J Vet Intern Med 2008;22:273–281

The Effects of Hydrocortisone on Systemic Arterial Blood Pressure and Urinary Protein Excretion in Dogs

S. Schellenberg, M. Mettler, F. Gentilini, R. Portmann, T.M. Glaus, and C.E. Reusch

Background: Hypertension and proteinuria are commonly recognized in dogs with spontaneous hypercortisolism. There is, however, little information regarding the effect of exogenous glucocorticoids on blood pressure (BP) and proteinuria and whether these changes are reversible.

Hypothesis: Hydrocortisone administration increases systemic BP and urinary protein excretion, and these effects are reversible after hydrocortisone withdrawal.

Animals: Six control dogs and 6 dogs treated with hydrocortisone.

Methods: BP, urine protein : creatinine ratio (UPC), microalbuminuria (MALB), urine albumin : creatinine ratio (UAC), and urine gel electrophoresis were evaluated before, during, and after administration of hydrocortisone (8 mg/kg PO q12h for 12 weeks) or placebo.

Results: BP and UPC increased substantially during hydrocortisone administration from 123 mmHg (range 114–136 mmHg) and 0.17 (0.15–0.28) to a maximum of 143 mmHg (128–148 mmHg) and 0.38 (0.18–1.78), respectively, on day 28. MALB developed in 4 dogs and UAC significantly increased in all dogs during hydrocortisone administration with the maximum on day 84. Both increases in BP and proteinuria were reversible and completely resolved within 1 month after stopping hydrocortisone administration. SDS-AGE revealed the proteinuria to be primarily albuminuria with a pronounced increase during hydrocortisone treatment. Furthermore, a protein of 25–30 kDa was found in male dogs, identified by mass spectrometry to be arginine esterase, the major secretory prostatic protein.

Conclusions and Clinical Importance: Long-term hydrocortisone treatment results in significant but only mild increases in systemic BP and urinary protein excretion, which are both reversible within 1 month after discontinuation of hydrocortisone.

Key words: Albuminuria; Arginine esterase; Hypercortisolism; Hypertension; Proteinuria.

Hypertension is a frequent finding in humans and dogs with exogenous or endogenous glucocorticoid excess. The prevalence of systemic hypertension in human patients with spontaneous (endogenous) hypercortisolism (HC) has been reported to be approximately 80%.[1] In experimental studies in humans, glucocorticoids have been shown to increase systolic (SAP) as well as diastolic (DAP) arterial blood pressure.[2,3] Substantial increases in SAP were identified within 24 hours of cortisol administration, but iatrogenic glucocorticoid-induced hypertension appears to be less severe than in the natural disease.[2] The prevalence of hypertension in dogs with spontaneous HC is similar at 59–86%.[4,5] When treating dogs with spontaneous HC, adequate suppression of cortisol secretion results in decreasing blood pressure (BP); however, hypertension is still common in the population of well controlled HC.[4] In contrast, in experimental dogs treated with hydrocortisone for several weeks, SAP was not significantly higher compared with placebo-treated dogs.[6]

From the Clinic for Small Animal Internal, Medicine, Vetsuisse Faculty University of Zurich, Winterthurerstr, Zurich, Switzerland (Schellenberg, Mettler, Glaus, Reusch); the Veterinary Clinical Department, Alma Mater Studiorum, University of Bologna, Ozzano Emilia, Bologna, Italy (Gentilini); and the Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland (Portmann). *An abstract of this study has been presented at the 2006 ACVIM Forum in Louisville, KY (J Vet Int Med 20, 741).*

Corresponding author: Prof Dr Claudia E. Reusch, Clinic for Small Animal Internal Medicine, Vetsuisse Faculty University of Zurich, Winterthurerstr. 260 CH-8057 Zurich, Switzerland; e-mail: creusch@vetclinics.unizh.ch.

Submitted July 5, 2007; Revised September 19, 2007; Accepted October 8, 2007.

10.1111/j.1939-1676.2007.0039.x

Proteinuria is also commonly observed in dogs with HC. The incidence of pathological proteinuria, defined as a urine protein : creatinine ratio (UPC) greater than 1.0, has been reported to be 44–75%.[4,7] Experimentally, long-term administration of prednisolone resulted in proteinuria of glomerular origin.[8] The reversibility of proteinuria in spontaneous HC is controversial. Whereas Ortega et al found a significant decrease in UPC in dogs with well-controlled HC, Hurley and Vaden did not.[4,7]

Much of the existing information about the effects of glucocorticoids on BP and proteinuria has been generated by experimental studies that used prednisolone for the induction of proteinuria and hypertension. However, in spontaneous HC, the endogenous glucocorticoid hydrocortisone seems to be the major cause of clinical and laboratory abnormalities.[9]

In view of the paucity of knowledge on the effect of hydrocortisone on systemic BP, urinary protein excretion, and particularly the reversibility of such changes in dogs, the purpose of this study was to induce experimental HC with hydrocortisone as an attempt to most closely mimic the natural disease. The specific goals then were first, to assess the developement and severity of hypertension, second, to quantitate and characterize the glucocorticoid-induced proteinuria, third, to assess the association between urinary protein excretion and BP, and fourth, to study the reversibility of these changes.

Material and Methods

This randomized, placebo-controlled study was approved by the Cantonal Veterinary Office (Canton of Zurich, Zurich, Switzerland).

Dogs

The study was conducted with twelve 3.5-year-old Beagle dogs, 6 intact males and 6 intact females, with a body weight ranging from 10.4 to 16.6 kg (median 12.9 kg). The dogs were determined to be healthy on the basis of physical examination, CBC, serum biochemistry profile, urinalysis, urine culture, UPC ratio, and indirect BP measurement.

Study Design

HC was induced by hydrocortisone, the synthetic glucocorticoid most similar to cortisol. The dosage was chosen extrapolating from previous studies inducing I-HC in dogs.[6,8] Dogs were randomly allocated to 2 groups of 6 dogs. Dogs in the control group received a placebo gelatin capsule PO q12h, whereas dogs in the treatment group received hydrocortisone[a] at a median dose of 8.5 mg/kg (range of 7.5–9.6 mg/kg) PO q12h for 84 days (I-HC group). Dogs were examined before (d0) and 1 (d1), 5 (d5), 28 (d28), 56 (d56), and 84 (d84) days after starting treatment, and 1 (d1p), 5 (d5p), 28 (d28p), 56 (d56p), and 84 (d84p) days after withdrawal of hydrocortisone and placebo, respectively. Dogs were observed for typical cortisol-induced clinical abnormalities, including PU/PD and hair loss, but only subjectively; ie water intake and urine production were not quantitated and it was not attempted to use a scoring system for the skin abnormalities. On d0, d28, d56, d84, and d1p, an ACTH stimulation test was performed in all dogs by obtaining samples for determination of cortisol before and 1 hour after IM injection of 0.25 mg of synthetic ACTH.[b] Cortisol concentrations were determined by use of a previously validated chemiluminescence method (ADVIA Centaur® System, Bayer [Schweiz] AG, Zurich, Switzerland).[10]

BP Measurement

Before the beginning of the study, dogs were acclimated to the BP measurement procedure on 12 different days, to minimize excitement and anxiety during the study.[c] Systolic (SAP), diastolic (DAP), and mean (MAP) arterial pressure were measured with an indirect oscillometric device.[d] An inflatable cuff of approximately 40% of the tail circumference was placed directly around the base of the tail without clipping hair.[11] Before recording BP, dogs were placed in left lateral recumbency and allowed to acclimate to the surroundings for at least 10 minutes, and the 1st BP readings were discarded. For data analysis, the arithmetic mean of 10 measurements was used.

Urinary Samples

Urine samples were collected from each dog by use of ultrasound-guided cystocentesis for routine urinalysis, urine culture, and UPC, and for assessing microalbuminuria (MALB), urine albumin : creatinine ratio (UAC), and urine protein electrophoresis.

Assessment of Proteinuria

UPC Ratio. Total urine protein levels were assayed on an autoanalyzer by an immunoturbidimetric method. Urine creatinine concentrations were determined by a commercial autoanalyzer-based kinetic Jaffe reaction and UPC ratios were calculated.

MALB and UAC Ratio. Urine albumin concentration was determined by 2 different methods. MALB was assayed semiquantitatively by means of the canine E.R.D.-Health Screen test[e] after urine samples were normalized to a urine-specific gravity of 1.010 to allow comparison among samples. The amount is indicated as none (negative), small (+), moderate (++), and high (+++). To quantitate albuminuria, a modified human immunoturbidimetric assay[f] validated for the dog was used as described previously,[12] and UAC ratios were calculated. To assess sensitivity and specificity of the MALB, 2 different UAC cut-offs were chosen, first the mean + 2 SD obtained in our control dogs, and second 0.03, the cut-off for pathologic microalbuminuria in people.[13]

Urine Protein Electrophoresis. Urinary proteins were separated by sodium dodecyl sulfate agarose gel electrophoresis (SDS-AGE) with the Hydragel proteinurie kit.[g14] Briefly, 20 μL of a 10 g/L SDS solution were added to 80 μL of urine. Five microliters were loaded to each well (5 wells/gel) and submitted to electrophoresis in an SDS-imidazole pH 7.0 buffer. After the gel was completely dried at 80 °C for 20 minutes, it was immersed for 30 minutes in an aqueous staining solution (acid violet) and destained in 2 successive aqueous baths before it was immersed in a glycerol aqueous solution and dried at 80 °C (15 minutes).

Characterization of the Male-Specific Protein. Gel protein bands were cut with a scalpel and repeatedly washed in 25 mM NH_4HCO_3 with subsequent shrinking of the gel pieces through addition of 50% acetonitrile in 25 mM NH_4HCO_3. The proteins were digested overnight by adding 19 ng/μL sequencing grade modified trypsin[h] in 47 mM Tris (pH 9). Peptides were extracted from the gel by sonification in buffer A (2% acetonitrile, 0.1% trifluoroacetic acid). For liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS) analysis, the protein supernatant was run over a C18 column eluting the peptides at 600 nL/min for 45 minutes by means of a gradient of 95% buffer A to 60% buffer B (80% acetonitrile, 0.1% trifluoroacetic acid). Peptides were ionized by electrospray ionization and directly injected into the massspectrometer LCQ Deca XP[i] recording their mass-to-charge ratio. After each full MS spectrum the 2 most intense peaks were automatically selected for fragmentation of these peptides and recording of the MSMS signals (fragments). All MSMS spectra were submitted to Mascot[15] for identification of the proteins.

Statistical Analysis. Results were analyzed by use of nonparametric statistical methods,[j,k] and reported as median and ranges. Differences within 1 group were tested by use of Friedman's repeated measures test followed by Dunn's posttests. Differences between the groups were tested by use of the Mann-Whitney *U*-test. Differences were considered significant at values of $P \leq .05$.

Results

Induction of HC

In this model, long-term hydrocortisone administration effectively created HC. All dogs receiving hydrocortisone developed clinical signs (polyuria, polydipsia, no regrowth of clipped hair within 1 month, and thinning of the ventral abdominal skin with prominent subcutaneous veins) and laboratory abnormalities (stress leukogram, increased alkaline phosphatase activity, isosthenuria) consistent with cortisol excess. During medication, dogs in the I-HC group had significantly higher baseline plasma cortisol concentrations than dogs in the control group (eg d56: 3 hours post pill 30.7 mcg/dL [13.3–57.6] versus 0.5 mcg/dL [0.1–2.5]). Finally, 36 hours after hydrocortisone withdrawal, dogs in the I-HC group failed to respond to ACTH stimulation; median plasma cortisol concentrations before and after ACTH adminis-

Table 1. Median values and ranges of systolic (SAP), diastolic (DAP) and mean arterial blood pressure (MAP) in control and hydrocortisone-treated dogs at the different time points.

	SAP (mmHg)		DAP (mmHg)		MAP (mmHg)	
	Controls	I-HC	Controls	I-HC	Controls	I-HC
	Median (Range)	Median (Range)	Median (Range)	Median (Range)	Median (Range)	Median (Range)
d0	128 (122—142)	123 (114–136)	63 (55–83)	69 (53–78)	90 (78–104)	90 (81–101)
d1	128 (123–135)	130 (118–136)	68 (63–75)	70 (66–78)	89 (82–95)	91 (84–99)
d5	126 (117–148)	133 (122–144)	66 (56–77)	75 (64–81)	89 (80–98)	94 (82–103)
d28	129 (116–149)	143 (128–148)[a]	70 (65–83)	81 (77–90)[a]	94 (83–107)	102 (95–109)[a]
d56	130 (122–145)	137 (134–139)[a]	72 (63–86)	82 (74–82)[a]	96 (84–105)	103 (96–105)[a]
d84	127 (119–144)	131 (129–141)	66 (59–81)	80 (71–81)[a]	89 (80–106)	98 (94–104)[a]
d1p	129 (118–141)	130 (124–144)	66 (54–75)	78 (65–84)	91 (80–103)	98 (89–103)
d5p	133 (117–152)	124 (106–136)	73 (53–86)	70 (55–76)	94 (77–115)	88 (77–98)
d28p	131 (123–145)	124 (117–128)	66 (60–86)	69 (61–81)	91 (86–105)	87 (84–97)
d56p	126 (118–140)	124 (116–130)	70 (59–81)	70 (61–82)	91 (85–104)	87 (79–101)
d84p	130 (121–143)	127 (117–133)	67 (60–79)	68 (62–77)	89 (79–107)	91 (85–95)

d*x*, day *x* of hydrocortisone treatment: d*x*p, day *x* after hydrocortisone treatment.
[a]$P < .05$ versus d0.

tration were 0.25 mcg/dL (0.1–1.4 mcg/dL) and 0.45 mcg/dL (0.1–2 mcg/dL), respectively.

BP

Initially, median SAP, DAP, and MAP were 128 mmHg (122–142 mmHg), 63 mmHg (55–83 mmHg), and 90 mmHg (78–104 mmHg) in the control group and 123 mmHg (114–136 mmHg), 69 mmHg (53–78 mmHg), and 90 mmHg (81–101 mmHg) in the I-HC group, respectively. Hydrocortisone, but not placebo treatment, resulted in a progressively increasing SAP, DAP, and MAP that peaked on day 28 (Table 1). The increase in SAP above baseline was significant on day 28 ($P < .01$) and 56 ($P < .05$), but not on d84. Increases in levels of DAP and MAP were significant on d28, d56, and d84. SAP, DAP, and MAP decreased to baseline values within the 1st 5 days after hydrocortisone withdrawal, and did not change over the following 11 weeks (Table 1).

Proteinuria

UPC Ratio. Initially, UPC was below 0.4 in all dogs (0.17; 0.13–0.38). No change in UPC was seen in control dogs throughout the study (Table 2). In the I-HC group, UPC ratios increased significantly ($P < .0001$) during hydrocortisone administration to a peak on day 28. UPC ratios on days 28 and 84 were significantly higher compared with days 1 and 2 (Fig 1). Compared with the control group, UPC ratios in the I-HC group were significantly higher on days 28 and 56. Only 2 out of 6 dogs in the hydrocortisone group developed clinically significant proteinuria defined as UPC ratio > 0.5,[16] with maximal values in these 2 dogs of 1.78 and 1.63. One of these dogs (UPC ratio 1.78) showed pyuria with 8–12

Table 2. Median values, ranges, and *P*-values (control versus I-HC dogs) of urine protein:creatinine (UPC) and urine albumin : creatinine (UAC) ratios in control dogs and dogs of the I-HC group at the different time points.

	UPC			UAC		
	Controls	I-HC		Controls	I-HC	
	Median (Range)	Median (Range)	*P*-Value	Median (Range)	Median (Range)	*P*-Value
d0	0.21 (0.13–0.38)	0.17 (0.15–0.28)	0.937	0.017 (0.005–0.082)	0.015 (0.009–0.023)	0.937
d1	0.15 (0.12–0.32)	0.12 (0.09–0.22)	0.240	0.014 (0.006–0.060)	0.013 (0.010–0.028)	0.818
d5	0.18 (0.10–0.23)	0.15 (0.08–0.28)	0.937	0.013 (0.004–0.048)	0.016 (0.005–0.043)	1.000
d28	0.19 (0.13–0.29)	0.38 (0.18–1.78)[a]	0.026	0.024 (0.004–0.067)	0.045 (0.012–0.795)	0.240
d56	0.19 (0.11–0.30)	0.30 (0.20–0.51)	0.041	0.016 (0.005–0.079)	0.083 (0.021–0.339)[b]	0.015
d84	0.22 (0.10–0.36)	0.33 (0.21–1.63)[a]	0.065	0.020 (0.003–0.103)	0.133 (0.034–1.251)[b,c]	0.026
d1p	0.24 (0.09–0.34)	0.34 (0.20–1.03)	0.180	0.023 (0.003–0.070)	0.101 (0.011–0.725)	0.041
d5p	0.24 (0.12–0.30)	0.24 (0.19–0.67)	0.699	0.035 (0.002–0.078)	0.037 (0.016–0.311)	0.589
d28p	0.20 (0.08–0.36)	0.17 (0.11–0.31)	0.937	0.020 (0.002–0.093)	0.010 (0.005–0.032)	1.000
d56p	0.18 (0.09–0.36)	0.18 (0.14–0.22)	0.937	0.018 (0.004–0.113)	0.013 (0.003–0.025)	0.699
d84p	0.25 (0.10–0.35)	0.20 (0.13–0.30)	0.699	0.027 (0.007–0.104)	0.014 (0.005–0.037)	0.485

d*x*, day *x* of hydrocortisone treatment: d*x*p, day *x* after hydrocortisone treatment.
[a]$P < .05$ versus d1 and d5.
[b]$P < .05$ versus d1.
[c]$P < .05$ versus d0.

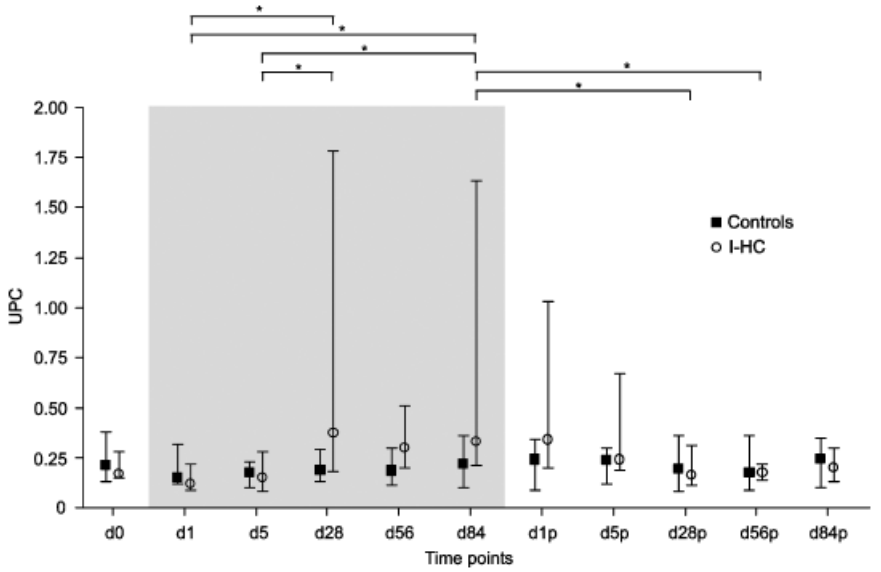

Fig 1. Change in urine protein : creatinine ratios (UPC) in 12 dogs before, during, and after administration of placebo (n = 6) or hydrocortisone (n = 6). An asterisk (*) indicates significant difference between the different time points. The gray background indicates the hydrocortisone or placebo treatment period.

leucocytes, results of urine cultures were negative in both these (and all the other dogs throughout the study), and urine electrophoresis indicated a strong band in the molecular weight range of 65–70 kDa corresponding to albumin in both dogs. On d84, the dog with pyuria again showed an increased UPC ratio of 1.63, this time without pyuria, a negative urine culture, and still a single band in the molecular weight range of 65–70 kDa.

In both groups there was a significant, but weak correlation between UPC ratios and SAP ($r = 0.611$, $P = .0001$ in control dogs and $r = 0.634$, $P = .0005$ in the I-HC group). After hydrocortisone withdrawal, UPC gradually decreased, reaching pretreatment values within 1 month (Table 2).

Table 3. Median values, ranges, and *P*-values (male versus female dogs) of urine protein:creatinine ratios (UPC) in all male and female dogs at the different time points.

	UPC		
	Male Dogs	Female Dogs	
	Median (Range)	Median (Range)	*P*-Value
d0	0.28 (0.18–0.38)	0.15 (0.13–0.16)	0.002
d1	0.19 (0.11–0.32)	0.13 (0.09–0.15)	0.041
d5	0.22 (0.16–0.28)	0.12 (0.08–0.14)	0.002
d28	0.30 (0.23–1.78)	0.17 (0.13–1.63)	0.065
d56	0.28 (0.20–0.49)	0.20 (0.11–0.51)	0.394
d84	0.33 (0.21–1.63)	0.24 (0.10–0.34)	0.180
d1p	0.34 (0.20–1.03)	0.20 (0.09–0.39)	0.065
d5p	0.29 (0.19–0.67)	0.19 (0.12–0.28)	0.026
d28p	0.30 (0.12–0.36)	0.11 (0.08–0.17)	0.009
d56p	0.22 (0.16–0.36)	0.14 (0.09–0.19)	0.004
d84p	0.29 (0.21–0.35)	0.14 (0.10–0.21)	0.002

d*x*, day *x* of hydrocortisone treatment: d*x*p, day *x* after hydrocortisone treatment.

Male dogs consistently had a higher UPC ratio than female dogs, except on d28, d56, and d84 under hydrocortisone treatment, ie, at times when urinary protein excretion was affected by hydrocortisone administration (Table 3).

MALB and UAC Ratio. Before starting treatment, all dogs in the I-HC group were found to be negative for MALB, whereas 2 male dogs in the control group were positive (++). During treatment, 4 dogs in the I-HC group developed variable degrees of MALB (+, ++, or +++) on days 28, 56, and 84, but not consistently. One month after stopping hydrocortisone administration, MALB was no longer detectable in these 4 dogs. One dog that was negative for MALB during hydrocortisone administration was positive (++/+) on days 1 and 5 after hydrocortisone withdrawal. The 2 dogs in the control group that were positive before the start of treatment remained positive, and all other control dogs remained negative throughout the study.

UAC ratios before treatment were 0.017 (0.005–0.082) in the control group and 0.015 (0.009–0.023) in the I-HC group. No change in UAC was seen in control dogs throughout the study (Table 2). UAC ratios gradually increased during hydrocortisone administration (Fig 2), reaching a maximum on day 84, which indicated a 13-fold (2.5–73.6) increase. UAC ratios were significantly higher on day 84 compared with baseline and day 1, and on day 56 compared with day 1. After stopping hydrocortisone administration, UAC progressively decreased and returned to baseline values within 28 days. UAC ratios in the 2 control dogs that were positive in the MALB test throughout the study ranged between 0.044 and 0.098 (median 0.067) and between 0.048 and 0.113 (median 0.081).

Correlation between the MALB Test and UAC Ratio. There was a moderate and significant correlation between UAC and the MALB concentrations ($r = 0.719$,

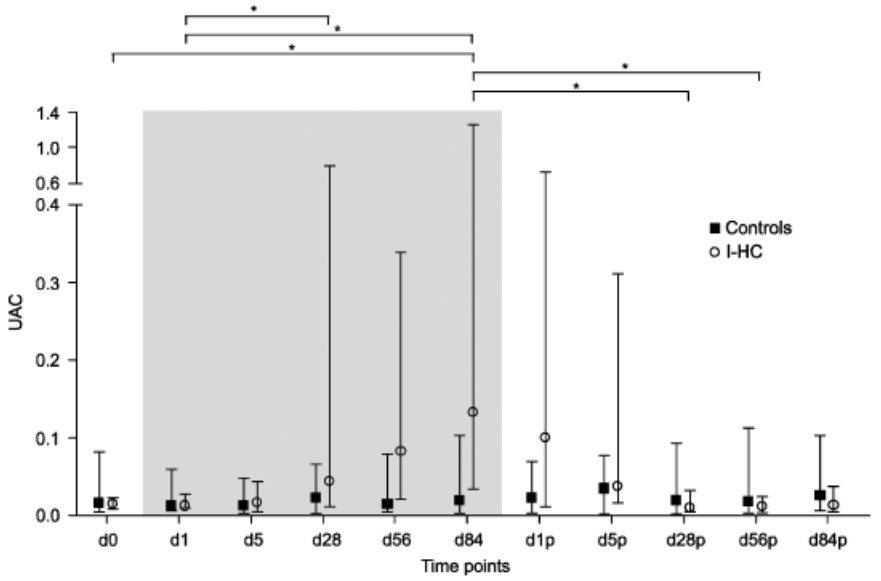

Fig 2. Change in urine albumin : creatinine ratios (UAC) in 12 dogs before, during, and after administration of placebo (n = 6) or hydrocortisone (n = 6). An asterisk (*) indicates significant difference between the different time points. The gray background indicates the hydrocortisone or placebo treatment period.

$P < .0001$). Overall, 105 samples had a negative MALB result, 31 had a + result, 4 a ++ result, and 4 a +++ result. UAC ratios in the different categories of MALB were 0.002–0.131 (median 0.012) for negative, 0.044–0.242 (0.079) for low positive, 0.164–0.725 (0.325) for medium positive, and 0.242–1.251 (1.023) for strong positive results. At a UAC cut-off of 0.078 (mean + 2 SD in control dogs), sensitivity and specificity of the MALB were 81 and 88%, respectively. At a UAC cut-off of 0.03, sensitivity and specificity of the MALB were 64 and 100%, respectively.

Urine Protein Electrophoresis. In the urine of the dogs, 2 distinct bands were found by SDS-AGE, one in the molecular weight range of 65–70 kDa corresponding to albumin and the other in the range of 25–30 kDa. The patterns in these dogs were characterized by either no band, an isolated band of 65–70 kDa, an isolated band of 25–30 kDa, or both bands (Fig 3A). Each dog of the I-HC group developed a band or showed a pronounced increase in the density of the band in the range of 65–70 kDa. After stopping hydrocortisone administration, the intensity of the albumin band decreased starting on day 5, and patterns were similar to baseline in each dog at d28p. A representative example of an SDS-AGE for one of these dogs is depicted in Figure 3B.

The band in the range of 25–30 kDa was present in urine samples from only male dogs. Each male dog showed this band during the study period, but not on every occasion. The sequence analysis of the tryptic peptides by LC-MSMS resulted in a sequence coverage

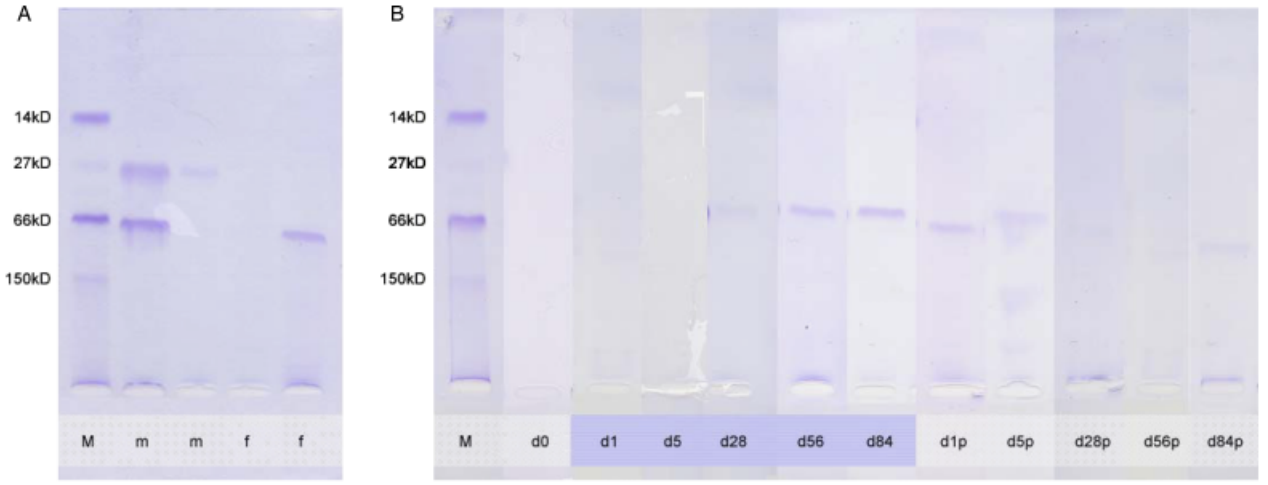

Fig 3. Analysis of urine protein patterns by agarose gel electrophoresis followed by staining with acid violet. The different protein patterns found in urine samples obtained from dogs in the control group (**A**). The change in urine protein pattern in a hydrocortisone treated female dog at the different time points (**B**). M, marker; m, male dog; f, female dog; Lxy, Lane xy.

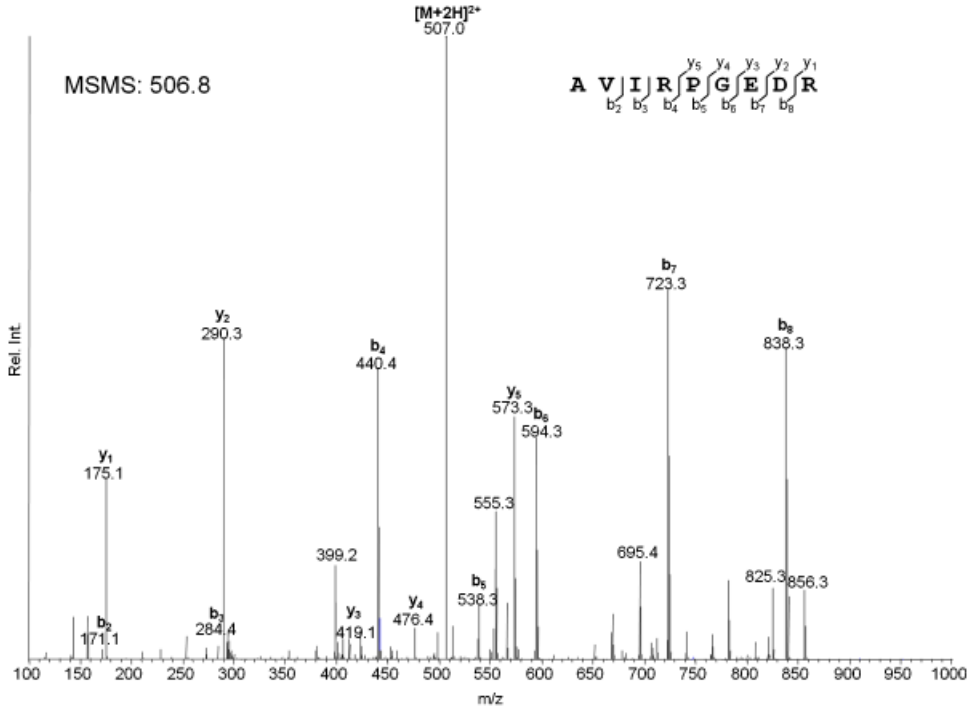

Fig 4. Representative MSMS spectrum of the tryptic peptide AVIRPGEDR of canine ariginine esterase (ESTA_CANFA) identified by Mascot. The major fragments generated are labeled and the y- and b- fragments detected are indicated in the sequence.

of 6–16%. The partial sequences AVIRPGEDR (Fig 4) and SHDLMLLHLEEPAK of canine arginine esterase were sequenced in all tested protein bands with a Mascot significance threshold of 0.05 searching against the full Uniprot database. In some of the samples, MSMS spectra matching the peptides SFIHPLYK and VMPHLMWIK were found. There was no additional significant Mascot hit for another protein in this band.

Discussion

Hydrocortisone administration consistently increased BP in the dogs in this study. Whereas in humans a significant rise in SBP has been described within as little as 24 hours of commencing cortisol administration,[2,17] the rise in our dogs was not significant on days 1 and 5, but only on day 28. In contrast to dogs with spontaneous HC, where hypertension is a common finding and may be marked,[18] no dog in our study developed increased BP to the point of hypertension defined as SAP >160 mmHg.[19] As a matter of fact, the increase in SAP averaged only 20 mmHg (12–21 mmHg) within 1–2 months of hydrocortisone administration and thereafter even tended to decrease during the treatment period. Recently, categorization of BP has been recommended because of the risk of developing subsequent target organ damage. Applying this new classification scheme of hypertension, all dogs stayed in the lowest risk category (BP $<$ 150/95 mmHg).[20]

This discrepancy may have different causes. First, glucocorticoids may not be a relevant contributor to hypertension in spontaneous HC, second, the prevalence of hypertension in spontaneous HC may have been overestimated, and third, our model may not closely resemble spontaneous HC. In dogs with spontaneous HC, hormones other than cortisol may be responsible for the development of hypertension. Although in HC hypercortisolemia is considered to be of primary pathophysiological importance in dogs, excess production of aldosterone and precursors of the glucocorticoid and mineralocorticoid pathways, ie, 17a-OH-pregnenolone, 17a-OH-progesterone, 11-deoxycortisol, 21-deoxycortisol, and corticosterone have been reported.[10,21,22] Of these, certainly aldosterone but also various precursors may play a role in the development of hypertension.[23,24] BP and the prevalence of hypertension may have been overestimated in clinical studies on HC. It has been shown that BP values strongly depend on acclimation to the measurement procedure,[25,26] with decreasing values over several days of measurements. Therefore, if BP measurements are obtained on a single day only, false high readings may result and hypertension may be overdiagnosed. A limitation of our model may be that our experimental dogs were only 3.5 years old, whereas spontaneous HC is typically a disease of middle-aged to older dogs. BP has been shown to increase with age[27]; thus, confounding factors related to age, eg, atherosclerosis and obesity, may play a role in developing hypertension in spontaneous HC, whereas in our experimental healthy dogs excessive hydrocortisone was the only variable. Also, in spontaneous HC the cortisol excess may be more gradual but present for a much longer time. In this respect it is interesting to note, however, that blood pressure did not continue to increase in the 3rd month of hydrocortisone administration but tended, rather, to decrease, suggesting that there is no linear relationship between duration of HC and increasing BP. Finally, with administration of hydrocortisone q12h we

created only 2 plasma peaks of cortisol, whereas in spontaneous HC there are multiple peaks throughout the day.[28]

As indicated, not only was the increase in BP mild, but also SAP again started to decrease after day 28. An explanation for this finding may be that chronic glucocorticoid treatment results in some metabolic adaptation. Glucocorticoid receptor (GR) downregulation in reaction to GC therapy was demonstrated in vitro and in vivo,[29,30] but the possible mechanism of this receptor downregulation is poorly understood. There is evidence for an enhanced receptor degradation and a modulated GR expression.[31,32] A recent study indicated that in humans with endogenous HC not a GR downregulation but a diminished ligand affinity might partially protect the cells from the high cortisol levels.[33] Another explanation for this finding may be that measurements on days 56 and 84 fell in the months of July and August, ie, the hottest months of the year. High temperatures result in vasodilatation, decreasing peripheral vascular resistance and reduction in BP.[34] Also, extracellular volume because of water loss by panting and impaired urine concentrating ability may have been lower in dogs with HC. However, all dogs in the study had free access to water and a cool environment, hematocrit was not different over time between groups, and thus there was no reason to suspect hypovolemia.

In all our experimental dogs, BP returned to basal values shortly after cessation of hydrocortisone administration. In contrast, in dogs and humans with spontaneous HC, hypertension often persists despite appropriate treatment and improvement of other clinical signs.[4,35,36] This again indicates that hypertension in spontaneous HC is not just caused by the cortisol excess per se. The persistence of hypertension in spontaneous HC could be the result of irreversible peripheral vascular remodeling followed by increased wall stiffness and increased vascular resistance. This hypothesis is supported by the finding that patients with a history of hypertension of < 5 years were more likely to become normotensive after adrenalectomy.[37] However, the factors that influence persistence of hypertension have not been studied in either humans or dogs.

In addition to an increased BP, we observed increased urinary protein excretion in hydrocortisone treated dogs. The increases in UPC ratios in most dogs were mild and smaller compared with dogs with spontaneous HC[4] or dogs receiving prednisone for 42 days.[8] The increase in proteinuria is thought to be primarily of glomerular origin, because there was only albuminuria as determined by SDS-AGE. Nevertheless, proteins other than albumin that may have been in the urine of our dogs were lost because the increase in UAC does not account for the increase in UPC; ie, UPC was higher than UAC; thus, SDS-AGE was not sensitive enough to detect small amounts of multiple various proteins.

Possible causes of increased glomerular proteinuria include increased intraglomerular pressure and damage to the glomerular basement membrane. Increased BP and a significant albeit weak positive correlation between SAP and UPC in our dogs support hypertension as being 1 causative mechanism of proteinuria. Even though systemic hypertension does not directly translate into increased intraglomerular pressure, glucocorticoids have also been shown to increase renal plasma flow and glomerular filtration rate in dogs.[38–40] Thus, glucocorticoids may mediate proteinuria by hemodynamic alterations, resulting in an increase in glomerular pressure. Altered renal histomorphology was found in dogs treated with prednisone for 42 days.[8] The most consistent finding was mild to moderate hypercellular glomerular tufts, suggestive of mesangial proliferation. Other findings were glomerular adhesions and moderately thickened Bowman's capsules. Electron microscopy was characterized by occasional mild segmental thickening of basement membranes, fusion of visceral cell foot processes, and glomerular adhesions. Lack of pretreatment renal histologic examination, lack of a control group, and lack of follow-up histologic examination after discontinuation of glucocorticoids make it difficult to say whether these changes are a result of glucocorticoid administration and whether histologic changes are reversible. Because in our study no renal histologic examinations were performed, our results cannot answer the question of whether the quickly reversible proteinuria was because of either functional or morphological changes, or both. In spontaneous HC, proteinuria may or may not significantly decrease when the disease is well controlled.[4,7,41] Presumably, increased intraglomerular pressure in HC will lead to reversible or irreversible morphologic changes, depending on degree and chronicity.

The 17% prevalence (2/12) of MALB in the healthy dogs in this study before any treatment is in accordance with other studies reporting a prevalence in healthy dogs ranging between 15 and 19%.[42,1] It is important to note that there may be significant day-to-day variations of up to 30–80% in daily urinary albumin exretion in humans.[43] This variation is dependent on posture, exercise, and dietary factors such as protein intake.[44,45] Data from human patients indicate that MALB is associated with an increased cardiovascular risk in hypertensive patients[46,47] and the nondiabetic population.[48,49] In our 2 healthy dogs follow-up assays were positive throughout the study, and they are therefore thought to be microalbuminuric; however, the significance of this finding is unknown. The most important consideration when assessing MALB should probably be the context of where this result is obtained; ie, is it any patient or a patient at risk of end organ damage? In this respect, the authors are unaware of any data that associate MALB with increased morbidity or mortality in dogs.

MALB can be quantitated by different means. ELISA, nephelometry, and immunoturbidimetry are complicated, time consuming, and expensive laboratory techniques unsuitable for routine use. Recently, the E.R.D. HealthScreen® test, a simple and fast test for the semiquantitative detection of MALB, has been commercialized. In the present study, to verify the accuracy of MALB observed in healthy dogs and in dogs with iatrogenic HC as measured by this simple screening test, we performed immunoturbidimetry as a quantitative reference method. In human medicine, an UAC ratio

>0.03 is considered abnormal and indicative of pathological MALB.[13] Unfortunately, few data exist on the reference range of UAC in healthy dogs, but in a previous study the mean UAC ratio in 10 healthy dogs was 0.04 ± 0.08.[m] The 2 healthy dogs with MALB were several times above the level of 0.078 and thus correctly identified with the screening test. Based on our findings, the E.R.D. HealthScreen test appears to be an efficient and fast method for the semiquantititative detection of MALB in dogs with good correlation to a quantitative laboratory method.

Higher UPC in male dogs when urinary protein excretion is not affected by hydrocortisone administration is explained by the distinct protein band in the molecular weight range of 25–30 kDa, identified as arginine esterase. This enzyme, the major secretory protein of the prostatic gland, enters the bladder by reflux from the proximal urethra.[50,51] An isolated band in the same molecular weight range has recently been described as free light chains and linked to male and female dogs exposed to or infected with certain infectious agents, ie *Leishmania infantum*, *Ehrlichia canis*, and *Babesia canis*.[52] On the basis of our results, this protein band is a physiological finding in urine samples of intact male dogs, ie, argininge esterase, and does not necessarily represent free light chains in every case.

In conclusion, our study showed that long-term hydrocortisone administration induces mild increase in BP and mild proteinuria mainly characterized by albuminuria, suggesting proteinuria of glomerular origin. Both hypertension and proteinuria were reversible and completely restored within 1 month after cessation of, ie, hydrocortisone administration. The higher UPC ratio in intact male dogs compared with female dogs can be explained by the physiological presence of arginine esterase.

Footnotes

[a] Hydrocortisone tablets, Hotz Pharmacy, Kusnacht, Switzerland

[b] Synacthen, Novartis Pharma Schweiz AG, Bern, Switzerland

[c] Schellenberg S, Reusch CE, Glaus TM. Effect of adaptation on indirect blood pressure measurement in conscious untrained beagle dogs. Congress Proceedings 15th ECVIM-CA Congress, Glasgow, Scotland, September 1–3, 2005

[d] SDI Vet/BP 6000; SDI, Waukesha, WI

[e] Canine E.R.D. HealthScreen Test, Heska Corp, Fort Collins, CO

[f] Microalbumin OSR6167 Olympus system reagent, Olympus Diagnostica GmbH, Clare, Ireland

[g] Hydragel 5 Proteinurie, semiautomated Hydrasys, Sebia, Fulda, Germany

[h] Promega AG, Duebendorf, Switzerland

[i] Thermo Finnigan, San Jose, CA

[j] SPSS 11.0 for Windows, SPSS Inc, Chicago, IL

[k] GraphPad Prism 4, Graphpad Software Inc, San Diego, CA

[l] Jensen WA, Grauer GF, Andrews J, et al. Prevalence of microalbuminuria in dogs. Proceeding of the American College of Veterinary Internal Medicine annual meeting, Denver, CO, May 23–26, 2001

[m] Mazzi A, Fracassi F, Gentilini F, et al. Urinary protein to creatinine ratio and albumin to creatinine ratio in dogs with diabetes mellitus and pituitary dependent hyperadrenocorticism. Congress Proceedings 16th ECVIM-CA Congress, Amsterdam, the Netherlands, September 14–16, 2006

References

1. Melby JC. Clinical review. 1: Endocrine hypertension. J Clin Endocrinol Metab 1989;69:697–703.

2. Kelly JJ, Martin A, Whitworth JA. Role of erythropoietin in cortisol-induced hypertension. J Hum Hypertens 2000;14:195–198.

3. Brotman DJ, Girod JP, Posch A, et al. Effects of short-term glucocorticoids on hemostatic factors in healthy volunteers. Thromb Res 2006;118:247–252.

4. Ortega TM, Feldman EC, Nelson RW, et al. Systemic arterial blood pressure and urine protein/creatinine ratio in dogs with hyperadrenocorticism. J Am Vet Med Assoc 1996;209:1724–1729.

5. Cowgill LD. Clinical significance, diagnosis, and management of systemic hypertension in dogs and cats. Partners Pract 1991; 4:2–3.

6. Martinez NI, Panciera DL, Abbott JA, et al. Evaluation of pressor sensitivity to norepinephrine infusion in dogs with iatrogenic hyperadrenocorticism. Pressor sensitivity in dogs with hyperadrenocorticism. Res Vet Sci 2005;78:25–31.

7. Hurley KJ, Vaden SL. Evaluation of urine protein content in dogs with pituitary-dependent hyperadrenocorticism. J Am Vet Med Assoc 1998;212:369–373.

8. Waters CB, Adams LG, Scott-Moncrieff JC, et al. Effects of glucocorticoid therapy on urine protein-to-creatinine ratios and renal morphology in dogs. J Vet Intern Med 1997;11:172–177.

9. Feldman EC, Nelson RW. Canine and Feline Endocrinology and Reproduction, 3rd ed. St Louis, MO: WB Saunders; 2004: 253–264.

10. Wenger M, Sieber-Ruckstuhl NS, Muller C, et al. Effect of trilostane on serum concentrations of aldosterone, cortisol, and potassium in dogs with pituitary-dependent hyperadrenocorticism. Am J Vet Res 2004;65:1245–1250.

11. Vincent IC, Michell AR, Leahy RA. Non-invasive measurement of arterial blood pressure in dogs: A potential indicator for the identification of stress. Res Vet Sci 1993;54:195–201.

12. Gentilini F, Dondi F, Mastrorilli C, et al. Validation of a human immunoturbidimetric assay to measure canine albumin in urine and cerebrospinal fluid. J Vet Diagn Invest 2005;17: 179–183.

13. Keane WF, Eknoyan G. Proteinuria, albuminuria, risk, assessment, detection, elimination (PARADE): A position paper of the National Kidney Foundation. Am J Kidney Dis 1999;33: 1004–1010.

14. Le Bricon T, Erlich D, Bengoufa D, et al. Sodium dodecyl sulfate-agarose gel electrophoresis of urinary proteins: Application to multiple myeloma. Clin Chem 1998;44:1191–1197.

15. Perkins DN, Pappin DJ, Creasy DM, et al. Probability-based protein identification by searching sequence databases using mass spectrometry data. Electrophoresis 1999;20:3551–3567.

16. Lees GE, Brown SA, Elliott J, et al. Assessment and management of proteinuria in dogs and cats: 2004 ACVIM forum consensus statement (small animal). J Vet Int Med 2005;19: 377–385.

17. Chin-Dusting JP, Ahlers BA, Kaye DM, et al. L-arginine transport in humans with cortisol-induced hypertension. Hypertension 2003;41:1336–1340.

18. Goy-Thollot I, Pechereau D, Keroack S, et al. Investigation of the role of aldosterone in hypertension associated with spontaneous pituitary-dependent hyperadrenocorticism in dogs. J Small Anim Pract 2002;43:489–492.

19. Stepien RL, Rapoport GS, Henik RA, et al. Comparative diagnostic test characteristics of oscillometric and Doppler ultrasonographic methods in the detection of systolic hypertension in dogs. J Vet Intern Med 2003;17:65–72.

20. Brown S, Atkins C, Bagley R, et al. Guidelines for the identification, evaluation, and management of systemic hypertension in dogs and cats. J Vet Intern Med 2007;21:542–558.

21. Behrend EN, Kemppainen RJ, Boozer AL, et al. Serum 17-alpha-hydroxyprogesterone and corticosterone concentrations in dogs with nonadrenal neoplasia and dogs with suspected hyperadrenocorticism. J Am Vet Med Assoc 2005;227:1762–1767.

22. Sieber-Ruckstuhl NS, Boretti FS, Wenger M, et al. Evaluation of different cortisol precursors in dogs with pituitary-dependent hyperadrenocorticism and in healthy dogs. Vet Rec, in press.

23. Rijnberk A, Kooistra HS, van Vonderen IK, et al. Aldosteronoma in a dog with polyuria as the leading symptom. Domest Anim Endocrinol 2001;20:227–240.

24. Connell JM, Jamieson AJ, Davies E, et al. 11 Beta-hydroxylase activity in glucocorticoid suppressible hyperaldosteronism: Lessons for essential hypertension? Endocr Res 1996;22: 691–700.

25. Remillard RL, Ross JN, Eddy JB. Variance of indirect blood pressure measurements and prevalence of hypertension in clinically normal dogs. Am J Vet Res 1991;52:561–565.

26. Schellenberg S, Glaus TM, Reusch CE. The effect of adaptation over multiple days on indirect blood pressure measurement in conscious and untrained beagle dogs. Vet Rec 2007;161: 418–421.

27. Bodey AR, Michell AR. Epidemiological study of blood pressure in domestic dogs. J Small Anim Pract 1996;37:116–125.

28. Hanson JM, Kooistra HS, Mol JA, Teske E, Meij BP. Plasma profiles of adrenocorticotropic hormone, cortisol, alpha-melanocyte-stimulating hormone, and growth hormone in dogs with pituitary-dependent hyperadrenocorticism before and after hypophysectomy. J Endocrinol 2006;190:601–609.

29. Silva CM, Powell-Oliver FE, Jewell CM, et al. Regulation of the human glucocorticoid receptor by long-term and chronic treatment with glucocorticoid. Steroids 1994;59:436–442.

30. Knutsson U, Stierna P, Marcus C, et al. Effects of intranasal glucocorticoids on endogenous glucocorticoid peripheral and central function. J Endocrinol 1995;144:301–310.

31. Dong Y, Poellinger L, Gustafsson JA, et al. Regulation of glucocorticoid receptor expression: Evidence for transcriptional and posttranslational mechanisms. Mol Endocrinol 1988;2:1256–1264.

32. Burnstein KL, Bellingham DL, Jewell CM, et al. Autoregulation of glucocorticoid receptor gene expression. Steroids 1991;56:52–58.

33. Huizenga NA, De Herder WW, Koper JW, et al. Decreased ligand affinity rather than glucocorticoid receptor down-regulation in patients with endogenous Cushing's syndrome. Eur J Endocrinol 2000;142:472–476.

34. Miki K, Morimoto T, Nose H, et al. Canine blood volume and cardiovascular function during hyperthermia. J Appl Physiol 1983;55:300–306.

35. Etxabe J, Vazquez JA. Morbidity and mortality in Cushing's disease: An epidemiological approach. Clin Endocrinol (Oxford) 1994;40:479–484.

36. Baid S, Nieman LK. Glucocorticoid excess and hypertension. Curr Hypertens Rep 2004;6:493–499.

37. Sapienza P, Cavallaro A. Persistent hypertension after removal of adrenal tumours. Eur J Surg 1999;165:187–192.

38. Baas JJ, Schaeffer F, Joles JA. The influence of cortisol excess on kidney function in the dog. Vet Q 1984;6:17–21.

39. Hall JE, Morse CL, Smith MJ, et al. Control of arterial pressure and renal function during glucocorticoid excess in dogs. Hypertension 1980;2:139–148.

40. Kubota E, Hayashi K, Matsuda H, et al. Role of intrarenal angiotensin II in glucocorticoid-induced renal vasodilation. Clin Exp Nephrol 2001;5:186–192.

41. Koh JM, Kim JY, Chung YE, et al. Increased urinary albumin excretion in Cushing's syndrome: Remission after correction of hypercortisolaemia. Clin Endocrinol (Oxford) 2000;52:349–353.

42. Gary AT, Cohn LA, Kerl ME, et al. The effects of exercise on urinary albumin excretion in dogs. J Vet Intern Med 2004;18: 52–55.

43. Dyer AR, Greenland P, Elliott P, et al. Evaluation of measures of urinary albumin excretion in epidemiologic studies. Am J Epidemiol 2004;160:1122–1131.

44. Hansen HP, Hovind P, Jensen BR, et al. Diurnal variations of glomerular filtration rate and albuminuria in diabetic nephropathy. Kidney Int 2002;61:163–168.

45. van Acker BA, Stroomer MK, Gosselink MA, et al. Urinary protein excretion in normal individuals: Diurnal changes, influence of orthostasis and relationship to the renin-angiotensin system. Contrib Nephrol 1993;101:143–150.

46. Jensen JS, Feldt-Rasmussen B, Borch-Johnsen K, et al. Microalbuminuria and its relation to cardiovascular disease and risk factors. A population-based study of 1254 hypertensive individuals. J Hum Hypertens 1997;11:727–732.

47. Bigazzi R, Bianchi S, Baldari D, et al. Microalbuminuria predicts cardiovascular events and renal insufficiency in patients with essential hypertension. J Hypertens 1998;16:1325–1333.

48. Yudkin JS, Forrest RD, Jackson CA. Microalbuminuria as predictor of vascular disease in non-diabetic subjects. Islington Diabetes Survey. Lancet 1988;2:530–533.

49. Kuusisto J, Mykkanen L, Pyorala K, et al. Hyperinsulinemic microalbuminuria. A new risk indicator for coronary heart disease. Circulation 1995;91:831–837.

50. Chapdelaine P, Dube JY, Frenette G, et al. Identification of arginine esterase as the major androgen-dependent protein secreted by dog prostate and preliminary molecular characterization in seminal plasma. J Androl 1984;5:206–210.

51. Isaacs WB, Coffey DS. The predominant protein of canine seminal plasma is an enzyme. J Biol Chem 1984;259:11520–11526.

52. Bonfanti U, Zini E, Minetti E, et al. Free light-chain proteinuria and normal renal histopathology and function in 11 dogs exposed to *Lleishmania infantum*, *Ehrlichia canis*, and *Babesia canis*. J Vet Intern Med 2004;18:618–624.