cells, and Meibomian and lacrimal glands.<sup>1</sup> Maintenance and distribution of the tear film is an important mechanism for protecting the cornea, and thus, for preserving the eye and vision.<sup>2</sup>

Metabolites such as glucose, urea, and creatinine can be detected in the lacrimal fluid (LF) as has been determined in several human studies. An important and clinically useful aspect is the relationship between the concentrations of these metabolites in the LF and their corresponding plasma values. Access to LF might be easier and faster than blood, especially in fractious or unstable feline patients. The mean basal tear production in people averages at approximately

1.2  $\mu\text{L}/\text{min}$ ,<sup>3</sup> and external stimulation can raise the tear production between 100 and 300 percent,<sup>3,4</sup> based on which a sufficient sample volume can be expected for analysis.

There are only few studies evaluating the correlation between clinical chemical variables in serum and tears in companion animals. Semiquantitative assessment of LF urea nitrogen in cats and dogs,<sup>5</sup> semiquantitative assessment of LF glucose in dogs,<sup>6</sup> and quantitative analyses of LF urea nitrogen and creatinine in horses<sup>7</sup> have been reported to date.

We hypothesized that LF and plasma glucose, creatinine, and urea nitrogen concentrations are closely correlated in cats. Thus, the aim of this study was to quantify glucose, creatinine, and urea nitrogen concentrations in LF obtained from cats either with normal or increased plasma concentrations of these metabolites.

## 2 | MATERIALS AND METHODS

The study was designed and conducted at the Small Animal Clinic, University of Leipzig (Germany), and was reviewed and approved by the responsible local authority for animal welfare (No. A07/12) according to the German Protection of Animals Act (§8, passage 7, phrase 1, no. 2; Sächsisches Staatsministerium für Soziales und Verbraucherschutz, Landesdirektion Chemnitz/Sachsen, Veterinärwesen und Lebensmittelüberwachung).

A total of 96 cats of different breeds, ages, and health status were prospectively included in the study. All cats were client-owned pet cats, of which 90 cats were hospitalized patients at the clinic or cats receiving outpatient care. The remaining six cats were clinically healthy animals that were owned by veterinary students.

All animals included in the study underwent a complete physical examination and slit lamp ophthalmological examination (SL 14, Kowa Company Ltd, Tokyo, Japan). Cats diagnosed with diseases of the eyelids, conjunctiva or cornea were excluded from the study.

Peripheral venous blood samples were collected into heparin tubes from cats in the morning after food was withheld for twelve hours. Sampling of LF was very closely timed with the collection of venous blood samples. Both procedures were performed on the same cat by the same veterinarian and by ensuring that cats (all unsedated) were handled as unexciting as possible and in a calm environment.

For LF sampling, three small polyurethane sponges (Pele Tim, REF 2250, VOCO GmbH, Cuxhaven, Germany) were carefully placed in the ventral fornix of each eye using small forceps (Figure 1), left in place for one minute, and were then carefully removed and transferred to

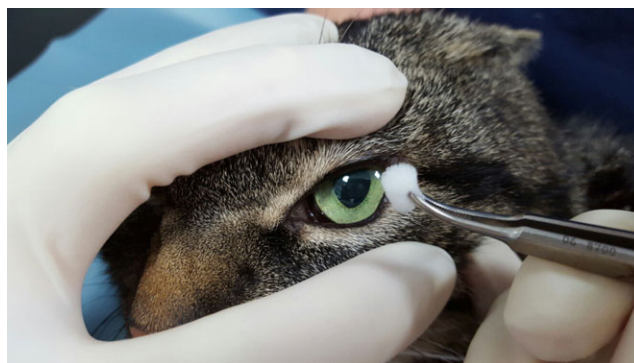
a small sample tube (Figure 2). Care was taken to manipulate the eyelids as little as possible, and a lubricant (Vis-lube® TRB Chemedica AG, Munich, Germany) was applied after removing the sponges.

To harvest the LF from the sponges, the bottom of the small sample tube was cut open, and the tube was inserted into a larger sample collection tube. The latter was then centrifuged at 16 100 g (Microcentrifuge 5415 D, Eppendorf AG, Hamburg, Germany), and the fluid retrieved from the sponge was used for further analyses.

Concentrations of LF and plasma glucose, creatinine, and urea nitrogen were measured using an automated chemistry analyzer (Roche Hitachi 912, Roche Diagnostics GmbH, Mannheim, Germany). The lower detection limits of the assays are: glucose = 0.11 mmol/L, urea nitrogen = 0.83 mmol/L, creatinine = 18  $\mu\text{mol}/\text{L}$ . The cats were assigned to three (A, B, and C) of six different groups (Table 1) based on the interpretation of the respective plasma metabolite concentration in light of the validated laboratory reference interval.

If the harvested LF volume from an individual cat was not sufficient for analysis of all three metabolites, this animal was included in only one or two of the six groups. Because of significant change in the permeability of the conjunctival membrane in patients with renal failure<sup>8,9</sup> and to exclude the possibility of falsely increased LF glucose concentrations in cats with azotemia, the LF in cats with a plasma creatinine concentration  $>141 \mu\text{mol}/\text{L}$  and concurrent hyperglycemia was analyzed only for urea.

Statistical analyses were performed using commercially available software packages (JMP® v13.0, SAS Institute Inc., Cary, NC, USA; GraphPad Prism® v7.0, GraphPad Software, San Diego, CA, USA; and MedCalc© v18.5, MedCalc Software bvba, Ostend, Belgium). Data were evaluated for normality and equal variances using a Shapiro-Wilk test and a Brown-Forsythe test, respectively. Descriptive statistics are presented as medians and ranges



**FIGURE 1** Polyurethane sponges were carefully placed in the ventral fornix of each eye using small forceps



**FIGURE 2** Polyurethane-sponges in the small sample tube

**TABLE 1** Groups and numbers (n) of cats with assignments based on the interpretation of the respective plasma analyte concentrations

Group	Group characteristic	Interval or cut-off	n
A1	Cats with normoglycemia	3.05–5.55 mmol/L	21
A2	Cats with hyperglycemia	>5.55 mmol/L	39
B1	Cats with normal blood urea nitrogen (BUN)	≤12.5 mmol/L	45
B2	Cats with increased BUN	>12.5 mmol/L	27
C1	Cats with normal plasma creatinine	53–141 μmol/L	32
C2	Cats with increased plasma creatinine	>141 μmol/L	16

(continuous data) or counts and percentages (categorical data).

First, the possibility of differences in LF concentrations between both eyes were evaluated for each metabolite by calculating the coefficient of variation for both measurements ( $CV\% = \text{standard deviation [SD]} \times 100\% / \text{mean concentration}$ ), calculating a Spearman rank correlation coefficient  $\rho$ , and by performing a Passing-Bablok regression as well as a Bland-Altman analysis using the absolute differences between both eyes.

The possibility of a correlation between the metabolite concentrations in plasma and the corresponding LF values (based on the sample volume obtained), measured in a combined sample from both eyes or the calculated mean of

both left and right LF concentrations) was tested by calculation of a Spearman rank correlation coefficient  $\rho$  and using a Passing-Bablok regression as well as a Bland-Altman analysis. A Wilcoxon rank-sum test served to compare metabolite concentrations between LF and plasma, and a receiver operating characteristic curve analysis (with a Youden index for optimum cut-off determinations) was used to determine sensitivities and specificities to detect cats with increased plasma metabolite levels.

### 3 | RESULTS

Breeds of cats included in the study were Domestic Shorthair (DSH,  $n = 76$ ), Persian (7), British Shorthair (4), Maine Coon (3), Birman (2), Norwegian Forest cat (2), Siamese (1), and Burmese cat (1). The age of the cats at the time of the study ranged from one to 18 years (median: 7 years). There were 68 males (50 male-castrated) and 28 females (16 female-spayed) cats.

Reasons for presenting the cats at the clinic were surgical procedures ( $n = 10$ ), feline lower urinary tract disease (FLUTD, 20), neurological diseases (3), intestinal diseases (9), cardiovascular diseases (2), neoplastic disease (1), intoxication (2), trauma (2), diabetes mellitus (16), renal diseases (19), pancreatitis (2), fibroadenomatosis (1), anemia (1), and fever (2). Six healthy student-owned cats were also included in the study.

Analyte concentrations in plasma and LF could be compared for glucose in 60 cats, for urea nitrogen in 72 cats, and for creatinine in 48 cats (Table 1).

There were no signs of ocular irritation or pain in any of the cats during LF sampling.

#### 3.1 | Glucose concentrations in LF and blood plasma

Glucose concentrations were measured in LF from both eyes of 40 cats and were highly correlated (Figure 3A) with CV% ranging from 0%–72% (median: 22%). Passing-Bablok analysis revealed a proportional bias ( $\beta = 1.56$ ; 95%CI: 1.28–1.80) but not a constant bias ( $\alpha = -0.10$ ; 95%CI:  $-0.26$ – $0.04$ ) between the higher and lower LF glucose concentrations. The difference between LF glucose concentrations was plotted against their mean (Bland-Altman plot; Figure 3B) following a common log transformation as the differences were related to the mean ( $P < 0.0001$ ). A moderate correlation between glucose in blood plasma and tear levels (Figure 4A) was detected. The LF glucose concentration was significantly lower than the corresponding plasma concentrations: only 13% (median; range: 4%–33%) of the blood glucose concentration was measured in the LF. The Passing-Bablok test identified

only a proportional bias ( $\beta = 0.16$ ; 95%CI: 0.12-0.20) between plasma and LF glucose concentrations ( $\alpha = -0.22$ ; 95%CI:  $-0.51$ - $0.08$ ). The difference between plasma and LF glucose concentrations (log transformed) was plotted against their mean (Figure 4B).

Glucose concentrations in the LF were significantly higher in cats with hyperglycemia (median: 1.50 mmol/L, range: 0.24-6.61 mmol/L) compared to cats with a normal blood glucose concentration (median: 0.58 mmol/L, range: 0.28-4.73 mmol/L;  $P < 0.0001$ ). Sensitivity and specificity of a LF glucose concentration  $>0.78$  mmol/L to detect hyperglycemia were 77% (95%CI: 61%-89%) and 81% (95%CI: 58%-95%), respectively (area under the curve [AUC]: 80%, 95%CI: 68%-92%).

### 3.2 | Urea nitrogen concentrations in LF and blood plasma

Urea nitrogen concentrations were measured in the LF from both eyes of 42 cats and were highly correlated (Figure 5) with CV% ranging from 0%-51% (median: 4%) and no bias detected in the Passing-Bablok test ( $\alpha = 0.27$ , 95%CI:  $-0.08$ - $0.61$ ;  $\beta = 1.03$ , 95%CI: 1.00-1.07).

There was a very strong correlation of the urea nitrogen concentration in blood plasma and the corresponding tear levels ( $\rho = 0.97$ , 95%CI: 0.95-0.98;  $n = 72$ ;  $P < 0.0001$ ), and the LF urea nitrogen concentration was 109% (median; range: 30%-195%) of the plasma value (Figure 6). The Passing-Bablok regression showed a slight proportional bias ( $\beta = 1.07$ ; 95%CI: 1.03-1.10) between plasma and LF urea nitrogen concentrations ( $\alpha = 0.30$ ; 95%CI:  $-0.09$ - $0.70$ ).

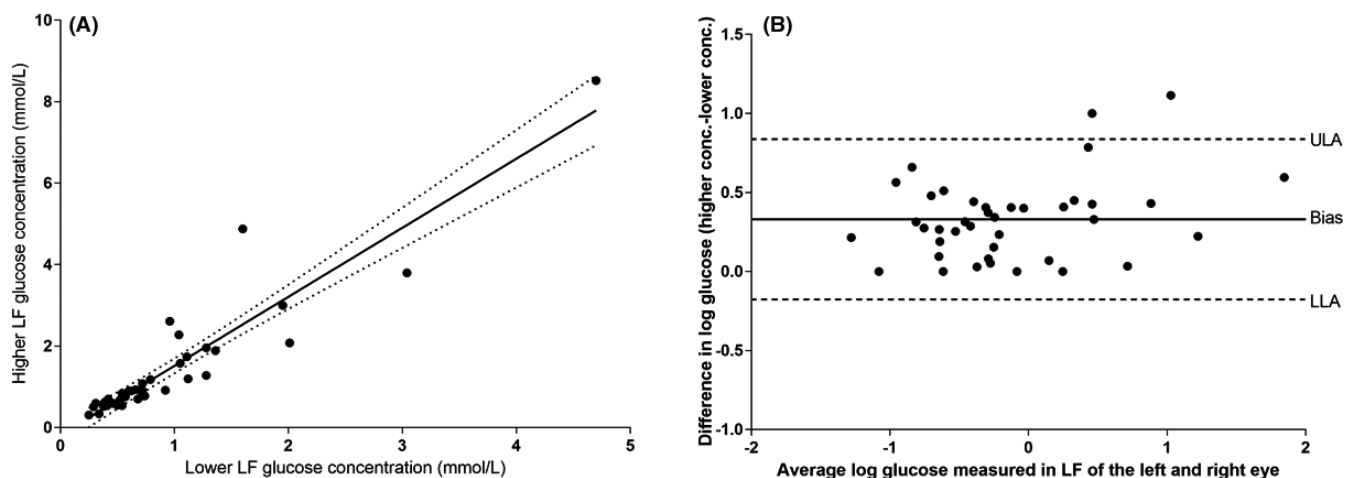
In cats with increased BUN concentrations the mean LF content was significantly higher (median: 40.0 mmol/L,

range: 14.5-131.6 mmol/L) than in cats with normal BUN concentrations (median: 8.4 mmol/L, range: 1.6-14.2 mmol/L;  $P < 0.0001$ ). Sensitivity and specificity of a LF urea nitrogen concentration  $>14.4$  mmol/L to detect an increased BUN were 100% (95%CI: 87%-100%) and 100% (95%CI: 92%-100%), respectively (AUC 100%).

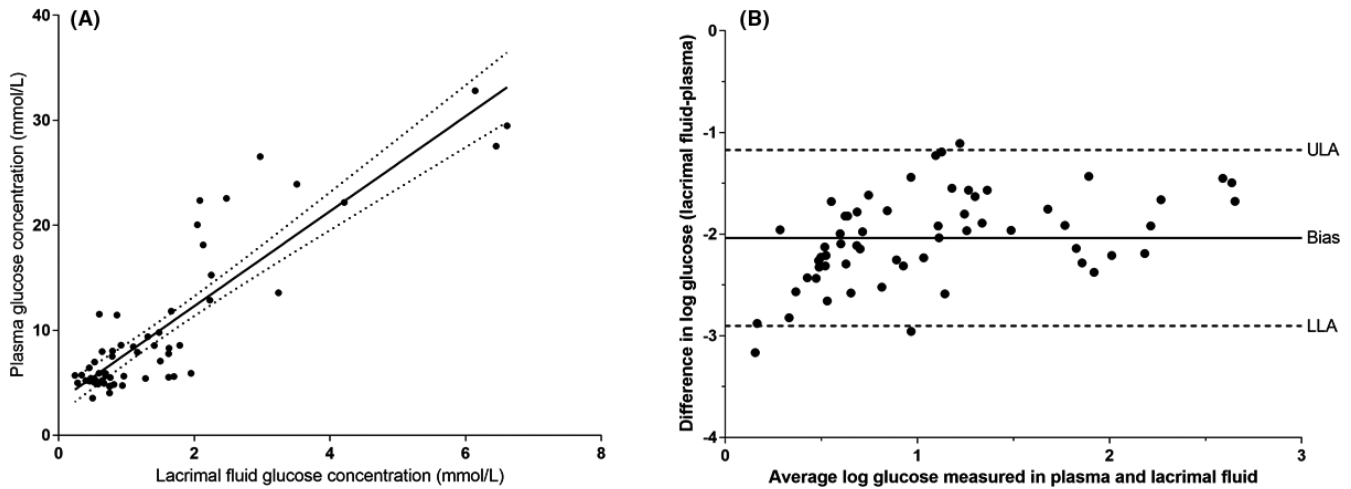
### 3.3 | Creatinine concentrations in LF and blood plasma

The creatinine concentrations in tears were much lower than those in plasma, with LF creatinine being detectable in only 12/48 (25%) cats. Hence, a comparison of the LF creatinine concentrations between both eyes (available from only 5 cats) or with the corresponding plasma creatinine concentration (paired data available from 12 cats, but 6 of those cats were also hyperglycemic) was not possible.

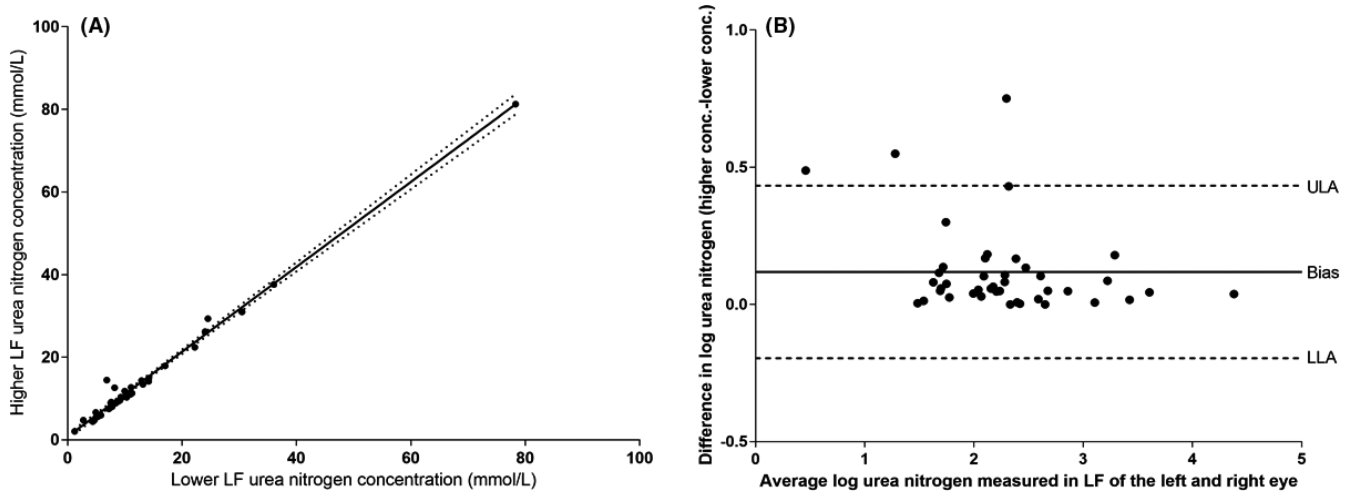
In 31 of the 32 cats (97%) with a normal plasma creatinine level, the LF creatinine concentration was below the detection limit of the assay (18  $\mu\text{mol/L}$ ). Also, in 5 of the 16 cats (31%) with an increased plasma creatinine concentration, there was no creatinine detectable in the LF. While one cat with a plasma creatinine concentration of 195  $\mu\text{mol/L}$  showed an LF level of 37  $\mu\text{mol/L}$  in one eye and 42  $\mu\text{mol/L}$  in the other eye, another cat with a blood creatinine concentration of 229  $\mu\text{mol/L}$  had no detectable creatinine any of the two eyes. Also, one cat with a plasma creatinine concentration of 409  $\mu\text{mol/L}$  had only a detectable LF creatinine (42  $\mu\text{mol/L}$ ) in one eye, whereas in the other eye creatinine could not be detected. In all cats with a plasma creatinine concentration  $\geq 254$   $\mu\text{mol/L}$  corresponding LF concentrations were detected.



**FIGURE 3** Comparison of LF glucose measurements in both eyes ( $n = 40$ ). A, Glucose concentrations in the LF from the left and right eye were highly correlated (Spearman  $\rho = 0.94$ , 95%CI: 0.89-0.97;  $P < 0.0001$ ). B, The mean difference (bias) and the lower (LLA) and upper (ULA) limit of agreement (dashed horizontal lines) were calculated as 0.330,  $-0.177$ , and 0.837 (log scale)



**FIGURE 4** Comparison of LF and plasma glucose concentrations ( $n = 60$ ). A, A moderate correlation was detected between glucose concentrations in blood plasma and LF ( $\rho = 0.74$ , 95%CI: 0.59-0.84;  $P < 0.001$ ). B, Bias, LLA, and ULA were calculated as  $-2.038$ ,  $-2.903$ , and  $-1.173$  (log scale)



**FIGURE 5** Comparison of urea nitrogen concentrations measured in both eyes ( $n = 42$ ). A, Urea nitrogen levels in the LF from both eyes were highly correlated (Spearman  $\rho = 0.94$ , 95%CI: 0.89-0.97;  $P < 0.0001$ ). B, Bias, LLA, and ULA were calculated as  $0.118$ ,  $-0.196$ , and  $0.433$  (log scale)

## 4 | DISCUSSION

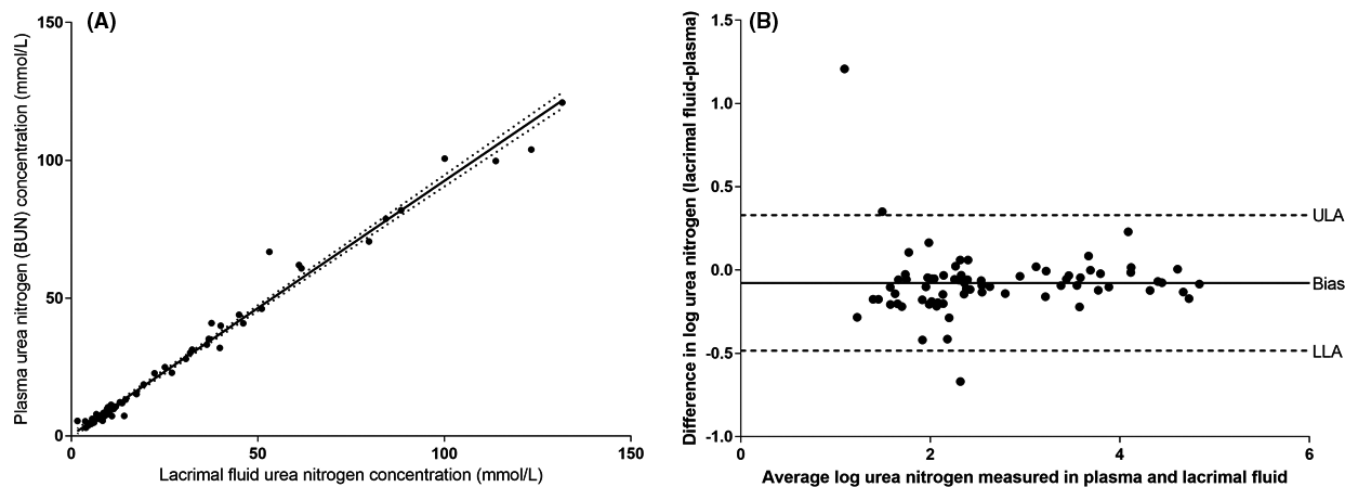
This study evaluated the LF concentrations of glucose, urea nitrogen, and creatinine in cats with normal or increased corresponding plasma values. The collection of LF with small polyurethane sponges is a minimally invasive method which can be easily performed even in fractious cats and would be clinically very useful.

In the cats included in this study, no difference in urea nitrogen concentration was detected between the LF of the left eye and the LF of the right eye, whereas there were discrepancies between glucose measurements (especially with high glucose concentrations) between both eyes. The LF creatinine contents could not be compared in the cats of this study because creatinine concentrations were below the

lower limit of quantification of the assay ( $18 \mu\text{mol/L}$ ) in a large number of cases. There are only very limited published data to compare our findings, but inconsistent results have been reported concerning the measurement of LF glucose in people.<sup>10-12</sup> Studies comparing the LF contents of all three metabolites (glucose, urea nitrogen, creatinine) between both eyes do not exist in the veterinary literature, and neither are there any reports in the human literature about the relationship between LF urea and creatinine concentrations measured in either of the two eyes. Our findings support the recommendation that—except for urea nitrogen—measurement of metabolites in the LF should be performed in specimens collected from both eyes.

In the cats included in this study, only a moderate correlation between glucose values but a strong correlation





**FIGURE 6** Comparison of LF and plasma urea nitrogen concentrations ( $n = 72$ ). A, A moderate correlation was detected between urea nitrogen concentrations in blood plasma and LF ( $\rho = 0.97$ , 95%CI: 0.95-0.98;  $P < 0.001$ ). B, Bias, LLA, and ULA were calculated as  $-0.077$ ,  $-0.484$ , and  $0.330$  (log scale)

between the urea nitrogen concentrations in LF and in blood plasma were found. Also, a significant proportional bias between LF and plasma glucose concentrations was detected, whereas the proportional bias in urea nitrogen levels was small. Together with the diagnostic accuracy (sensitivity, specificity) of LF levels to detect cats with abnormally increased plasma metabolite concentrations, these results suggest that urea nitrogen can be reliably detected in tears but that LF and plasma levels cannot be used interchangeably (ie, measurements cannot be directly compared and a separate reference interval for LF urea nitrogen concentrations is needed). Measurement of LF glucose, on the other hand, is less reliable to detect hyperglycemia in cats, but this may also vary depending on the etiology of hyperglycemia (ie, stress hyperglycemia vs. diabetes mellitus/prediabetes). However, the findings for LF glucose in this study are consistent with those in diabetic dogs, where LF glucose concentrations were higher than the LF glucose levels in normal dogs.<sup>6</sup> The strong correlation between the LF and plasma urea nitrogen concentrations were seen in the cats of our study is consistent with the results in humans, dogs, and horses.<sup>6,7,13-16</sup> This finding leads us to conclude that the biochemical analysis of LF has clinical utility for the diagnosis of an increased BUN in humans, horses, dogs, and cats.

The possibility of a correlation between the creatinine values in LF and blood plasma could not be statistically analyzed. LF creatinine concentrations were invariably detected and could only be measured in cats with very high plasma creatinine concentrations ( $\geq 254 \mu\text{mol/L}$ ). This leads us to conclude that measurement of creatinine in tears is not reliable or useful in cats. The reason for this is assumed to be a significant change in the permeability of the conjunctival membrane in patients with renal failure.<sup>8,9</sup>

Our results contrast those of Kang et al (1988) that found a correlation between the LF creatinine concentration and the corresponding plasma levels in human patients, but our results agree with those in horses where no correlation was found between these variables.<sup>7</sup> Also, to prevent false positive results, eight cats with azotemia in this study were excluded for measurement of glucose concentrations in the tears and the LF was analyzed only for urea in these cats.

Overall, only approximately 13% of the blood glucose concentration could be measured at the same time in the LF of the cats in this study. In humans, it has been reported there is a lag time between blood glucose concentrations being reflected in excreted fluid glucose concentrations,<sup>17</sup> suggesting that the measurement of LF glucose is not a reliable alternative to blood glucose monitoring in intensive care patients or in patients requiring insulin therapy.<sup>18</sup> Based on the current investigation, it is presumed that this is true for cats as well.

In conclusion, measurement of LF urea nitrogen concentrations in cats appears to be reliable and might have the potential to be clinically useful. Measurement of LF glucose concentrations is less reliable but may still be useful in some cats. However, further studies determining the clinical utility of measuring LF urea nitrogen and glucose in cats and other companion animals are warranted. Creatinine is not reliably detected in the LF in cats, but this variable should also be evaluated in other companion animal species.

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