Cross-Latitudinal, Seasonal and Diurnal Comparisons in Thyroid Hormone Concentrations in Sled Dogs

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Abstract: Problem Statement: Hypothyroidism has been a common disorder in humans and dogs. Amongst a sundry of functions, the thyroid gland plays a key role in metabolism and consequently, hypothyroidism has been a common diagnosis for a decrease in performance in sled dogs. Research has indicated that a variety of environmental factors influence thyroid hormone production, of which, light exposure, climate, latitude, exercise and season demonstrate pronounced effects. Sled dogs are exposed to many of these variables and often display thyroid hormone levels that are clinically below normal ranges. **Approach:** This study took a cross-latitudinal naturalistic approach in determining the effects of day length, time of day, season, climate and exercise on thyroid hormone function in sled dogs. In the process, appropriate reference ranges for racing sled dogs were established that correlate with other studies involving working dogs. **Results:** There was a clear indication that thyroid hormones play an integral role in thermoregulation and are greatly affected by environmental cues. Unexpectedly, sled dogs in the sub Arctic were not more prone to hypothyroidism but in fact, had higher levels of most thyroid hormones than dogs residing at lower latitudes. **Conclusion:** An evolutionary adaptation may account for up-regulation of thyroid function in times of environmental extremes. Consequently, the normal range for most thyroid hormones in sled dogs is lower than clinical standards.

Key words: Sled dogs, diurnal, thyroid hormones, seasonality, Alaska, exercise

INTRODUCTION

Thyroid hormone production is regulated by the hypothalamic-pituitary-thyroid hormone negative-feedback axis[14]. The main form of thyroid hormone produced by the thyroid gland is thyroxine (T_4). Extrathyroid tissues regulate the deiodization of the prohormone T_4 to the active form of the hormone, triiodothyronine (T_3). Small increases in T_4 and T_3 suppress the production and secretion of thyroid stimulating hormone (cTSH) through alterations in nuclear receptor binding, mRNA transcription and protein synthesis. Thyroid hormones, in serum, are either in a free form or bound to carrier proteins, such as thyroid hormone binding globulin (TBG), transthyretin, albumin and apolipoproteins. Small concentrations of free hormone are in a dynamic equilibrium with thyroid hormone bound to these carrier proteins. Both free and bound thyroid hormone reversibly enters interstitial fluid from circulating blood in a tissue dependant manner. Free hormone subsequently moves into the cell and either binds to cytosol-binding proteins and nuclear receptors, or is metabolized. Previous studies have shown that dogs have a higher concentration of free T_4 than humans and a lower binding affinity between T_4 and binding proteins. In addition, dogs, when compared to humans, exhibit a wider range of daily fluctuations in serum T_4[6,8].

Hypothyroidism is the most common endocrine disorder in dogs, often caused by autoimmune destruction or idiopathic atrophy of the thyroid gland. Replacement therapy involves oral administration of exogenous L-thyroxine (L-T_4)[6]. Despite this relatively easy solution to a common problem, the complexity of the thyroid hormone regulation and feed back systems

can lead to complications. Hypothryoidism affects many organ systems making it difficult to diagnose upon initial inspection. Compounding this diagnostic ambiguity on non-specific abnormalities is the uncertainty derived from canine T_4 test results. Euthyroid dogs can often have low or borderline T_4 levels because of normal fluctuations in serum T_4, age, breed variations, other illnesses, or interfering medications[9].

Documented deviations in human thyroid hormones have resulted from seasonal changes[12,18], exercise[16], diurnal variations[8,22] and possibly as a consequence of light[12]. Cold environments are known to heighten thyroid activity[18], but the mechanism is uncertain. Fluctuations in thyroid hormone are not limited to seasonal cycles but a diurnal cycle is also present. In humans, serum T_4 concentrations reach a high between 10:00 and 14:00 and a low around 2:00. A reverse trend is seen for cTSH. In contrast, dogs exhibit more random variations in thyroid hormone throughout the day due to decreased protein binding affinity and half-life differences[8]. Hoh and Oh[7] did not report any diurnal variation in T_3 but found that Total T_4 (TT4) and Free T_4 (FT4) peaked between 11:00 and 14:00. Bruner *et al*.[1] reported no diurnal variations in cTSH in hypothyroid and euthyroid dogs, collected over a 12 h sample period.

Sled dogs sampled before and after competing in a long distance race were sampled for T_3, T_4, total protein and albumin concentrations which all decreased significantly from pre-race to post-race. Conditioned sled dogs often display below the normal reference range for thyroid hormones[16,11]. Other breeds, such as sight hounds, also have lower than normal serum thyroid hormone concentrations[6]. Based on all the variables associated with racing sled dogs and reported thyroid hormone levels, Lee *et al*.[11] suggested lowering the reference ranges for the sled dog. In this study, we address the question of whether sled dogs exposed to more extreme daylight and temperature are more susceptible to hypothyroidism as judged by serum thyroid hormone levels. We present data from a cross-latitudinal and cross-seasonal study, looking at both exercising and non-exercising sled dogs.

MATERIAL AND METHODS

Animals: Alaskan huskies, *Canis lupis familiaris*, raised in Fairbanks, Alaska (Latitude, 65°N) or North Creek, New York (Latitude 45°) were used as test subjects. The Institutional Animal Use and Care Committee at the University of Alaska Fairbanks approved this study (#03-45). The test subjects were privately owned, typical Alaskan husky sled dogs from indistinguishable bloodlines. Nineteen dogs in NY, designated as the study dogs, were separated by the kennel owners into 2 groups, balanced for age, sex and ability. In New York there were 7 control (CON) and 12 exercise (EX) dogs. Similarly 24 sled dogs in Alaska were separated into 2 equal groups of 12. One dog was eliminated from the NY CON group prior to the termination of the study due to a diagnosis with lymphoma. The average age of the dogs in AK was 3.9 with 55% males. The average age of the dogs in NY was 3.6 with 65% males. Housing arrangements consisted of 2-m chains on which the dogs were tethered for the duration of the study (6 months). Each dog had access to his or her own house.

Diet: To insure that the dogs were acclimated to the diet, they were maintained on the study diet for 2 months preceding the study. All dogs were fed a measured amount of Purina Pro Plan Performance® daily (approximately 450 g). The amount varied slightly throughout the study for each dog in order to maintain ideal body condition. Ideal body condition is defined as easily palpable ribs and vertebral spinal processes, with a slight depression between the wings of the ileum[10,21]. During the acclimation period, the dogs were fed once a day in the morning. During the actual experiment the dogs were fed 12 h prior to blood collection to insure that the dogs were in a post-absorptive state.

Exercise: All exercising sled dogs were in a training program developed by the kennel owner, which focused on events that took place during the winter and spring. Kennels were selected based on congruency in distance trained, average speed and performance ability. No exercise was performed at least 12 h prior to sampling. CON dogs were not involved in any formal exercise program throughout the duration of the study.

Blood sampling: All dogs were bled on the winter and summer solstices. Average ambient temperature in New York on the summer sampling date was 18.7 and -11.3°C in the winter. Likewise, average ambient temperature in Alaska on the summer sampling date was 23.0 and -13.6°C in the winter. On each of the solstice's blood was drawn at 2:00, 8:00, 10:30 and 17:00. At the equinox blood was drawn at 17:00. These times were chosen based on reported fluxes in thyroid hormones[12,7]. Blood was drawn by venipuncture from the jugular into three 5 mL vacutainer tubes containing no anticoagulant. Serum was obtained by centrifugation at 2500 X g for 10 min, transferred into freezer vials, flash frozen in liquid nitrogen and stored at -70°C until they were analyzed.

Endocrine assays: Endocrine assays for this study were performed at the Endocrine Section, Diagnostic Center for Population and Animal Health, Michigan State University. Commercially available radioimmunoassay kits, validated for use in canine serum, were utilized for assay of total thyroxine (TT4[17], free thyroxine by equilibrium dialysis (FT4[4] and free triiodothyronine (FT3)[2]. Serum concentrations of total triiodothyronine (TT3) were measured using an in-house charcoal-separation radioimmunoassay, of which the procedure[20] and validation for dogs[15] were previously reported. Thyroid stimulating hormone (cTSH) was measured with a commercially available immunoradiometric assay kit, with previously reported performance data for the laboratory[17].

Statistics: Data was analyzed using repeated measures analysis of variance.[a] The dogs were nested within region and type of exercise. There were repeated measurements on the same dogs for the 2 seasons and 4 sampling times. Due to the double repeated measures, seasons and sampling times, a direct product covariance structure was used with an unstructured covariance matrix for the season and a compound symmetry covariance matrix for the hours sampled.

RESULTS

Diurnal trends: Significant TT4 diurnal trends were observed in dogs from NY in the summer, NY in the winter and AK in the summer. New York summer values displayed an increasing trend from 17:00-10:30, with a significant peak at 10:30, where as in the winter the opposite trend was observed with a significant peak at 17:00. No significant diurnal trend was observed in the winter in AK sled dogs but a trend existed that was opposite AK summer values. In the summer, levels of TT4 culminated with a peak at 2:00 (Fig. 1, Table 1).

Significant FT4 diurnal trends were observed in the summer and winter in dogs from NY. In the summer the FT4 trend mimicked that of TT4, with an increasing trend peaking at 10:30. In the winter, levels fluctuated throughout the day, with significant elevations occurring at 17:00 and 8:00. No significant diurnal trends were observed in AK (Fig. 2, Table 1).

Like TT4, significant TT3 diurnal trends were observed in dogs from NY in the summer, NY in the winter and AK in the summer. Though diurnal variations followed similar trends in both summer and winter in NY with peaks at 8:00, more exaggerated fluctuations were observed in the winter. No significant diurnal trend was observed in the winter in AK, but summer values peaked at 2:00 (Fig. 3, Table 1).

Significant FT3 diurnal trends were observed in dogs from NY in the winter, AK in the winter and AK in the summer. Although, no significant diurnal trend was observed in the summer in NY, in the winter a peak in serum FT3 levels persisted between 10:30 and 17:00.

Table 1: Mean and SEM of thyroid hormones in sled dogs located in Alaska or New York

NY winter	mean±sem	NY summer	mean±sem	AK winter	mean±sem	AK summer	mean±sem
Serum free T$_4$							
17:00	12.5±1.3[C,G]	17:00	7.71±0.9[a,c,E]	17:00	12.7±1.0	17:00	11.2±1.1[A]
2:00	7.17±1.1[b,g,h]	2:00	10.3±1.4[b,e,f]	2:00	14.0±1.0[B]	2:00	10.7±0.8
8:00	11.5±1.7[H]	8:00	13.4±1.4[e]	8:00	13.3±0.9	8:00	10.7±0.7
10:30	9.02±1.8[d,g]	10:30	15.1±1.4[D,e,F]	10:30	12.1±1.6	10:30	12.2±1.0
Serum free T$_3$							
17:00	5.16±0.3[A,D,k]	17:00	3.93±0.3[d]	17:00	3.95±0.3[a,H]	17:00	3.93±0.3[e]
2:00	4.73±0.2[k]	2:00	4.42±0.2	2:00	4.61±0.3[h,I]	2:00	4.43±0.4[E,F,G]
8:00	4.18±0.3[K,l]	8:00	3.56±0.2	8:00	4.38±0.3[C,h,J]	8:00	3.56±0.3[c,f]
10:30	5.01±0.4[B,L]	10:30	3.78±0.2	10:30	3.61±0.3[b,I,j]	10:30	3.78±0.3[g]
Serum total T$_4$							
17:00	19.3±1.4[H]	17:00	14.7±2.1[a,F]	17:00	21.5±2.0[E]	17:00	22.8±1.6[A]
2:00	13.9±1.4[h]	2:00	18.2±2.4[b,f,G]	2:00	18.5±2.0[b,e]	2:00	25.7±1.6[B,a]
8:00	15.9±1.6[c]	8:00	23.7±2.2[C,f,g,d]	8:00	20.8±2.0	8:00	19.3±1.6[a,b]
10:30	14.7±1.0[d,h]	10:30	24.8±1.7[D,f,g]	10:30	18.7±2.0[e]	10:30	20.5±1.9[b]
Serum total T$_3$							
17:00	1.16±0.09[B,I]	17:00	0.97±0.07	17:00	1.37±0.07[b]	17:00	0.97±0.06[e]
2:00	0.99±0.06[C,i,j]	2:00	0.93±0.08[h]	2:00	1.41±0.05[c,G]	2:00	1.04±0.03[e,F]
8:00	1.35±0.07[i,J]	8:00	1.04±0.06[A,H]	8:00	1.38±0.07	8:00	0.86±0.05[a,E]
10:30	1.09±0.06[D,j]	10:30	1.02±0.06	10:30	1.30±0.07[d,g]	10:30	0.89±0.05[f]
Serum thyroid stimulation hormone							
17:00	13.8±1.3	17:00	10.4±0.4[H]	17:00	11.9±0.7[g]	17:00	13.5±1.8[F]
2:00	12.6±1.7[D]	2:00	5.89±1.3[d,h]	2:00	12.5±0.8[C,g]	2:00	9.39±0.6[c,f]
8:00	13.8±1.4[B]	8:00	7.93±1.4[b]	8:00	9.42±0.5[b,G]	8:00	10.9±0.8[f]
10:30	12.7±2.1[e]	10:30	6.41±1.3[a,E,h]	10:30	11.8±0.8[g]	10:30	10.8±0.6[A,f]

Values represent the diurnal variation on the winter and summer solstice. Significance is established with p≤0.05 and significance is shown with letter superscripts. Capital letter superscripts refer to the values in which the lower case superscripts are compared
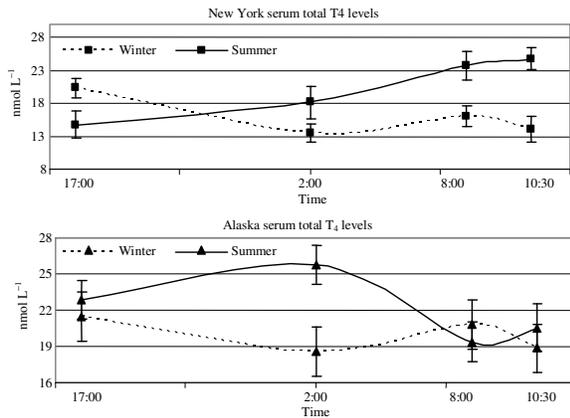
Fig. 1: Serum total T_4 (TT4) in sled dogs living in New York or Alaska collected on the winter and summer solstice
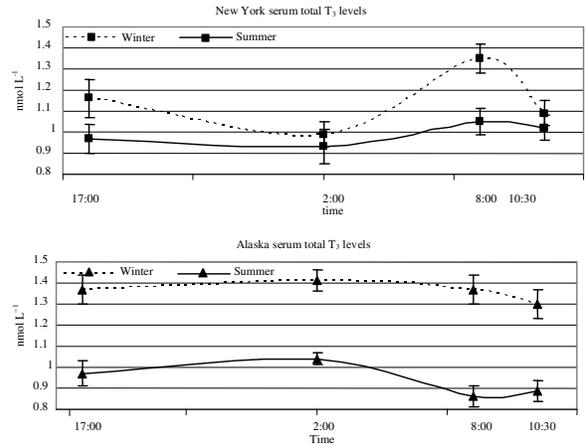

Fig. 2: Serum free T_4 (FT4) in sled dogs living in New York or Alaska collected on the winter and summer solstice

Fig. 3: Serum total T_3 (TT3) in sled dogs living in New York or Alaska collected on the winter and summer solstice
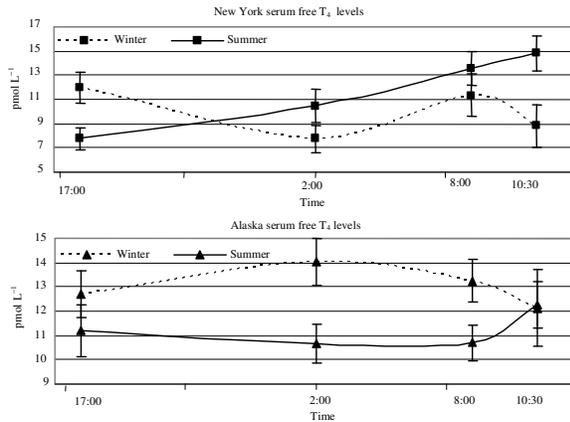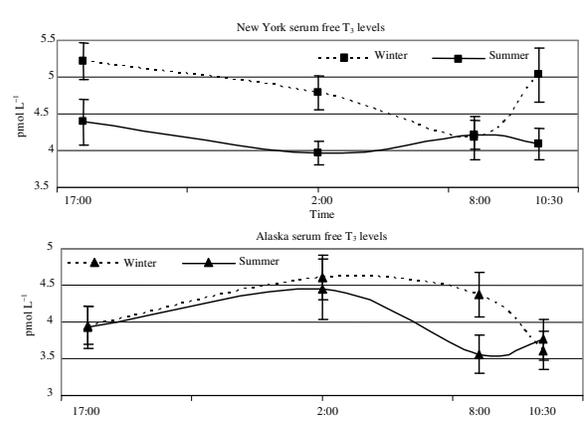

Fig. 4: Serum free T_3 (TT3) in sled dogs living in New York or Alaska collected on the winter and summer solstice

Summer values in AK followed a similar trend as was seen in TT3 levels, with a more pronounced peak occurring at 2:00. In the winter the peak persisted between 2:00 and 8:00 (Fig. 4, Table 1).

Significant cTSH diurnal trends were observed in dogs from NY in the summer, AK in the winter and AK in the summer. Serum cTSH in the summer in both NY and AK fluctuated thoughout the day and followed the same trend with elevations occurring at 17:00 and 8:00. No significant diurnal variation was observed in NY winter, but definite variations were observed in serum cTSH levels in dogs in AK in the winter that mirrored AK summer values. There was a significant lull in AK winter values at 8:00 (Fig. 5, Table 1).

Seasonal trends: Comparable seasonal trends were observed in serum TT4 levels in both AK and NY, summer values were higher than winter. Both locations displayed contrasting trends in the winter verses summer, with daily peaks and lull at opposing times (Fig 1, Table 1).

Significant seasonal variations in FT4 occurred in dogs located in AK, but not in NY. In contrast with TT4, serum FT4 levels in Alaskan sled dogs were significantly higher in the winter than in the summer. Opposing seasonal trends were observed, with peaks and lulls at opposite times of the day (Fig. 2, Table 1).

Significant seasonal variations in TT3 occurred in dogs located in AK, but not in NY and again, Alaskan sled dogs were higher in the winter than in the summer.
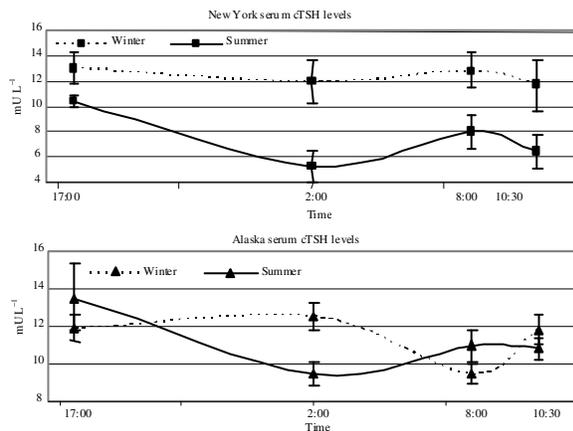
Fig. 5: Serum thyroid stimulating hormone (cTSH) in sled dogs living in New York or Alaska collected on the winter and summer solstice

Alaskan sled dogs displayed similar trends in both the winter and summer, with significantly higher values at all of the sampling times in the winter compared to summer (Fig. 3, Table 1).

In contrast with TT3, significant seasonal variations in FT3 occurred in dogs located in NY, but not in AK and again winter values were higher than summer. Though seasonal trends appear to mirror each other in NY, peaks and lulls do not. All sample times in the winter in NY display significantly higher values with the exception of 8:00 (Fig. 4, Table 1).

Significant seasonal variations in cTSH occurred in dogs located in NY, again, winter values were higher than summer. Though overall significant seasonal differences were not observed in AK, there appears to be opposing diurnal trends in winter and summer. In sled dogs in NY, winter cTSH values were significantly higher than summer at all sample times (Fig. 5, Table 1).

Latitude trends: Overall, Alaska values were significantly higher than New York for most of the thyroid hormones measured. More specifically, serum TT4, FT4 and TT3 levels in sled dogs in the winter living in AK were all greater than serum levels in NY, regardless of season. Much less of a variation in thyroid hormone levels was seen between latitudes in the summer. The only variables that were not impacted by latitude were serum FT3 and cTSH. Several individual sampling times differed between latitudes (Table 1).

Exercise: Exercise did not impact most of the variables measured (FT4, TT3, FT3). However, TT4 values at 17:00 were significantly greater than 10:30 in exercising dogs, while the 10:30 mean was significantly greater than the 17:00 mean in non-exercising dogs, regardless of location or time of year. Additionally, non-exercising sled dogs had significantly greater cTSH.

DISCUSSION

In all mammals, the thyroid gland is a central regulating gland that controls overall metabolic rate as well as stimulating cell and tissue growth. Due to the thyroid's role in body temperature regulation, studies have examined the effects of cold temperatures on thyroid hormone levels. While some published reports show an increase in T_3 in winter months, others show a decrease of T_3 and T_4, making it difficult to generalize and the field remains unclear because of confounding seasonal and diurnal factors. Our results create a complicated picture that illustrates a dynamic impact on thyroid hormone levels by season and environmental stimuli.

Availability of data on diurnal fluctuations in dogs is limited, especially when factoring in variables such as different day length. Reports show similar variation in T_3 and T_4 levels throughout the day in dogs as in humans, with the peak of T_3 and T_4 levels for humans between 10:00 and 14:00[18] and the peak of T_4 levels in dogs between 11:00 and 14:00[1,7]. While sled dogs in NY for the current study displayed T_4 levels in the summer that were comparable to previous reports (10:30)[7], winter trends were the opposite. Free T_3 for NY dogs in the winter was the only T_3 value that resembled previous findings in humans (10:30-17:00). Alaska experiences 21 h of daylight on the summer solstice and interestingly, sled dogs displayed a peak in TT4, FT3 and TT3 that was shifted to 2:00 a.m. when light is still present. In the winter in Alaska, when there is only 3.5 h of low light, there is no diurnal variation in FT4 and TT4 levels. These findings, clearly illustrate that diurnal variation in thyroid hormones are impacted by day length.

Climate has been an area of interest as a possible influence on thyroid hormone production. Reed et al.[19] described an increase in cTSH, a decrease in T_3 and no change in T_4 in human, euthyroid males during the first 42 weeks of the subjects' stay in Antarctica. This has been described as the polar T_3 syndrome. Xu et al.[24] found similar results but a smaller difference in free T_3 and a decrease in total T_4. Maes et al.[13] reported a significant annual cycle in total T_3, with higher values in the winter and fall. Our results, akin to[13], showed higher levels of all but TT4 in the winter than summer. Additionally, sled dogs living in the sub Arctic had

Table 2: Comparison of thyroid hormone panels in determining a normal range in sled dogs

REFERENCES	TT4 nmol L^{-1}	TT3 nmol L^{-1}	FT4 pmol L^{-1}	FT3 pmol L^{-1}	cTSH mU L^{-1}
MSU	15-67	1-2.5	6-42	4.5-12	0-37
Lee et al. 2004 pre-race (122 dogs)	21±10 26%	N/A	14±6.5 18%	N/A	18±10 6%
Lee et al. 2004 post-race	7.7±3.7 95%	N/A	9.8±5.4 44%	N/A	13±6.4 0%
Present Study (43 dogs)	20±7.8 31%	1.1±0.33 30%	11±2.5 13%	4.3±1.2 67%	12.4±4.9 0.3%
Suggested normal range for sled dogs	5-40	0.8-1.6	3-24	2-6.5	5-17

Michigan State University (MSU) accepted canine reference range for thyroid hormones is given. Values for means and standard deviations for previous and current sled dog studies followed by the percent of dogs that fell outside of the MSU reference range are shown. Based on means, medians and standard deviations in sled dog thyroid studies, a new reference range is suggested

overall higher thyroid hormone concentration, than the sled dogs living in lower latitudes, especially in the winter. Unlike the Antarctica studies, the dogs in this study were already acclimated to their environments. We examined Alaskan sled dogs that have been bred and raised in the circumpolar north. Thyroid hormones are integral in thermoregulation and therefore it make sense that an acclimated animal would have elevated thyroid hormone production in the coldest part of the year and at a location where mean winter temperatures are lower.

Exercise did not appear to be a major influence in FT3, TT3, or FT4 in this study. Total T_4 levels, however, were significantly higher at 17:00 than at 10:30 in exercising sled dogs, while the opposite was seen in the non-exercise dogs, regardless of season or location. Sled dogs are often exercised early in the day to avoid the heat. This reverse trend may indicate that exercise temporarily suppresses TT4. Another indication that exercise may suppress thyroid function was reduced cTSH levels observed in the winter in exercise dogs compared with non-exercise dogs, regardless of location. Lee *et al*.[11] and Panciera *et al*.[16] reported reduced thyroid hormone production (TT4, FT4 and cTSH) at the completion of a long distance race that were compared with pre-race and off-season values. The sled dogs participating in the above study were long distance, endurance sled dogs. The current study used sprint-type sled dogs that exercise at a higher intensity but for a much shorter duration. Additionally, these sled dogs were not sampled following an intense period of exercise, so the levels are more reflective of the basal concentrations in physically fit sled dogs. Any suppression of thyroid function or hormone production that occurred as a result of exercise, in the current study, was temporary and did not extend throughout the day.

As clearly illustrated by the fluctuating thyroid hormone levels throughout the day, across season and latitudes, diagnosing deficiencies in thyroid hormone production is not an easy task. In the past, hypothyroidism has been a common diagnosis for a non-specific lack or decrease in performance in sled

dogs. Lee *et al*.[11] suggested that reference ranges for sled dogs might be lower than for other breeds. Our results support this conclusion. The percentage of sled dogs in the aforementioned study and the current study that displayed lower than normal range for all thyroid hormone levels was between 13-95%, depending on exercise and the variable measured. The Animal Health Diagnostic Laboratory Endocrine Diagnostic Section at Michigan State University has established the current reference ranges used by clinicians. Based on breed variability that has been reported in other working dogs, we have suggested a new reference range for sled dogs that strongly agrees with[11] (Table 2). In addition, for diagnostic and research purposes we suggest that a fixed time of day for sampling be agreed upon by the veterinary community.

Most competitive racing sled dog teams come from similar and overlapping bloodlines. Historically, sled dogs have been selected for endurance, tenacity, conformation, toughness and speed. Their ancestors were part of a subsistence culture, often performing in a feast or famine economy. This may have given sled dogs an evolutionary advantage in an extreme environment that has allowed for an adaptation to less-than-ideal conditions, a reflection that can be seen by exceptional feats of athleticism with thyroid hormone concentrations lower than the general populace. Contrary to expectations, sled dogs residing in Alaska do not appear to be more susceptible to hypothyroidism. Quite the opposite, during the time of intense competitions, the dark of the winter and plummeting temperatures, the mechanisms of thermoregulation compensated and maintained appropriate levels.

CONCLUSION

Sled dogs are truly amazing animals and continue to prove to be a remarkable model for research related to environmental impacts, endocrinology, nutrition and exercise physiology. Thyroid problems afflict a great number of humans and dogs and it is clear that thyroid function is sensitive to environmental stimuli. For

future research, standardizing sampling time would improve comparative studies and advance the field. Dogs are becoming a good indicator species for the human environment[5] and baseline studies will also serve in monitoring the effects of thyroid hormone-disrupting chemicals[3,23].

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