Research in Veterinary Science 95 (2013) 59–68

Contents lists available at SciVerse ScienceDirect

Research in Veterinary Science

journal homepage: www.elsevier.com/locate/rvsc

Characterization and quantification of the influence of season and gender on plasma chemistries of Hermann's tortoises (*Testudo hermanni*, Gmelin 1789)

Alexandra Scope [a,*], Ilse Schwendenwein [b], Günther Schauberger [c]

[a] The Clinic for Avian, Reptile, and Fish Medicine, University of Veterinary Medicine Vienna, Veterinaerplatz 1, A 1210 Vienna, Austria
[b] The Central Laboratory, University of Veterinary Medicine Vienna, Veterinaerplatz 1, A 1210 Vienna, Austria
[c] Unit for Molecular Physiology and Biophysics, University of Veterinary Medicine Vienna, Veterinaerplatz 1, A 1210 Vienna, Austria

ARTICLE INFO

Article history:
Received 26 November 2012
Accepted 27 February 2013

Keywords:
Clinical chemistry
Reference limits
Seasonal influence
Influence of sex
Fitted function
Reptile

ABSTRACT

The present study is the first to monitor plasma chemistries of a group of 30 Hermann's tortoises (HTs) over two summer seasons to characterize and quantify seasonal and gender-related influences. The following analytes were analyzed: ALT, ALP, AST, BA, CA, CHO, CK, LDH, GLU, GLDH, P, TP, TRIG, urea and uric acid. Two-way repeated-measures ANOVA, mean values, confidence intervals were calculated. The overall results showed distinct patterns and somewhat unexpected results concerning the plasma chemistry changes induced by season and gender in a majority of routine biochemical analytes. TRIG, CHO, CA, and P were significantly higher in females. AST, ALT, BA, LDH and GLDH showed an increase in males in midsummer. ALP showed a significant and analogous seasonal variation for both sexes. GLU showed a moderate increase during midsummer. Uric acid and urea concentrations showed a distinct seasonal variation. For some analytes, fitted curves could be calculated describing the seasonal variation.

1. Introduction

Hermann's tortoises (*Testudo hermanni*) (HTs) are small- to medium-sized tortoises endemic in Southeast Europe. Before conservation regulations came into effect, hundreds of thousands of these tortoises were taken from their habitat and exported to Western Europe and the United States (Tüjrkozan et al., 2008; Ljubisavljević et al., 2011). A cold and wet climate is unsuitable for the outdoor husbandry and breeding of these animals. In Austria, summers in general are sufficiently warm and dry for the outdoor housing and breeding of HTs, and most are kept primarily in outdoor enclosures.

As in other species, haematological and biochemical blood tests are used for the diagnosis of disease and monitoring of health in these animals (Kölle and Hoffmann, 1996; Campbell, 2006). It is highly likely that external factors such as environmental conditions have a greater influence on the physiology and health status of ectothermic vertebrates than on that of endotherms (Campbell, 2006; Kimble and Williams, 2012). It has previously been reported that species, age, gender, nutritional status, season, and physiologic status influence the blood chemistries of reptiles (Gilles-Baillien, 1969; Samour et al., 1986; Lawrence, 1987; Kölle et al., 2001; Mathes et al., 2006), but standardized studies to quantify these changes are missing.

The activity periods of HTs during the summer are related to day length and temperature. In summer, males are very active, and often interact with each other and engage in combat. In eastern Austria, female tortoises start nesting in June when temperatures average 18 °C. From two to 12 eggs may be laid in holes in the ground. Some females may produce more than one clutch per year. All of these seasonal activities are known to influence physiological variables of tortoises (Hutton and Goodnight, 1957; Lawrence, 1987). In their countries of origin, HTs hibernate as temperatures fall in autumn. Hibernation is also recommended for tortoises in captivity (Kölle et al., 2001). The recommended temperature for hibernation of HTs is between 4 and 7 °C, low enough to reduce their metabolism. Overwintering, of course, also has a major influence on different physiologic variables (Ultsch, 1989; DeNardo, 1996).

The interpretation of laboratory data is accomplished by comparison of results with reference intervals. Reference intervals depend on the population and analytical methods, and thus are highly variable (Geffré et al., 2009). The situation is further complicated by the fact that recruiting an appropriate reference population is difficult in rare or uncommonly encountered species. Though Hermann's tortoises are not rare, published reference ranges differ to such an extent that interpretation is difficult (Göbel and Spörle, 1992; Kölle et al., 2001; Mathes et al., 2006). The lack of appropriate reference intervals for a species might be overcome by referencing an individual's analytical values to values determined in the same individual

* Corresponding author. Address: Clinic for Avian, Reptile and Fish Medicine, Department for Farm Animals and Veterinary Public Health, University of Veterinary Medicine Vienna, Veterinaerplatz 1, A 1210 Vienna, Austria. Tel.: +43 1 25077 5151; fax: +43 1 25077 5192.
E-mail address: alexandra.scope@vetmeduni.ac.at (A. Scope).

0034-5288/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.rvsc.2013.02.017

when healthy. The present study is the first to monitor plasma chemistries of a group of HTs over two activity periods and to characterize and quantify seasonal influences. The data revealed distinct patterns and unexpected results concerning the plasma chemistry changes induced by season and gender.

2. Materials and methods

The study was performed with 30 (15 male, 15 female) members of a group of approximately 45 HTs. The animals were kept outdoors, approximately 30 km east of Vienna (48.11°N, 16.47°E), Austria, in an area of approximately 1000 m^2 with grassland, bushes, hedges, caves and a small and shallow lagoon. The monthly mean temperatures for the months of the active period between April and October were 10.6, 15.1, 18.3, 20.1, 19.7, 16.0, and 10.7 °C, respectively; the monthly amounts of precipitation for these months were 51, 61, 74, 62, 60, 45, and 41 mm, respectively, with an annual total of 610 mm. Feed consisted of naturally occurring field herbs, hay, leaves, flowers, seasonally available fruits such as greengages (*Prunus domestica*) and wild cherries (*Prunus avium*), and occasionally small animals, such as earthworms and snails. The diet was sometimes supplemented with fresh lettuce and cut grass, especially in the dry summer time. Hibernation of the animals occurred from the end of October to March/April in an earth basement kept at approximately 5–8 °C, in transparent boxes filled with bark mulch and dry foliage. Blood sampling was performed on the first day of de-hibernation in April and thereafter every 5–6 weeks until hibernation, resulting in seven blood samplings per activity period. Because of the unexpected results in 2006, the study was repeated in 2007 to evaluate the reproducibility of results, and data on two additional variables were collected. All animals included in the study were clinically healthy and had a body mass between 1000 and 2300 g. On every sampling day, a short clinical examination was performed and the body mass was registered. One milliliter of blood was sampled from the dorsal coccygeal vein using heparinized syringes. The time from first handling of the animal to blood draw was kept at less than 5 min to minimize stress. The blood was cooled at 8 °C until centrifugation at 1500*g* for 10 min. The plasma was stored at −20 °C until analysis. All specimens from each sampling day were thawed on the same day at room temperature, stirred thoroughly, and analyzed within the same run. Plasma chemistries were measured by a fully selective chemistry analyzer (Hitachi 911®, Roche Diagnostics, Vienna, Austria). Assays were applied according to manufacturers' recommendations; methods are listed in Table 1. Quality control was performed by analyzing two levels of control material before each run.

The following 13 analytes were determined in 2006: alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), calcium (CA), cholesterol (CHO), creatinine kinase (CK), lactate dehydrogenase (LDH), glucose (GLU), glutamate dehydrogenase (GLDH), inorganic phosphorus (P), total protein (TP), triglycerides (TRIG), and uric acid. To validate the data of 2006, the same parameters were collected again in 2007, and bile acids (BA) and urea were added. To facilitate discussion, the analytes were sorted into related groups. Within the first group, certain metabolites and minerals (TRIG; CHO, CA, P) are summarized. Group 2 consisted of AST, ALT, LDH, GLDH, CK, ALP, and BA, the third group summarized four metabolites, uric acid, GLU, and TP.

A two-way (gender) repeated-measures (months) ANOVA was performed for the measured plasma concentration or activity. For all analytes except ALT and TP, a two-stage transformation was performed to obtain normally distributed values in accordance with the method described by Solberg (1995), which is the IFCC-recommended method to determine reference intervals. Normality was tested by the Jarque–Bera test at the $p = 0.05$ level.

The seasonal variation was fitted with a Gaussian function represented by the equation $C(t) = C_0 + C_p \exp[\frac{-1}{2}((t - t_p)/t_s)^2]$, with the concentration/enzyme activity as a function of time $C(t)$. The time t is given in months. The shape of the curve is given by four parameters. The concentration/activity C_0 represents the pre- and post-hibernation concentrations/activities, which were assumed to be identical. The peak concentration C_p describes the increase from baseline value C_0 during the non-hibernation phase. The maximum concentration/activity of the active phase is given by the sum $C_0 + C_p$, which is reached at the peak time t_p, given in months of the year. The form parameter t_s describes the steepness of the curve, with lower values representing steeper seasonal increases. The functions were only presented for those analytes/activations for which the parameters were significant ($p < 0.05$). Outliers were tested by the Grubbs test on a level of significance of $p = 0.05$.

3. Results

The plasma concentrations and enzyme activities are depicted graphically (Figs. 1–3) by box-and-whisker plots, using the median value and the lower and upper quartiles for the box and the 10%- and 90%-quantiles for the whiskers. Data from female animals are shown in black and data from males in grey. If the fitting of a seasonal function was statistically significant, then the curve was depicted in the figures.

The results of the ANOVA are presented in Tables 2, 4 and 6. The median and the lower (2.5% quantile) and upper limit (97.5% quantile) of the confidence interval for the plasma concentration/activity were calculated. The median and the confidence intervals are marked in bold if the stratification by gender and month was statistically significant (Tables 3, 5 and 7). The seasonal variation was tested against the post-hibernation concentration/activity in April (C_4) and against the pre-hibernation concentration/activity in October (C_{10}).

3.1. Group 1: TRIG, CHO, CA, and P

The values of all analytes of this group were significantly higher in females than in males; only for P, there were no differences during September and October. The seasonal variation can be described with a fitted function (Fig. 1). The earliest maximum was found for P in June, whereas the peak of CA occurred approximately 3 weeks later. The latest peaks were found for TRIG and CHO.

The seasonal variation was highest for TRIG, with the peak value in July ($C_0 + C_p$ = 608 mg/dL) at triple the pre/post-hibernation concentration (C_0 = 200 mg/dL). The pre/post-hibernation concentration for TRIG in females (C_0 = 200 mg/dL) was about three times the mean concentration for the male animals (C_{all} = 66 mg/dL). The maximum concentration of TRIG in females ($C_0 + C_p$ = 608 - mg/dL) was approximately 9.2 times higher than the median value for the active phase in males (C_{all} = 66 mg/dL) (Table 3).

The pre/post-hibernation concentration of CHOL in females (C_0 = 115 mg/dL) was approximately the same as the mean value for the active phase for the male animals (C_{all} = 114 mg/dL), with the increase at peak concentration at approximately 117% of the pre/post-hibernation concentration. The maximum concentration in female animals ($C_0 + C_p$ = 249 mg/dL) was approximately 2.2 times higher than the mean value for the active phase in male animals (C_{all} = 114 mg/dL) (Table 3).

The pre/post hibernation concentration for CA in females (C_0 - = 2.37 mmol/L) was about the same as the mean concentration in male animals (C_{all} = 2.67 mmol/L) (Table 3). As expected, the CA values of the females increased significantly during the summer months (Table 2).