Plasma Vitamin D Metabolites and C-Reactive Protein in Stage-Stop Racing Endurance Sled Dogs

J.W. Spoo, R.L. Downey, C. Griffitts, R.J. Horst, C.B. Levine, R.M. Childs, and J.J. Wakshlag

Background: Dogs are a unique model for examining the effects of exercise on vitamin D status because of their lack of vitamin D synthesis by UV exposure. In addition, the inflammatory response may be associated with hypovitaminosis D.

Objectives: To investigate the effects of several days of endurance exercise on plasma vitamin D (25-(OH)D₃, 24,25-(OH)₂D₃, and 1,25(OH)₂D₃) and serum C-reactive protein (CRP) concentrations in stage-stop racing sled dogs.

Animals: 12 racing sled dogs and 8 control dogs.

Methods: Blood was collected before the race and immediately after racing on days 2 and 8. Plasma vitamin D metabolites and serum CRP concentrations were measured.

Results: Racing dogs showed a significant increase in 25(OH)D₃ on day 2 (P = .027) and day 8 of the race (P < .001), whereas no increases were observed in control dogs. The plasma concentration of 24,25(OH)₂D₃ showed a significant increase by day 8 (P < .001). There were no significant changes in 1,25(OH)₂D₃ concentrations across all time points and groups. Racing dogs had significantly increased CRP concentrations by day 2 (39.3 ± 30.1 μg/mL; P < .001).

Conclusions and Clinical Importance: Increases in vitamin D metabolites as well as increases in CRP concentrations were observed in racing sled dogs. This finding was contrary to the hypothesis that decreases in vitamin D status in athletes may be related to the acute phase inflammatory response during exercise. In addition, the increased 24,25(OH)₂D₃ concentrations compared to what is observed in other species suggests metabolic variations in dogs that lead to enhanced disposal of vitamin D.

Key words: Endocrinology; Metabolism; Nutrition; Physiology; Sports medicine; Vitamins and minerals.

The effects of vitamin D on human athletic performance, has been examined as early as the 1930s. More recently, there has been widespread concern about vitamin D deficiency in humans. As a result, many have examined 25(OH)D₃ status in human athletes and evaluated the associated links to injury and performance, as well as muscle strength and neuromuscular function in humans.

Vitamin D, in its active form 1,25(OH)₂D₃, is tightly regulated by parathyroid hormone activation of renal 1α-hydroxylase activity, is 100-fold lower in concentrations than 25(OH)D₃, and has marked influence over calcium homeostasis. Recently, it has been observed to play roles in neoplasia and muscle development, and nearly all organ systems have receptors for 1,25(OH)₂D₃. In the dog, vitamin D concentrations have, status has been investigated and found to be decreased in protein losing enteropathy, renal disease, neoplasia, and cardiovascular disease. Vitamin D concentrations have been found to be low in human endurance athletes; it is difficult to determine if these lower concentrations are related to the exercise, shifts in dietary patterns or a lack of ultraviolet exposure.

Abbreviations:
- 1,25 (OH) D₃: 1,25-dihydroxyvitamin D₃
- 24,25 (OH) D₃: 24,25-dihydroxyvitamin D₃
- 25 (OH) D₃: 25-hydroxyvitamin D₃
- CRP: C-reactive protein
- CYP24: cytochrome p450 24 hydroxylase
- GI: gastrointestinal
- HPLC: high pressure liquid chromatography

In addition, research suggests that in inflammatory conditions, in which 25(OH)D₃ concentrations decrease, there often is a concomitant increase in C-reactive protein (CRP) concentrations. Endurance sled dogs have been shown to mount a robust CRP response after exercise, that is 3- to 5-fold higher than that observed in human marathon runners. This increase in CRP without chronic disease allows examination of the associations between hypovitaminosis D and increased CRP concentrations to determine if chronic inflammation drives hypovitaminosis D as a result of increased exercise, increased gastrointestinal (GI) or renal losses, or both.
To date, no study has investigated the vitamin D status of the canine athlete with a steady dietary intake of vitamin D$_2$ (ergocalciferol) and D$_3$ (cholecalciferol). Results obtained could provide critical information as to the influence of exercise on serum 25(OH)D$_3$ concentrations in endurance athletes, both human and dog. 5-8 In addition, 24,25(OH)D$_3$ concentrations in dogs have not been examined and will provide valuable information related to renal 24 hydroxylase activity (CYP 24) and its relationship with vitamin D metabolism. 9

We assessed the complete vitamin D (25(OH)D$_3$, 24,25(OH)D$_3$, and 1,25(OH)D$_3$) and CRP status of control and racing sled dogs competing in a multi-day stage race on days 0, 2, and 8 of the event.

Materials and Methods

Animals

Dogs (16) from 2 teams (but the same kennel) participating in the 2014 International Pedigree Stage-Stop Sled Dog Race were enrolled in the study. The study was approved by the Cornell University Institutional Animal Care and Use Committee. The kennel owner signed a client consent form before study initiation. All dogs had full health examinations before the race, and they were deemed healthy. Dogs selected for analysis participated in 5 of the 8 days of the race with all dogs racing on days 1 and 8 of the racing schedule, with variable racing on the days between. Dogs were rested on day 3 as a scheduled travel day, and because of weather and trail conditions also were rested on day 7. The average racing time for each stage of the race was 3.5–5 hours of continuous running with no relevant resting periods, and carrying a lightweight sled with 80–95 kg including musher and supplies. Of the 12/16 racing dogs that had a complete sample set on days 0, 2, and 8; 7 were male and 5 were female, averaging 4.3 years old. Control racing dogs that had a complete sample set on days 0, 2, and 8; 7 were male and 2 were female, with an average age of 3.6 years. All dogs were weighed immediately before the race on day 0 and immediately after the race on day 8 using a scale with a single investigator holding the dogs at both time points and subtracting the investigator’s weight at both time points to obtain a final weight for each dog to the nearest pound, which then was converted to kilograms.

Blood Sampling and Analysis

Blood was sampled on day 0 between 12 and 1 PM, day 2 immediately after racing between 2 and 3 PM and day 8 between 2 and 3 PM. At each time-point, 6 mL of whole blood was obtained by cephalic venipuncture using a 22 gauge needle into a 5 mL lithium heparin tube. Blood samples were protected from light and immediately centrifuged at 4,000 x g for 10 minutes. Three aliquots of plasma were immediately stored on dry ice until transportation to the investigators’ lab within 48 hours where they were immediately placed into a –80°C freezer.

25(OH)D$_3$ and 24,25(OH)D$_3$

Serum/plasma samples (100 µL) were aliquoted into 12 x 75 mm borosilicate glass in preparation for analysis of 25(OH)D$_3$. Samples were spiked with deuterated 25(OH)D$_3$ followed by protein precipitation using the ZnSO$_4$/methanol method. 10 25(OH)D$_3$ was extracted with hexane, dried and reconstituted with liquid chromatography-mass spectrometry grade methanol and water. Quantification was achieved by liquid chromatography-tandem mass spectrometry unit using an Agilent 1290 high pressure liquid chromatography (HPLC) unit coupled to an Agilent 6460 Triple-quad mass spectrometer after the procedure outlined by Agilent. Samples were prepared and analyzed for 24,25(OH)D$_3$ in the same manner as 25(OH)D$_3$, except 200 µL of sample was extracted and deuterated 24,25(OH)D$_3$ was used as an internal standard. 30 The inter- and intra-assay coefficients of variation were <12% for both assays.

Total 1,25(OH)$_2$D

Total 1,25(OH)$_2$D was assayed using the competitive radioimmunoassay procedure described by Hollis et al. 31 Inter- and intra-assay coefficients of variation for 1,25(OH)$_2$D were 8 and 11%, respectively.

In addition, a representative food sample was taken from the day 4 feeding and was immediately frozen and shipped to the investigators’ laboratory and stored at –80°C. This feed sample (150 g) then was sent to the same nationally certified laboratory for similar vitamin D analysis as performed on the plasma samples. 6 Feed samples were processed according to the methods of Phillips et al, which included saponification and solvent extraction. 32 One-hundred and fifty grams of sample was sent for proximate analysis of protein, fat, ash, fiber, and moisture at a commercial analysis laboratory. 33 Details on the constituents in the meal were obtained from the kennel owner while making the feed on site.

Vitamin D metabolite analysis was performed for the feed and serum samples after purification by a semipreparative normal-phase HPLC, and quantification with a reverse-phase HPLC/mass spectroscopy technique utilizing cholecalciferol as the internal standard for quantification. 32

C-Reactive Protein ELISA

A canine C-reactive protein kit that has been validated for use on canine serum was used. The kit was used according to the manufacturer’s suggestions with all samples from the same dog being performed on the same plate in duplicate. All postexercise samples were diluted 1 : 5 as suggested by the manufacturer to ensure that concentrations fell within the linear portion of the standard curve (5–100 µg/mL).

Data and Statistical Analysis

Initial dog weights before and after the race were analyzed using a paired Student’s t-test. Vitamin D metabolites and CRP concentrations across the 2 populations of racing and control dogs were assessed for normality utilizing the Shapiro-Wilk test. After normality was confirmed for all vitamin D metabolites (1,25(OH)$_2$D$_3$, 25(OH)D$_3$, and 24,25(OH)D$_3$) results were analyzed across the control and racing dogs using a 2-way analysis of variance with Tukey’s posthoc analysis over time and group. Not all CRP data was normally distributed, therefore all data was transformed before analysis of variance and no residual outliers were removed. Furthermore, Pearson’s correlates were examined assessing 25(OH)D$_3$ and CRP status across all racing dogs to establish whether there is an association between serum 25(OH)D$_3$ and CRP concentrations at days 2 and 8. P values of .05 were considered significant.

Results

Dogs and Exercise

Of the 16 dogs from which blood was initially obtained, only 12 ran in 5 of the 6 days of racing over the 8-day time period. The other 4 dogs participated in
4 or fewer days and were excluded from final analysis. The total distance each dog ran over the 8 days was between 218 and 236 miles. The average weight of the 12 dogs before the race on day 0 was 25.2 ± 2.1 kg, whereas after day 8 racing the average was 25.0 ± 2.0 kg, which was not statistically different. The control dogs’ average weight on day 0 was 26.5 ± 2.5 kg and on day 8 it was 26.9 ± 2.3 kg, which was not statistically significant.

**Vitamin D Metabolites**

Vitamin D metabolites concentrations were different from resting during days 2 and 8 of the event (Table 1). Dogs competing in the race had a significant increase in 25(OH)D3 concentrations on day 2 of the race ($P = .027$) and a further increase in 25(OH)D3 concentration on day 8 of the race ($P < .001$). When comparing control and racing dogs, there was no difference on day 0 ($P = .09$), whereas on day 2 ($P = .006$) and day 8 ($P < .001$) there was a significant increase compared to controls. The plasma concentration of 24,25(OH)D3 did not change significantly in the racing dogs from the start of the race to day 2 ($P = .65$), but did significantly increase by day 8 ($P < .001$). Differences in 25(OH)D3 across days in the control group were not significant, whereas the racing group had higher 24,25(OH)D3 concentrations on day 8 ($P = .004$) and no significant differences on days 0 ($P = .081$) and 2 ($P = .062$) when compared to the control group. There were no significant changes in 1,25(OH)D3 concentration across all time points in the racing group. The 1,25(OH)D3 concentrations in the control dogs decreased from days 0 to 2 ($P = .014$) and 8 ($P = .016$), whereas there was only a significant difference between control and racing dogs at day 0 ($P < .001$).

**Feed Analysis**

Complete analysis of the feed can be found in supplemental section with tabular comparison to National Research Council established recommended daily intake values per 1000 kcals. Vitamin D analysis showed ergocalciferol content of <25 IU/kg dry matter and approximately 1,919 IU/kg dry matter of cholecalciferol.

**C-Reactive Protein**

Plasma CRP concentrations were significantly different between control dogs and racing dogs, and there were significant changes from day-to-day within the 2 groups (Fig 1). The control group started with a concentration that was higher than that of the racing group ($19.4 ± 14.8$ μg/mL and $10.7 ± 4.8$ μg/mL, respectively), but this difference was not statistically significant. The control group’s CRP concentrations decreased over the 8 days and were significantly lower on day 8 as compared to day 0 (day 2: 11.6 ± 4.5 μg/mL; day 8: −8.3 ± 3.2 μg/mL; $P = .023$). The racing group had a significant increase in concentration from racing h/o day 2 (39.3 ± 30.1 μg/mL; $P < .001$). The concentrations on day 8 were statistically decreased from day 2 concentrations ($P < .001$). Concentrations on day 8 (17.2 ± 10.5 μg/mL), although increased from day 0, were not significantly higher.

Pearson’s correlates were performed on days 2 and 8 to examine the associations between increases in CRP and 25(OH)D3 concentrations in racing dogs. Pearson’s correlates for these associations were $R = 0.11$ on day 2 and $R = 0.10$ on day 8 and were not significantly different, hence negative associations between increasing CRP and decreasing vitamin D concentrations were not observed in this population.

**Discussion**

Vitamin D concentrations in the racing dogs responded in a manner contrary to the hypothesis with significant increases during exercise. Studies of human athletes have evaluated various vitamin D metabolites over the course of a competitive season, or using mean concentrations in a group of athletes, and not the effects of daily exercise. One hypothesis for the increases in vitamin D concentrations is the aerobic nature of the exercise performed by sled dogs and the subsequent mobilization of fat stores during exercise, which may result in vitamin D release during lipolysis of adipose stores, liver fat stores, or both. Interestingly, the dogs in this study experienced no significant change in weight between the start of the race and the end of the race making lipolysis and weight loss less likely a contributing factor, but increased adipose tissue turnover still may be

**Table 1.** 25(OH)D3, 24,25(OH)2D3, and 1,25(OH)2D3 mean ± SD at days 0, 2, and 8 (D0 – day 0, D2 – day 2, D8 – day 8) in racing and the control subjects during an 8-day multi-stage sled canine race.

|                    | Control D0 | Control D2 | Control D8 | Racing D0 | Racing D2 | Racing D8 |
|--------------------|------------|------------|------------|-----------|-----------|-----------|
| 25(OH)D3 (ng/mL)   | 57 ± 13    | 55 ± 11    | 57 ± 13    | 67 ± 9    | 72 ± 11a,b| 87 ± 16a,b|
| 24,25(OH)2D3 (ng/mL)| 54 ± 12    | 54 ± 13    | 55 ± 12    | 66 ± 13   | 67 ± 15   | 76 ± 18a,b|
| 1,25(OH)2D3 (pg/mL)| 157 ± 30   | 127 ± 33a  | 129 ± 34b  | 122 ± 34b | 119 ± 26  | 121 ± 22  |

Significant differences are indicated as follows: a indicates a significant difference between racing and control groups on day 0 ($P < .05$); b indicates a significant difference between day 0 only ($P < .05$).
A significant difference between racing and control groups on day 2 (P < .05). *Indicates a significant difference between racing and control groups on day 2 (P < .05).

Fig 1. Racing and control canine serum C-reactive protein (CRP) concentrations. Mean ± SD of CRP concentration at Rest (days 0), day 2 and day 8 in racing sled dogs (n = 12) and the control dogs (n = 8) during an 8-day multi-stage sled dog race. "Indicates a significant difference between racing and control groups on day 2 (P < .05)." *Indicates a significant difference between day 0 within the same group (P < .05).

involved. Although controversial, adipose tissue can store vitamin D, but studies in humans have shown that changes in body fat composition do not lead to an increase in serum 25(OH)D3 concentrations during weight loss.35

A second possibility would be an increase in dietary intake of vitamin D3. Here again, the dog driver verified that the approximate amount of provided feed was not changed in response to the race. Because the control dogs were fed approximately 20% less without a decrease in serum 25(OH)D3 concentration, the likelihood that any modest increase in intake influenced the racing dogs is unlikely.

Further proposed reasoning for the increased vitamin D concentrations revolve around the endocrine responses to exercise. The anabolic hormone insulin-like growth factor-1 (IGF-1) can increase during exercise because of hepatic synthesis and may be related to vitamin D increases. Although vitamin D increases during exercise are contrary results of a human study,37,38 younger athletes might experience an increase in IGF-1 which is directly correlated with increases in 25(OH)D3.39,40 Information regarding IGF-1 and IGF-1 binding proteins in canine foods in compliance with the American Association of Feed Control Officials.20 The meal these dogs consumed contained approximately 45% of the calories as commercial kibble45 and approximately 55% as a mix of beef, poultry, organ meat, and fish oil. Therefore, the excessive 24,25(OH)D3 concentrations were unlikely to have arisen from the diet. This poses the question of whether the amounts of cholecalciferol in commercial pet foods could be considered over-supplementation, because dogs in general have higher concentrations of serum 25(OH)D3 than humans.1,5,19-21 The dogs studied were athletes, and their vitamin D metabolism may be different than that of the average household companion dog. This situation warrants further examination into how cholecalciferol is metabolized in non-working companion dogs.

A decrease in vitamin D concentration may be associated with a reciprocal increase in CRP.25,26 Such was not the case in our study in which increases in 25(OH)D3 concentrations in the face of increases in CRP...
concentrations were observed. Overtraining syndrome and chronic fatigue is common in humans\textsuperscript{37,50} but little is known about this phenomenon in the canine athletes.\textsuperscript{51} The CRP response in exercising sled dogs has been well established.\textsuperscript{29,52,53} In these races, dogs ran >50 miles per day for 5–10 consecutive days. The CRP concentrations observed in previous studies of endurance sled dogs were between 100 and 200 µg/mL concentrations which are well above that observed in marathon runners, who show ranges from 15 to 30 µg/mL within 24 hours after cessation of exercise.\textsuperscript{54–56} The dogs in this study ran ≤50 miles on any given day (average, 40 miles) which might be the reason for the lower CRP concentrations, making them more similar to a marathon runner. None of the dogs in this study (control or racing) had run in the 36 hours before the start of the race. For many of the dogs, the last run occurred 3 days before and consisted of a 20–22 mile run. On day 0, the CRP concentrations in both groups (with the exception of 1 outlier in the exercise group and 2 in the control group) were approximately 10–20 µg/mL which is higher than the average sedentary dog.\textsuperscript{57} Previous studies in sled dogs showed that there appears to be a chronic increase in CRP concentrations at rest and that sprinting dogs tend to have lower CRP concentrations than endurance sled dogs.\textsuperscript{58} By day 2, we saw similar increases in CRP concentrations as previously reported in sled dogs, but not to the same magnitude. In fact, the increases observed on day 2 were similar to what is observed in human marathon athletes.\textsuperscript{51,54} We did not see the increase in CRP concentration expected on day 8, but this likely was because of day 7 of the race being canceled because of weather conditions, and all dogs were rested on day 7 and then competed on day 8. This interval presumably allowed recovery of CRP concentrations to near baseline for most dogs and we would have expected a similar increase to that observed on day 2 if we had collected blood 24 hours after the day 8 event.\textsuperscript{29}

Conclusion

In summary, the dogs in this study showed significant increases in vitamin D concentrations as well as increases in CRP concentrations. This finding was contrary to our hypothesis that decreases in vitamin D concentrations in athletes may be related to inflammation associated with exercise. This study highlights the need for further studies of vitamin D metabolism, particularly as it relates to athletic performance. Concentrations can fluctuate in a short period of time, and this fluctuation may be related to activity and potentially the nutritional status. In addition, the pronounced increase in 24,25(OH)\textsubscript{2}D\textsubscript{3} concentrations suggest that the metabolism of vitamin D in dogs may be different from other species or that dietary concentrations are high suggesting increased conversion for excretion from the body, which requires further study in normal sedentary canine populations. The results of this study also showed that CRP concentrations will decrease to 10–20 µg/mL 24–48 hours after peak concentration, but often remain higher than what is observed in sedentary dogs.

Footnotes

\textsuperscript{a} Heartland Assays, Ames, IA  
\textsuperscript{b} Dairy One, Ithaca, NY  
\textsuperscript{c} Canine C-reactive protein ELISA, Tridelta Development limited, Maynooth, Ireland  
\textsuperscript{d} Redpaw 32/20, Franklin, WI

Acknowledgments

This project was supported by Internal Grant Monies from Cornell University. We thank Terry and Buddy Streeper for allowing us to work with their dogs for this study. We also thank the Pedigree Stage-Stop Veterinary Team for their help with sample collections during the race.

Conflict of Interest Declaration: The authors disclose no conflict of interest.

Off-label Antimicrobial Declaration: The authors declare no off-label use of antimicrobials.

References

1. Cannell JJ, Hollis BW, Sorenson MB, et al. Athletic performance and vitamin D. Med Sci Sports Exerc 2009;41:1102–1110.
2. Holick MF. Vitamin D deficiency. N Engl J Med 2007;357:266–281.
3. Holick MF. The vitamin D deficiency pandemic and consequences for nonskeletal health: Mechanisms of action. Mol Aspects Med 2008;29:361–368.
4. Hanley DA, Davison KS. Vitamin D insufficiency in North America. J Nutr 2005;135:332–337.
5. Angeline ME, Gee AO, Shindle M, et al. The effects of vitamin D deficiency in athletes. Am J Sports Med 2013;41:461–464.
6. Willis KS, Peterson NJ, Larson-Meyer DE. Should we be concerned about the vitamin D status of athletes? Int J Sport Nutr Exerc Metab 2008;18:204–224.
7. Willis KS, Smith DT, Broughton KS, Larson-Meyer DE. Vitamin D status and biomarkers of inflammation in runners. Open Access J Sports Med 2012;3:35–42.
8. He C-S, Handzlik M, Fraser WD, et al. Influence of vitamin D status on respiratory infection incidence and immune function during 4 months of winter training in endurance sport athletes. Exerc Immunol Rev 2013;19:86–101.
9. Magee PJ, Pourshahidi LK, Wallace JM, et al. Vitamin D status and supplementation in elite irish athletes. Int J Sport Nutr Exerc Metab 2013;23:441–448.
10. Powers S, Nelson WB, Larson-Meyer E. Antioxidant and Vitamin D supplements for athletes: Sense or nonsense? J Sports Sci 2011;29(Suppl 1):S47–S55.
11. Peeling P, Fulton SK, Binnie M, Goodman C. Training environment and Vitamin D status in athletes. Int J Sports Med 2013;34:248–252.
12. Mastaglia SR, Seijo M, Muzio D, et al. Effect of vitamin D nutritional status on muscle function and strength in healthy women aged over sixty-five years. J Nutr Health Aging 2011;15:349–354.
13. Stockton KA, Mengersen K, Paratz JD, et al. Effect of vitamin D supplementation on muscle strength: A systematic review and meta-analysis. Osteoporos Int 2011;22:859–871.

14. Dhesi JK, Jackson SHD, Bearne LM, et al. Vitamin D supplementation improves neuromuscular function in older people who fall. Age Ageing 2004;33:589–595.

15. Zhu K, Austin N, Devine A, et al. A randomized controlled trial of the effects of vitamin D on muscle strength and mobility in older women with vitamin D insufficiency. J Am Geriatr Soc 2010;58:2063–2068.

16. Bischoff-Ferrari HA, Dietrich T, Orav EJ, et al. Higher 25-hydroxyvitamin D concentrations are associated with better lower-extremity function in both active and inactive persons aged > or =60 y. Am J Clin Nutr 2004;80:752–758.

17. Ceglia L, Chiu GR, Harris SS, Araujo AB. Serum 25-hydroxyvitamin D concentration and physical function in adult men. Clin Endocrinol (Oxf) 2011;74:370–376.

18. Demay MB. Physiological insights from the vitamin D receptor knockout mouse. Calcif Tissue Int 2013;92:99–105.

19. Kraus MS, Rassnick KM, Wakshlag JJ, et al. Relation of vitamin D status to congestive heart failure and cardiovascular events in dogs. J Vet Intern Med 2014;28:109–115.

20. Wakshlag JJ, Rassnick KM, Malone EK, et al. Cross-sectional study to investigate the association between vitamin D status and cutaneous mast cell tumours in Labrador retrievers. Br J Nutr 2011;106:560–563.

21. Galler A, Tran JL, Krammer-Lukas S, et al. Blood vitamin D levels in dogs with chronic kidney disease. Vet J 2012;192:226–231.

22. Mellanby RJ, Mellor PJ, Roulois A, et al. Hypocalcaemia associated with low serum vitamin D metabolite concentrations in two dogs with protein-losing enteropathies. J Small Anim Pract 2005;46:345–351.

23. How KL, Hazewinkel HA, Mol JA. Dietary vitamin D dependence of cat and dog due to inadequate cutaneous synthesis of vitamin D. Gen Comp Endocrinol 1994;96:12–18.

24. Hincliff KW, Olson J, Crusberg C, et al. Serum biochemical changes in dogs competing in a long-distance sled race. J Am Vet Med Assoc 1993;202:401–405.

25. Waldron JL, Ashby HL, Cornes MP, et al. Vitamin D: A negative acute phase reactant. J Clin Pathol 2013;66:405–410.

26. Reid D, Toole BJ, Knox S, et al. The relation between acute changes in the systemic inflammatory response and plasma 25-hydroxyvitamin D concentrations after elective knee arthroplasty. Am J Clin Nutr 2011;93:1006–1011.

27. Castle LM, Poirmans JR, Leclercq R, et al. Some aspects of the acute phase response after a marathon race, and the effects of glutamine supplementation. Eur J Appl Physiol Occup Physiol 1997;75:47–53.

28. Weight LM, Alexander D, Jacobs P. Strenuous exercise: Analogous to the acute-phase response? Clin Sci (Lond) 1991;81:677–683.

29. Wakshlag JJ, Stokol T, Geske SM, et al. Evaluation of exercise-induced changes in concentrations of C-reactive protein and serum biochemical values in sled dogs completing a long-distance endurance race. Am J Vet Res 2010;71:1207–1213.

30. Polson C, Sarkar P, Incelden B, et al. Optimization of protein precipitation based upon effectiveness of protein removal and ionization effect in liquid chromatography–tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 2008;785:263–275.

31. Hollis BW, Kamerdjian QJ, Kurkowski A, et al. Quantification of circulating 1,25-dihydroxyvitamin D by radioimmunoassay with 125I-labeled tracer. Clin Chem 1996;42:586–592.

32. Phillips KM, Craig Byrdwell W, Ezler J, et al. Development and validation of control materials for the measurement of vitamin D3 in selected US foods. J Food Comp Anal 2008;21:527–534.

33. Beitz DC. Nutrient requirements and dietary nutrient concentrations. In: Beitz, 3rd ed. National Research Council Nutrient Requirements of Dogs and Cats. Washington, DC: National Academies Press; 2006:359.

34. Norman AW. The vitamin D endocrine system. Physiologist 1985;28:219–232.

35. Pramyothin P, Biancuzzo RM, Lu Z, et al. Vitamin D in adipose tissue and serum 25-hydroxyvitamin D after roux-en-Y gastric bypass. Obesity (Silver Spring) 2011;19:2228–2234.

36. Puthucheary Z, Skipworth JRA, Rawal J, et al. Genetic influences in sport and physical performance. Sports Med 2011;41:845–859.

37. Bell NH, Godsen RN, Henry DP, et al. The effects of muscle-building exercise on vitamin D and mineral metabolism. J Bone Miner Res 1988;3:369–373.

38. Klausen T, Breum L, Sorensen HA, et al. Plasma levels of parathyroid hormone, vitamin D, calcitonin, and calcium in association with endurance exercise. Calcif Tissue Int 1993;52:205–208.

39. Eliakim A, Nemet D. Interval training and the GH-IGF-I axis—an old look into an old training regimen. J Pediatr Endocrinol Metab 2012;25:815–821.

40. Ameri P, Giasti A, Boschetti M, et al. Interactions between vitamin D and IGF-I: From physiology to clinical practice. Clin Endocrinol (Oxf) 2013;79:457–463.

41. Burr JR, Reinhart GA, Swenson RA, et al. Serum biochemical values in sled dogs before and after competing in long-distance races. J Am Vet Med Assoc 1997;211:175–179.

42. de Brito Galvao JF, Nagode LA, Schenck PA, Chew DJ. Calcitriol, calcidiol, parathyroid hormone, and fibroblast growth factor-23 interactions in chronic kidney disease. J Vet Emerg Crit Care (San Antonio) 2013;23:134–142.

43. Davis MS, Willard MD, Williamson KK, et al. Sustained strenuous exercise increases intestinal permeability in racing Alaskan sled dogs. J Vet Intern Med 2005;19:34–39.

44. Ogunkulade WB, Boucher BJ, Bustin SA, et al. Vitamin D metabolism in peripheral blood mononuclear cells is influenced by “Betel Nut” and vitamin D status. J Clin Endoc Metab 2006;91:2612–2617.

45. Horst RL, Littledietke ET. Comparison of plasma concentrations of vitamin D and its metabolites in young and aged domestic animals. Comp Biochem Physiol B 1982;73:485–489.

46. Horst RL, Littledietke ET, Riley JL, Napoli IL. Quantitation of vitamin D and its metabolites and their plasma concentrations in five species of animals. Anal Biochem 1981;116:189–203.

47. Eichner ER. Overtraining: Consequences and prevention. J Sports Sci 1995;13:S41–S48.

48. Fry RW, Morton AR, Keast D. Overtraining in athletes. An update. Sports Med 1991;12:32–65.

49. Budgett R. Overtraining syndrome. Br J Sports Med 1990;24:231–236.

50. Kreher JB, Schwartz JB. Overtraining syndrome: a practical guide. Sports Health 2012;4:128–136.

51. Fallon KE. The acute phase response and exercise: The ultramarathon as prototype exercise. Clin J Sport Med 2001;11:38–43.

52. Kenyon CL, Basaraba R, Bohn AA. Influence of endurance exercise on serum concentrations of iron and acute phase proteins in racing sled dogs. J Amer Vet Med Assoc 2011;239:1201–1207.

53. Yazwinski M, Milizio JG, Wakshlag JJ. Assessment of serum myokines and markers of inflammation associated with exercise in endurance racing sled dogs. J Vet Intern Med 2013;27:371–376.
54. Strachan AF, Noakes TD, Kotzenberg G, et al. C reactive protein concentrations during long distance running. Br Med J (Clin Res Ed) 1984;289:1249–1251.
55. Howatson G, McHugh MP, Hill JA, et al. Influence of tart cherry juice on indices of recovery following marathon running. Scand J Med Sci Sports 2010;20:843–852.
56. Scherr J, Braun S, Schuster T, et al. 72-h kinetics of high-sensitive troponin T and inflammatory markers after marathon. Med Sci Sports Exerc 2011;43:1819–1827.
57. Otae K, Sugimoto T, Jinbo T, et al. Physiological levels of C-reactive protein in normal canine sera. Vet Res Commun 1998;22:77–85.
58. Wakshlag JJ, Koaus MS, Gelzer AR, Downey RL, Vacchani P. The influence of high-intensity moderate duration exercise on cardiac troponin 1 and C-reactive protein in sled dogs. J Vet Intern Med 2010;1388–1392.

Supporting Information

Additional Supporting Information may be found online in Supporting Information:

Table S1. Calculated nutritional analysis of total diet nutrient consumption per 1,000 kcals compared to the National Research Council (NRC) recommended daily allowance (RDA) of nutrients for dogs, based on 1000 kilocalories.