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Serum concentrations of leptin and adiponectin in dogs with chronic kidney disease

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Abstract

Background: An imbalance in adipokines is associated with the progression of chronic kidney disease (CKD) in humans. However, alterations in adipokines in dogs with CKD remain unclear.

Objectives: To examine whether adipokine concentrations in serum differ between healthy dogs and dogs with CKD and to determine the correlation between serum adipokine concentrations and CKD severity in dogs.

Animals: Twenty dogs with CKD and 10 healthy dogs.

Methods: In this cross-sectional study, serum concentrations of leptin, adiponectin, interleukin (IL)-6, IL-10, IL-18, and tumor necrosis factor (TNF)-α were measured in healthy dogs and dogs with CKD, which were classified according to the International Renal Interest Society guidelines.

Results: Serum leptin concentrations were positively correlated with systolic arterial blood pressure ($r = .41$), creatinine concentrations ($r = .39$), and symmetric dimethylarginine concentrations ($r = .73$). Serum adiponectin concentrations (median [range]) in CKD dogs with borderline or non-proteinuric (20.25 [14.9-45.8] ng/mL) were significantly higher than those in proteinuric CKD dogs (13.95 [6.4-22.1] ng/mL; $P = .01$). Serum IL-6 (median [range]; 43.27 [24.30-537.30] vs 25.63 [6.83-61.03] pg/mL; $P = .02$), IL-18 (median [range]; 25.98 [11.52-280.55] vs 10.77 [3.53-38.45] pg/mL; $P = .01$), and TNF-α (median [range]) concentrations (11.44 [8.54-38.45] vs 6.105 [3.97-30.68] pg/mL; $P = .02$) were significantly different between proteinuric and borderline or non-proteinuric CKD dogs.

Conclusions and Clinical Importance: leptin and adiponectin concentrations in serum might be associated with severity of CKD and proteinuria in dogs with CKD, respectively.

KEYWORDS
adipokine, canine, creatinine, hypertension, proteinuria

Abbreviations: BCS, body condition score; CI, confidence interval; CKD, chronic kidney disease; GFR, glomerular filtration rate; IL, interleukin; LAR, leptin-to-adiponectin ratio; SAP, systolic arterial blood pressure; SDMA, symmetric dimethylarginine; TNF, tumor necrosis factor; UPC, urinary protein-to-creatinine.

Dongjoon Choi and Taesik Yun contributed equally to this study.

1 | INTRODUCTION

Chronic kidney disease (CKD) is the most common renal disease in older dogs.[1] The kidneys play key roles in maintaining homeostasis, such as regulation of blood pressure, electrolytes, acid-base status, and endocrine function. Thus, CKD progression affects numerous metabolic functions and quality of life.[1] A negative prognostic indicator for dogs with CKD is persistent proteinuria,[2] which causes alterations in the immune system, hormonal status, and electrolyte metabolism.[3] Further, a urinary protein-to-creatinine (UPC) ratio greater than 0.5 is associated with reduced survival and greater hazard ratio in dogs with CKD.[4]

Leptin and adiponectin are the most well-characterized adipokines in veterinary medicine.[5] Leptin regulates the immune system, energy homeostasis, and insulin sensitivity and is an important mediator of inflammatory processes and immune-mediated diseases.[5,6] In contrast, adiponectin has multifunctional roles, including enhancement of insulin sensitivity, regulation of inflammation, and inhibition of atherosclerosis progression by suppressing tumor necrosis factor (TNF)-α.[5,7] In addition, pro-inflammatory cytokines such as interleukin (IL)-1, IL-6, IL-8, IL-10, and TNF-α originate from adipose tissue and are therefore considered to be adipokines.[5]

Recent studies have examined the association between serum/plasma adipokine concentrations and CKD in humans.[7-9] A positive correlation between plasma leptin concentration and prevalence of CKD in adult humans has been identified.[8] In addition, plasma adiponectin concentration is negatively correlated with the degree of proteinuria,[10] a negative prognostic factor of CKD, although adiponectin is clearly increased in humans with nephrotic syndrome.[11] Serum adiponectin concentration is positively associated with case fatality rate in humans with CKD.[9] However, there is a paucity of literature on circulating adipokine concentrations in dogs with CKD.

This study hypothesized that serum leptin concentration would be associated with the severity of CKD and serum adiponectin concentration would be associated with the severity of proteinuria. The objective of this study was to examine the relationship between concentrations of adipokines, including leptin, adiponectin, and pro-inflammatory cytokines such as IL-6, IL-10, IL-18, and TNF-α, and the severity of CKD in dogs.

2 | MATERIALS AND METHODS

2.1 | Case selection

A total of 89 dogs with CKD were enrolled between July 2017 and May 2021. Informed consent was obtained from the owners. This study was approved by the Ethics Committee of Chungbuk National

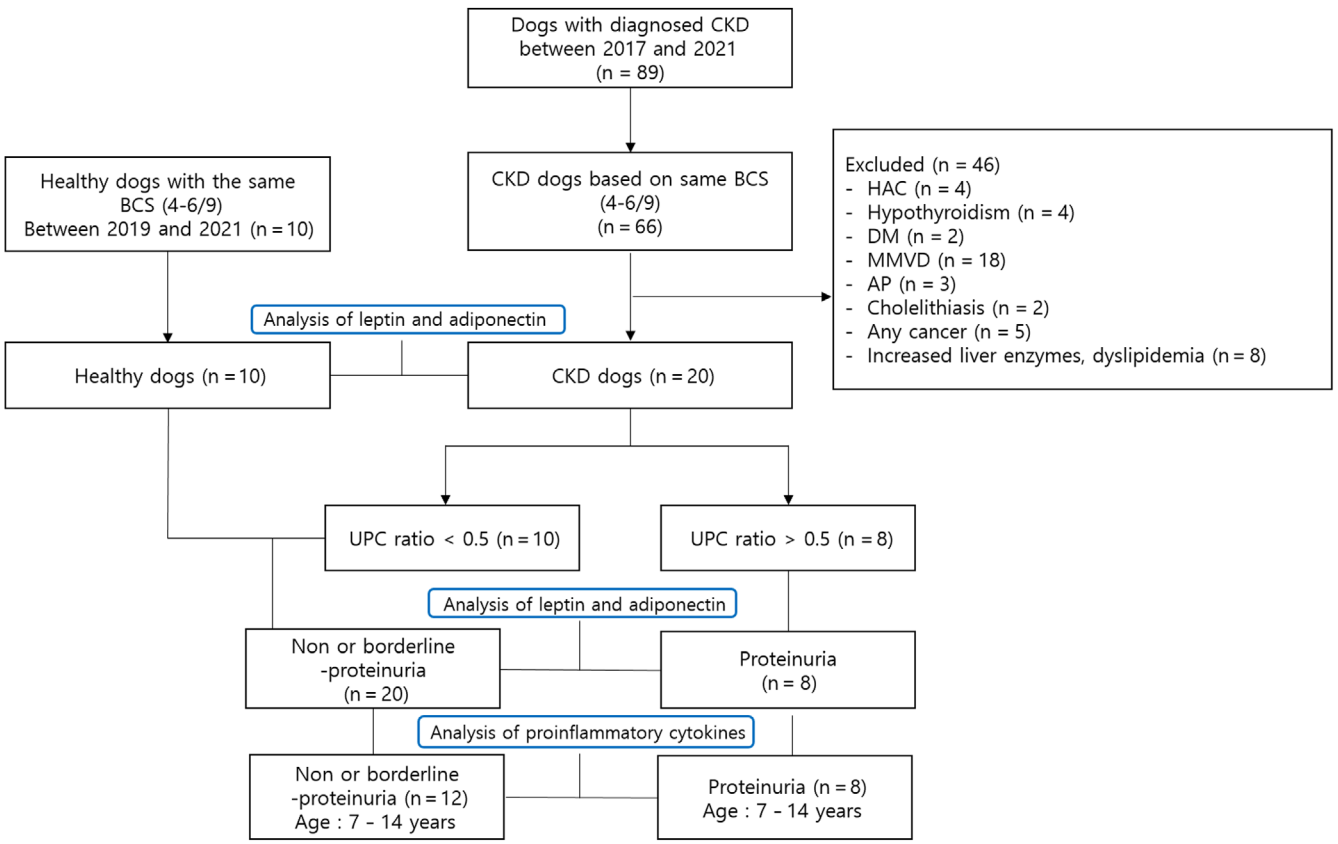

FIGURE 1 Flow diagram of the enrollment of cases in the present study. AP, acute pancreatitis; BCS, body condition score; CKD, chronic kidney disease; DM, diabetes mellitus; HAC, hyperadrenocorticism; MMVD, myxomatous mitral valve disease; UPC, urinary protein-to-creatinine

TABLE 1 Summary of subject characteristics of dogs in the present study

			Dogs with CKD (n = 20)	
	Healthy dogs (n = 10)	Dogs with CKD (n = 20)	IRIS 1 & 2 (n = 11)	IRIS 3 & 4 (n = 9)
Age, years (median; range)	7 (4-15)	10 (3-15)	13 (7-15)	8 (3-14)
Sex (IF/SF/IM/CM)	0/5/1/4	1/9/3/7	0/5/2/4	1/4/1/3
Breed				
Maltese	5	3	3	0
Poodle	1	2	2	0
Mixed breed	1	3	2	1
Others	3	12	4	8
BCS (median; range)	4.5 (4-6)	4 (4-6)	5 (4-6)	4 (4-5)

Note: Data are presented as medians (ranges).
Abbreviations: BCS, body condition score; CKD, chronic kidney disease; CM, castrated male; IF, intact female; IM, intact male, IRIS, International Renal Interest Society; SF, spayed female.

University (CBNUA-1567-21-01). A flow diagram of case enrollment is presented in Figure 1. Given the potential correlation between adipokines and body fat mass in dogs,[5] 23 dogs that were underweight (body condition score [BCS] < 4/9) or obese (BCS > 6/9) based on a 9-point scale,[12] as evaluated by 2 veterinary clinicians, were excluded from enrollment. Exclusion criteria were based on prior diagnosis of concurrent diseases known to affect circulating adipokine concentrations, including hyperadrenocorticism (n = 4), diabetes mellitus (n = 2), hypothyroidism (n = 4), myxomatous mitral valve disease (n = 18), acute pancreatitis (n = 3), cholelithiasis (n = 2), and any cancer (n = 5).[13-17] Subjects with elevated liver enzyme activities (increased alanine aminotransferase or alkaline phosphatase activities more than 3 times of the upper margin) or dyslipidemia that could potentially affect adipokines were also excluded (n = 8). Ten healthy, client-owned dogs with the same BCS (between 4 and 6) were recruited as controls. Dogs with no clinical signs were considered clinically healthy if there were no abnormal findings on the following examinations and tests: physical examination (general appearance, body condition, mentation, posture and gait, hydration status, and vital signs), blood analyses (complete blood count, serum biochemistry profiles, electrolytes), urinalyses (urine dipstick test, sedimentation, UPC), survey radiography (abdominal radiography with or without thoracic radiography), and abdominal ultrasonography. A final total of 30 dogs (10 healthy dogs and 20 dogs with CKD) were enrolled in this cross-sectional study. Among 20 dogs with CKD, 8 had proteinuria (UPC > 0.5). To evaluate the association of pro-inflammatory cytokines with proteinuria and non-proteinuria in dogs with CKD, the difference in age between the proteinuria and non-proteinuria subgroups was evaluated to identify the potential effects of aging on pro-inflammatory cytokine concentrations.[18,19]

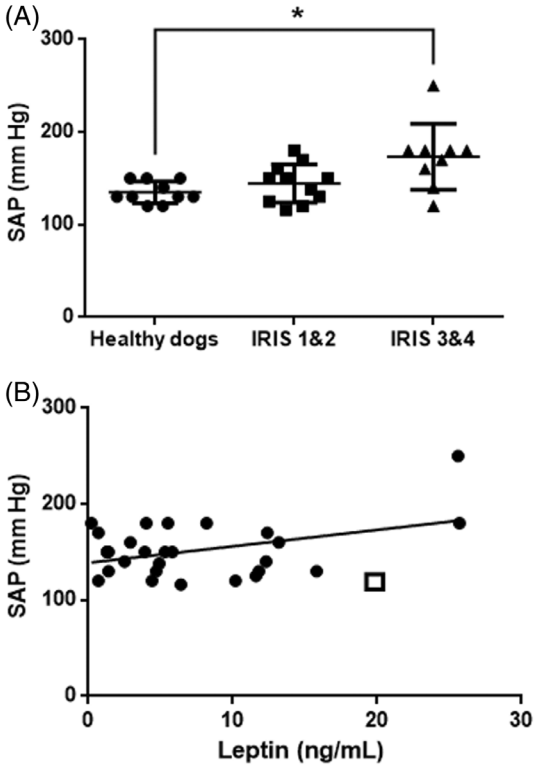

FIGURE 2 Comparison of SAP (A; $P = .01$) in healthy dogs and dogs with CKD according to the IRIS guidelines. Linear associations between the concentrations of leptin and SAP (B; $r = .41$, $P = .03$) are presented. Outliers >1.5 interquartiles from the medians are shown as squares. CKD, chronic kidney disease; IRIS, International Renal Interest Society; SAP, systolic arterial blood pressure. *Adjusted $P < .02$ (Kruskal-Wallis test)

2.2 | Diagnosis and grouping of CKD and proteinuria

CKD was diagnosed based on increases in serum creatinine and symmetric dimethylarginine (SDMA) concentrations and morphologic abnormalities compatible with CKD based on ultrasonographic examination, as previously described.[20-22] The following examinations were performed in all dogs with CKD: physical examination, complete blood count, urinalysis, serum biochemical analysis, and diagnostic imaging including survey radiography and ultrasonography. Systolic arterial

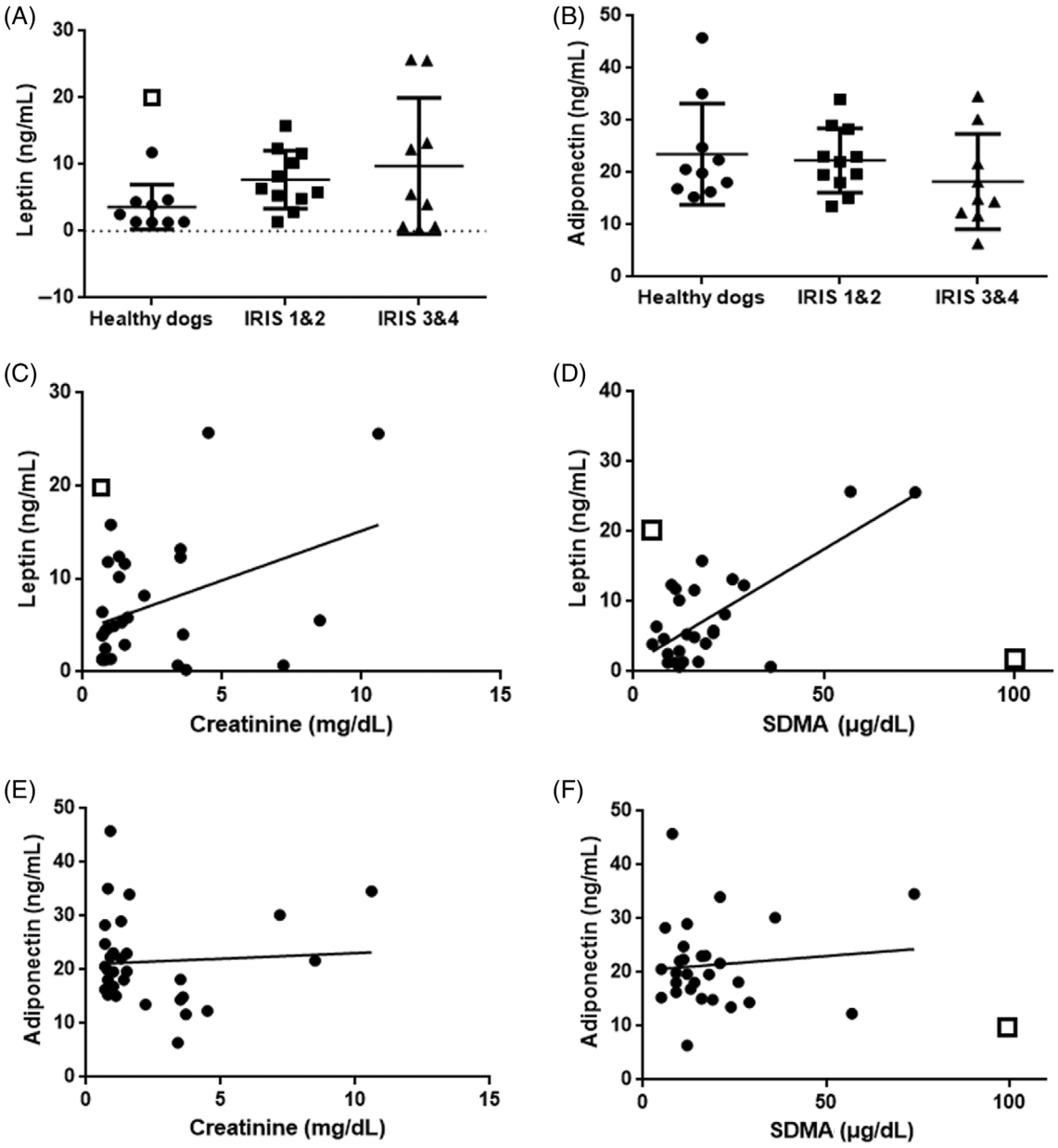

FIGURE 3 Serum concentrations of leptin (A) and adiponectin (B) in healthy dogs and dogs with CKD according to the IRIS guidelines. Linear associations between the concentrations of leptin and creatinine (C; $r = .39$, $P = .03$) and SDMA (D; $r = .73$, $P < .001$) are presented. No associations between the concentrations of adiponectin and creatinine (E) and SDMA (F) are presented. Outliers >1.5 interquartiles from the medians are shown as squares. CKD, chronic kidney disease; IRIS, International Renal Interest Society; SDMA, symmetric dimethylarginine

blood pressure (SAP) was measured using an ultrasonic Doppler flow detector (Model 811 B, Parks Medical Electronics, Inc., Aloha, Oregon). Serum biochemistry was conducted using a biochemical analyzer (Hitachi 7020, Hitachi High-Technologies Co., Tokyo, Japan), UPC ratio and SDMA were measured using a biochemistry analyzer (Catalyst One, IDEXX Laboratories, Westbrook, Maine), and urine specific gravity was measured using a pocket refractometer (PAL-USG, ATAGO. CO., LTD., Tokyo, Japan).

To compare serum adipokine concentrations in healthy dogs and dogs with CKD, 20 dogs diagnosed with CKD were classified into 4 groups according to the International Renal Interest Society (IRIS) guidelines.[20] Dogs with serum SDMA persistently greater than 14 µg/dL and less than 18 µg/dL were assigned to IRIS stage 1.[20] Dogs were also assigned to IRIS stage 1 if 1 or more of the following diagnostic findings were identified: persistent renal proteinuria (UPC ratio > 0.5) and ultrasonographic abnormalities consistent with CKD (eg, loss of cortico-medullary junction, irregular contours, and decreased renal size).[20,22] Dogs with mildly increased (1.4-2.8 mg/dL), moderately increased (2.9-5.0 mg/dL), and severely increased (> 5.0 mg/dL) creatinine concentration were assigned to IRIS stage 2, 3, and

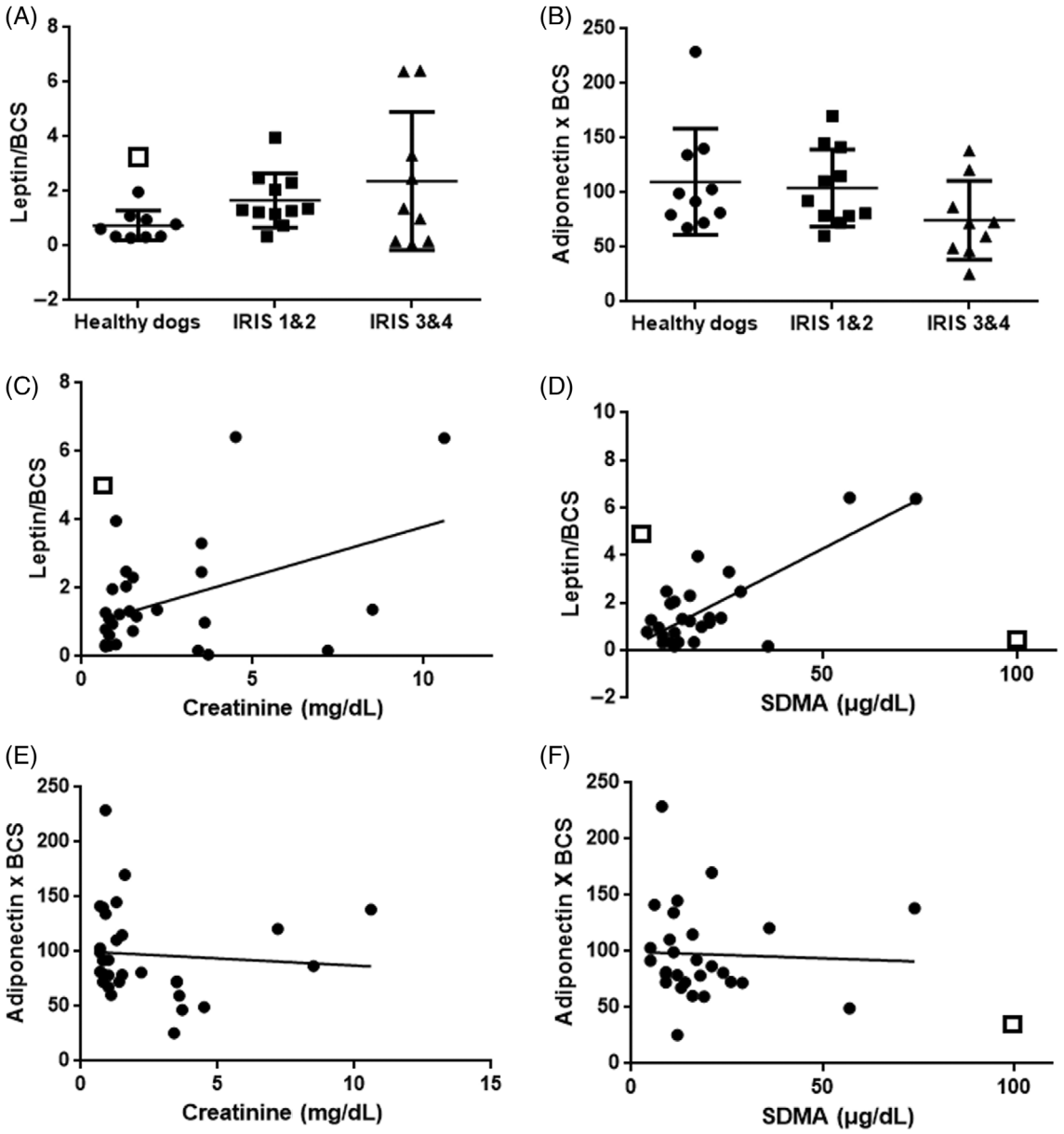

FIGURE 4 The value of leptin/BCS (A) and adiponectin × BCS (B) in healthy dogs and dogs with CKD according to the IRIS guidelines. Linear associations between leptin/BCS values and creatinine concentrations (C; $r = .44$, $P = .02$) and SDMA concentrations (D; $r = .77$, $P < .001$) are presented. No associations between adiponectin × BCS values and creatinine (E) and SDMA concentrations (F) are presented. Outliers >1.5 interquartiles from the medians are shown as squares. BCS, body condition score; CKD, chronic kidney disease; IRIS, International Renal Interest Society; SDMA, symmetric dimethylarginine

4, respectively.[20] The classification of proteinuria in dogs with CKD was based on IRIS guidelines[20]: the non-proteinuric, borderline proteinuric, and proteinuric groups had a UPC < 0.2, between 0.2 and 0.5, and >0.5, respectively.

2.3 | Analyses of serum adipokine concentrations

Dogs were fasted for at least 12 hours before venipuncture. Blood was obtained from the peripheral vein or jugular vein into plain tubes. Serum was separated from clotted whole blood by centrifugation at 1200*g* for 10 minutes within 1 hour of blood collection. One portion of serum was used for blood analysis measurements. The remainder was frozen at −80°C until the assay and then batch analyzed. The following adipokines were analyzed: leptin, adiponectin, IL-6, IL-10, IL-18, and TNF-α.

Serum leptin concentrations were analyzed using a canine-specific ELISA kit (Canine Leptin ELISA, Millipore Co., Billerica, Massachusetts), which had intra-assay and interassay variabilities of 4% and 6%, respectively, and a sensitivity of 0.4 ng/mL, according to the

manufacturer's protocol. Serum adiponectin concentrations were analyzed using a canine-specific ELISA kit (Canine Adiponectin ELISA, MyBioSource Co., San Diego, California), which had intra-assay and interassay variabilities of <8% and < 12%, respectively, and a sensitivity of up to 0.5 ng/mL, according to the manufacturer's protocol. Serum leptin and adiponectin concentrations in all samples, standards, and controls were assayed in duplicate. Optical density was determined with an automated microplate reader (ELx 808, BioTek Instruments Inc., Winooski, VT, USA) at 450 nm.

Serum IL-6, IL-10, IL-18, and TNF-α concentrations were analyzed with a Milliplex MAP kit (Canine Cytokine/Chemokine MAGNETIC kit, Millipore Co., Billerica, Massachusetts). Intra-assay and inter-assay variabilities of these assays were <4% and <17%, respectively, and the assay sensitivities for IL-6, IL-10, IL-18, and TNF-α were 3.7, 8.5, 5.8, and 6.1 pg/mL, respectively, according to the manufacturer's protocol. The assays were quantified with a Luminex system (Luminex 200, Luminex Co., Billerica, Massachusetts).

Circulating leptin concentration is positively correlated with BCS, whereas adiponectin concentration is negatively correlated with BCS.[5] Accordingly, the value of leptin/BCS was derived by dividing serum leptin concentrations by BCS, and the value of adiponectin × BCS was derived by multiplying serum adiponectin concentrations by BCS to minimize the effect of BCS on serum leptin and adiponectin concentrations. The leptin-to-adiponectin ratio (LAR) was derived by dividing serum leptin concentrations by serum adiponectin concentrations.

2.4 | Statistical analyses

Data were analyzed using commercially available statistical software (Prism 6, GraphPad Software Inc., La Jolla, California). All data were expressed as medians (ranges). *P*-values were calculated using 2-tailed tests, and 95% confidence intervals (CIs) for differences between medians were evaluated. The D'Agostino-Pearson omnibus test was performed to determine whether data were normally distributed. *P*-values < .05 were considered statistically significant. The Kruskal-Wallis test was used to evaluate differences in SAP and serum concentrations of leptin and adiponectin, as well as differences in leptin/BCS, adiponectin × BCS value, and LAR among 3 groups (healthy, IRIS 1&2, and IRIS 3&4). If a significant difference was detected, a pairwise comparison was performed using the Mann-Whitney *U*-test with Bonferroni-adjusted *P*-values, for which a value of $P < .02$ was considered statistically significant. The correlations between SAP and adipokine concentrations were evaluated using Pearson or Spearman's correlation tests. The concentrations of adiponectin, leptin, IL-6, IL-10, IL-18, and TNF-α and age were compared between 2 groups (proteinuric group and non-proteinuric, borderline proteinuric group) using the Mann-Whitney *U*-test. The correlations of adipokine (leptin or adiponectin) concentrations with creatinine concentration, SDMA concentration, and UPC ratio, as well as the correlations of leptin/BCS, adiponectin × BCS value, and LAR with creatinine and SDMA concentrations, were evaluated using Pearson or Spearman's correlation tests.

3 | RESULTS

3.1 | Subject characteristics and differences in SAP between dogs with CKD and healthy dogs

The characteristics of the dogs are presented in Table 1. Significant differences were observed in median SAP among the healthy, IRIS stage 1&2, and IRIS stage 3&4 groups ($P = .02$; Figure 2A). Significant differences of SAP were observed among the healthy, IRIS stage 1&2, and IRIS stage 3&4 groups ($P = .02$; Figure 2A). Systolic arterial blood pressure (median [range]) in the IRIS 3&4 group (180.0 [120.0-250.0] mm Hg) was significantly higher than that in healthy dogs (130.0

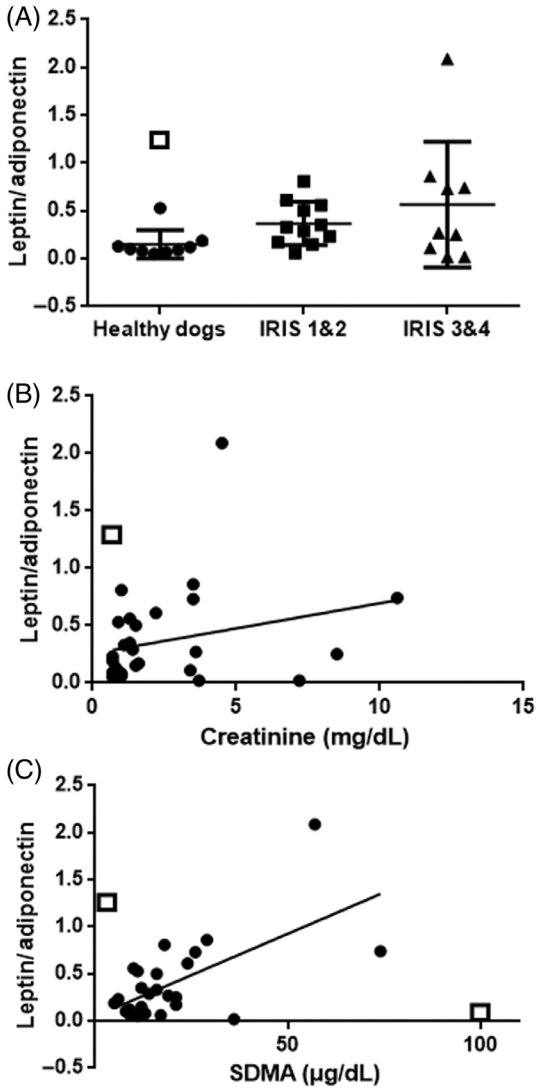

FIGURE 5 The LAR in healthy dogs and dogs with CKD according to the IRIS guidelines (A). There is no association between LAR and creatinine concentrations (B). Spearman's correlation between LAR and SDMA concentrations (C; $r_s = .46$, $P = .02$) is presented. Outliers >1.5 interquartiles from the medians are shown as squares. CKD, chronic kidney disease; LAR, leptin-to-adiponectin ratio; IRIS, International Renal Interest Society; SDMA, symmetric dimethylarginine

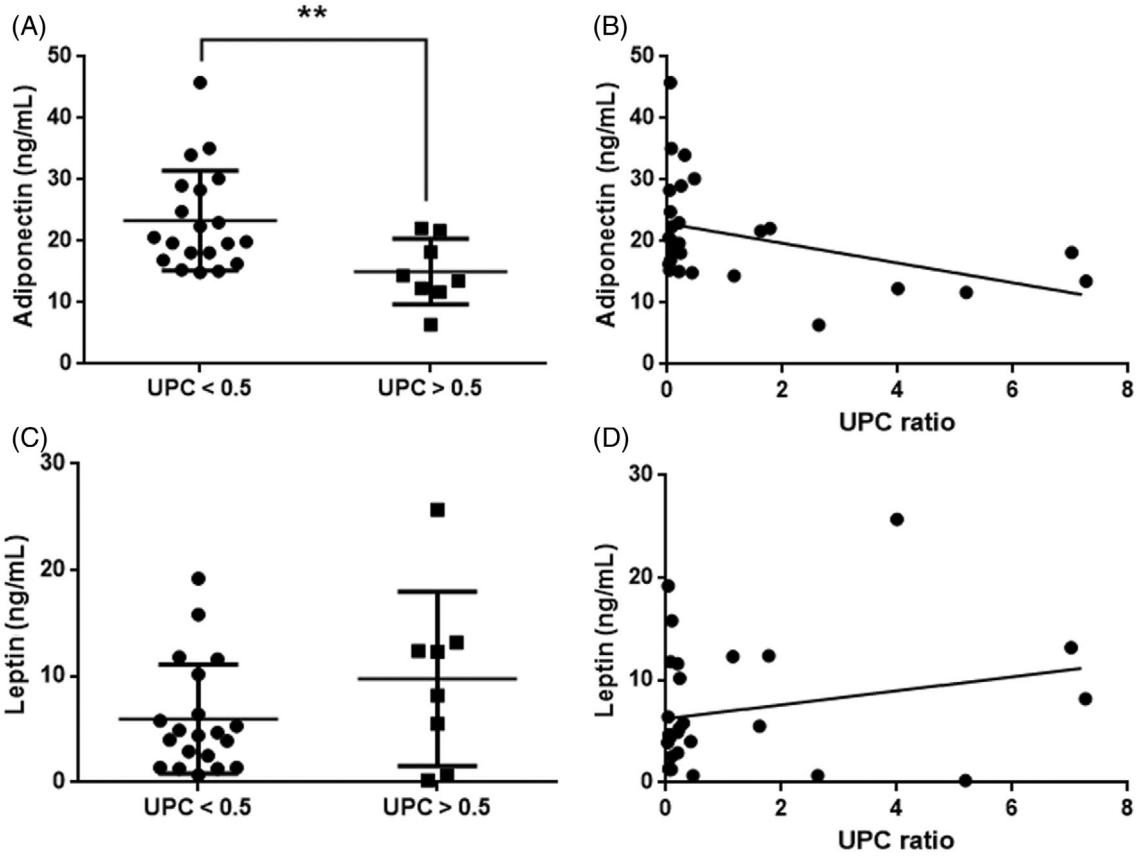

FIGURE 6 Comparisons of circulating concentrations of adiponectin (A; $P = .01$) and leptin (C) between borderline or non-proteinuric and proteinuric CKD dogs. Linear association between UPC ratio and adiponectin concentrations (B; $r = .41$, $P = .03$) is presented. There is no correlation between UPC ratio and leptin concentrations (D). CKD, chronic kidney disease; UPC, urinary protein-to-creatinine. $^{**}P < .01$ (Mann-Whitney *U*-test)

[120.0-150.0] mm Hg; $P = .01$). Systolic arterial blood pressure was positively correlated with leptin concentrations ($r = .41$, 95% CI: 0.05 to 0.68, $P = .03$; Figure 2B). No significant correlations were observed between SAP and concentrations of adiponectin ($P = .95$) or pro-inflammatory cytokines, including IL-6 ($P = .12$), IL-10 ($P = .06$), and TNF-α ($P = .06$). Systolic arterial blood pressure was significantly correlated with IL-18 concentrations ($r_s = .57$, 95% CI: 0.15 to 0.82, $P = .01$).

3.2 | Concentrations of leptin and adiponectin in healthy dogs and dogs with CKD

No significant differences were noted in serum leptin concentrations ($P = .12$; Figure 3A) and adiponectin concentrations ($P = .24$; Figure 3B) among groups. Leptin concentrations were positively correlated with creatinine ($r = .39$, 95% CI: 0.03 to 0.66, $P = .03$; Figure 3C) and SDMA ($r = .73$, 95% CI: 0.48 to 0.87, $P < .001$; Figure 3D) concentrations. No correlation was observed between creatinine and adiponectin concentrations ($P = .75$; Figure 3E) or between SDMA and adiponectin concentrations ($P = .61$; Figure 3F).

3.3 | Differences in leptin/BCS and adiponectin × BCS values between dogs with CKD and healthy dogs

No significant differences were observed in leptin/BCS and adiponectin × BCS values among groups ($P = .09$ and $P = .09$, respectively; Figure 4A, B). Leptin/BCS was positively correlated with creatinine ($r = .44$, 95% CI: 0.09 to 0.70, $P = .02$; Figure 4C) and SDMA ($r = .77$, 95% CI: 0.56 to 0.89, $P < .001$; Figure 4D) concentrations. No correlations were noted between adiponectin × BCS values and concentrations of creatinine ($P = .68$; Figure 4E) or SDMA ($P = .83$; Figure 4F).

3.4 | Differences in LAR between dogs with CKD and healthy dogs

No significant difference was observed in LAR among groups ($P = .07$; Figure 5A). There was no correlation between creatinine concentrations and LAR ($P = .17$; Figure 5B). However, LAR was positively correlated with SDMA concentrations ($r_s = .46$, 95% CI: 0.08 to 0.72, $P = .02$; Figure 5C).

3.5 | Differences between non-proteinuric and proteinuric dogs with CKD

Adiponectin concentrations (median [range]) were significantly lower in CKD dogs with proteinuria (13.95 [6.4-22.1] ng/mL) than in those with borderline or non-proteinuria (20.25 [14.9-45.8] ng/mL; $P = .01$; Figure 6A). Adiponectin concentrations were negatively correlated with UPC ratio ($r = .41$, 95% CI: −0.68 to −0.04, $P = .03$; Figure 6B). No significant difference was noted in leptin concentrations between the groups ($P = .26$; Figure 6C). There was no correlation between leptin concentrations and UPC ratio ($P = .24$; Figure 6D). Serum TNF-α concentrations (median [range]) were significantly higher in proteinuric CKD dogs (11.44 [8.54-38.45] pg/mL) than in borderline or non-proteinuric CKD dogs (6.105 [3.97-30.68] pg/mL; $P = .02$; Figure 7A). Serum IL-6 concentrations (median [range]) were significantly higher in proteinuric CKD dogs (43.27 [24.30-537.30] pg/mL) than in borderline or non-proteinuric CKD dogs (25.63 [6.83-61.03] pg/mL; $P = .02$; Figure 7B). No significant difference was observed in median IL-10 concentrations between the groups ($P = .64$; Figure 7C). Serum IL-18 concentrations (median [range]) were significantly higher in proteinuric CKD dogs (25.98 [11.52-280.55] pg/mL) than in borderline or non-proteinuric CKD dogs (10.77 [3.53-38.45] pg/mL; $P = .01$; Figure 7D). No significant difference was observed in age between the groups ($P = .58$).

4 | DISCUSSION

The present study examined the relationship between circulating adipokine concentrations and severity of CKD in dogs. The dogs with CKD were staged based on creatinine and SDMA concentrations and substaged based on SAP and proteinuria.[20] Circulating leptin concentrations were positively correlated with SAP, creatinine concentrations, and SDMA, which are markers of CKD in dogs. Conversely, circulating adiponectin concentrations were negatively correlated with UPC ratio in dogs. Additionally, the concentrations of most pro-inflammatory cytokines, including TNF-α, IL-6, and IL-18, were significantly higher in proteinuric dogs than in borderline or non-proteinuric dogs. Collectively, these results might suggest that adipokine imbalances are associated with the severity of CKD in dogs.

Correlation analysis revealed positive correlations between leptin concentrations and SAP, serum creatinine concentrations, and SDMA, which are indicative of renal function in dogs. Further, SAP was significantly higher in the IRIS 3&4 group than in healthy dogs. Leptin elevates sympathetic nervous system activity, contributing to upregulation of renal sodium reabsorption.[23] Consequently, it might cause systemic hypertension and aggravate renal dysfunction.[23] However, leptin is predominantly removed from the circulation by the kidneys.[24,25] Therefore, it could also be possible that elevated serum leptin concentration might be a consequence, rather than a cause, of

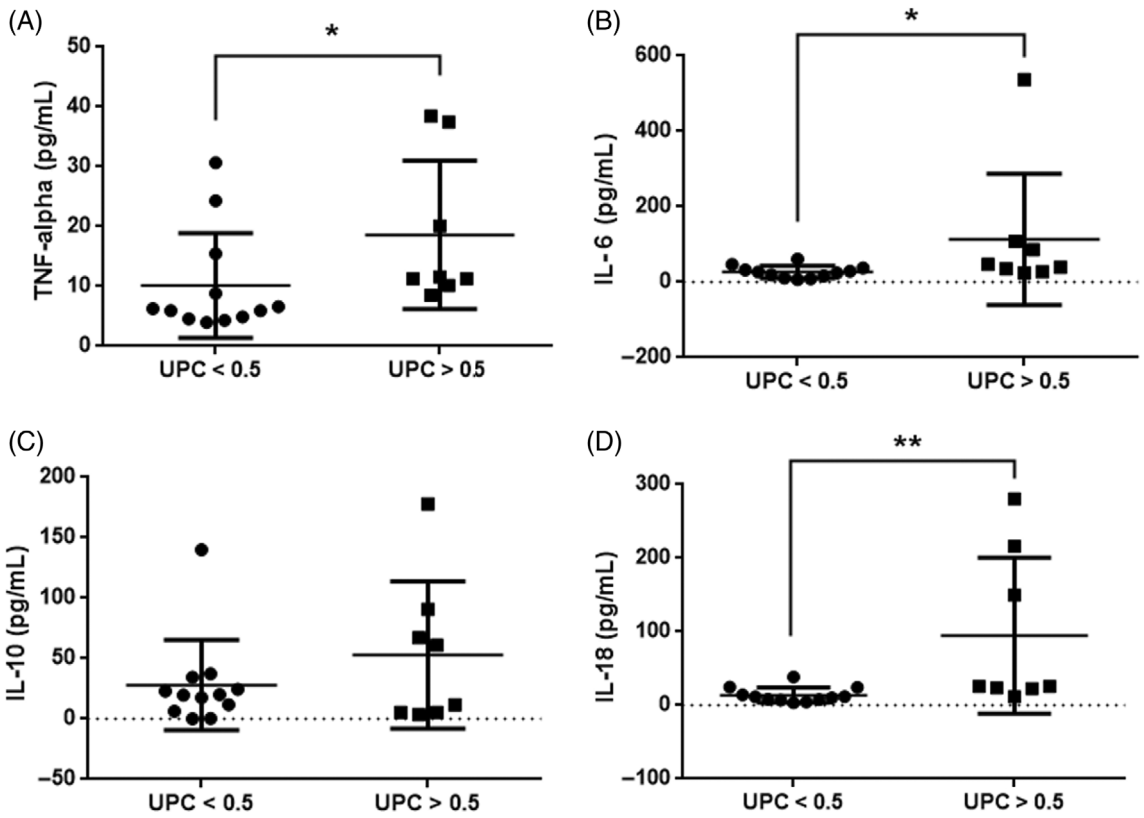

FIGURE 7 Comparisons of serum TNF- α (A; $P = .02$), IL-6 (B; $P = .02$), IL-10 (C), and IL-18 (D; $P = .01$) between borderline or non-proteinuric (aged 7-14 years, n = 12) and proteinuric CKD dogs (aged 7-14 years, n = 8). CKD, chronic kidney disease; IL, interleukin; TNF, tumor necrosis factor; UPC, urinary protein-to-creatinine. *$P < .05$, **$P < .01$ (Mann-Whitney U-test)

deterioration in renal function due to CKD progression. In addition, leptin acts as a hormone that stimulates the hypothalamus to reduce appetite.[26] In other words, hyporexia in CKD might be due to increased serum leptin concentration as well as azotemia. Therefore, it was challenging to delineate the causal relationship between elevated serum leptin concentration and severity of CKD in this study. Notably, although leptin concentrations were correlated with creatinine concentrations and SDMA, there was no significant difference in serum leptin concentrations among healthy, IRIS stage 1&2, and IRIS stage 3&4 groups. In human medicine, glomerular filtration rate (GFR) is used for staging and classifying CKD into 5 stages.[27] Progression of CKD is characterized by a decrease in GFR, and these changes are associated with plasma leptin concentrations in humans.[8] However, GFR is not routinely measured in dogs in clinical practice. In this regard, creatinine concentration is used as a surrogate biomarker for staging and artificially classifying CKD into 4 stages according to IRIS guidelines.[20] Although SDMA concentrations can be used to classify CKD, staging based on SDMA concentrations was not conducted in the present study due to the small number of patients. Measurement of GFR, rather than creatinine concentrations, remains the gold standard for evaluating CKD severity, but its application is limited in veterinary medicine. It was suspected that the serum leptin concentrations were not significantly different among the study groups despite significant correlations of leptin concentrations with creatinine and SDMA concentrations because of inappropriate classification of CKD in some dogs.

There is a relationship between hypoadiponectinemia and progression of renal damage in human patients with type 2 diabetes.[7,23] However, in the present study, there was no correlation between adiponectin and serum concentrations of creatinine or SDMA, in contrast to the results obtained for leptin. In vivo studies in rats exposed to intermittent and chronic hypoxia demonstrated that injections of adiponectin reduced renal cell apoptosis, suggesting that adiponectin attenuated endoplasmic reticulum stress and generation of reactive oxygen species in renal tissue.[28] However, CKD is paradoxically associated with increased serum adiponectin concentrations, which are positively associated with death in human patients with CKD.[9] The nature of this paradox in CKD remains unclear. Potential factors underscoring these effects include low renal clearance or a compensatory mechanism for increasing damage caused by renal insufficiency.[7,9] One study demonstrated that adiponectin was weakly affected by kidney function and was more strongly associated with metabolic disorders.[29] These inconsistent findings highlight the complex relationship between adiponectin concentrations and CKD severity. Furthermore, adiponectin has multiple isoforms, and biological functions vary based on molecular weight: low (trimer), medium (hexamer), and high (multimer). In humans, high-molecular weight adiponectin has the most potent biological effects, and different adiponectin isoforms might be involved in the pathogenesis of CKD.[30,31] However, the different behavior of adiponectin isoforms in the context of CKD cannot be elucidated because only total serum adiponectin concentration was measured in the present study.

Body condition score is positively correlated with circulating leptin concentrations and negatively correlated with adiponectin concentrations.[5] Since our study used data from dogs with BCS 4 to 6, additional analyses were performed based on leptin/BCS and adiponectin × BCS to exclude the effect of BCS. There were significant correlations between leptin/BCS and concentrations of creatinine or SDMA. In contrast, adiponectin × BCS and concentrations of creatinine or SDMA were not significantly correlated. These findings suggest that BCS does not affect the relationship between CKD severity and imbalances in adipokines in dogs. Nevertheless, it is possible that weight loss in CKD affects serum leptin concentrations via negative feedback[32] or by affecting creatinine concentrations, which are positively correlated with muscle mass.[33]

It has been demonstrated that LAR is positively associated with CKD, reflecting a stronger positive correlation between CKD and leptin compared to that with adiponectin.[34] In humans, LAR is known to be a more accurate predictor of cardiovascular diseases compared to leptin alone. In a study of patients with obesity, LAR was positively correlated with pulse wave velocity, which is a potential atherogenic index in humans; however, no such correlation was observed with leptin alone.[35] Chronic kidney disease in dogs and cardiovascular disease in humans share a similar pathophysiology involving endothelial damage.[34,36] In the present study, leptin concentrations were correlated with both creatinine and SDMA concentrations. In contrast, LAR was not correlated with creatinine concentrations but was significantly correlated with SDMA concentrations. Compared to creatinine, SDMA is a more sensitive and earlier biomarker of a decrease in GFR.[21] Collectively, these findings suggest that LAR might be a more accurate predictor of CKD compared to leptin alone in dogs, similar to findings in humans.

Proteinuria reflects renal endothelial dysfunction, which results in altered production of vasoactive mediators, increased permeability, and chronic inflammatory responses.[37,38] The decrease in adiponectin concentration was reported to be greater in humans with nephrotic proteinuria than in those without nephrotic proteinuria.[10] Similarly, in the present study, there was a significant inverse correlation between serum adiponectin concentrations and UPC ratio. Adiponectin deficiency might be associated with endothelial damage.[39] Damage to the endothelial wall might lead to accumulation of adiponectin in the vascular wall, which consumes circulating adiponectin and might contribute to hypoadiponectinemia.[10] An inverse correlation between serum adiponectin concentration and GFR has been revealed,[40] but increased serum concentration of adiponectin has also been identified in humans with nephrotic syndrome.[11] Although the exact mechanism is not known, increased concentration of urinary adiponectin might contribute to altered glomerular permeability.[41] Furthermore, the urinary concentration of adiponectin showed a positive correlation with the urinary albumin-to-creatinine ratio, and the serum concentration of adiponectin revealed a negative correlation with the urinary albumin-to-creatinine ratio.[42] Therefore, further studies with creatinine-adjusted adiponectin concentrations in urine samples are needed to identify the relationship between adiponectin and proteinuria.

Additionally, adiponectin inhibits TNF-α and is negatively correlated with pro-inflammatory cytokines such as IL-6.[43,44] In this study, median concentrations of pro-inflammatory cytokines (TNF-α, IL-6, IL-10, and IL-18) were not significantly different between proteinuric

dogs and borderline or non-proteinuric dogs (data not shown). However, after age-matching based on the ages of proteinuric dogs with CKD (7-14 years) to minimize the potential effects of aging on pro-inflammatory cytokine concentrations,[18,19] the concentrations of the majority of pro-inflammatory cytokines, including TNF-α, IL-6, and IL-18, were significantly higher in proteinuric dogs than in borderline or non-proteinuric dogs. Based on those findings, hypoadiponectinemia might cause endothelial dysfunction associated with proteinuria by exacerbating inflammatory status because of decreased inhibition of TNF-α or pro-inflammatory cytokines.[37,43,44]

This study has several limitations. First, GFR, which is the gold standard for evaluating CKD severity was not analyzed. Rather, we used creatinine concentrations as a surrogate biomarker for staging CKD, which was artificially classified into 4 stages according to IRIS guidelines. In addition, SDMA, which is a more sensitive and earlier biomarker of reduced GFR compared to creatinine, was also measured. Second, due to the small number of animals with CKD, dogs with IRIS stage 1 and 2 and those with IRIS stage 3 and 4 were grouped into single groups, which could have contributed to false negative results. Moreover, the weak correlation between each variable might be due to the small sample size and strict inclusion and exclusion criteria. Third, the BCS, a semiquantitative method for evaluating body composition, was used to prevent the effect of adiposity on the concentration of adipokines. Due to the semiquantitative characteristics of BCS, dual-energy X-ray absorptiometry, which is the gold standard for composition analysis, might be considered to analyze the effect of adiposity on serum adipokine concentrations more accurately although it could be hard to perform in clinical settings.[45] Fourth, the concentration of adiponectin might be affected by the medications that were prescribed for CKD. It is known that angiotensin II receptor type 1 blockers and angiotensin-converting enzyme inhibitors increase the concentration of adiponectin in humans.[46] In the present study, some dogs with CKD (5/20; enalapril [n = 2], stage 1 with proteinuria, stage 2 with non-proteinuria; benazepril [n = 2], stage 3 with borderline proteinuria, stage 4 with proteinuria; benazepril + telmisartan [n = 1], stage 2 with proteinuria) were receiving angiotensin-converting enzyme inhibitors (benazepril or enalapril) or angiotensin II receptor type 1 blockers (telmisartan) at the time of this study. Another limitation is that our study was not a longitudinal cohort study. Chronic kidney disease is a disease that progresses over time, without complete recovery. Therefore, it has been unable to track alterations in adipokine concentrations with the management and treatment of CKD. It was also difficult to investigate the causal relationship between alterations in adipokine concentrations and progression of CKD in this study. Further studies that chart the progression of CKD in individual dogs are warranted.

5 | CONCLUSIONS

The present study suggests that severity of CKD and proteinuria are associated with imbalances of adipokines in dogs. In particular, circulating concentrations of leptin are positively associated with systemic hypertension and aggravation of renal dysfunction. In contrast, circulating concentrations of adiponectin are negatively associated with the severity of proteinuria. The current findings provide a basis for future research on the effects of adipokine imbalances on CKD progression. Further studies are required to investigate the progression of CKD caused by other diseases that alter serum adipokine concentrations.

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CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Approved by the IACUC at Chungbuk National University (CBNUA-1567-21-01).

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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