Japanese Journal of Veterinary Research 70(3&4): 117-123, 2022

JJVR

SHORT COMMUNICATION Experimental Research

Intraday secretion pattern in serum fibroblast growth factor-23 concentration in healthy dogs and cats

Hirosumi Miyakawa[1,*)], Huai-Hsun Hsu[1)], Mizuki Ogawa[1)], Ryota Akabane[1)], Yuichi Miyagawa[1)] and Naoyuki Takemura[1)]

[1)] Laboratory of Veterinary Internal Medicine II, Nippon Veterinary and Life Science University, 1-7-1 Kyonan-cho Musashino-shi, Tokyo, 180-8602, Japan

Received for publication, January 23, 2022; accepted, June 27, 2022

Abstract

Intraday variation, circadian rhythm and postprandial effects of FGF-23 were examined in five healthy beagles and five cats. Blood samples were collected every 4 hr. The animals were fed at 08:00 and 20:00. In dogs, FGF-23 had a significant intraday variation and circadian rhythm and decreased after meals in comparison with the fasting state, but not significant. The intraday coefficient variation (±standard deviation) of FGF-23 in dogs was 9.1 ± 2.6. In cats, FGF-23 did not have significant intraday variations and circadian rhythms. Postprandial FGF-23 levels in dogs decreased, although the range of variation might not be of clinical importance. The evaluation of FGF-23 does not need to be considered for the effect of the sampling timing in cats.

Key Words: Mineral metabolism, Nephrology

Fibroblast growth factor (FGF)-23 is a phosphaturic hormone that is released from osteocytes in response to increased blood levels of phosphorus and calcitriol.[5)] Recently, FGF-23 has been shown as factor associated with mineral metabolic disorder of chronic kidney disease (CKD) in dogs and cats.[6,12)] Blood FGF-23 concentration increases along with decreased glomerular filtration rate (GFR) in patients with CKD. In dogs and cats with CKD, increase of blood FGF-23 concentration occurs earlier as compared with that of parathyroid hormone (PTH) and phosphorus.[6,12)] Thus, FGF-23 has been noticed as an earlier marker of mineral metabolic disorder in canine and feline CKD.

Blood phosphorus, calcium and PTH levels have been routinely measured for the evaluation of mineral metabolism in patients with CKD.[5)] Intraday variations or circadian rhythms are observed in these parameters.[10,14)] In particular, the blood concentration of phosphorus increases after a meal.[7)] However, most studies in humans have not reported an intraday variation, circadian rhythm or postmeal effect in circulating FGF-23 levels.[8,9)] The determination of the presence of intraday variation and postprandial effect of FGF-

* Corresponding author: Hirosumi Miyakawa
Laboratory of Veterinary Internal Medicine II, Nippon Veterinary and Life Science University, 1-7-1 Kyonan-cho Musashino-shi, Tokyo, 180-8602, Japan
Tel: +81-422-31-4151, Fax: +81-422-33-2094
E-mail: d1809@nvlu.ac.jp
doi: 10.14943/jjvr.70.3-4.117

Table 1. Mean dietary composition as provided by the manufacturer

	Dog[a]	Cat[b]
Protein	23.8	33.7
Fat	16.1	21.4
Crude fiber	1.7	1.4
Carbohydrate	53.3	37.8
Calcium	0.77	0.79
Phosphorus	0.70	0.78
Sodium	0.29	0.32
Potassium	0.75	0.85
Magnesium	0.096	0.088
Vitamin D	794 IU/kg	642 IU/kg

All compositions except for vitamin D are represented with dry matter %. [a] Hill's Science Diet Adult Small Bites Adult dog dry food (Hill's-Colgate [Japan] Ltd.). [b] Hill's Science Diet Adult Chicken Recipe cat dry food (Hill's-Colgate [Japan] Ltd.).

23 may be important to obtain an appropriate interpretation of the measurements. However, no studies have evaluated the intraday variations in FGF-23 in dogs and cats.

Thus, the purpose of this study was to evaluate the intraday variation, circadian rhythm and postmeal effect of serum FGF-23 concentration in healthy dogs and cats.

This study included five clinically healthy beagles (four intact males and one spayed female) and five domestic short-haired cats (two intact males, one castrated male and two intact females) from our laboratory. Median age (range) of dogs was 2.0 years (1.9–7.9 years). Median body weight (range) of dogs was 12.6 kg (10.8–13.3 kg). Median age (range) of cats was 9.6 years (8.3–9.7 years). Median body weight (range) of cats was 3.9 kg (3.1–4.7 kg). This study was approved by the Animal Experiments Committee of Nippon Veterinary and Life Science University (acceptance no. 2019S-74). Animals were considered healthy based on history, clinical sign, physical examination, complete blood cell count, plasma biochemistry profile and urinalysis. All cats were confirmed to have normal serum total thyroid hormone concentration. Animals were housed in individual cages. They were fed commercial maintenance dry foods twice per day (at 08:00 and 20:00) for 7 days before the study. On the day of the experiment, dogs and cats were fed after blood samples were obtained at 08:00 and 20:00. We used Science Diet Adult small bites (Hill's-Colgate (Japan) Ltd., Tokyo, Japan) and Science Diet Adult chicken (Hill's-Colgate (Japan) Ltd.) for dogs and cats, respectively. Table 1 presents the characteristics of each food. The amount of food intake was based on calories needed for the animals to maintain their body weight. Daily calorie intake (mean ± standard deviation [SD]) was 804 ± 109 kcal in dogs and 191 ± 13 kcal in cats. The animals were provided water ad libitum, and were not administered any agents during the study period.

The first blood samples were taken at 08:00 and then at 4-hr intervals thereafter (12:00, 16:00, 20:00, 00:00, 04:00 and day 2 at 08:00). Blood samples were obtained from the jugular, cephalic or saphena vein, and collected into heparin tubes (0.5 ml) and tubes containing serum separator (1.5 ml). The heparinized plasma samples were centrifuged at 2700 × g for 5 min, and the supernatant were used to measure the plasma total calcium concentration by a biochemical automatic analyzer (Fuji Dry Chem 4000 V, FUJIFILM VET Systems Co., Ltd., Tokyo, Japan). Serum samples were separated by centrifugation at 1181 × g for 5 min, and stored at -30°C. Thereafter, serum samples were submitted to an external commercial laboratory (FUJIFILM VET Systems Co., Ltd.) for analysis of FGF-23, intact PTH and phosphorus. Serum FGF-23 concentrations were measured by sandwich enzyme linked immuno sorbent assay (MedFrontier FGF23, Minaris Medical Co., Ltd., Tokyo, Japan), which we previously reported in dogs and cats.[11,12] Serum intact PTH concentration was analyzed using chemiluminescent enzyme immunoassay (Siemens Immulyze intact PTH III, Siemens Healthcare Diagnostics K.K., Tokyo, Japan) in both dogs and cats. Serum phosphorus concentration was measured using enzyme method (L type wako

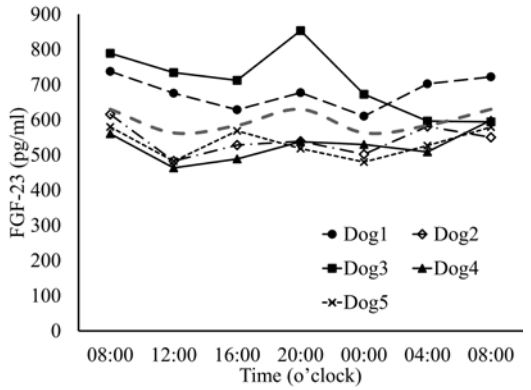

Fig. 1. Intraday variation of serum fibroblast growth factor (FGF)-23 in each dog. The dashed grey curved line represents the estimated cosine function.

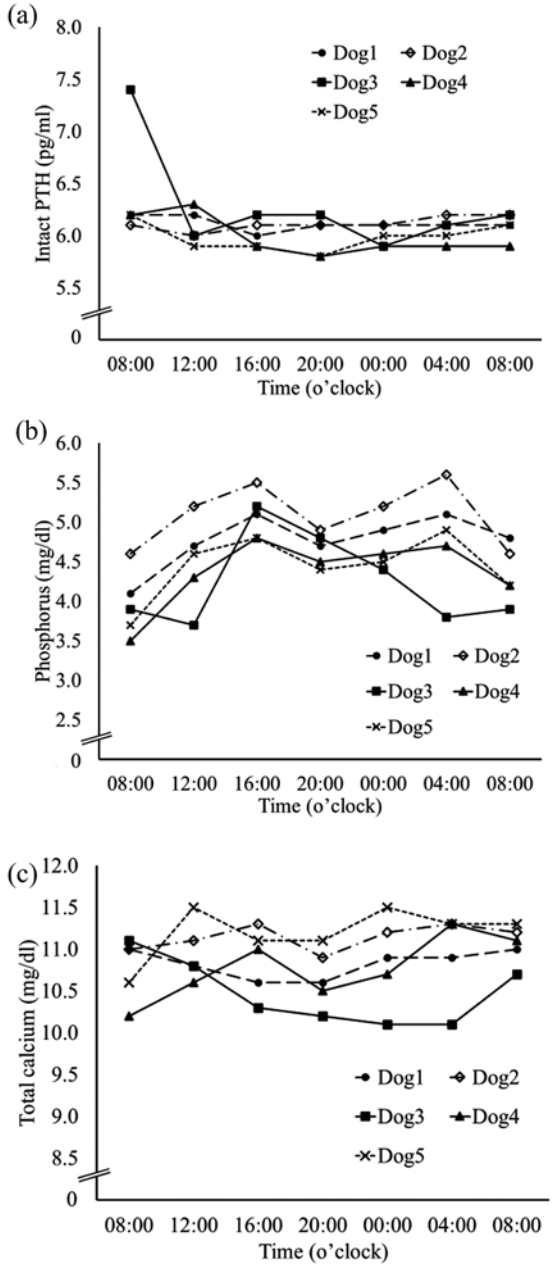

Fig. 2. Intraday variation of serum intact parathyroid hormone (PTH) (a), phosphorus concentration (b) and plasma total calcium concentration (c) in each dog.

inorganic phosphorus, FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan).

We performed statistical analysis using a commercial software package, SPSS version 24 for Windows (IBM Japan Co., Ltd., Tokyo, Japan) and statistical free software, version 4.1.1 R for Windows (https://www.R-project.org/). $P < 0.05$ was considered statistically significant. For each parameter, the intraday coefficient of variations (CV) was calculated by dividing the SD by the mean of the individual animals. We used the Friedman test to evaluate the presence or absence of the intraday variations. Since the Friedman test cannot detect presence or absence of intraday variation periodicity, we used cosine fitting to evaluate the presence or absence of a circadian rhythm[4] The mean measurement on each time was fitted by the following equation using a least-squares method: $y = M + A \cos (2\pi t/P+\varphi)$, where y is the tested mean variable, M is the mean of oscillation, A is the amplitude, t is the collected time of the sample, P is the period of one cycle (12 hr) and φ is the acrophase, the period of which ranged from the start to the wave peak. Then, if the A of the wave form was significantly greater than zero using the F test, it was determined that there was significant rhythmicity in the variable. The measurement between each time was compared using the Wilcoxon signed rank test.

Fig. 1 and 2 present the measurements at each time point in dogs. Although the Friedman test revealed a significant variation in the serum FGF-23 concentrations ($P = 0.016$), there was no significant difference in FGF-23 level between times. For the periodic analysis, there was a significant circadian rhythm in the serum FGF-23 concentration ($P = 0.029$). As can be seen by Fig.

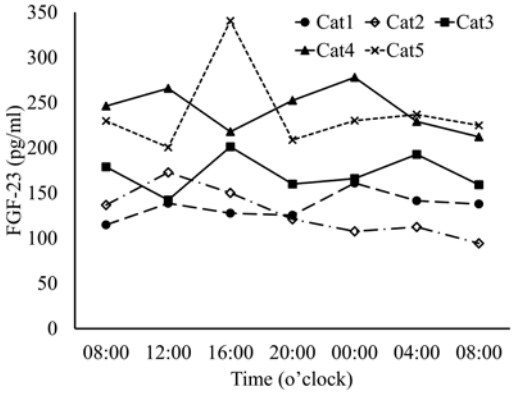

Fig. 3. Intraday variation in serum fibroblast growth factor (FGF)-23 in each cat.

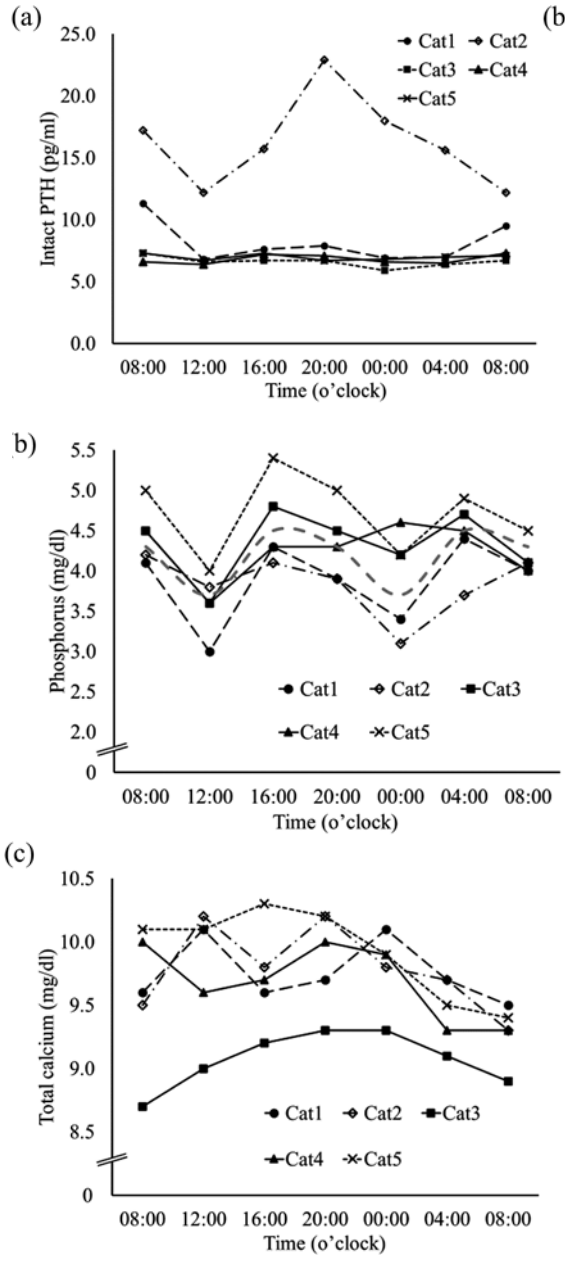

Fig. 4. Intraday variation of serum intact parathyroid hormone (PTH) (a), phosphorus concentration (b) and plasma total calcium concentration (c) in each cat. The dashed grey curved line represents the estimated cosine function.

1, peaks of FGF-23 were subjectively observed at the fasting state (08:00 and 20:00). The mean intraday CV (±SD) of FGF-23 was 9.1 ± 2.6. There was neither a significant variation (Friedman test, $P = 0.66$) nor significant circadian rhythm ($P = 0.14$) in the serum intact PTH concentration. The serum PTH concentration was not significantly different between times (Fig. 2a). Although the serum phosphorus concentration did not have a significant circadian rhythm ($P = 0.15$), the results of the Friedman test showed a significant variation in phosphorus level ($P = 0.003$). However, there was no significant difference in serum phosphorus concentration between times (Fig. 2b). There was neither a significant variation (Friedman test, $P = 0.78$) nor significant circadian rhythm ($P = 0.28$) in the plasma total calcium concentration. The plasma calcium concentration was not significantly different between times (Fig. 2c). The mean intraday CVs (±SD) of PTH, phosphorus and calcium were 3.1 ± 2.9, 9.5 ± 2.5 and 2.6 ± 1.1, respectively.

Fig. 3 and 4 present the measurements obtained at each time point in cats. A significant circadian rhythm and variation were not observed in serum FGF-23 concentration ($P = 0.19$ and Friedman test; $P = 0.32$). In addition, there were no significant differences in FGF-23 between time points. The changing tendency in FGF-23 was not observed between the fasting and postmeal state. The mean intraday CV (±SD) of FGF-23 was 14.8 ± 5.2. Although Friedman test determined significant variation in serum PTH concentration ($P = 0.019$), there was no significant circadian rhythm ($P = 0.66$) in serum intact PTH concentration. Serum PTH concentration was not significantly different between times (Fig. 4a).

Serum phosphorus concentration had a significant circadian rhythm (P = 0.02) and variation (P = 0.014). The serum phosphorus concentration was not significantly between times. (Fig. 4b). Although the results of the Friedman test indicated a significant variation in plasma total calcium concentration (P = 0.012), total calcium did not have a significant circadian rhythm (P = 0.58). The plasma calcium concentration was not significantly different between time points (Fig. 4c). The mean intraday CVs (± SD) of PTH, phosphorus and calcium were 11.8 ± 9.1, 10.2 ± 1.8 and 3.0 ± 0.5, respectively.

Fibroblast growth factor-23 downregulates the expression of sodium phosphorus cotransporter in the proximal renal tubules, resulting in increased urinary fractional excretion of phosphorus.[5] Increased blood phosphorus level is one factor that stimulates FGF-23 secretion.[1] It is well known that circulating phosphorus levels increase after a meal.[7] Given the physiological effect of FGF-23, it is predicted that the secretion of FGF-23 increases in response to postprandial elevated blood phosphorus levels, resulting in phosphaturia and maintenance of phosphate homeostasis. Therefore, based on this theory, it is presumed that the postprandial blood FGF-23 concentration increases in comparison with the fasting state. However, most studies in humans have not observed a postprandial effect on FGF-23.[8,9] In addition, we observed the peak FGF-23 level in dogs in the fasting state (08:00 and 20:00). Although the difference was not significant, the serum FGF-23 concentration decreased 4-hr after a meal in all dogs (Fig. 1). This result suggests that FGF-23 measurements can need to be carefully interpreted in dogs, due to the presence of a variation in the time at which the sample is obtained. However, the intraday CV in the serum FGF-23 concentration was similar to the intra-assay and interassay CV that we previously reported in dogs.[12] Thus, there may be no clinical importance in the diurnal variation of FGF-23 because the intraday variation remains within the measurement deviation. In a previous study that included healthy men, the FGF-23 level significantly decreased after a meal in comparison with the fasting state.[13] The results of the study in humans are consistent with the variation in FGF-23 observed in dogs in our study. The study in humans did not reveal the cause of the decreased circulating FGF-23 level after a meal, and our study also could not explain this mechanism. On the other hands, the FGF-23 level in cats did not show a significant diurnal variation and was not different between the postprandial and fasting states. These findings are consistent with those from previous studies in healthy cats and humans.[3,8,9,14] Thus, it is likely that, when measuring FGF-23 in healthy cats, not much consideration is required regarding the time at which the sample is collected and the postmeal effects.

A previous study showed that plasma PTH concentrations in dogs had a peak in the morning before meal,[10] although the serum PTH concentrations in the present study did not have intraday variations in dogs. Also, postprandial blood phosphorus concentration has been known to increase in comparison with the fasting state in both dogs and cats.[7,14] However, the serum phosphorus concentration in the postmeal period did not increase from the fasting state in the present study, in especially cats. The reason may be the lack of statistical power by small sample in the present study. A recent study in healthy cats indicated that the presence or absence of increased postmeal blood phosphorus concentration depended on the dietary content of inorganic phosphorus.[3] The present study did not investigate the dietary phosphorus composition of the cats' food. Thus, we could not determine whether the composition of dietary phosphorus affected the result observed in cats in the present study.

In conclusion, the postmeal serum FGF-23 concentration in healthy dogs can decrease as compared with the fasting state, but the variation range might not carry any clinical importance. In healthy cats, because there is no

significant intraday variation, circadian rhythm or postprandial effect of FGF-23, there is no need to consider the effect of the timing of the obtained sample for the evaluation of FGF-23.

Acknowledgment

We wish to thank students of the Laboratory of Veterinary Internal Medicine II at Nippon Veterinary and Life Science University for supporting our study. We thank FUJIFILM VET Systems for the analysis of FGF-23, intact PTH and phosphorus. In addition, the authors would like to thank Enago (www.enago.jp) for English language review.

Conflict of interest

Yuichi Miyagawa is in receipt of speaker honoraria from FUJIFILM VET Systems Co., Ltd.

References

1) Antoniucci DM, Yamashita T, Portale AA. Dietary phosphorus regulates serum fibroblast growth factor-23 concentrations in healthy men. J Clin Endocrinol Metab 91, 3144–3149, 2006.
2) Berndt T, Thomas LF, Craig TA, Sommer S, Li X, Bergstralh EJ, Kumar R. Evidence for a signaling axis by which intestinal phosphate rapidly modulates renal phosphate reabsorption. Proc Natl Acad Sci U S A 104, 11085–11090, 2007.
3) Coltherd JC, Staunton R, Colyer A, Thomas G, Gilham M, Logan DW, Butterwick R, Watson P. Not all forms of dietary phosphorus are equal: an evaluation of postprandial phosphorus concentrations in the plasma of the cat. Br J Nutr 121, 270–284, 2019.
4) Cornelissen G. Cosinor-based rhythmometry. Theor Biol Med Model 11, 16, 2014.
5) Foster JD. Update on mineral and bone disorders in chronic kidney disease. Vet Clin North Am Small Anim Pract 46, 1131–1149, 2016.
6) Geddes RF, Finch NC, Elliott J, Syme HM. Fibroblast growth factor 23 in feline chronic kidney disease. J Vet Intern Med 27, 234–241, 2013.
7) Gilor S, Gilor C. Common laboratory artifacts caused by inappropriate sample collection and transport: how to get the most out of a sample. Top Companion Anim Med 26, 109–118, 2011.
8) Isakova T, Xie H, Barchi-Chung A, Smith K, Sowden N, Epstein M, Collerone G, Keating L, Jüppner H, Wolf M. Daily variability in mineral metabolites in CKD and effects of dietary calcium and calcitriol. Clin J Am Soc Nephrol 7, 820–828, 2012.
9) Ix JH, Anderson CA, Smits G, Persky MS, Block GA. Effect of dietary phosphate intake on the circadian rhythm of serum phosphate concentrations in chronic kidney disease: a crossover study. Am J Clin Nutr 100, 1392–1397, 2014.
10) López I, Aguilera-Tejero E, Estepa JC, Bas S, Mayer-Valor R, Jiménez A, Rodríguez M. Diurnal variations in the plasma concentration of parathyroid hormone in dogs. Vet Rec 157, 344–347, 2005.
11) Miyakawa H, Hsu HH, Ogawa M, Akabane R, Miyagawa Y, Takemura N. Serum fibroblast growth factor-23 concentrations in young and mature adult cats with chronic kidney disease. J Feline Med Surg 24, 815–820, 2022.
12) Miyakawa H, Nagatani Y, Ogawa M, Nagakawa M, Sakatani A, Akabane R, Miyagawa Y, Takemura N. Fibroblast growth factor-23 as an early marker of CKD-mineral bone disorder in dogs: preliminary investigation. J Small Anim Pract 61, 744–751, 2020.
13) Nishida Y, Taketani Y, Yamanaka-Okumura H, Imamura F, Taniguchi A, Sato T, Shuto E, Nashiki K, Arai H, Yamamoto H, Takeda

E. Acute effect of oral phosphate loading on serum fibroblast growth factor 23 levels in healthy men. Kidney Int 70, 2141–2147, 2006.
14) Reynolds BS, Brosse C, Jeunesse E, Concordet D, Lefebvre HP. Routine plasma biochemistry analytes in clinically healthy cats: within-day variations and effects of a standard meal. J Feline Med Surg 17, 468–475, 2015.