Journal of the American Association for Laboratory Animal Science
Copyright 2018
by the American Association for Laboratory Animal Science

Vol 57, No 3
May 2018
Pages 291–294

Influence of Isoflurane Anesthesia on Plasma Thyroxine Concentrations in Black-tailed Prairie Dogs (*Cynomys ludovicianus*)

David Eshar,[1*] Sara M Gardhouse,[2] and Hugues Beaufrere[3]

Anesthesia can affect measured thyroxine (total T4) concentrations in humans and animals, but its effect in black-tailed prairie dogs (*Cynomys ludovicianus*) has not yet been studied. We used isoflurane to anesthetize 12 prairie dogs for 60 min. Blood samples were obtained from each animal immediately after anesthesia induction and at 30 and 60 min and used for analysis of plasma T4 concentration. The plasma T4 concentration (mean ± 1 SD) was significantly decreased from baseline (3.49 ± 0.52 µg/dL) at both 30 min (3.24 ± 0.52 µg/dL) and 60 min (3.27 ± 0.65 µg/dL) after induction. Compared with baseline, some of the T4 trends were inconsistent between animals, and individual variability in response was responsible for 86% of the overall variability. Regardless of the observed change under isoflurane anesthesia, all measurements in all prairie dogs and at all time points (2.4 to 4.4 µg/dL) were within the reported normal plasma T4 reference range for this species. In conclusion, isoflurane anesthesia appears to cause a significant but inconsistent reduction in plasma T4 concentrations in black-tailed prairie dogs, but because values remain within normal basal levels, the clinical importance of this effect is likely minimal.

Abbreviations: T4, thyroxine; T3, triiodothyronine

DOI: 10.30802/AALAS-JAALAS-17-000154

Black-tailed prairie dogs (*Cynomys ludovicianus*) are a nonhibernating burrowing member species of the order Rodentia and family Sciuridae.[16] They are a keystone species in the grasslands of North America and the most common prairie dog species in zoologic collections, in research facilities, and as privately owned pets.[16] Due to their propensity for hepatobiliary diseases and various biliary similarities to humans, black-tailed prairie dogs are often used as models for related research.[2,15,18] Chemical immobilization is frequently required for examination and diagnostic testing of prairie dogs, with inhalant anesthetic agents commonly used for anesthesia.[7,11] Isoflurane is a gas anesthetic commonly used in many rodent procedures and has generally been recommended for use in prairie dogs.[7,11] However, a comparative report showed that rodents and other small mammals had a significantly (10% to 22%) higher anesthesia-related mortality rate than other anesthetized species.[4] Changes in T4 concentration (and other thyroid hormones) can lead to unstable anesthesia and increase the associated risks.[3,5,12,19,23,24,26,27] To our knowledge, the potential effect of anesthesia on thyroid function has not been explored in prairie dogs.

Thyroxine (T4) is one of several hormones produced by the thyroid gland. It is the major precursor of other iodine-containing thyroid hormones that control the rate of cell metabolism and other physiologic processes, such as urinary water absorption.[1,28] Estimation of the total T4 concentration is considered a sensitive screening test to assess thyroid function but can have low specificity due to influence by other factors.[14,20] Anesthesia and surgery, in general, can affect measured thyroid markers.[3,5,12,19,23,24,26] In dogs, halothane has been suggested to reduce serum concentrations of T4, triiodothyronine (T3), and reverse triiodothyronine, and isoflurane reportedly decreases serum T4 and T3 concentrations.[9,27] In rats, ether increased measured serum T4 concentrations.[22] However, a more recent study in rats showed that ether, CO_2/O_2, methoxyflurane, and isoflurane decreased serum T4 and T3 concentrations.[6] In that study, a comparison between conscious and isoflurane-anesthetized rats showed a lower mean T4 of 24% in males and 27% in females.[6]

The goal of the current study was to assess the effect of isoflurane anesthesia on plasma T4 concentrations in black-tailed prairie dogs. We hypothesized that isoflurane would significantly decrease measured T4 plasma concentrations.

Materials and Methods

Animals. Clinically healthy zoo-raised, intact, male black-tailed prairie dogs (*Cynomys ludovicianus*; *n* = 12; age, 6 mo; weight [mean ± 1 SD], 651 ± 85 g) were included in this study. The animals were a new group kept in quarantine before placement in a zoologic collection. They were group-housed in a concrete-lined room bedded with hay and were maintained at a constant temperature range (21 to 23 °C). Water and grass hay were offered freely, and the rest of the provided diet consisted of a mix of vegetables and commercial rodent blocks (Rodent Breeder 6F, Mazuri Exotic Pet Food, Richmond, IN). Several plastic dog kennels (40 in. × 27 in. × 30 in.) and PVC tubing were provided for enrichment and hiding. The prairie dogs were acclimated to the new environment for 3 wk before the beginning of the study. This study was approved by the IACUC of Kansas State University (protocol no. 3792.2) and the Ethics Committee of the participating zoo.

Received: 27 Nov 2017. Revision requested: 13 Dec 2017. Accepted: 08 Feb 2018.
[1]Department of Clinical Sciences, College of Veterinary Medicine, Kansas State University, Manhattan, Kansas, and [2]Health Sciences Center and [3]Department of Clinical Studies, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada
[*]Corresponding author. Email: deshar@vet.k-state.edu

One week before the study, the prairie dogs underwent health evaluation under isoflurane (IsoFlo, Abbott Laboratories, North Chicago, IL) anesthesia, including a complete physical examination, accurate body weight, identification by microchipping, CBC analysis, and plasma biochemistry. All prairie dogs were determined to be healthy prior to the start of the study.

Experimental design and sample collection. Anesthesia in each prairie dog was chamber-induced by using 5% isoflurane gas in 2 L/min O_2. After induction, each prairie dog was maintained under general anesthesia for 60 min by using a tight-fitting, small face mask and nonrebreathing circuit, with 2.0% isoflurane delivered in O_2 at 1.5 L/min. The animals were allowed to breathe spontaneously. Body temperature (approximately 37 °C) was monitored rectally by using a handheld digital thermometer and maintained by using a warm-water blanket. Vital signs were monitored by using a stethoscope, Doppler ultrasonic flow detector (model 811B, Parks Doppler System, Parks Medical Electronics, Aloha, OR), and a pulse oximeter (model N20PA, Nellcor Handheld Pulse Oximeter, Covidien, Dublin, Ireland). Lactated Ringer solution (30 mL) was administered subcutaneously before discontinuing anesthetic delivery. Venous blood samples (0.5 mL) were collected from either the jugular vein or cranial vena cava by using a 1.0-mL syringe (Kendall Monoject 1-mL syringe, Tyco Healthcare Group, Mansfield, MA) and 25-gauge, 16-mm needle (Hypodermic needle, Exelint, Los Angeles, CA). Samples were placed into lithium heparin-coated blood collection tubes (0.5-mL BD Microtainer, Becton Dickinson, Franklin Lakes, NJ). Blood samples were collected at 3 time points for each animal: immediately after anesthesia induction (baseline, 0 min) and at 30 and 60 min thereafter.

T4 analysis. Immediately after collection, 100 µL of blood was removed from each collection tube by using the standard Abaxis pipette and placed into a total T4 rotor (T4 and Cholesterol Panel, Abaxis, Union City, CA). This test is available for use in small mammals and is operated by a veterinary bench-top biochemistry analyzer (Vetscan VS2, Abaxis). Reference intervals obtained by using this assay were previously reported for this species,[8] and a full precision and validation study performed on guinea pigs produced excellent results (r_i = 0.95; mean coefficient of variation, 4.2% [range for T4 in Guinea pigs, 0 to 15.7], n = 90).[10] In the current study, each T4 rotor was used within 15 min of removal from the refrigerator and immediately after opening its protective pouch. Tests were analyzed immediately after rotor filling and according to the manufacturer directions. Prior to this study, the analyzer was functioning appropriately on a routine basis, and its software was regularly updated as provided by the manufacturer. All samples were run by the same operator (DE).

Statistical analysis. A linear mixed model was used to analyze the data, with T4 values as the outcome variable, time as a fixed variable, and individual prairie dog as a random variable. Assumptions of the model (normality and homoscedasticity of the residuals and linearity) were checked on the residual plot as well as a quantile plot. An α value of 0.05 was used for statistical significance. R statistical programs (R core team, Vienna, Austria) were used for analysis.

Results

Isoflurane anesthesia was induced within less than 5 min for each prairie dog. All animals performed well under the 60-min of isoflurane anesthesia, with no significant changes in vital parameters and body temperature throughout the procedure. Recovery was fast (less than 10 min) and uneventful in all of the animals.

Plasma T4 concentrations (mean ± 1 SD) at 30 min (3.24 ± 0.52 µg/dL) and 60 min (3.27 ± 0.65 µg/dL) were significantly lower than baseline levels (decreased from the baseline (3.49 ± 0.52 µg/dL; P = 0.008 and P = 0.016, respectively; Figure 1). Marked intrasubject variability (Figure 2) accounted for 86% of the overall variability that was detected in this study. The overall measurements for all animals at all time points (minimum to maximum, 2.4 to 4.4 µg/dL) were within the reported reference interval (0.6 to 8.0µg/dL) for this species, for which animals also were sampled under isoflurane anestehsia.[8]

Discussion

We investigated the effect of isoflurane on plasma T4 concentration in black-tailed prairie dogs during 60 min of isoflurane anesthesia. The data indicate that isoflurane significantly but inconsistently decreased T4 levels in this species. These changes likely have little clinical importance, given that all T4 measurements at all time points were within the reported reference interval for this species.

In this study, plasma T4 concentrations were measured by using an enzyme immunoassay in a veterinary bench-top biochemistry analyzer. We chose to use this analyzer because it provides immediate patient-side results and requires only a small volume of blood.[10] This analyzer has been used before in research in black-tailed prairie dogs both in field[29] and laboratory settings.[7,8,13,18] In addition to enzyme immunoassays, thyroid hormones can be measured by using radioimmunoassays, fluorescence immunoassays, time-resolved fluorescence immunoassays, and chemiluminescent immunoassay.[20,21,28] Radioimmunoassays are considered the reference standard, but results obtained by using radioimmunoassays can vary due to differences in reagents, machinery, and handling techniques.[20] In people, the accuracy and precision of enzyme immunoassays were equivalent to radioimmunoassays.[17]

Comparing the findings from the current study with other studies might be challenging due to differences in the use of anesthetic protocols and surgery. In veterinary medicine, the only 2 species previously tested are dogs and laboratory rats, and in both, the measured thyroid hormones serum concentration was decreased after isoflurane anesthesia.[6,27] The rats under brief anesthesia were compared with control animals,[6] and the dogs were tested over multiple time points until 338 h after procedures.[27] The prairie dogs in our study were tested at 3 time points during brief (60 min) isoflurane-only anesthesia, to assess the effect on the thyroid as a potential risk factor for this type of anesthesia. Future studies should include a prolonged testing period and unanesthetized control animals.

Although a significant decrease in plasma T4 concentrations was detected in this study, this response was inconsistent between and within the prairie dogs, as has also been noted in humans, dogs, and rats. This inconsistency of results from multiple species suggests that thyroid function in black-tailed prairie dogs might not be as sensitive to the influence of isoflurane as the study results imply. Potential causes for a decrease in measured blood concentrations of thyroid hormones include altered protein binding, increased metabolism of T4, increased intracellular T4 transport, and decreased thyroid hormone secretion.[14,27] Although evaluating T4 levels is considered a good basic screening test for thyroid function, a panel of thyroid hormones (for example, T4, T3, free T4, thyroid stimulating hormone) needs to be tested to fully assess the effect of an anesthetic on thyroid function.[20]

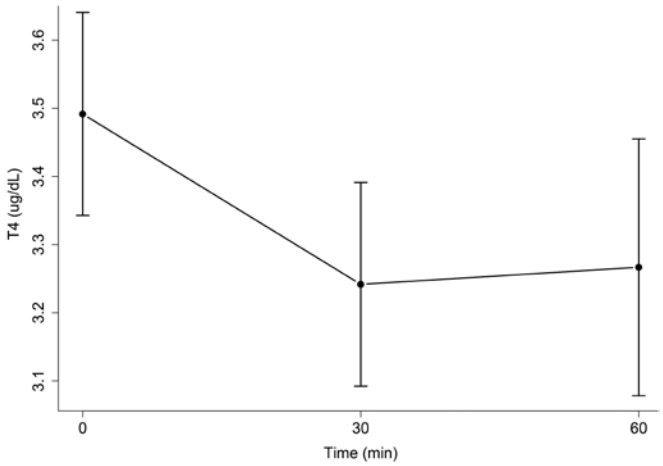

Figure 1. Plasma T4 concentrations (mean± 1 SD) in black-tailed prairie under isoflurane anesthesia. Plasma T4 concentration measurements were significantly decreased at 30 min ($P = 0.008$) and 60 min ($P = 0.016$) compared with baseline levels.

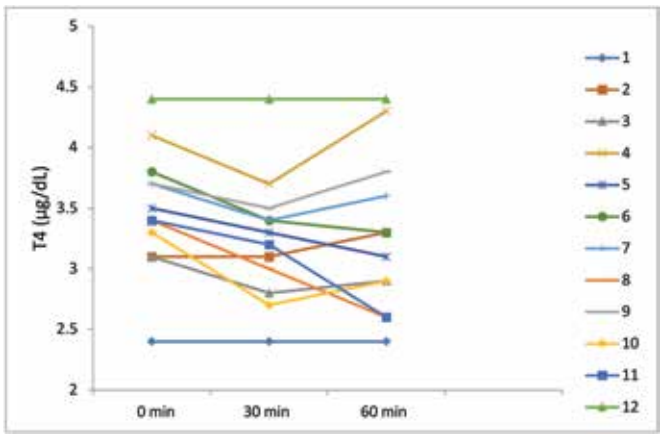

Figure 2. Plasma T4 concentrations in individual isoflurane-anesthetized black-tailed prairie dogs are highly variable.

The limitations of the current study include a small sample size. Although we tested only 12 prairie dogs, this number seems appropriate number when compared with the numbers of animals used in similar studies.[6,27] In addition, we tested only juvenile intact males. In rats, males showed a stronger decrease in T4 and T3 measurements under inhalation anesthesia compared with female rats.[6] Future studies should evaluate the effect of sex and age on T4 measurements of black-tailed prairie dogs under isoflurane anesthesia.

In conclusion, the data from this study suggest that 60 min of isoflurane anesthesia does not have clinically important or consistent effects on measured plasma T4 concentrations in black-tailed prairie dogs and thus is not likely a risk factor in this species. Because alternative immobilization protocols for prairie dogs include other gas and injectable anesthetic drugs,[25] separate studies need to be performed to evaluate the potential effect of each of these drugs on thyroid function in this species.

Acknowledgments

This study was funded by an internal Department of Clinical Sciences Research Grant, College of Veterinary Medicine, Kansas State University. We thank Dr Louden Wright, Dr Christina McCullough, Christine Hackworth, and the Sunset Zoo keepers and staff for their assistance with this study.

References

1. **Banta MR, Holcombe DW.** 2002. The effects of thyroxine on metabolism and water balance in a desert-dwelling rodent, Merriam's kangaroo rat (*Dipodomys merriami*). J Comp Physiol B **172:**17–25. https://doi.org/10.1007/s003600100222.
2. **Beisele M, Shen Z, Parry N, Mobley M, Taylor NS, Buckley E, Abedin MZ, Dewhirst FE, Fox JG.** 2011. *Helicobactermarmotae* and novel *Helicobacter* and *Campylobacter* species isolated from the livers and intestines of prairie dogs. J Med Microbiol **60:**1366–1374. https://doi.org/10.1099/jmm.0.032144-0.
3. **Börner U, Klimek M, Schoengen H, Lynch J, Peschau C, Schicha H.** 1995. The influence of various anesthetics on the release and metabolism of thyroid hormones: results of 2 clinical studies. Anesth Analg **81:**612–618.
4. **Brodbelt DC, Blissitt KJ, Hammond RA, Neath PJ, Young LE, Pfeiffer DU, Wood JL.** 2008. The risk of death: the confidential enquiry into perioperative small animal fatalities. Vet Anaesth Analg **35:**365–373. https://doi.org/10.1111/j.1467-2995.2008.00397.x.
5. **Court MH, Dodman NH, Norman WM, Seeler DC.** 1988. Anaesthetic management of small animal patients with endocrine disease. Br Vet J **144:**323–342. https://doi.org/10.1016/0007-1935(88)90062-0.
6. **Deckardt K, Weber I, Kaspers U, Hellwig J, Tennekes H, van Ravenzwaay B.** 2007. The effects of inhalation anaesthetics on common clinical pathology parameters in laboratory rats. Food Chem Toxicol **45:**1709–1718. https://doi.org/10.1016/j.fct.2007.03.005.
7. **Eshar D, Mason D, Avni-Magen N, Kaufman E, Paz A, Beaufrère H.** 2017. Evaluation of the effects of sternal versus lateral recumbency on trends of selected physiologic parameters during isoflurane anesthesia in zoo-housed black-tailed prairie dogs (*Cynomys ludovicianus*). J Zoo Wildl Med **48:**388–393. https://doi.org/10.1638/2016-0192R2.1.
8. **Eshar D, Nau MR, Pohlman LM.** 2017. Plasma thyroxine (T4) concentration in zoo-kept black-tailed prairie dogs (*Cynomys ludovicianus*). J Zoo Wildl Med **48:**116–120. https://doi.org/10.1638/2016-0073.1.
9. **Ferguson DC.** 1988. The effect of nonthyroidal factors on thyroid function tests in dogs. Compendium on continuing education for the practicing veterinarian **10:**1365–1377.
10. **Fredholm DV, Cagle LA, Johnston MS.** 2012. Evaluation of precision and establishment of reference ranges for plasma thyroxine using a point-of-care analyzer in healthy guinea pigs (*Cavia porcellus*). Journal of exotic pet medicine **21:**87–93. https://doi.org/10.1053/j.jepm.2011.11.004.
11. **Gardhouse SM, Eshar D, Bello N, Mason D.** 2015. Venous blood gas analytes during isoflurane anesthesia in black-tailed prairie dogs (*Cynomys ludovicianus*). J Am Vet Med Assoc **247:**404–408. https://doi.org/10.2460/javma.247.4.404.
12. **Halevy S.** 1980. Effects of anesthesia and surgery on thyroid function. Contemp Anesth Pract **3:**55–90.
13. **Higbie CT, Eshar D, Bello NM.** 2015. Evaluation of three point-of-care meters and a portable veterinary chemistry analyzer for measurement of blood glucose concentrations in black-tailed prairie dogs (*Cynomys ludovicianus*). Am J Vet Res **76:**532–539. https://doi.org/10.2460/ajvr.76.6.532.
14. **Ho WM, Wang YS, Tsou CT, Lin WH, Liao SQ, Hershman JM, Wong KC.** 1989. Thyroid function during isoflurane anesthesia and valvular heart surgery. J Cardiothorac Anesth **3:**550–557. https://doi.org/10.1016/0888-6296(89)90151-8.
15. **Holzbach RT.** 1984. Animal models of cholesterol gallstone disease. Hepatology **4 5 Suppl:**191S–198S. https://doi.org/10.1002/hep.1840040836.
16. **Hoogland JL, James DA, Watson L.** 2009. Nutrition, care, and behavior of captive prairie dogs. Vet Clin North Am Exot Anim Pract **12:**255–266. https://doi.org/10.1016/j.cvex.2009.01.013.
17. **Kaplan LA, Chen IW, Gau N, Fearn J, Maxon H, Volle C, Stein EA.** 1981. Evaluation and comparison of radio-, fluorescence, and enzyme-linked immunoassays for serum thyroxine. Clin Biochem **14:**182–186. https://doi.org/10.1016/S0009-9120(81)91212-1.
18. **Keckler MS, Gallardo-Romero NF, Langham GL, Damon IK, Karem KL, Carroll DS.** 2010. Physiologic reference ranges for

captive black-tailed prairie dogs (*Cynomys ludovicianus*). J Am Assoc Lab Anim Sci **49:**274–281.

19. **Kehlet H.** 1982. The modifying effect of general and regional anesthesia on the endocrine-metabolic response to surgery. Reg Anesth Pain Med **7:**S38–S48.
20. **Kemppainen RJ, Birchfield JR.** 2006. Measurement of total thyroxine concentration in serum from dogs and cats by use of various methods. Am J Vet Res **67:**259–265. https://doi.org/10.2460/ajvr.67.2.259.
21. **Lurye JC, Behrend EN, Kemppainen RJ.** 2002. Evaluation of an in-house enzyme-linked immunosorbent assay for quantitative measurement of serum total thyroxine concentration in dogs and cats. J Am Vet Med Assoc **221:**243–249. https://doi.org/10.2460/javma.2002.221.243.
22. **Michajlovskij N, Knopp J.** 1973. Effect of some anaesthetics on the level of free thyroxine in human and rat blood sera. Endokrinologie **62:**90–94.
23. **Murkin JM.** 1982. Anesthesia and hypothyroidism: a review of thyroxine physiology, pharmacology, and anesthetic implications. Anesth Analg **61:**371–383. https://doi.org/10.1213/00000539-198204000-00012.
24. **Oyama T, Wakayama S.** 1988. The endocrine responses to general anesthesia. Int Anesthesiol Clin **26:**176–181. https://doi.org/10.1097/00004311-198802630-00002.
25. **Pelizzone I, Vitolo GD, D'Acierno MA, Stefanello D, Forlani A, Broich G.** 2016. Lateral approach for excision of maxillary incisor pseudo-odontoma in prairie dogs (*Cynomys ludovicianus*). In Vivo **30:**61–67.
26. **Traynor C, Hall GM.** 1981. Endocrine and metabolic changes during surgery: anaesthetic implications. Br J Anaesth **53:**153–160. https://doi.org/10.1093/bja/53.2.153.
27. **Wood MA, Panciera DL, Berry SH, Monroe WE, Refsal KR.** 2009. Influence of isoflurane general anesthesia or anesthesia and surgery on thyroid function tests in dogs. J Vet Intern Med **23:**7–15. https://doi.org/10.1111/j.1939-1676.2008.0216.x.
28. **Yin D, Cui D, Gao F, He R, He Y, Liu Y, Shen D, Wu M.** 2008. A rapid and sensitive chemiluminescent immunoassay of total thyroxin with DMAE· NHS-Labeled. J Immunoassay Immunochem **29:**257–265. https://doi.org/10.1080/15321810802119075.
29. **Yoder CA, Miller LA.** 2010. Effect of GonaCon™ vaccine on black-tailed prairie dogs: immune response and health effects. Vaccine **29:**233–239. https://doi.org/10.1016/j.vaccine.2010.10.055.