Boretti et al. BMC Veterinary Research (2025) 21:480
https://doi.org/10.1186/s12917-025-04876-9

BMC Veterinary Research

RESEARCH

Open Access

Effects of long-term high-dose prednisolone in Beagle dogs on structural and functional responses of the HPA Axis

Felicitas S. Boretti[1], Livia Harburger[1], Barbara Riond[2], Elisabeth Neubert[1], Regina Hofmann-Lehmann[2], Stefan Unterer[1], Matthias Dennler[3] and Nadja S. Sieber-Ruckstuhl[1*]

Abstract

Background Long-term glucocorticoid administration can suppress the hypothalamic-pituitary-adrenal (HPA) axis, increasing the risk of adrenal insufficiency upon withdrawal, with an unpredictable recovery timeline. This study provides a standardized evaluation of a > 200-day prednisolone administration regimen in dogs by assessing its effect on HPA axis through adrenocorticotropic hormone (ACTH) stimulation tests, ACTH dynamics monitoring, and adrenal ultrasonography. Four healthy Beagle dogs received once-daily oral prednisolone from days 1–181 (median dose: 2 mg/kg for days 1–150; median dose: 0.25 mg/kg for days 151–181). From days 182–208, prednisolone (median dose: 0.25 mg/kg) was given on alternate days before discontinuation on day 209.

Results All dogs developed clinical signs of hypercortisolism after 150 days of high-dose prednisolone administration. The ACTH stimulation test indicated a reduced cortisol response after 150 days of high-dose administration, though only one dog exhibited cortisol levels below 138 nmol/L. Unexpectedly, endogenous ACTH concentrations were significantly increased on day 150 but had returned to baseline levels by day 183. ACTH curves showed a short-lived suppression, with levels declining within 6 h of each prednisolone dose and rebounding to baseline by 12 h. Ultrasonography revealed significant adrenal downsizing by day 150, with a reduction in the maximal diameter of the caudal pole of both adrenal glands (left: 15–42%, right: 13–26%). By day 211, adrenal size had significantly re-increased compared to day 150.

Conclusions Prolonged, once-daily high-dose prednisolone administration caused overt clinical hypercortisolism and pronounced adrenal shrinkage in dogs; however, most retained a normal functional reserve despite these structural changes. The rapid normalization of ACTH and only mild reduction in cortisol responsiveness indicate that once-daily dosing may allow sufficient off-drug intervals to maintain near-intact HPA function. Nevertheless, the absence of a twice-daily comparison group limits any definitive conclusions about the role of dosing frequency in preventing severe HPA axis suppression.

Keywords Glucocorticoid therapy, Endocrine suppression, ACTH stimulation test, Adrenal atrophy, Canine

*Correspondence:
Nadja S. Sieber-Ruckstuhl
nsieber@vetclinics.uzh.ch
[1]Clinic for Small Animal Internal Medicine, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland
[2]Clinical Laboratory, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland
[3]Clinic of Diagnostic Imaging, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland

Background

The administration of glucocorticoids (GCs) is a cornerstone in the management of various inflammatory and immune-mediated diseases in both humans and animals. However, chronic use of GCs can lead to suppression of the hypothalamic-pituitary-adrenal (HPA) axis due to negative feedback mechanisms acting on both the hypothalamus and pituitary gland [1–3]. This feedback results in diminished secretion of corticotropin-releasing hormone (CRH) and adrenocorticotropic hormone (ACTH), ultimately reducing adrenal cortisol production. Suppression of the HPA axis is particularly concerning during chronic administration, as it increases the risk of adrenal insufficiency upon GC withdrawal [4]. Although the HPA axis typically recovers after discontinuation of GCs, the recovery period can be variable. The extent and duration of HPA axis suppression is often unpredictable and depend on several factors, including the type, dose, and duration of glucocorticoid administration [1–4].

Chronic glucocorticoid exposure can also lead to structural changes in the adrenal glands, such as atrophy, which may further delay functional recovery [5]. Ultrasonographic evaluation of the adrenal glands is a valuable non-invasive method to monitor such changes, providing real-time insights into potential size reductions associated with long-term GC use. However, since ultrasonography only assesses structural changes, it cannot determine the functional reserve capacity of the adrenal glands. To complement this, the ACTH stimulation test is a key tool for evaluating adrenal gland reserve, allowing for a direct assessment of the adrenal glands' capacity to respond to ACTH and produce cortisol [1]. This test provides essential functional information about adrenal recovery. Additionally, the measurement of endogenous ACTH concentrations offers a valuable means of understanding the pituitary gland's role in HPA axis recovery.

In veterinary medicine, numerous studies have explored the effect of GCs on the HPA in dogs [6–24]. However, most of these studies focus on short-term GC administration (up to 35 days) or single-dose administration. Only one study has investigated HPA axis suppression and canine ACTH (cACTH) dynamics following GC administration lasting longer than 35 days [4]. However, in this study the glucocorticoid regimen (dose and duration) and tapering strategy were inconsistent, leading to variability in HPA axis recovery outcomes [4]. Additionally, not all dogs underwent an alternate-day tapering process, a method believed to facilitate more rapid HPA axis recovery by stimulating endogenous ACTH secretion [25], and cACTH day-curves were not specifically evaluated [4].

By addressing these limitations, this study provides a more standardized investigation of prolonged glucocorticoid administration in dogs, incorporating a consistent immunosuppressive GC regimen, controlled tapering process, and detailed cACTH profiling to offer new insights into the dynamics of HPA axis recovery.

To achieve this, the study aims to: (1) assess adrenal suppression and subsequent recovery by measuring plasma ACTH levels and performing ACTH stimulation tests at various time points before, during, and after > 200 days of oral prednisolone administration; (2) monitor adrenal gland size using ultrasonographic examinations to detect potential structural changes associated with chronic glucocorticoid administration; and (3) establish a timeline for the normalization of endogenous cortisol production and ACTH levels following prednisolone cessation. Our working hypotheses were as follows:

(1) Prolonged once-daily administration of high-dose prednisolone (> 150 days) will induce complete HPA-axis suppression, reflected by markedly reduced endogenous ACTH, a severely blunted or absent cortisol response to exogenous ACTH, and pronounced adrenal atrophy on ultrasonography. (2) Despite tapering to an alternate-day regimen and final withdrawal, basal and stimulated cortisol concentrations—as well as endogenous ACTH—will remain subnormal for an extended period, indicating a protracted functional suppression of adrenal steroidogenesis. (3) Ultrasonography will demonstrate persistently reduced adrenal dimensions during and after drug withdrawal, implying that morphological restitution lags appreciably behind any functional recovery of the HPA axis.

Results

All four healthy Beagle dogs developed clinical signs consistent with hypercortisolism during high-dose prednisolone administration. Polyuria and polydipsia appeared shortly after the initiation of therapy. After several weeks, additional signs such as muscle atrophy, abdominal distension, and delayed regrowth of clipped hair became evident. Following discontinuation of prednisolone, polyuria and polydipsia resolved rapidly, whereas the other clinical signs gradually improved over the course of several weeks.

Standardized GC regimen

All dogs were treated with oral prednisolone at a median dose of 2 mg/kg once-daily until day 150. From days 151 to 181, the dosage was reduced to median 0.25 mg/kg once-daily. From days 182 to 208, all dogs received a median dose of 0.25 mg/kg every other day (EOD), with the final dose administered on day 208. A detailed overview of the prednisolone administration protocol is provided in Fig. 1.

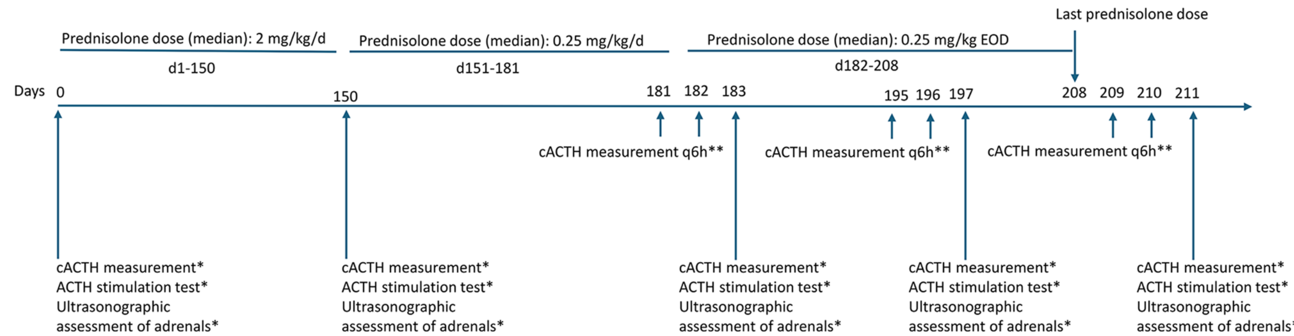

Fig. 1 Standardized prednisolone administration regimen and list of performed procedures. Abbreviations: ACTH, adrenocorticotropic hormone; cACTH, canine ACTH; EOD, every other day. *During prednisolone treatment, the daily dose was administered after the ACTH stimulation test. **During prednisolone treatment, the dose was given in the morning after the first blood sample

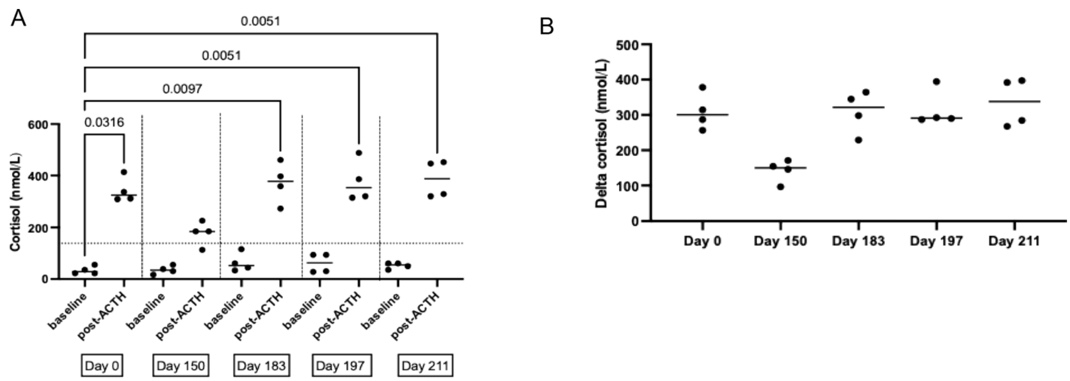

Fig. 2 Baseline, post-ACTH, and delta cortisol concentrations. Scatter plot of cortisol concentrations. The black line represents the median at each time point. The dotted line represents the cut-off for a normal cortisol response. All possible pairwise comparisons were performed, and all significant differences are indicated with a line and the corresponding p-value. **A**:Baseline and post-ACTH cortisol concentrations at different time points during the prednisolone administration and its tapering phase. **B**:Delta cortisol concentrations (post-ACTH cortisol– baseline cortisol) at different time points

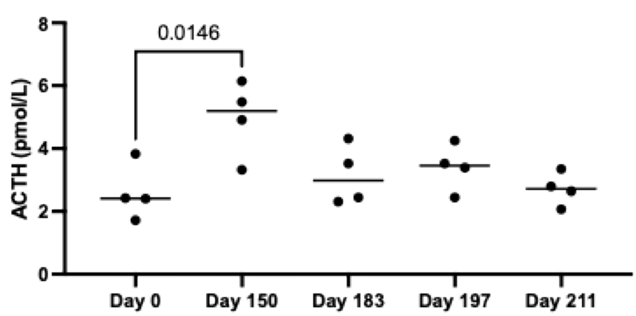

Fig. 3 ACTH concentrations. Scatter plot of ACTH concentrations before and during prednisolone administration and the tapering phase. The line represents the median at each time point. All significant changes are indicated with a line and the corresponding p-value

ACTH stimulation test results

On day 0, stimulated cortisol levels were significantly higher than baseline values. After 150 days of once-daily high-dose prednisolone administration, the cortisol response to ACTH was blunted, and the post-ACTH increase was no longer statistically significant (day 150, Fig. 2A). No significant difference was observed in post-ACTH cortisol concentrations or delta cortisol between day 150 and day 0 (Fig. 2A and B).

In 3 of 4 dogs the post-ACTH cortisol concentration on day 150 was > 138 nmol/L and considered a normal cortisol response (Fig. 2A).

Throughout the study period, baseline cortisol concentrations did not differ significantly between time points. Similarly, post-ACTH cortisol concentrations remained statistically unchanged across most time points. The only additional significant differences were observed between post-ACTH cortisol levels on days 183, 197, and 211 and baseline cortisol on day 0 (Fig. 2A).

Endogenous ACTH measurements

On day 0 the median endogenous ACTH concentration was 2.4 pmol/L (1.7–3.8 pmol/L) (Fig. 3). The ACTH concentrations on day 150 were significantly higher compared to day 0 (Fig. 3). The ACTH concentration on days 183, 197, and 211 were not different compared to day 0 (Fig. 3). The 48-hour ACTH curves showed a significant decrease in ACTH concentrations consistently 6 h after prednisolone administration, but this effect diminished 6 h later (Fig. 4A and B).

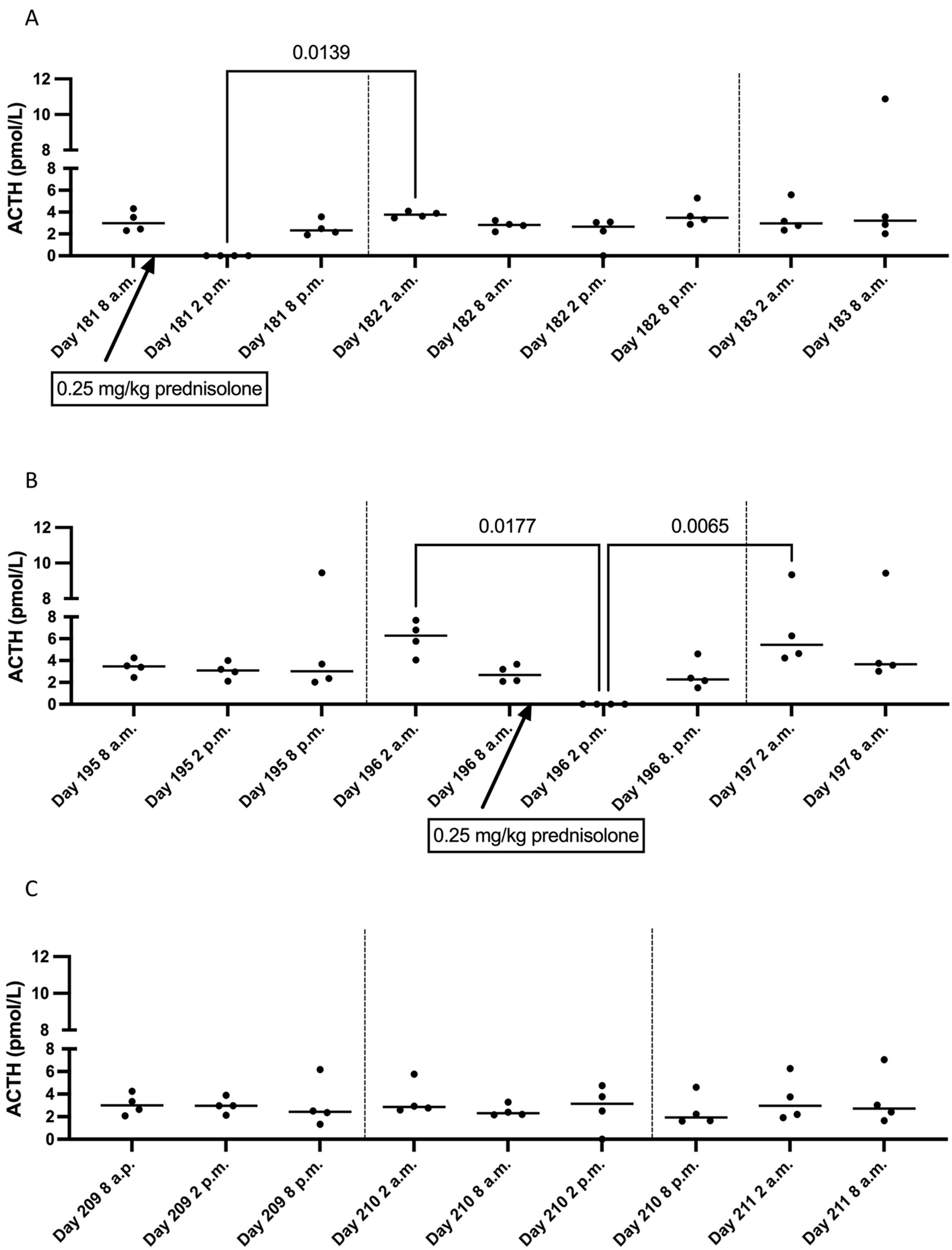

Fig. 4 ACTH dynamics monitoring. Scatter plot of ACTH concentrations. The line represents the median at each time point. All significant changes are indicated with a line and the corresponding p-value. **A**:48-hour period after 30 days of once-daily 0.25 mg/kg prednisolone administration. **B**:48-hour period after 13 days of every other day prednisolone administration. **C**:48-hour period after 27 days of every other day prednisolone administration

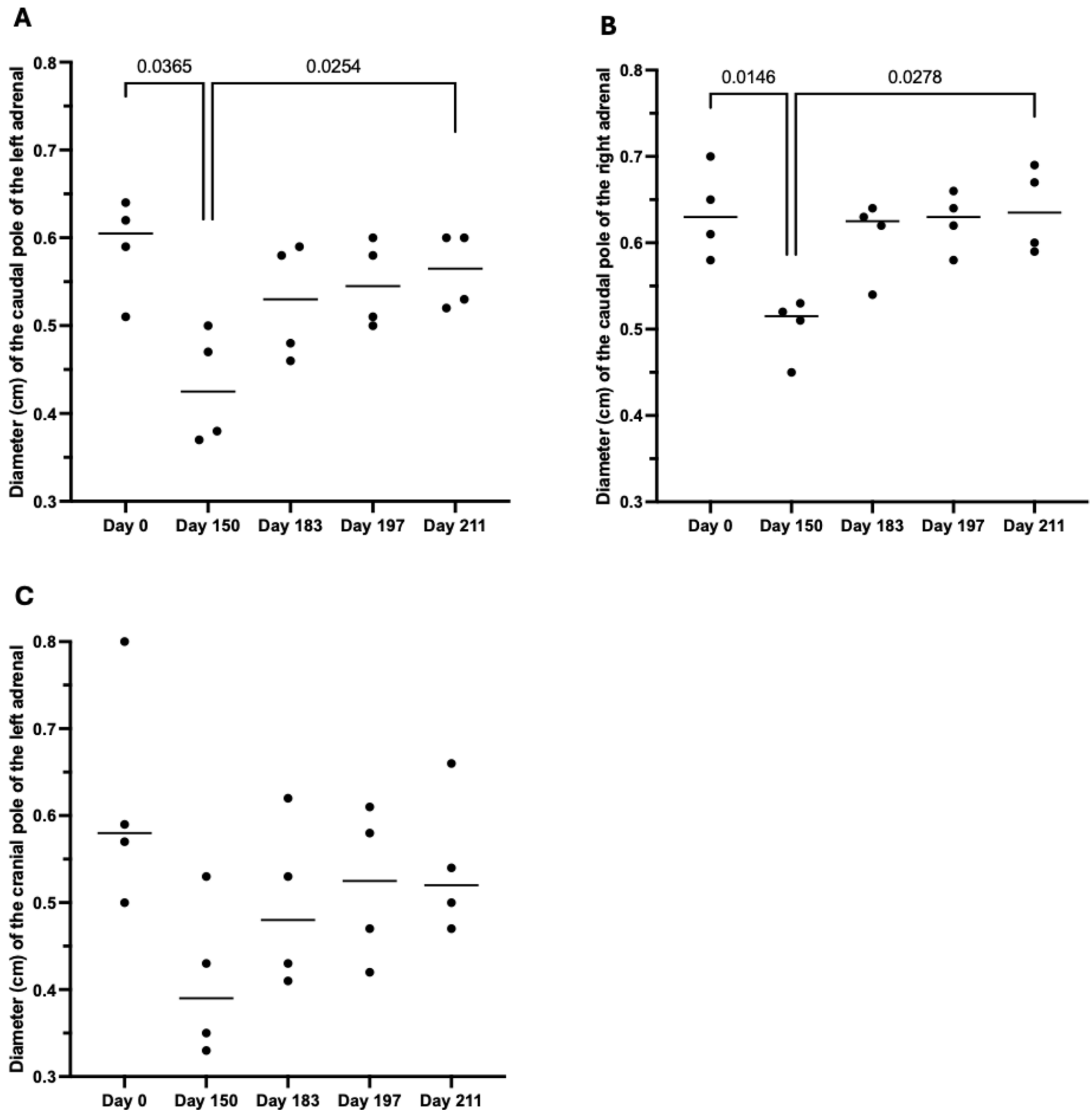

Fig. 5 Adrenal gland measurements. Measurements of the diameter (cm) of the caudal pole of the left (**A**) and the right (**B**) and the cranial pole of the left (**C**) adrenal gland during the prednisolone administration and its tapering phase. All significant changes are indicated with a line and the corresponding p-value

Ultrasonographic assessment of the adrenal glands

Both adrenal glands could be visualized sonographically in all dogs at all time points. None of the dogs showed ultrasonographic abnormalities at day 0. The diameter of the caudal pole of the *left* adrenal gland was significantly decreased on day 150 after prednisolone administration compared to day 0 (Fig. 5A). On day 211 the size had re-increased and was significantly larger compared to day 150 (Fig. 5A). The percentage of atrophy of the diameter of the caudal pole on day 150 compared to day 0 ranged between 15 and 42% (median: 25%).

The diameter of the caudal pole of the *right* adrenal gland was significantly decreased on day 150 after prednisolone administration compared to day 0 (Fig. 5B). On day 211 the size had re-increased and was significantly larger compared to day 150 (Fig. 5B).

The percentage of atrophy of the diameter of the caudal pole on day 150 compared to day 0 ranged between 13 and 26% (median: 22%).

The diameter of the cranial pole was assessed only for the *left* adrenal gland. The percentage of atrophy of the diameter of the cranial pole on day 150 compared to day 0 ranged between 10 and 57% (median: 29%), but this change was not statistically significant (Fig. 5C).

Discussion

Our study investigated the effects of prolonged, high-dose prednisolone administration on the HPA axis in healthy Beagle dogs, focusing on both structural and functional outcomes.

A central observation was the minimal functional impact on the HPA axis already before transitioning to an alternate-day dosing regimen, as all except one dog maintained normal cortisol responses to ACTH stimulation. While prior research often documents more pronounced suppression under prolonged or more frequent GC dosing our once-daily high-dose prednisolone regimen appeared to allow a prolonged "off-drug" window each day [4, 15]. This interval may reduce overall HPA axis suppression compared to dosing schedules that yield persistently elevated GC concentrations, as has been already shown for alternate-day dosing [25]. A prolonged HPA axis suppression has been reported after a single dose of long-acting triamcinolone or dexamethasone, suggesting that drug formulation and drug pharmacokinetics, in addition to dosing frequency, plays a critical role in the duration of adrenal suppression [9, 16].

That once-daily dosing possibly leads to a reduced HPA axis suppression than a more frequent dosing regimen, is further supported by our ACTH dynamic curves. ACTH levels measured 24 h after the last high-dose prednisolone administration on day 150 were significantly higher than baseline, which might initially seem paradoxical given the expected suppression of pituitary output during chronic GC exposure. However, examining cACTH every six hours over 48 h revealed a trough shortly after dosing, followed by a rapid rebound once the direct suppressive effects of prednisolone waned. This pattern suggests that a once-daily regimen, especially with a GC possessing an intermediate half-life, allows sufficient pituitary "escape" to avoid substantial adrenal suppression in most dogs. A previous study evaluated ACTH suppression in healthy Beagle dogs treated with 1 mg/kg prednisolone every other day [14]. In contrast to our findings, ACTH suppression in their study lasted approximately 18 to 24 h. Their administration duration was considerably shorter, which may explain this discrepancy. A longer administration period could lead to physiological adaptations, such as more rapid metabolism. Evaluating ACTH dynamics in dogs receiving twice-daily prednisolone would have been valuable but was not part of our study.

Recent research challenged the assumption that alternate-day dosing significantly accelerates HPA axis recovery after prolonged GC administration. The authors reported no significant difference in recovery times between dogs in which the dose had been tapered using an alternate-day dosing schedule and those that did not have this dosing regimen [4]. Unfortunately, the study did not specify whether the dogs were treated once- or twice-daily and only client-owned dogs had been included without standardization of the treatment protocol [4]. Therefore, it is not possible to determine from their study data whether the dogs that exhibited prolonged HPA suppression were dosed more frequently than those that showed a faster recovery [4].

Our study showed a significant decrease in adrenal gland size in all dogs during high-dose prednisolone administration, as observed through ultrasonographic evaluation. The diameter of the caudal pole of the left adrenal gland was below 0.5 cm in all except one dog. This finding aligns with known effects of glucocorticoid administration, which can induce adrenal atrophy due to negative feedback mechanisms suppressing pituitary ACTH secretion and reducing endogenous cortisol production [5]. Although we observed comparable structural changes to those previously described, the majority of dogs maintained a normal cortisol response to the ACTH stimulation test. Therefore, the reduced adrenal size did not necessarily reflect a clinically relevant impairment of cortisol secretion. This discrepancy underscores that morphological and functional adjustments may not proceed in parallel: once the suppressive effects of GCs lessen, hormone levels can normalize before structural changes fully revert. Consequently, ultrasonography, while valuable for detecting size alterations, does not reliably indicate the functional status of the adrenal glands. It should not replace the ACTH stimulation test in routine clinical practice when evaluating potential adrenal insufficiency.

There are several limitations to our study: one is the small number of dogs, which may limit the generalizability of the findings. Another is that the study was conducted exclusively on healthy, purpose-bred Beagle dogs, without the inclusion of dogs from different breeds or those with concurrent diseases, which could potentially influence the results. Therefore, the experimental setting does not fully replicate the clinical scenarios seen in veterinary practice, where GCs are commonly administered to dogs with underlying conditions such as immune-mediated diseases or chronic inflammation. These conditions may alter the response of the HPA axis to glucocorticoid administration, and the presence of systemic illness could affect both the extent of HPA axis suppression and the timeline for recovery. As such, the results observed in healthy dogs may not fully reflect the outcomes in dogs with pre-existing conditions requiring long-term glucocorticoid administration. However, the controlled experimental nature of the study can be considered useful and important for standardization, as exactly this heterogeneity is seen if dogs of a general population are used in a study, making interpretation of the observed data difficult. Moreover, without standardization, too many factors influence the results, which makes it impossible to draw a general conclusion. Finally, including an additional group of dogs treated with twice-daily prednisolone would have allowed for a direct comparison of HPA axis recovery between dosing regimens.

Conclusion

Our study provides valuable insights into the effects of prolonged high-dose prednisolone administration on the HPA axis in healthy Beagle dogs. While significant adrenal atrophy was observed via ultrasonography, adrenal function, as assessed by ACTH stimulation tests, remained largely intact when a once-daily treatment protocol was used. Therefore, it appears more accurate to speak of maintaining “functional integrity” rather than of a true “recovery,” given that marked functional suppression did not develop in the first place. Our study underscores the importance of considering both dosing regimens and drug pharmacokinetics when managing long-term glucocorticoid therapy to minimize adrenal insufficiency risks. Future investigations comparing once- vs. twice-daily regimens, exploring various GC formulations and schedules, and including clinically affected dogs will help refine strategies to balance therapeutic efficacy with the risk of HPA axis suppression.

Materials and methods

Dogs

Animals and ethics statement

Four Beagle dogs, which were purpose-bred and owned by the Vetsuisse Animal Facility of the University of Zurich, were housed in groups at the research unit of the Vetsuisse Faculty, University of Zurich, where all experiments were conducted. Since the animals are property of our institution, obtaining informed consent from external owners was not applicable.

The group consisted of two intact males and two castrated males, aged between 6 and 10.5 years (median age: 8.4 years), with body weights ranging from 14 to 20.8 kg (median body weight: 17.6 kg). All dogs had a body condition score (BCS) of 5/9 and were deemed healthy based on the results of a thorough physical examination, no observed clinical signs, no preexisting illnesses or administration except deworming and all dogs had a normal CBC and serum biochemistry panel. During the experiment, physical examinations were performed at regular intervals.

The study design and protocols were reviewed by the Office for Animal Welfare and 3R of the University of Zurich and approved by the Cantonal Veterinary Office of Zurich, operating under the supervision of the Federal Food Safety and Veterinary Office FSVO Bern, Switzerland (permission number ZH018/2023). All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Study design

Prednisolone administration

All dogs were orally treated with once-daily (administered in the morning) prednisolone from day 1 to 181. From day 182 to day 208 prednisolone was given on alternate days until it was stopped on day 209 (Fig. 1).

Times point of different procedures (Fig. 1)

On days 0, 150, 183, 197 and 211, blood samples for cACTH measurement were collected between 8:00 and 9:00 AM, followed immediately by an ACTH stimulation test. If the dogs were still receiving prednisolone, the daily dose was administered after completion of the ACTH stimulation test. Subsequently, ultrasonographic assessment of the adrenal glands was performed.

On days 181, 182, 195, 196, 209 and 210, blood samples for cACTH measurement were collected every 6 h. The first sample was collected at 8:00 AM (days 181, 195, 209), followed by samples every 6 h over a 48-hour period (in total 9 samples). If the dogs were still receiving prednisolone, the daily dose was administered in the morning after the first blood sample had been drawn.

Analytical procedures

For the ACTH stimulation test, blood samples were taken before and 60 min after IV injection of 5 µg/kg synthetic ACTH (Synacthen, Future Health Pharma GmbH, Wetzikon, Switzerland). Serum cortisol concentrations were measured by a competitive immunoassay (DPC Immulite 2000 (new antibody), Siemens Schweiz AG, Zurich, Switzerland). A post-ACTH cortisol concentration > 138 nmol/L was considered a normal cortisol response. For the determination of plasma endogenous ACTH, blood was collected (before ACTH administration) into chilled EDTA-coated tubes placed on ice and centrifuged at 4 °C. Plasma ACTH concentrations were determined by a 2-site solid-phase chemiluminescent immunometric assay (DPC Immulite 2000, Siemens Schweiz AG, Zurich, Switzerland), previously validated in dogs [26, 27]. Plasma and serum were stored (a maximum of 7.5 months) at -80 °C until batch analysis.

Ultrasonographic examination of the adrenal glands

Both adrenals were assessed by ultrasonography on day 0, 150, 183, 197 and 211. A board-certified radiologist (MD) performed all examinations with the dog in lateral and dorsal recumbency using a GE Logiq E10 with a microconvex 10 transducer (Scil Animal Care Company, Viernheim, Germany). Images and videos of both adrenal glands in a longitudinal section (maximal diameter and length) were stored on the picture archiving and communication system (IntelliSpace PACS 4.4 Radiology, Philips Healthcare, Horgen, Switzerland). Under the supervision of the radiologist, a qualified veterinarian (LH) obtained linear measurements of the adrenal glands (caudal and cranial pole of left adrenal gland, caudal pole of right adrenal gland) on a workstation of the picture archiving and communication system. The percentage atrophy of the caudal pole of both adrenal glands or the cranial pole of the left adrenal gland in each dog was calculated according to the following formula: percentage of atrophy = 100 x [(measurement t0– measurement t1) / measurement t0] (Pey et al. 2013) [5].

Statistical analyses

Statistical analysis was performed by commercial software using nonparametric tests (GraphPad Prism 10, GraphPad Software, San Diego, California; SPSS, Statistical Package for the Social Science, Software Packets for Windows, Version 24). Data are expressed as median and range. To analyze several time points during the follow-up period, the Friedman's repeated measures test and Dunn's multiple comparisons post-test were used. If only two time points were compared, the Wilcoxon matched-pairs signed rank test was used. For all statistical analyses, values of $P < 0.05$ were considered significant.

Post hoc power and effect size estimates were calculated using G*Power 3.1.

For post-ACTH cortisol, the time effect was significant ($F(4,12) = 20.08$, $p < 0.001$), with a robust effect size (partial η^2 = 0.87, Cohen's $f = 2.59$). Based on this, the achieved power exceeded 0.90 with a sample size of $n = 4$. For endogenous ACTH, the time effect was also significant ($F(4,12) = 6.75$, $p = 0.0044$), with a large effect size (partial η^2 = 0.69, Cohen's $f = 1.50$), again corresponding to adequate power (> 0.80). These analyses indicate that, despite the small sample size, the study was sufficiently powered to detect the observed within-subject changes over time. Nevertheless, the limited sample size should be acknowledged, and replication in larger cohorts is recommended to confirm the robustness and generalizability of these findings. With a greater number of animals, it is likely that additional statistically significant effects might have become apparent.

Abbreviations

HPA	Hypothalamic-pituitary-adrenal
ACTH	Adrenocorticotropic hormone
GCs	Glucocorticoids
CRH	Corticotropin-releasing hormone
EOD	Every other day

Acknowledgements

The authors thank the staff at the research unit of the Vetsuisse Faculty of the University of Zurich.

Author contributions

FSB and NSR participated in the conceptual development and design of the study. FSB, NSR, LH, EN and DM performed the animal study as well as sample and data collection. BR and RHL performed the laboratory analysis. NSR, FSB and SU performed statistical evaluation and data interpretation. FSB and NSR wrote the first draft of the manuscript, with all authors involved in reviewing. All authors read and approved the final version of the manuscript.

Funding

This study was part of a larger study which was founded by the Swiss National Foundation (SNF 208101).

Data availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical approval and consent to participate

The study design and protocols were reviewed by the Office for Animal Welfare and 3R of the University of Zurich and approved by the Cantonal Veterinary Office of Zurich, operating under the supervision of the Federal Food Safety and Veterinary Office FSVO Bern, Switzerland (permission number ZH018/2023). Four Beagle dogs, which were purpose-bred and owned by the Vetsuisse Animal Facility of the University of Zurich, were housed in groups at the research unit of the Vetsuisse Faculty, University of Zurich, where all experiments were conducted. Since the animals are property of our institution, obtaining informed consent from external owners was not applicable. All applicable national, and/or institutional guidelines for the care and use of animals were followed.

Consent of publication

Not applicable.

Competing interests

The authors declare no competing interests.

Received: 21 February 2025 / Accepted: 13 June 2025
Published online: 22 July 2025

References

1. Reusch CE. Glucocorticoid administration. In: Feldman EC, Nelson RW, Reusch CE, Scott-Moncrieff JC, Behrend E, editors. Canine and feline endocrinology. 4th ed. St. Louis: Elsevier; 2015. p. 555–74.
2. Mendoza-Cruz AC, Wargon O, Adams S, Tran H, Verge CF. Hypothalamic-pituitary-adrenal axis recovery following prolonged prednisolone administration in infants. J Clin Endocrinol Metab. 2013;98(12):E1936–40. https://doi.org/10.1210/jc.2013-2649. Epub 2013 Sep 30. PMID: 24081733.
3. Schlaghecke R, Kornely E, Santen RT, Ridderskamp P. The effect of long-term glucocorticoid administration on pituitary-adrenal responses to exogenous corticotropin-releasing hormone. N Engl J Med. 1992;326(4):226–30. https://doi.org/10.1056/NEJM199201233260403. PMID: 1309389.
4. Del Baldo F, Corsini A, Tardo AM, Tirolo A, Sapignoli A, Tumbarello M, Vasylyeva K, Fracassi F. Hypothalamic-pituitary-adrenal axis recovery after intermediate-acting glucocorticoid treatment in client-owned dogs. J Vet Intern Med. 2024 Mar-Apr;38(2):942–50. https://doi.org/10.1111/jvim.16979. Epub 2024 Feb 6. PMID: 38321784; PMCID: PMC10937487.
5. Pey P, Daminet S, Smets PM, Duchateau L, Travetti O, Saunders JH. Effect of glucocorticoid administration on adrenal gland size and sonographic appearance in beagle dogs. Vet Radiol Ultrasound. 2012 Mar-Apr;53(2):204-9. https://doi.org/10.1111/j.1740-8261.2011.01884.x. PMID: 22092685.
6. Hoppers S, Drake E, Frank LA, Sun X. Effect of topical florfenicol, terbinafine and Mometasone furoate ear drops on adrenocortical function in healthy small breed dogs with bilateral otitis externa. Vet Dermatol. 2022;33(3):192–e54. https://doi.org/10.1111/vde.13051. Epub 2021 Dec 14. PMID: 34904763.
7. Bugbee AC, Smith JR, Ward CR. Effect of dexamethasone or synthetic ACTH administration on endogenous ACTH concentrations in healthy dogs. Am J Vet Res. 2013;74(11):1415-20. https://doi.org/10.2460/ajvr.74.11.1415. PMID: 24168307.
8. Cohn LA, DeClue AE, Reinero CR. Endocrine and immunologic effects of inhaled fluticasone propionate in healthy dogs. J Vet Intern Med. 2008 Jan-Feb;22(1):37–43. https://doi.org/10.1111/j.1939-1676.2007.0011.x. PMID: 18289287.

9. Abraham G, Demiraj F, Ungemach FR. Comparison of the hypothalamic-pituitary-adrenal axis susceptibility upon single-dose i.m. depot versus long-acting i.v. triamcinolone acetonide administration: a direct pharmacokinetic correlation. J Endocrinol. 2006;191(2):491-6. https://doi.org/10.1677/joe.1.06991. PMID: 17088419.
10. Stroup ST, Behrend EN, Kemppainen RJ, Smith-Carr S. Effects of oral administration of controlled-ileal-release budesonide and assessment of pituitary-adrenocortical axis suppression in clinically normal dogs. Am J Vet Res. 2006;67(7):1173-8. https://doi.org/10.2460/ajvr.67.7.1173. PMID: 16817739.
11. Abraham G, Gottschalk J, Ungemach FR. Evidence for ototopical glucocorticoid-induced decrease in hypothalamic-pituitary-adrenal axis response and liver function. Endocrinology. 2005;146(7):3163–71. https://doi.org/10.1210/en.2005-0080. Epub 2005 Mar 31. PMID: 15802495.
12. Tumulty JW, Broussard JD, Steiner JM, Peterson ME, Williams DA. Clinical effects of short-term oral budesonide on the hypothalamic-pituitary-adrenal axis in dogs with inflammatory bowel disease. J Am Anim Hosp Assoc. 2004 Mar-Apr;40(2):120-3. https://doi.org/10.5326/0400120. PMID: 15007047.
13. Church DB, Emslie DR, Watson AD. Plasma cortisol concentrations following cortisone infusion in dogs before and after treatment with cortisone acetate. Aust Vet J. 1999;77(10):671-3. https://doi.org/10.1111/j.1751-0813.1999.tb13163.x. PMID: 10590797.
14. Brockus CW, Dillon AR, Kemppainen RJ. Effect of alternate-day prednisolone administration on hypophyseal-adrenocortical activity in dogs. Am J Vet Res. 1999;60(6):698–702. PMID: 10376895.
15. Moore GE, Hoenig M. Duration of pituitary and adrenocortical suppression after long-term administration of anti-inflammatory doses of prednisone in dogs. Am J Vet Res. 1992;53(5):716–20. PMID: 1326243.
16. Kemppainen RJ, Sartin JL, Peterson ME. Effects of single intravenously administered doses of dexamethasone on response to the adrenocorticotropic hormone stimulation test in dogs. Am J Vet Res. 1989;50(11):1914-7. PMID: 2559631.
17. Moriello KA, Fehrer-Sawyer SL, Meyer DJ, Feder B. Adrenocortical suppression associated with topical otic administration of glucocorticoids in dogs. J Am Vet Med Assoc. 1988;193(3):329–31. PMID: 2846483.
18. Zenoble RD, Kemppainen RJ. Adrenocortical suppression by topically applied corticosteroids in healthy dogs. J Am Vet Med Assoc. 1987;191(6):685-8. PMID: 2824410.
19. Kemppainen RJ, Sartin JL. Effects of single intravenous doses of dexamethasone on baseline plasma cortisol concentrations and responses to synthetic ACTH in healthy dogs. Am J Vet Res. 1984;45(4):742–6. PMID: 6329041.
20. Kemppainen RJ, Thompson FN, Lorenz MD. Use of a low dose synthetic ACTH challenge test in normal and prednisone-treated dogs. Res Vet Sci. 1983;35(2):240-2. PMID: 6314462.
21. Regnier A, Toutain PL, Alvinerie M, Periquet B, Ruckebusch Y. Adrenocortical function and plasma biochemical values in dogs after subconjunctival treatment with Methylprednisolone acetate. Res Vet Sci. 1982;32(3):306–10. PMID: 6285435.
22. Kemppainen RJ, Thompson FN, Lorenz MD. Effects of dexamethasone infusion on the plasma cortisol response to cosyntropin (synthetic ACTH) injection in normal dogs. Res Vet Sci. 1982;32(2):181-3. PMID: 6281869.
23. Kemppainen RJ, Lorenz MD, Thompson FN. Adrenocortical suppression in the dog given a single intramuscular dose of prednisone or triamcinolone acetonide. Am J Vet Res. 1982;43(2):204-6. PMID: 6283956.
24. Bhargava AS, Staben P, Siegmund F, Schöbel C, Günzel P. Effect of intratracheal and oral application of corticosteroids on the adrenal function test in beagle dogs after ACTH-stimulation. Arch Toxicol Suppl. 1979;(2):425-9. https://doi.org/10.1007/978-3-642-67265-1_50. PMID: 223526.
25. Chastain CB, Graham CL. Adrenocortical suppression in dogs on daily and alternate-day prednisone administration. Am J Vet Res. 1979;40(7):936–41. PMID: 507496.
26. Rodríguez Piñeiro MI, Benchekroun G, de Fornel-Thibaud P, Maurey-Guenec C, Garnier F, Rosenberg D. Accuracy of an adrenocorticotropic hormone (ACTH) immunoluminometric assay for differentiating ACTH-dependent from ACTH-independent hyperadrenocorticism in dogs. J Vet Intern Med. 2009 Jul-Aug;23(4):850-5. https://doi.org/10.1111/j.1939-1676.2009.0328.x. Epub 2009 Jun 1. PMID: 19496909.
27. Scott-Moncrieff JC, Koshko MA, Brown JA, Hill K, Refsal KR. Validation of a chemiluminescent enzyme immunometric assay for plasma adrenocorticotropic hormone in the dog. Vet Clin Pathol. 2003;32(4):180-7. https://doi.org/10.1111/j.1939-165x.2003.tb00333.x. PMID: 14655102.

Publisher's note