




Urine protein:creatinine ratio in growing kittens: new insight

Razão proteína:creatinina urinária em filhotes felinos em crescimento: nova visão

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ABSTRACT: This study aimed to evaluate the urinary excretion of protein employing the urine protein:creatinine ratio in growing health kittens and included twelve clinically healthy multi-mixed-breed kittens, seven males and five females from three different litters. Urine collections started when the kittens completed the fourth week of life. The study followed a controlled longitudinal experimental model, with 14 repeated measures, one per week. A total of 168 urine samples from 12 healthy growing kittens were analyzed. UPC ratio median value was 0.56 (confidence interval 0.51 - 0.62), revealing urinary protein excretion more remarkable than is expected for healthy adult cats. Two-way repeated-measures ANOVA results indicated no statistically significant differences in urine protein:creatinine ratio values concerning the kittens' ages and genders. Additionally, there was a striking fluctuation of urine protein: creatinine ratio values for all kittens over the 14 weeks (coefficient of variation 53.3%). Urine protein:creatinine ratio of healthy growing kittens changes unexpectedly, and most values reach magnitudes considered pathological for adult cats, suggesting a clinically irrelevant condition. Since the fluctuation of the UPC is not related to age (from the fourth to the seventeenth week of age), to interpret of UPC of growing kittens, in practice or research, it is mandatory to repeat the measures, at least three times, at one week apart, to discharge pathologic event.

KEYWORDS: physiological proteinuria; UPC; cat; urine.

RESUMO: Este estudo teve como objetivo avaliar a excreção urinária de proteínas empregando a razão proteína:creatinina urinária (RPCU) em filhotes felinos saudáveis em crescimento e incluiu doze animais sem raça definida clinicamente saudáveis, sendo sete machos e cinco fêmeas de três ninhadas diferentes. As coletas de urina iniciaram quando os gatinhos completaram a quarta semana de vida. O estudo seguiu um modelo experimental longitudinal controlado, com 14 medidas repetidas, uma por semana. Um total de 168 amostras de urina dos 12 felinos saudáveis em crescimento foram analisadas. O valor mediano do índice RPCU foi de 0,56 (intervalo de confiança 0,51 - 0,62), revelando excreção urinária de proteínas mais notável do que o esperado para gatos adultos saudáveis. Os resultados da ANOVA de medidas repetidas bidirecionais não indicaram diferenças estatisticamente significativas nos valores da RPCU em relação às idades e ao sexo dos gatinhos. Além disso, houve uma flutuação importante nos valores da RPCU para todos os gatinhos ao longo das 14 semanas (coeficiente de variação de 53,3%). A proporção proteína:creatinina urinária de filhotes felinos saudáveis em crescimento muda inesperadamente, e a maioria dos valores atinge magnitudes consideradas patológicas para gatos adultos, sugerindo uma condição clinicamente irrelevante. Como a oscilação da RPCU não está relacionada à idade (da quarta à décima sétima semana de vida), para interpretação da RPCU de gatinhos em crescimento, na prática ou na pesquisa, é obrigatório repetir as medidas, pelo menos três vezes, em com intervalo de uma semana, para descartar evento patológico.

PALAVRAS-CHAVE: proteinúria fisiológica; RPCU; gato; urina.

INTRODUCTION

The presence of urinary proteins in healthy cats may not be pathological (Giraldi *et al.*, 2020). Therefore, measuring urine protein concentration is admittedly essential for diagnosing and following kidney diseases.

To estimate the presence of protein in the urine, the first tool to be performed is the dipstick test. For dogs, the measurement of protein by dipstick test together with the urine specific gravity is reliable to guide the decision to assess or not a quantitative test (Zatelli *et al.*, 2010). However, the same

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procedure does not work for cats because the dipstick test is not accurate for protein detection in this species (Pérez-Accino *et al.*, 2020). Therefore, urinary protein must be analyzed by quantitative methods, such as the Coomassie brilliant blue (CBB) or the Pyrogallol red-molybdate (PRM), the most common colorimetric tests in use.

Urine protein:creatinine ratio (UPC) from a urine spot sample accurately estimates urinary protein loss in cats (Grauer, 2007). Reference values for UPC available for adult cats, including borderline proteinuria, ranges from 0.2 to 0.4 (International Renal Interest Society, 2023). However, enough data are lacking to establish any agreement on UPC in kittens. Therefore, this study aimed to access the UPC of healthy kittens in a controlled longitudinal experiment whose methodology is applicable in clinical practice or research.

MATERIAL AND METHODS

The project has been approved by the Animal Ethics and Welfare Committee from the Federal University of Santa Catarina, protocol number 9616120417.

The study included 12 clinically healthy multi-mixed-breed growing kittens, seven males and five females, from three litters of different parents. Each pregnant mother stayed housed in an individual comfortable cage before natural delivery. Mothers were removed after weaning, and the litters remained until the end of the experiment, being fed ad libitum with water and commercial kittens' diet (39% of crude protein). In addition, kittens were allowed to exercise and play once a day on the room floor for one hour.

All kittens assigned to this study remained healthy during the entire experimental period and have endoparasite control

with praziquantel and pyrantel pamoate at 30 and 45 days of age. Immediately after the sample collection period, core vaccination protocol started, and the kittens were adopted.

Urine sampling was performed once a week, starting on the thirtieth day after birth (fourth week) and completed in the seventeenth week. Samples were obtained by stimulation and gentle compression of the urinary bladder, always at night (from 7:00 pm to 10:00 pm). Uncentrifuged urine samples, ranging from 0.5 to 2.0 mL, were stored in microtubes at -20°C for a month. The urine protein and creatinine analysis were done with the commercial kits pyrogallol red-molybdate method and modified Jaffé test (both from Labtest Diagnostica[®]), respectively. The analyses were performed in duplicate for both analytes using semi-automatic biochemical analyzer, and arithmetic means were used to calculate the UPC.

The study design is a controlled longitudinal experiment with repeated measures (RM). The statistical analysis, including descriptive statistics, two-way RM ANOVA (main factors: gender and age), and graphics, were performed using the GraphPad Prism version 9.3.0 (463) for Windows (GraphPad Software, San Diego, California USA).

RESULTS

The statistical analysis included all the 168 UPC ratios obtained. Results of descriptive statistics (Table 1) show high coefficients of variation (CV) for males (51.1%) and females (54.9%). In addition, both male and female sets of repeated measures had striking fluctuations in UPC values over the 14 weeks (Figure 1).

ANOVA results (Table 2) show that the interaction gender x age was not statistically significant. Likewise, testing the

Table 1. Descriptive statistic data of urine protein:creatinine ratio (UPC) from 12 healthy, multi-mixed-breeds kittens, from 4th to 17th weeks of age. Urine was sampled once a week at 14-time points.

UPC	Male (n= 7 x14)	Female (n= 5x14)	All (n= 12x14)
Minimum	0.18	0,23	0.18
25% Percentile	0.37	0.40	0.38
Median	0.54	0.60	0.56
75% Percentile	0.77	0.91	0.83
maximum	1.94	1.96	1.96
Range	1.76	1.73	1.78
Confidence level of median	96.7%	95,9%	96.3%
Lower confidence limit	0.47	0.50	0.51
Upper confidence limit	0.61	0.73	0.62
Mean (Std. Deviation)	0.61 (0.31)	0.70 (0.38)	0.65 (0.35)
Mean (Std. Error of mean)	0.61 (0.032)	0.70 (0.05)	0.65 (0.03)
Mean confidence level	95%	95%	95%
Lower confidence limit	0.55	0.61	0.60
Upper confidence limit	0.67	0.79	0.70
Coefficient of variation	51.1%	54.9%	53.3%

gender or age as a source of UPC variation resulted as statistically not significant.

The UPC fluctuation (Figure 2) shows an apparent chaotic up-and-down pattern over the weeks not influenced by the analyzed sources of variations. Each of the 12 kittens presents this up-and-down pattern.

DISCUSSION

The studied kittens presented UPC values ranging from 0.18 to 1.96, which seems pathological since to be non-proteinuric, adult cats must have UPC <0.2. However, in a contest of healthy kittens presenting relevant fluctuations of UPC

values, the physiological cause is more reasonable to explain the events.

Although the reference value for elderly and geriatric cats is no different to that of adult felines (Mortier *et al.*, 2023a), when we think about kittens, this can be considered different. In a pioneering study of growing kittens, Hoskins *et al.* (1991) found UPC similar to that observed in healthy adult cats. Although the authors randomly composed experimental groups (7 to 14 kittens for each week of age, out of 29 kittens available for the study), the statistical design implied the absence of actual repeated measures due to the dissociation between the evaluated groups. Thus, it may not have

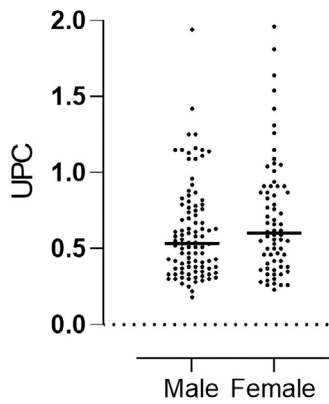


Figure 1. Scatter dot plotter with median of urine protein:creatinine ratio (UPC) from seven males and five females, multi-mixed-breed healthy kittens, evaluated in 14 time-points (once a week) from 4th to 17th weeks of age.

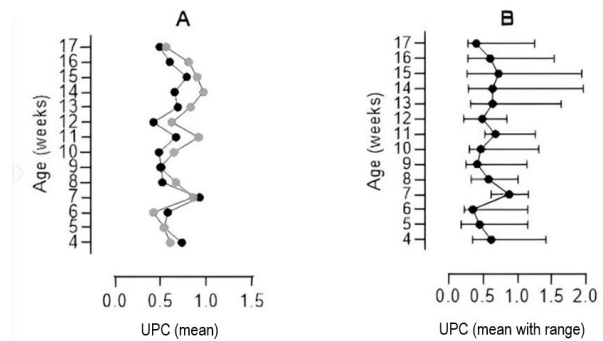


Figure 2. (A) Means of urine protein:creatinine ratio (UPC) from the male set (black symbols) and female set (grey symbols) longitudinal data. (B) Median with maximum and minimum values of longitudinal data. From 12 multi-mixed-breed healthy kittens (7 male and 5 female), evaluated in 14 time-points (once a week), from 4th to 17th weeks of age (n= 168).

Table 2. Two-way RM ANOVA (alpha = 0.05) of urine protein:creatinine ratio (UPC) data from 12 healthy multi-mixed-breeds kittens (7 males and 5 females), from the 4th to 17th weeks of age. Urine was sampled once a week at 14-time points.

Source of variation	% of total variation	P value		P value summary	
Gender x Age	3.91	0.8492		ns	
Gender	1.62	0.3195		ns	
Age	14.9	0.0983		ns	
Kittens	14.7	0.0023		**	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Gender x Age	0.779	13	0.069	F (13, 130) = 0.602	0.8492
Gender	0.322	1	0.322	F (1, 10) = 1.10	0.3195
Age	2.98	13	0.229	F (2.97, 29.7) = 2.30	0.0983
Kittens	2.94	10	0.294	F (10, 130) = 2.95	0.0023
Residual	12.9	130	0.100		
Difference between row means					
Mean of male	0.610				
Mean of female	0.699				
Difference between means	-0.0888				
SE of difference	0.0848				
95% CI of difference	-0.278 to 0.100				

been possible to capture the subject's significant variations of UPC values. There was no such bias in the present study, which allowed the identification of large fluctuations in the UPC manifested in all 12 kittens over the 14 weeks of evaluation. To the authors' knowledge, this statistic design bringing natural UPC behavior for healthy growing kittens has not been reported.

Interesting findings in children point out proteinuria as a common finding in routine testing (10%). However, in test repetition, the incidence drops to 1%. At basal condition, the UPC values must be <0.2 or $UPC <0.5$ if the child is 6-24 months old). In transient or functional proteinuria, the natural causal factors include age, exercise, stress, cold exposure, or idiopathic (Leung *et al.*, 2017). Despite the apparent difference between the species, it seems plausible to consider that the kittens also had transient proteinuria. Although the cause remains undetermined in the present study, it is probably a physiological event since all kittens grew up properly as expected.

Many factors can be causes of variation or even errors in measuring and interpreting the UPC ratio. Therefore, urinalysis, including sedimentoscopy, is helpful (International Renal Interest Society, 2023). However, at least 5 mL of urine is necessary to obtain a trustworthy urinalysis result for cats. Unfortunately, in the present study, urinalysis could not be performed due to the small urine volume (2 mL as maximum) collected to prevent bladder harm.

In this study, uncentrifuged animal urine samples were stored at -20°C for one month before processing, which is in accordance with a study that found that centrifugation of urine samples before analysis did not affect UPC values (Mortier *et al.*, 2023b). Furthermore, these authors obtained changes in UPC results with urine stored only after 6 months.

Cystocentesis is considered the ideal method for urine sample collection to assess proteinuria once it excludes proteins from the urethra and genital tract. Although, it can lead to microscopic hematuria in various degrees (Lees *et al.*, 2005). However, urine sampling by cystocentesis or manual bladder compression may not influence the quantification of urinary protein for cats (Vilhena *et al.*, 2015, Mortier *et al.*, 2023c). Despite that, cystocentesis was disregarded in the

present study to avoid iatrogenic harm, mainly considering that the subjects are kitten. Evermore, following the mother's care, kittens were conditioned to urinate with minimum vesical compression by the experimenter.

Although the kittens evaluated were very young in the first few weeks, cats are more active at night than most dogs. So then, in this study, to avoid unnecessary stress on kittens that might be sleeping during the day, we opted for weekly nocturnal collections, thus preventing additional behavior disturbance.

In a series of experiments to compare dye-binding methods-dependency to analyze urinary protein in cats, both PRM and CBB were precise. However, the CBB method tended to detect urine protein and UPC significantly higher than those detected by the PRM method (Giraldi *et al.*, 2018). However, besides differences in laboratory methods used for quantifying urinary proteins excretion, some other factors can generate dependencies on the variables used for UPC (e.g., food, collection time, environmental stress, water intake) (Mortier *et al.*, 2023d). In the present study, it should be considered that kittens were fed ad libitum but ate more at night when they were most active. Urine collections were performed at night from 7:00 pm to 10:00 pm, which may have been the cause of the high UPC values found. On the other hand, fluctuations in UPC values were present since the beginning of the experiment when the kittens were still under the mother's care.

Finally, the highlight of this study is the presentation of impacting data, which may lead to new and more enlightening research on the urinary excretion of proteins in growing kittens.

CONCLUSIONS

Urine protein:creatinine ratio of healthy growing kittens changes unexpectedly, and most values reach magnitudes considered pathologic for adult cats, suggesting a clinically irrelevant condition.

Since the fluctuation of the UPC is not related to age (from the fourth to the seventeenth week of age), to interpret of UPC of growing kittens, in practice or research, it is mandatory to repeat the measures, at least three times, at one week apart, to discharge pathologic event.

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