Effect of 1 mg/kg oral prednisolone on biochemical analytes in ten dogs: a cross-over study

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Abstract

Prednisolone is used for treatment of inflammatory, allergic, neoplastic, and immune-mediated diseases in dogs. As a glucocorticoid, prednisolone has biochemical effects, which may interfere with the interpretation of biochemistry test results. The aim of this study is to investigate the effects of prednisolone treatment in an anti-inflammatory dose on common biochemical analytes in dogs and to evaluate the clinical relevance of the changes. Ten beagle dogs, enrolled in a cross-over study, were treated with oral prednisolone (1 mg/kg 24 h) for 10 days. Blood samples were collected at day 0, 1, 3, 6, 9, 10, 12, 16, and 20. Data was analyzed using a general linear model with time and treatment as fixed factors. Pairwise comparisons were done between prednisolone and control period for each dog and sampling. Significant results were further evaluated for clinical relevance using laboratory-specific reference intervals and reference change values (RCVs), when available. Statistically significant changes were observed for ALP activity and iron concentration, which increased to levels exceeding the RCV, and several results were outside reference intervals. Phosphate and bile acids increased significantly, while amylase, lipase, and cholesterol decreased significantly, but with mean/median results remaining within reference intervals. Anti-inflammatory prednisolone treatment did not induce significant changes in ALT, GLDH, GGT, cPLI, glucose, or calcium. Treatment with an anti-inflammatory dose of prednisolone induced changes in several analytes. Only the increases in ALP and iron were of such magnitude that they are expected to affect the clinical interpretation of test results.

Keywords Canine · Glucocorticoids · Biochemistry · Laboratory analysis

Background

Glucocorticoids are widely used in veterinary medicine for the treatment of various inflammatory, allergic, immune-mediated, and neoplastic diseases. A British study from 2012 showed that approximately 15% of all dog consultations resulted in glucocorticoid therapy (O'Neill et al. 2012). Since glucocorticoids influence a wide variety of physiological and pathological processes in the body (Behrend and Kemppainen 1997), glucocorticoid therapy can affect laboratory test results. Knowledge of the extent and nature of these influences is therefore important in making the right clinical implications.

Therapeutic use of glucocorticoids has been implicated in causing a wide variety of serum biochemical abnormalities in treated dogs. The most commonly noted change is an increase in liver enzyme activity, such as alkaline phosphatase (ALP) and alanine aminotransferase (ALT) (Badylak and Van Vleet 1981; Braun et al. 1981; Cizinauskas et al. 2000; DeNovo and Prasse 1983; Dillon et al. 1980; Masters et al. 2018; Muñoz et al. 2017). Glucocorticoid use has also been associated with an increased activity in pancreatic enzymes (Fittschen and Bellamy 1984; Parent 1982) and with changes in analytes associated with metabolism, such as glucose and lipids (Wilcke and Davis 1982). In addition, corticosteroids have effects on bone and calcium metabolism and in people glucocorticoid-induced osteoporosis is a common side effect of long-term glucocorticoid therapy (van Brussel et al. 2009).

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Furthermore, previous studies indicate that iron metabolism is affected by glucocorticoid treatment (Braun et al. 1981; Harvey et al. 1987).

In earlier studies of glucocorticoid effects on biochemical variables, many different types of glucocorticoids and pharmaceutical preparations, in varying dosages and different routes of administration, have been used; dexamethasone (DeNovo and Prasse 1983; Ghubash et al. 2004; Parent 1982), prednisone (Badylak and Van Vleet 1981; Dillon et al. 1980; Masters et al. 2018; Moore et al. 1992; Muñoz et al. 2017; Tinklenberg et al. 2020), and 6α-methyl-prednisolone acetate (Braun et al. 1981). The influence of prednisolone, which is commonly used in the clinical setting for the treatment of various inflammatory or allergic diseases (Plumb 2011), on often used biochemical analytes has less commonly been assessed (Adamama-Moraitou et al. 2005; Cizinauskas et al. 2000; Melamies et al. 2012; Ohta et al. 2018). A study showed that in Britain, prednisolone was the most commonly prescribed oral synthetic glucocorticoid in dogs (Elkholly et al. 2019). Prednisone is rapidly metabolized by the liver to prednisolone, and it is generally assumed that prednisolone and prednisone are equivalent in terms of dosing when used as an oral drug in dogs (Reusch 2015). In one study, however, the relative bioavailability for prednisolone was only 65% after administration of prednisone compared to the administration of prednisolone (Colburn et al. 1976), which indicates that oral administration of prednisone in dogs may not result in the same systemic prednisolone concentrations as after prednisolone administration. Since effects of glucocorticoids on physiological processes are dependent on the synthetic glucocorticoid used, dosage, duration of therapy, and route of administration (Behrend and Kemppainen 1997; Wilcke and Davis 1982), the changes in biochemical analytes in dogs receiving anti-inflammatory doses of prednisolone may differ from the results in previous studies.

Most often, population-based reference intervals are used for interpreting laboratory test results, where a value below or above the reference range may implicate a pathological condition. However, dogs can have clinically relevant changes in laboratory test results even if they are within the reference intervals, and such changes may be missed by clinicians. RCV provides a tool for assessing the significance of serial measurements from one individual and is used to evaluate whether observed changes are larger than what could be expected to be caused only by biological and analytical variation. RCV therefore offers a measure for estimating if an individual patient exceeds its own normal range, while maybe still remaining within the population-based reference interval, which can improve the decision-making process in a clinical setting (Bugdayci et al. 2015).

The aim of this study was to investigate the effect of oral prednisolone treatment in a commonly used anti-inflammatory dosage (1 mg/kg body weight q 24 for 10 days) on 14 biochemical analytes in a crossover study in 10 beagles. An additional aim was

to evaluate if any such changes were expected to interfere with clinical interpretation using laboratory specific reference intervals and reference change values (RCV).

Material and methods

Study design

Ten beagle dogs were included in a cross-over study design. This design was chosen because it is statistically efficient and therefore requires fewer subjects than do non-crossover designs. Also, the influence of confounding covariates is reduced in a cross-over design since each subject serves as their own control. The dogs were allocated into either a control group or a treatment group, and after a washout period of 5 weeks, the groups were switched. One group consisted of four dogs and the other one of six dogs. For active treatment, 1 mg/kg (0.97–1.07 mg/kg) prednisolone (Prednisolon Pfizer, tablets) was administered orally to the dogs with their morning feed for ten consecutive days. Blood samples were collected in the morning, before drug administration and feeding, after 14 h of fasting at days 0, 1, 3, 6, 9, 10, 12, 16, and 20. On day 9, samples were collected more frequently throughout the day, by the same team, to follow the concentration–time course of prednisolone for a pharmacological part of the study. The same sampling schedule was used for the control group without treatment. In the beginning of the study, one dog showed signs of systemic disease. This dog was therefore excluded and a replacement dog was included. The sampling interval for the newly recruited dog as control differed slightly from the general sampling schedule and was as follows: days 0, 1, 3, 6, 8, 10, 14, and 16. For the statistics, day 8 was matched with day 9, day 14 was match with day 12, and day 20 is missing.

Subjects

The study was conducted with ten beagle dogs from a group of dogs, owned by the University of Agricultural Sciences in Uppsala, Sweden, that were used for educational and research purposes. The dogs were used to blood sampling and overall handling and were, throughout the study, housed in their regular groups and environment. The dogs included in the study were four females and six males, ranging in age between 2 and 12 years (mean 6.9 years) and in weight between 13 and 16 kg (mean 14.9). Exclusion criteria were steroid treatment during the last 12 weeks before the start of the trial and clinical signs of systemic disease.

Blood sampling

Venous blood was collected from the cephalic vein using a butterfly needle (Safety-Lok TM, $0.8 \times 19 \times 178$ mm), except for day 9 when more frequent samples were collected from

an indwelling catheter (Venflon 1.0 × 32 mm, Becton–Dickinson, Franklin Lakes, USA) for the pharmacological part of the study. Blood samples for biochemistry were collected into serum 4-mL vacutainer plastic gel tubes (SST II tubes, Becton–Dickinson, Franklin Lakes, USA). The blood samples were kept at room temperature for 30 min and then stored on ice until centrifuged in a refrigerated centrifuge at 2100*g* for 10 min within 2 h of collection. The separated serum samples were aliquoted into 1 mL cryo tubes (SARSTEDT AG &Co, Nümbrecht, Germany) and stored at − 80 °C until thawed and analyzed within 10 months.

Serum biochemistry analysis

Thirteen biochemistry analytes (alkaline phosphatase (ALP), amylase, alanine aminotransferase (ALT), bile acids, calcium, cholesterol, gamma-glutamyltransferase (GGT), glucose, glutamate dehydrogenase (GLDH), iron, lipase, phosphorus, unsaturated iron binding capacity (UIBC)) were analyzed using an automated chemistry analyzer (Architect C4000, Abbott Diagnostics). Methods and reagents are presented in Table 1.

Total iron-binding capacity (TIBC) and saturation was calculated according to the following formulas:

$$TIBC = serum iron + UIBC$$

$$\% saturation = (serum iron \div TIBC) \times 100$$

All 179 serum samples were analyzed on Architect c400 on the same day using the same reagent batches. Samples from one dog at a time were analyzed in a random order. Two levels of control material were run for each analyte, prior to, in the middle, and at the end of the analysis of the samples in the study.

Serum canine pancreatic immunoreactivity (cPLI) concentrations were analyzed at IDEXX laboratories in Ludwigsburg, Germany, using a commercial, previously validated, immunoassay, Spec cPL ® (Huth et al. 2010). This assay is a double-sandwich ELISA that utilizes 2 different monoclonal antibodies directed against canine pancreatic lipase. Samples were run in duplicate, and the mean was used. The measuring range was 30–2000 µg/L.

Data presentation and statistical analyses

Results are presented in figures as mean and standard deviation for analytes with normal distribution and with median and interquartile ranges for analytes that did not show normal distribution at each sampling. Enzyme results are presented in both µkat/L and U/L in text and figures.

Effect of treatment was evaluated to identify statistically significant changes caused by prednisolone treatment. A general linear model was used with time and treatment as fixed factors. Pairwise comparisons were done between prednisolone and control

Table 1 Methods used on the Architect c4000 for measurements of biochemistry analytes

Analyte	Method	Manufacturer
ALP	Para-nitrophenyl phosphate	Abbott
ALT	IFCC	Abbott
Amylase	CNPG3-substrate	Abbott
Bile acids	Enzymatic	DiaSystem Scandinavia AB
Calcium (total)	Arsenazo III	Abbott
Cholesterol	Enzymatic	Abbott
GGT	L-Gamma-glutamyl-3-karboxy-4-nitroanilinsubstrate	Abbott
Glucose	Hexokinase/G-6-PHD	Abbott
GLDH	DGKC	Cobas
Iron	Ferene	Abbott
Lipase	Quinone dye	Abbott
Phosphate	Phosphomolybdate	Abbott
UIBC	Ferene	Abbott

ALP alkaline phosphatase, *ALT* alanine aminotransferase, *GGT* gamma-glutamyltransferase, *GLDH* glutamate dehydrogenase, *UIBC* unsaturated iron-binding capacity

period for each dog and sampling. The repeated measure structure in the data was accounted for by modelling the error term with an autoregressive error structure. Tukey's method was used to adjust for multiple testing. The results of ALP, ALT, amylase, bile acids, cholesterol, GGT, GLDH, iron lipase, phosphate, and cPLI concentrations were log-transformed before statistical analysis to achieve normally distributed residuals. One outlier for GGT at day 20 was excluded to achieve normally distributed residuals. Diagnostic plots of the residuals were evaluated to ensure the assumptions of the statistical models had been met. JMP Pro 12 (SAS Institute Inc., Cary, NC, USA) was used for the statistical analysis. A level of $p < 0.05$ was used as a threshold for significance. In samples where the concentration was below the measuring range, the concentration was set to the lowest limit of the measuring range for the statistical analysis. This was applied for GGT 0.07 µkat/L (4.2 U/L) (32/179 samples), UIBC 4.5 µmol/L (18/179 samples), and cPLI 30 µg/L (11/179 samples).

To estimate mean percentage change of variables with significant changes, result for the treated dogs was compared with its own baseline value before treatment (day 0) and percentage change was calculated for each time point. The mean change of the 10 dogs was then calculated.

The clinical relevance of the statistically significant results was further evaluated using laboratory-specific reference intervals, which are shown in Figs. 1, 2, and 3, and RCVs when available.

RCV was calculated according to the following formula:

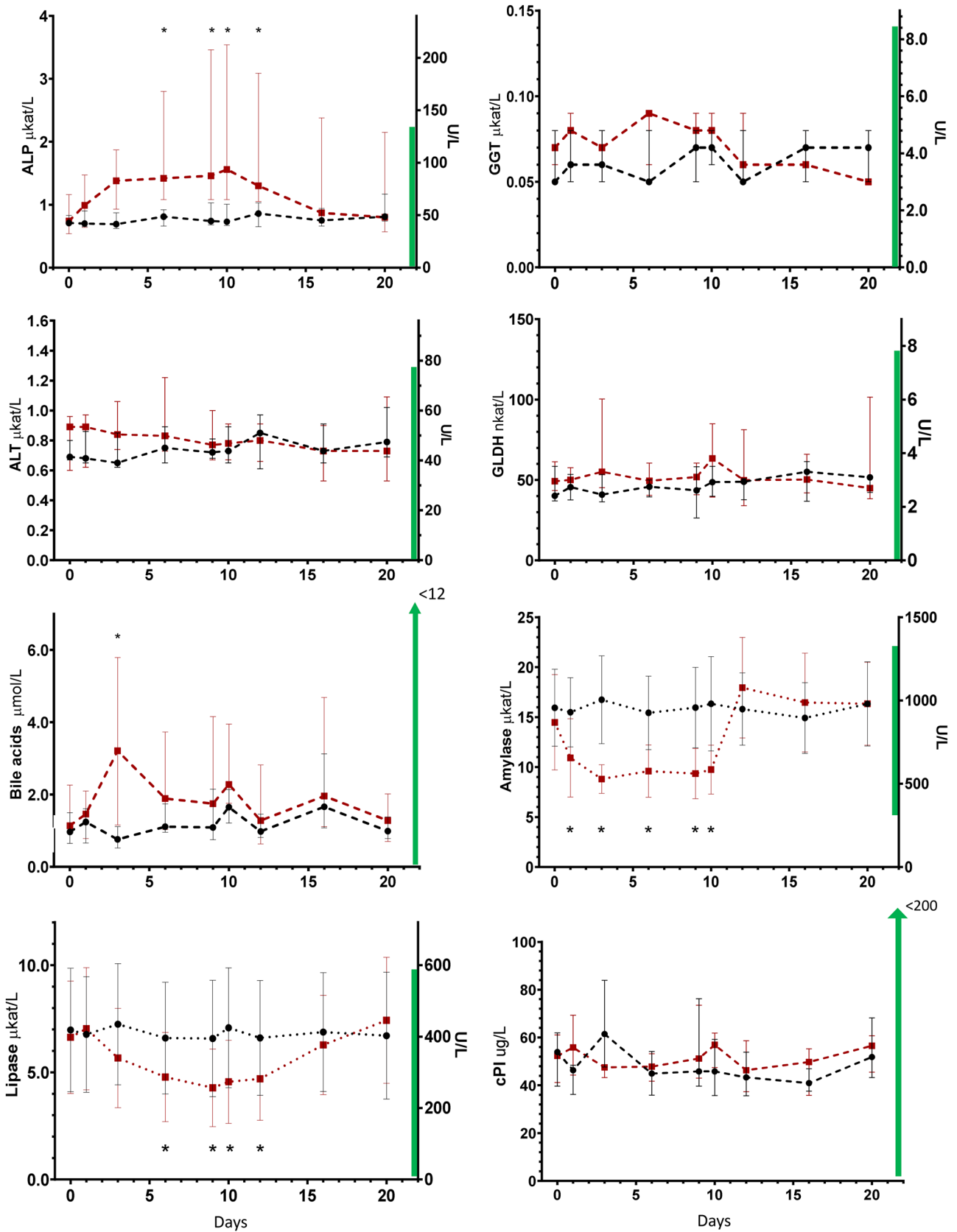$$2^{0.5} \times Z \times (CV_A^2 + CV_I^2)^{0.5}$$

◄**Fig. 1** Changes in ALP, GGT, ALAT, GLDH, bile acids, amylase, lipase, and cPL activity over 20 days in 10 beagle dogs (for ALT and GLDH 9 dogs), included in a cross-over study with oral prednisolone treatment 1 mg/kg q 24 for 10 days. Red lines show results for the dogs treated with prednisolone. Black lines show result for the same dogs when untreated. Results with dotted lines are displayed as mean values, and bars represent standard deviation. Results that did not have normal distribution (ALP, GGT, ALT, GLDH, bile acids, and cPL) are displayed with dashed lines as median values and interquartile ranges. Enzyme results are presented both in µkat/L to the left and U/L to the right. Reference intervals for each analyte are presented as a green line to the right in the graph. Results that were statistically different ($p < 0.05$) are marked with asterisks. Results below measuring range for GGT and cPL was set to the lowest limit of measuring range, GGT 0.07 µkat/L (4.2 U/L) and cPL 30 µg/L

where

$Z = 1.96$

CV_A = analytical variation

CV_I = intraindividual variation

The intraindividual variation (CV_I) was retrieved from the literature: iron (Jensen et al. 2003), ALP, cholesterol, and phosphorous (Ruaux et al. 2012). The analytic variation was determined from laboratory-specific intra-assay variation on dog samples.

One dog had ALT activity 16 times higher than the laboratory-specific reference interval and GLDH activity 10 times higher than the laboratory-specific reference interval, at the start of the treatment period. Results for ALT and GLDH for this dog, both as treated and as control, were therefore excluded from the study. The dog did not show clinical signs of systemic disease,

and the other variables for this dog followed the same pattern as the other dogs, and these results were therefore kept in the study.

Results

In the ten dogs, oral administration of prednisolone (1 mg/kg q 24 for 10 days) resulted in a statistically significant increase in serum ALP activity between day 6 and 12 compared to when the dogs were untreated. The increase was gradual with a maximum on day 10 with an increase of 130% compared with baseline values on day 0 (Fig. 1). All dogs had an increase in ALP that exceeded the RCV on days 6–12, and four dogs remained outside the RVC on the last measurement on day 20 (Table 2). Four out of ten dogs had increases in ALP activity that exceeded the reference interval during treatment. Two of these dogs remained above the reference interval at the last measurement point. No significant changes were noted in ALT, GLDH, and GGT activity (Fig. 1). Two dogs, however, demonstrated a gradual increase in ALT activity, with results slightly above the reference interval on days 12–20. Bile acids showed a significant increase, in dogs when receiving treatment, at day 3 with an increase of 155% compared to baseline values on day 0 (Fig. 1).

Serum amylase activity was significantly decreased in the dogs during the treatment period between days 1 and 10 (Fig. 1). There was a sudden decrease on day 1 with a 24% decline below baseline value. Mean decrease in amylase activity on days 3–10 were between 29 and 35% lower than baseline values. Lipase activity showed a gradual decrease and was significantly decreased on days 6–12 (Fig. 1). On day 9, the mean activity was 35% lower than baseline values.

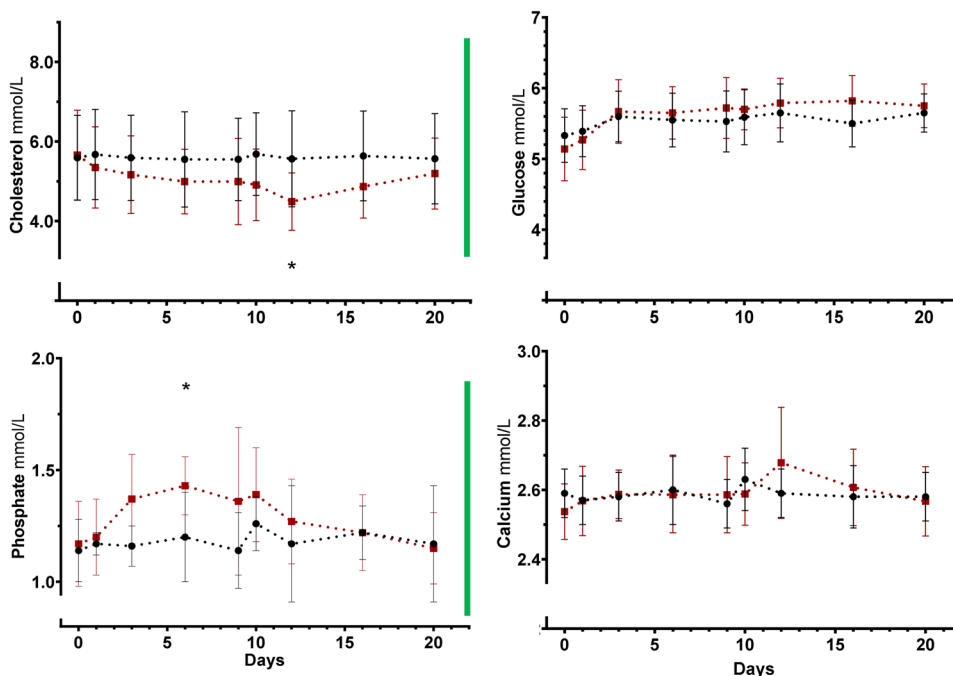**Fig. 2** Changes in cholesterol, glucose, phosphorus, and calcium concentration over 20 days in 10 beagle dogs included in a cross-over study with oral prednisolone treatment 1 mg/kg q 24 for 10 days. Red lines show results for the dogs treated with prednisolone. Black lines show result for the same dogs when untreated. Results with dotted lines are displayed as mean values, and bars represent standard deviation. Reference intervals for each analyte are presented as a green line to the right in the graph
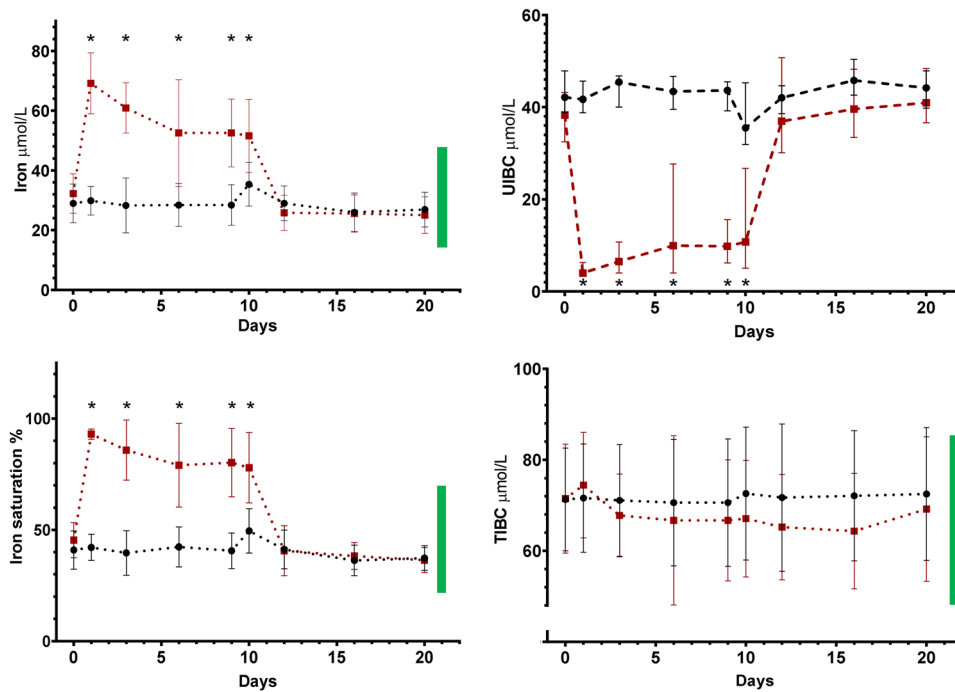
Fig. 3 Changes in iron concentration, UIBC, iron saturation and TIBC over 20 days in 10 beagle dogs, included in a cross-over study with oral prednisolone treatment1 mg/kg q 24 for 10 days. Red lines show results for the dogs treated with prednisolone. Black lines show result for the same dogs when untreated. Results with dotted lines are displayed as mean values and bars represent standard deviation. Results that did not have normal distribution (UIBC) are displayed with dashed lines as median values and interquartile ranges. Reference intervals for each analyte are presented as a green line to the right in the graph. Results that were statistically different ($p < 0.05$) are marked with asterisks. Results below measuring range for UIBC was set to the lowest limit of measuring range for 4.5 μmol/L both in the figure and in calculations of TIBC and iron saturation

After day 12, the activity gradually normalized. No significant changes were seen in cPLI (Fig. 1).

Serum cholesterol concentration showed a gradual decrease during treatment from day 1 but was only statistically significant on day 12 (Fig. 2). At day 12, the mean decrease was 35% compared with the baseline value, and at this measurement point, seven out of ten dogs exceeded the RCV (Table 2). No significant changes were noted in glucose concentration.

Serum phosphate concentration increased gradually in the treated dogs but was only significantly different on day 6, with a mean increase of 24% from baseline values (Fig. 2). All dogs remained within the RCV at this measurement point. No significant changes were noticed in serum calcium concentration (Fig. 2).

Serum iron concentration showed a significant increase on days 1–10, compared to when dogs were untreated. The increase was sudden and obvious already on day 1 with a 120% mean increase from the baseline value (Fig. 3). All dogs showed results exceeding the RCV on day 1 and 6 of 10 dogs remained outside the RCV until day 10 (Table 2). Iron concentration in all dogs was back to baseline values by day 12. Serum UIBC showed an opposite pattern compared to iron with a significant decrease between days 1 and 10 (Fig. 3). The decrease was sudden with a mean approximate 85% drop from baseline value on day 1. Iron saturation was above 90% in 9 of the 10 treated dogs on day 1, in 6 dogs on day 3, and 5 dogs on day 6 (Fig. 2). No significant changes were detected for TIBC.

Table 2 Number of the 10 prednisolone-treated dogs with results outside RCV (%) for analytes with statistically significant changes and with published biological variation data for dogs (Jensen et al. 2003; Ruaux et al. 2012)

	RCV %	Day 1	Day 3	Day 6	Day 9	Day 10	Day 12	Day 16	Day 20
ALP	37.3	1	9	10	10	10	10	5	4
Cholesterol	15.9	0	1	2	6	6	7	5	3
Phosphorous	35.2	0	2	0	1	1	0	0	0
Iron	49.4	10	9	8	6	6	0	0	0

RCV reference change value, *ALP* alkaline phosphatase

Discussion

This cross-over study, including 10 dogs, shows that short-time anti-inflammatory prednisolone treatment (1 mg/kg q 24 h) had variable effects on the measured analytes. Changes of both statistical and clinical relevance were detected in ALP and iron. Statistically significant changes were also observed in cholesterol, phosphate, amylase, lipase, and bile acids, but these changes are not expected to affect the clinical interpretation of test results. Anti-inflammatory prednisolone treatment induced a decrease in lipase and phosphate, which differ compared with earlier studies using higher doses of glucocorticoids (Parent 1982; Tinklenberg et al. 2020).

Prednisolone treatment induced a significant increase in serum ALP activity which is consistent with findings in previous studies of anti-inflammatory prednisone or prednisolone treatments (0.5–1 mg/kg) in dogs (Masters et al. 2018; Tinklenberg et al. 2020). All dogs in the treatment group had ALP activities exceeding RCV between days 6 and 12, and four dogs remained outside the RCV on the last measurement on day 20. RCV provides a tool in evaluating clinically diagnostic changes for analytes with large between dog variation where population-based reference intervals are insensitive (Bugdyaci et al. 2015). Therefore, we decided to include both reference intervals and RCV for analytes with published CV_I in this study of the pharmacological effects of prednisolone. Even if ALP activity exceeded RCV in all treated dogs, only four dogs had increased ALP activity compared with the laboratory-specific reference intervals.

Increased ALP activity for an extended period after discontinued glucocorticoid treatment has been reported previously, suggesting a continued overproduction of the enzyme after the glucocorticoid is removed (Badylak and Van Vleet 1981; Solter et al. 1994). According to a previous study (Ginel et al. 2002), the duration of increased ALP activity depends mainly on the type of glucocorticoid used, the dosage, and the duration of treatment. The results indicated that a 3-week period for short-acting glucocorticoids and more than 4 weeks for long-acting glucocorticoids may be required for ALP to return to baseline levels in dogs. In our study, four dogs remained outside the RCV at the last measurement point, suggesting that even a relatively short-term treatment in an anti-inflammatory dosage of prednisolone may result in prolonged increases in ALP in some dogs. Monitoring of the liver enzyme activities after tapering of the glucocorticoid dose may be warranted to help distinguish the effects of glucocorticoids from pathological conditions.

No statistically significant changes were noted in other liver enzymes, ALT, GLDH, or GGT activity, during treatment. Increased ALT and GGT activity is commonly noted in dogs treated with high doses of glucocorticoids (Badylak & Van Vleet 1981; Abdou et al. 2013; Solter et al. 1994) and in dogs with hyperadrenocorticism (Behrend et al. 2013; Huang et al. 1999). Anti-inflammatory doses of prednisone, on the other hand, induced no significant changes or mild changes with mean activities within reference intervals (Masters et al. 2018; Moore et al. 1992). Previous studies have, however, indicated that there are variations between individual dogs; in some dogs, the hepatic enzymes are elevated, whereas others have no change during glucocorticoid treatment (Badylak and Van Vleet 1981; Moore et al. 1992). Also, in our study, two dogs demonstrated an increase in ALT that exceeded the reference interval after treatment.

In our study, a significant, but transient increase in bile acid concentration was noted in dogs receiving treatment. The concentration was within the laboratory-specific reference interval for all treated dogs and hence would probably not have been detected in a clinical setting. In addition, the increase was only detected at sampling day 3, and it is therefore questionable if this finding was an effect of the treatment or rather an incidental finding.

In two earlier studies total serum lipase activity increased, in the absence of clinical or histopathological signs of pancreatitis, following IM treatment with dexamethasone (Parent 1982) and prednisone 4 mg/kg (Fittschen and Bellamy 1984). In our study using an anti-inflammatory dose with prednisolone, total lipase instead showed a mild gradual decrease during treatment, indicating that an anti-inflammatory dose of prednisolone causes reduced total lipase concentration in serum. Also, amylase activity decreased significantly after treatment, but still remained within reference intervals. A decrease in amylase is consistent with the findings in the two previously mentioned studies (Fittschen and Bellamy 1984; Parent 1982). The kidneys are the main route of amylase and lipase excretion (Stockham 2008), and since glucocorticoids enhance the glomerular filtration rate (Baylis and Brenner 1978; Baylis et al. 1990), the observed decrease could be the result of increased renal excretion of amylase and lipase. The decrease in total lipase and amylase activity caused by prednisolone treatment probably has minor effect on diagnostic interpretation, since clinical interest in amylase and lipase changes applies to high levels, even if the decreased activity potentially could obscure a mild increase in lipase and amylase.

cPLI concentrations did not show statistically significant changes during treatment in our study, which is consistent with the findings in a previous study where treatment of six dogs with prednisone (2.2 mg/kg q 24 for 28 days) did not cause statistically significant changes in cPLI or clinical signs of pancreatitis (Steiner et al. 2009). Contrasting results were seen in a study of dogs with naturally occurring hyperadrenocorticism, where cPLI concentrations were significantly higher than in healthy dogs (Mawby et al. 2014), suggesting that prolonged and presumably higher concentrations of endogenous glucocorticoids are needed to detect significant

increases in cPLI concentrations. Dogs with immune-mediated disease treated with prednisolone (2–2.2 mg/kg q 24) also showed significantly elevated cPLI concentrations without clinical signs of pancreatitis (Ohta et al. 2017), indicating that concurrent disease may influence the cPLI concentrations in dogs receiving glucocorticoid treatment.

Our results suggest that short-term treatment with anti-inflammatory doses of prednisolone does not excert diabetogenic effects. No significant changes in blood glucose were noted at any evaluation time during treatment. This is in accordance with previous studies where treatment with anti-inflammatory doses of prednisone (Masters et al. 2018; Moore and Hoenig 1993) and prednisolone (Wolfsheimer et al. 1986) did not result in increased blood glucose concentrations. However, increased glucose levels are a consistent finding in dogs with naturally occurring hyperadrenocorticism (Peterson et al. 1984) and treatment with a high dose of prednisone (4 mg/kg PO q 24) resulted in increased glucose concentrations (Tinklenberg et al. 2020). This suggests that immunosuppressive doses of prednisolone and possibly also treatment with prolonged duration could result in increased blood glucose levels.

Serum cholesterol showed a mild gradual decrease in treated dogs and was significantly decreased on day 12. The changes exceeded RCV for 7 of 10 dogs, but the cholesterol concentrations in all treated dogs were still within reference intervals. Our result is consistent with the results in a recent study where cholesterol was significantly decreased in dogs treated orally with prednisolone (1 mg/kg q 24 for 14 days) (Masters et al. 2018). In contrast, hypercholesterolemia is a common finding in dogs with naturally occurring hyperadrenocorticism, (Behrend and Kemppainen 1997) and it has also been reported in dogs treated with higher doses of prednisone (2–4 mg/kg) (Cizinauskas et al. 2000; Tinklenberg et al. 2020) and 6-alpha-methylprednisolone (4.4 mg/kg IM) (Braun et al. 1981). The reason for the different effects of glucocorticoids on cholesterol concentration is not known.

Serum iron concentration in our study increased with 63–205% already after 24 h following treatment. Previous studies involving prednisone (Harvey et al. 1987), methylprednisolone (Braun et al. 1981), and prednisolone (Adamama-Moraitou et al. 2005) have also reported an increase in serum iron concentration, but in these studies, the increase was slower and more progressive. In our study, UIBC, which indicates the total unused iron-binding sites on transferrin, was significantly decreased, while TIBC was unaffected. The total TIBC, of serum is an indirect measure of total serum transferrin concentration. The presented result with an increase in serum iron, unchanged TIBC but a decreased UIBC, therefore caused an increased iron transferrin saturation. The calculated percentage transferrin saturation was above 90% in 9 of the 10 treated dogs. This represents a pronounced increase compared to the laboratory-specific reference interval, which lies between 21 and 70% and compared to the control period where iron saturation was

between 26 and 68%. The mechanism behind glucocorticoid-induced increase in serum iron has not been established, but factors such as decreased storage in the liver or a decrease in iron uptake by macrophages have been discussed (Braun et al. 1981). Preliminary studies in humans treated with prednisolone also suggest that glucocorticoids might function as a hepcidin antagonist (Eisenga et al. 2017). The physiological significance of the observed increase in serum iron following glucocorticoid treatment is unclear (Harvey et al. 1987). Regardless of the clinical significance, the practitioner should be aware of this phenomenon when interpreting laboratory test results.

Calcium concentration remained unchanged during treatment, which is consistent with findings in a previous study where prednisolone (1 mg/kg q 48 for 5 weeks) administration did not result in significant changes in calcium or phosphate concentration (Kovalik et al. 2012). In our study, phosphate concentration showed a statistically significant increase in dogs receiving treatment. The increase was mild, within reference intervals and only two dogs were outside RCV. Short time treatment with an anti-inflammatory dosage of prednisolone does not seem to induce changes that affect diagnostic interpretation in neither calcium nor phosphate.

Previous publications on the effect of prednisolone on biochemical analytes have involved other dosages, less frequent sampling or fewer analytes than in the study presented here (Adama-Moraitou et al. 2005; Cizinauskas et al. 2000; Kovalik et al. 2012; Ohta et al. 2018). Our study describes the effects on a broad panel of commonly used biochemical analytes after treatment with prednisolone 1 mg/kg, which is clinically relevant since it is a common initial anti-inflammatory dosage regimen (Blois and Mathews 2017). The fact that a cross-over design was used, making the dogs act as their own control, is a strength of the study. One limitation of the study is that only beagle dogs were used. On the other hand, dogs were of both sexes and in all ages, which resembles the situation in a clinical setting. Dogs were treated for 10 days, which is a common starting dose for prednisolone. Long-term treatment is, however, often necessary in the clinical setting and the clinicopathological changes may have been different or there might have been greater changes with a more long-term glucocorticoid administration. Therefore, the results in this study cannot necessarily be extrapolated to long-term glucocorticoid treatment.

Conclusion

The presented study showed that 10 days of oral prednisolone treatment in an anti-inflammatory dose (1 mg/kg q 24 h) caused changes in ALP activity and iron concentration that were of such magnitude that they could influence the clinical interpretation. For the other studied analytes, no or only mild changes were observed, indicating that if biochemical

alterations are observed in these parameters in a dog receiving anti-inflammatory doses of prednisolone, other factors may be the underlying cause and further diagnostics may therefore be warranted.

Declarations

Ethical approval The study was approved by the Local Ethics Committee in Uppsala, Sweden (Dnr 5.8.18-07216/2017).

Conflict of interest The authors declare no competing interests.

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