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Hypocitraturia in Common Bottlenose Dolphins (*Tursiops truncatus*): Assessing a Potential Risk Factor for Urate Nephrolithiasis

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Numerous cases of urate nephrolithiasis in managed collections of common bottlenose dolphins (*Tursiops truncatus*) have been reported, but nephrolithiasis is believed to be uncommon in wild dolphins. Risk factors for urate nephrolithiasis in humans include low urinary pH and hypocitraturia. Urine samples from 94 dolphins were collected during April 2006 through June 2009 from 4 wild populations (n = 62) and 4 managed collections (n = 32). In addition, urine uric acid and pH were tested in a subset of these animals. Our null hypothesis was that wild and managed collection dolphins would have no significant differences in urinary creatinine, citrate, and uric acid concentrations and pH. Among urine samples from all 94 dolphins, the urinary levels (mean ± SEM) for creatinine, citrate, uric acid, and pH were 139 ± 7.6 mg/dL, 100 ± 20 mg citrate/g creatinine, 305 ± 32 mg uric acid/g creatinine, and 6.2 ± 0.05, respectively. Of the 4 urinary variables, only citrate concentration varied significantly between the 2 primary study groups; compared with wild dolphins, managed collection dolphins were more likely to have undetectable levels of citrate in the urine (21.0% and 81.3%, respectively). Mean urinary citrate concentrations for managed collection and wild dolphin populations were 2 and 150 mg citrate/g creatinine, respectively. We conclude that some managed collections of dolphins, like humans, may be predisposed to urate nephrolithiasis due to the presence of hypocitraturia. Subsequent investigations can include associations between metabolic syndrome, hypocitraturia, and urate nephrolithiasis in humans and dolphins; and the impact of varying levels of seawater ingestion on citrate excretion.

Abbreviation: MMP, Navy Marine Mammal Program.

Urate nephrolithiasis has been reported in marine mammals, including 14 common bottlenose dolphins (*Tursiops truncatus*) in a single population.[10,36,39,40] Ammonium acid urate nephrolithiasis, in particular, has been associated with anemia, high serum creatinine, high BUN, low glomerular filtration rate, dilated collecting ducts, and hydronephrosis in dolphins.[40] According to hundreds of necropsies conducted on wild dolphins over the past 20 y, nephrolithiasis appears to be primarily associated with managed collections and is not believed to be a significant disease in wild populations.[8] To date, the cause of urate nephrolithiasis in dolphins remains unknown.

Urate nephrolithiasis can be caused by uric acid, sodium acid urate, or ammonium acid urate calculi. Both uric acid and ammonium acid urate calculi have been reported in dolphins. However, archived dolphin calculi initially characterized as uric acid in the 1990s were retested by using polarizing light microscopy and recharacterized as pure ammonium acid urate.[40] Therefore, the number of dolphin ammonium acid nephroliths incorrectly characterized as uric acid is unknown.

In humans, urate nephrolithiasis, specifically uric acid nephrolithiasis, typically has been associated with high levels of uric acid in serum and urine and low urinary pH.[5,23] However, people with type 2 diabetes mellitus can have pure uric acid nephrolithiasis in the face of low serum and normal urinary uric acid levels. Urate nephroliths may develop in people with type 2 diabetes due to the presence of low urinary pH, decreased ammonium excretion, a compensatory response of hypocitraturia, and further acidification of urine due to an impaired ammonia excretory response. This mechanism presumably is driven by insulin resistance.[32]

Humans with recurrent uric acid calculi can be at risk of developing ammonium acid urate calculi.[35]Ammonium acid urate calculi can result due to uric-acid- and ammonium-enriched urine and hypocitraturia.[15,35] These calculi are endemic in Asian countries, and pure ammonium acid urate calculi are rare in industrialized countries.[28] Other risk factors for ammonium acid urate calculi include inflammatory bowel disease and morbid obesity.[35] In addition, urine-rich animal protein diets in humans have been associated with urate nephrolithiasis, including uric acid and ammonium acid urate calculi.[13]

Other animals susceptible to ammonium urate calculi include dogs, cats, and reptiles.[7,14,20] Dalmatians are particularly susceptible to uric and ammonium urate calculi formation due to a recessive gene that results in defective urate metabolism. In cats and

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non-Dalmatian dogs, the pathogenesis of this disease is not well known. Changes in protein metabolism due to altered protein intake are hypothesized as risk factors for urate calculi formation in these animals.[7,14]

To determine whether managed collections of dolphins have risk factors for urate nephrolithiasis similar to those found in other animals, especially humans, urine samples from 94 animals were collected during April 2006 through June 2009 from 4 wild populations living off the coasts of Florida, Georgia, Mississippi, and North Carolina and from 4 managed collections, including a predominance of animals from the Navy Marine Mammal Program (MMP). In addition, urine uric acid and pH were tested in a subset of animals. Our null hypothesis was that there would be no significant differences in urinary creatinine, citrate concentration, uric acid concentration, and pH between wild and managed collection dolphins.

Materials and Methods

Animal care and use. The MMP is AAALAC-accredited and adheres to the national standards.[1,26] As required by the Department of Defense, the MMP's animal care and use program is routinely reviewed by an institutional animal care and use committee and the Department of Defense Bureau of Medicine. Sampling of wild dolphins was approved by the institutional animal care and use committees at the Mote Marine Laboratory and the National Marine Fisheries Headquarters. Urine samples collected from wild dolphins were approved under National Marine Fisheries Service permits 522-1785 (issued to RSW, Sarasota, FL) and 932-1905/MA-009526 (issued to the National Marine Fisheries Marine Mammal Health and Stranding Response Program, Georgia).

Study population. A total of 94 common bottlenose dolphins were included in our study. Thirty-two (34%) study dolphins were from the following managed collections: Dolphin Quest in Hawaii (n = 2), Gulfworld in Texas (n = 3), The Mirage in Nevada (n = 3), and the Navy Marine Mammal Program in California (n = 24). Sixty two (66%) study dolphins were from the following wild, free-ranging populations: Beaufort, North Carolina (n = 7), Georgia coastline (n = 13), Indian River Lagoon, Florida (n = 21), and Sarasota Bay, Florida (n = 21). The median ages for the managed collection and wild dolphin populations were 24.6 y (range, 4 to 48 y) and 8.9 y (range, 2 to 24 y), respectively. Females represented 50% of both the managed collection and wild populations. All urine samples from managed collection dolphins were collected by using free-catch methods, whereas all urine samples from the wild dolphins were collected by using sterile catheterization.

Sample collection, storage, and preparation. Urine samples were obtained from managed collection bottlenose dolphins in 120-mL sterile plastic specimen cups using free catch methods. Samples from bottlenose dolphins in the wild were obtained during capture-release for population health assessments.[41] Urine was collected by aseptic catheterization. Regardless of source, fresh samples were kept cold and transferred into 10-mL cryovials and frozen at –80 °C. Urine samples were frozen for 20 and 918 d. Frozen urine for citrate analysis was thawed on ice, and standard acid base titration methods were used to adjust pH to 2.0 by using 6 M hydrochloric acid (Mallinckrodt Analytical, Phillipsburg, NJ).[34] Sample pH was analyzed by using a portable meter (model 51910 electrode and Sension 2 pH/ISE meter, Hach, Loveland, CO). Samples were refrozen and shipped on dry ice to the reference laboratory for analysis.

Urinalysis. At the reference laboratory, citric acid and creatinine were analyzed enzymatically (Modular P800, Roche Diagnostics, Indianapolis, IN). Reagents used were Citrate R1 and Citrate R2 (ARUP Laboratories, Salt Lake City, UT). Citrate:creatinine ratios were calculated. Uric acid and creatinine were analyzed on an automated system (model AU5400, Olympus, Tokyo, Japan), and uric acid:creatinine ratios were calculated.

Statistical methods. All statistics were conducted by using SAS software (version 9.2, SAS Institute, Cary, NC). Descriptive statistics of the study population were calculated by sampling location, including age, sex, urine collection method, and time between sample collection and submission; to test for potential confounders, these variables were compared between managed collections and wild, free ranging dolphins using a general linear model for continuous variables (Proc GLM; Class Managed_Wild; Model Age CollToSubmit_Time = Manage_Wild; Means Managed_Wild) and χ^2 analyses for urine collection method and sex. If citrate was lower than the limit of detection (less than 10 mg/dL), the value was assigned as zero. Mean, standard deviation, median, and ranges were determined by study population for urinary creatinine (mg/dL), citrate:creatinine ration (mg:g), uric acid:creatinine ratio (mg:g), and pH. Comparisons of means by using a general linear model were run to test for significant differences in urinary uric acid, creatinine, pH, and citrate between managed collection and wild dolphins (Proc GLM; Class Managed_Wild; Model [Urine Variables] = Managed_Wild; Means Managed_Wild). P values less than or equal to 0.01 were defined as significant for all tests.

Results

Fasting status. Urine was collected after an overnight fast from 19 of the 32 (59.4%) dolphins in managed collections. Because feeding status was unknown for wild, free-ranging dolphins, they were not included in the analysis of fasting versus nonfasting values. Compared with nonfasted animals, fasted dolphins were more likely to have higher mean urinary creatinine values (80 and 190 mg/dL, respectively; P = 0.0002) and lower urinary uric acid concentration (392 and 142 uric acid/g creatinine, P = 0.01). Fasted and nonfasted dolphins had no significant differences in urinary citrate concentrations (3.5 and 0 mg citrate/g creatinine, P = 0.08) or pH (6.2 and 6.4, P = 0.3).

Urinary values. Among urine samples from all 94 dolphins evaluated, the creatinine level (mean ± SEM) was 139 ± 7.6 mg/dL, uric acid concentration was 305 ± 32 mg uric acid/g creatinine, citrate concentration was 100 ± 20 mg citrate/g creatinine, and pH was 6.2 ± 0.05 . Only mean urinary citrate concentration varied significantly (P = 0.0003) between managed collection and free-ranging dolphins (2 and 150 mg citrate/g creatinine, respectively; Table 1). Of 32 managed collection dolphins, 26 (81.3%) animals had nondetectable urinary citrate (less than 10 mg/dL), compared with 13 (21%) wild dolphins (P < 0.0001). Urinary citrate levels among the 3 largest wild dolphin populations (Georgia coastline, 66 mg citrate/g creatinine; Indian River Lagoon, FL, 185 mg citrate/g creatinine; and Sarasota, FL, 129 mg citrate/g creatinine) did not differ significantly (P = 0.26).

Discussion

We report significantly lower concentrations of urinary citrate in bottlenose dolphins from managed collections compared with those from wild populations. Most of the managed collection animals included in this study were from the Navy Marine Mammal

Table 1. Comparison of urine values (mean ± SEM) among managed collection (n = 32) and free-ranging (n = 62) bottlenose dolphins

	Managed collection	Free-ranging	P
Citrate (mg citrate/g creatinine)	2 ± 1	150 ± 28	0.0003
Uric acid (mg uric acid/g creatinine)	236 ± 49	349 ± 41	0.08
Creatinine (mg/dL)	144 ± 16	137 ± 8	0.67
pH	6.3 ± 0.08	6.1 ± 0.06	0.15

Urine uric acid and pH comparisons based on 29 samples from managed collection dolphins and 47 from wild dolphins.

Program, a population with at least 14 cases of nephrolithiasis,[40] a disease that is rare in wild dolphin populations.[8]

In humans, urinary citrate serves as an effective alkalinizer and chelator of calcium ions, both of which decrease the risk of stone formation.[16] If urinary citrate is low, urinary pH may decrease or the amount of free calcium ions may increase, leading to uric acid or calcium oxalate stone formation, respectively. Therefore, low concentration of urinary citrate is associated with stone formation in humans[24,42] and can be the only metabolic abnormality in patients with nephroliths.[19] Hypocitraturia occurs in a wide range of kidney stone diseases, including those associated with renal tubular acidosis, renal hypercalciuria, and idiopathic nephrolithiasis and in people without acid–base disturbances.[31] Low urinary citrate has been attributed to excessive net tubular reabsorption of citrate, which may be a predisposing factor for nephrolithiasis.[30] Because of the high prevalence of both urate nephrolithiasis and hypocitraturia in the MMP population, hypocitraturia may a risk factor for nephrolithiasis in dolphins as it is in people.

Most studies involving hypocitraturia and nephrolithiasis focus on hypocitraturia as a risk factor for calcium oxalate calculi.[16,30,31,42] However, some people with urate nephrolithiasis also have hypocitraturia, which is believed to be a compensatory response to decreased ammonium excretion.[32] Therefore, hypocitraturia may be a direct cause of nephrolithiasis or an indicator of another cause of nephrolithiasis.

Nephrolithiasis in humans can lead to blocked ureters, hydronephrosis, and renal failure.[21] A nephrolithiasis case-control study conducted at the MMP demonstrated that dolphins with more than 20 nephroliths were significantly more likely to have high serum creatinine, high blood urea nitrogen, low glomerular filtration rate, and low urinary pH compared with dolphins with no evidence of nephroliths.[40] In addition, as can occur in humans, 2 advanced cases of nephrolithiasis in dolphins resulted in hydronephrosis and decreased renal function. As such, if hypocitraturia is a risk factor for nephrolithiasis in dolphins, determining why these animals become hypocitraturic may provide insight for disease prevention and treatment to better protect dolphin renal health.

The association between hypocitraturia and nephrolithiasis in humans has been well documented for more than 30 y, but research to determine why humans have hypocitraturia is ongoing. Citrate is reabsorbed with the aid of a sodium-citrate cotransporter.[27] Renal cortical m-aconitase appears to play a key role in regulating citrate urinary levels by increasing citrate reabsorption during chronic metabolic acidosis, causing hypocitraturia, and decreasing citrate reabsorption after alkali feeding, causing hypercitraturia.[18]

Managed collection dolphins receive a diet of high-quality, frozen–thawed fish, including mackerel, herring, capelin, and squid. Wild dolphins living off Sarasota, FL, feed on live, wild fish of at least 15 species.[4] Further, studies in humans have demonstrated that high-protein diets may increase a person's risk for hypocitraturia.[6] Given the potential effect of diet on urinary citrate levels, there is a need to formally compare the diets of wild and managed collection dolphins to assess potential differences in protein, fat, ash, and alkali content, which may influence urinary citrate concentrations.

In addition to differences in the actual diets of managed collection and wild dolphins, these 2 populations differ in how fish are physically fed and, in some institutions, how meals are provided. Dolphins from managed collections most often feed with their head out of the water, whereas wild dolphins feed while swimming under the water. Given the influence of acidic and alkalotic metabolic states on urinary citrate levels, the opportunity for wild dolphins to ingest high concentrations of sodium from seawater may explain why wild dolphins have urinary citrate. In humans, however, increased ingestion of sodium actually would lower urinary citrate levels.[17]

Feeding behavior studies on wild dolphins in Sarasota Bay have demonstrated that in-shore dolphins feed 3 to 30 times an hour over a minimum of an 8-h period; this behavior may be continuous over 24 h.[25] Dolphins from managed collections, including the MMP, often are fed on a schedule similar to that typical of humans, with 3 to 4 meals over 10 h, followed by a 14-h overnight fast. Previous studies have shown that dolphins can have significant changes in blood values during the fasting state, including a shift to a relative metabolic acidosis.[38] Chronic metabolic acidosis can lead to hypocitraturia in humans,[18] and daily changes to a metabolic acidic state similarly may predispose dolphins to hypocitraturia. In our current study, we did not find significant differences in urinary citrate levels between overnight-fasted and recently fed animals from managed collections; because of the unknown feeding status of wild dolphins in our study, we were unable to include them in this analysis. As such, further studies are needed to assess the potential long-term effects of overnight fasting and bulk meals on urinary citrate levels in bottlenose dolphins.

Other conditions in humans that may result in hypocitraturia include low urinary potassium and calcium,[11] gastrointestinal malabsorption,[30] medications that inhibit angiotensin converting enzyme (for example, Enalapril),[17] exposure to hot temperatures in the working environment,[3] and insulin resistance.[32] When evaluating potential reasons why managed collection dolphins may have hypocitraturia and wild dolphins do not, we feel that it is unlikely that dolphins from managed collections absorb citrate less well than do those in wild populations, given that the presence of citrate in dolphin diets is likely to be low. However, citrate perhaps is in the diet of wild and not managed collection dolphins. Further, there is a potential, though unlikely, for a genetic mutation that has been passed within managed collections associated with citrate malabsorption. However, most of the wild-caught dolphins at the MMP were originally in-shore dolphins from the Gulf of Mexico. We expect that these animals share common genes with wild dolphins included in this study, especially those living in Sarasota Bay, FL.

None of the managed collection dolphins were on medications associated with hypocitraturia in humans, so medications likely

are not a cause. However, most, if not all, dolphins in managed collections are provided vitamin supplements. It would be worthwhile to ensure that supplements were not associated with hypocitraturia. Wild bottlenose dolphins included in our study live in regions with periods of warm water temperatures, including the Gulf of Mexico. Managed collection dolphins in our study live in open water in San Diego Bay and Hawaii, as well as in semiclosed systems in Florida and a closed system in Las Vegas. Although the semiclosed and closed locations may experience high air temperatures during part of the year, the majority of managed collection animals included in this study lives in San Diego Bay, an area with air and water temperatures that do not exceed the environments of their wild counterparts. Therefore, we do not believe that high heat is the cause of hypocitraturia in managed collection dolphins. Further studies are needed to compare urinary potassium and calcium between managed collection and wild dolphins to assess these potential risk factors for hypocitraturia.

We previously reported that healthy, short-term fasted bottlenose dolphins have a diabetes-like metabolism, including increased serum glucose, platelets, and γ-glutamyltranspeptidase and decreased serum uric acid.[29,38] Similar to our findings in the MMP population of dolphins, which has pure urate nephrolithiasis, a diabetes-like metabolism, and hypocitraturia, recent studies have demonstrated associations among hypocitraturia, pure urate nephrolithiasis, and metabolic syndrome in humans.

A key parameter that we currently cannot address is the difference in type of urate nephrolithiasis that develops in dolphins and people with diabetes. People with type 2 diabetes are susceptible to uric acid calculi formation, whereas dolphins appear most susceptible to ammonium acid urate calculi formation. One reason for this difference may be the dietary differences between humans and dolphins. Despite having a common underlying etiology (for example, insulin resistance), the very high-protein fish diet of dolphins may make them more susceptible to ammonium acid urate stone formation because they may excrete higher levels of ammonium than do humans.

Obese people with diets high in purines had a high prevalence of hypocitraturia and uric acid calculi,[12] and insulin resistance appears to be the primary driver for both hypocitraturia and urate nephrolithiasis.[2,9,32,37] More specifically, people with diabetes appear to have impaired ammonia excretion, leading to escalating decreases in urinary pH and compensatory hypocitraturia. This response leads to uric acid stone formation without the need for high urine or serum uric acid concentrations.[2,32] Low urinary pH is a characteristic often shared among people with insulin resistance, hypocitraturia, and nephrolithiasis is often reported to be low urinary pH.[9]

In our study, urine uric acid concentrations did not differ between wild and managed collection dolphins, a finding that is consistent with normouricosuric finding in people with urate nephrolithiasis and diabetes. Unlike that in people with diabetes and urate nephrolithiasis, urinary pH did not differ between managed collection and wild dolphins. The lack of difference in urinary pH may not have been surprising in light of the type of nephrolith that appears to be dominant in managed collection dolphins, namely ammonium acid urate;[40] unlike pure uric acid nephroliths, this type of urate nephrolith can form independent of low urinary pH.[5,15] Although the potential link between diabetes and hypocitraturia among dolphins and humans is compelling, further studies are needed to assess genetic, metabolic, and evolutionary parallels between dolphins and in humans with metabolic syndrome.

In humans, decreased intake of animal protein resolved hypocitraturia and decreased stone formation.[12] Bottlenose dolphins ingest very high-protein diets, and the MMP dolphin diet, excluding moisture content, consists of 73.3% protein, 24.4% fat, and 2.2% carbohydrates.[38] Currently, feeding a true low-protein diet to dolphins is not a feasible treatment plan, but feeding them lower protein diets consisting of higher portions of squid and lower portions of high-protein fish (for example, mackerel) may be feasible. Supplementation with potassium citrate is a common and effective treatment for hypocitraturia in most humans,[43] and 60 mEq/day is the preferred alkalinizer specifically for humans with urate stones and hypocitraturia.[33] Use of high levels of citrate supplementation in dolphins may be explored as a treatment option, although early and unpublished results involving potassium citrate treatment (30 to 100 mEq orally daily) in dolphins at the MMP have not demonstrated marked changes in urinary pH or stone formation. Additional studies are needed to assess effects of various levels of potassium citrate treatment doses on urinary pH and citrate, as well as the ability for the dolphin gastrointestinal system to absorb oral potassium citrate. The ideal approach, however, will be to identify the underlying cause of hypocitraturia in dolphins to prevent possible subsequent stone formation.

Our study is limited by single measurements of urine variables (for example, urine concentrations). Most human studies measure urine uric acid and citrate over a 24-h period, and few use single readings to determine whether a study subject has abnormal urine uric acid or citrate levels.[19,30,42] To enable comparability between our study populations, we used concentrations correcting for creatinine, in lieu of raw values. Although 24-h urine values would be the most reliable values to compare between dolphin populations and with humans, acquisition of these samples is difficult in managed collections and not possible with wild dolphins. Nearly half of the urine samples from managed collection dolphins were taken after overnight fasting, and the feeding status of samples from wild dolphins were unknown. To address the possible effects of fasting on urine variables, we compared urinary variable values between fasting and nonfasting samples of managed collection dolphins; although fasting dolphins were more likely to have higher urinary creatinine and lower uric acid concentrations compared with those of nonfasting dolphins, significant differences in urinary citrate or urinary citrate concentration were not present. Greater diagnostic value could have been gained from this study had we included more tests, including urinary calcium, potassium, and phosphorous levels, and microscopic analysis to determine the presence and type of crystals that may have been present. Future studies should include additional indicators that reflect the metabolic state and presence of urate crystals at the time urine was collected.

In conclusion, dolphins from managed collections show a high prevalence of hypocitraturia. The presence of hypocitraturia may explain why some of these populations have numerous cases of urate nephrolithiasis. Further investigations are needed to assess the roles of diet, seawater ingestion, and the presence of a diabetes-like metabolism in causing hypocitraturia in dolphins. Such studies may not only protect the renal health of dolphins but may provide valuable insight into the etiologies of similar diseases in humans.

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References

1. **Animal Welfare Act as Amended.** 2007.7 USC §2131-2156.
2. **Abate N, Chandalia M, Cabo-Chan AV, Orson WM, Sakhaee K.** 2004. The metabolic syndrome and uric acid nephrolithiasis: novel features of renal manifestation of insulin resistance. Kidney Int **65:**386–392.
3. **Atan L, Andreoni C, Ortiz V, Silva EK, Pitta R, Atan F, Srougi M.** 2005. High kidney stone risk in men working in steel industry at hot temperatures. Urology **65:**858–861.
4. **Barros NB, Wells RS.** 1998. Prey and feeding patterns in resident bottlenose dolphins (*Tursiops truncatus*) in Sarasota Bay, Florida. J Mammal **79:**1045–1059.
5. **Berenyi M.** 1972. Models for the formation of uric acid and urate stones. Int Urol Nephrol **4:**199–204.
6. **Breslau NA, Brinkley L, Hill KD, Pak CY.** 1988. Relationship of animal protein-rich diet to kidney stone formation and calcium metabolism. J Clin Endocrinol Metab **66:**140–146.
7. **Cannon AB, Westropp JL, Ruby AL, Kass PH.** 2007. Evaluation of trends in urolith composition in cats: 5230 cases (1985–2004). J Am Vet Med Assoc **231:**570–576.
8. 2008. Personal communication.
9. **Daudon M, Traxer O, Conort P, Lacour B, Jungers P.** 2006. Type 2 diabetes increases the risk for uric acid stones. J Am Soc Nephrol **17:**2026–2033.
10. **Dennison S, Gulland F, Haulena M, Morais HE, Colgrove K.** 2007. Urate nephrolithiasis in a Northern elephant seal (*Mirounga angustirostris*) and a California sea lion (*Zalophus californianus*). J Zoo Wildl Med **38:**114–120.
11. **Domrongkitchaiporn S, Stitchantrkul W, Kockarkarn W.** 2006. Causes of hypocitraturia in recurrent calcium stone formers: focusing on urinary potassium excretion. Am J Kidney Dis **48:**546–554.
12. **Ekeruo WO, Tan YH, Young MD, Dahm P, Maloney ME, Mathias BJ, Albala DM, Preminger GM.** 2004. Metabolic risk factors and the impact of medical therapy on the management of nephrolithiasis in obese patients. J Urol **172:**159–163.
13. **Fellstrom B, Danielson BG, Karlstrom B, Lithell H, Ljunghall S, Vessby B.** 1983. The influence of high dietary intake of purine-rich animal protein on urinary urate excretion and supersaturation in renal stone disease. Clin Sci (Lond) **64:**399–405.
14. **Houston DM, Moore AEP, Favrin MG, Hoff B.** 2004. Canine urolithiasis: a look at over 16000 urolith submissions to the Canadian Veterinary Urolith Centre from February 1998 to April 2003. Can Vet J **45:**225–230.
15. **Klohn M, Bolle JF, Reverdin NP, Susini A, Baud A, Graber P.** 2004. Ammonium urate urinary stones. Urol Res **14:**315–318.
16. **Liangos O, Jaber BL.** 2008. Kidney stones, p 513–527. In: Byham-Gray LD, Burrowes JD, Chertow GM, editors. Nutrition in kidney disease. New York (NY): Humana Press.
17. **Melnick JZ, Preisig PA, Haynes S, Pak CYC, Sakhaee K, Alpern RJ.** 1998. Converting enzyme inhibition causes hypocitraturia independent of acidosis or hypokalemia. Kidney Int **54:**1670–1674.
18. **Melnick JZ, Srere PA, Elshourbagy NA, Moe OW, Preisig PA, Alpern RJ.** 1996. Adenosine triphosphate citrate lyase in hypocitraturia. J Clin Invest **98:**2381–2387.
19. **Menon M, Mahle CJ.** 1983. Urinary citrate excretion in patients with renal calculi. J Urol **129:**1158–1160.
20. **Miller HA.** 1998. Urinary diseases of reptiles: pathophysiology and diagnosis. Semin Avian Exotic Pet Med **7:**93–103.
21. **Moe OW.** 2006. Kidney stones: pathophysiology and medical management. Lancet **367:**333–344.
22. **Moe OW, Abate N, Sakhaee K.** 2002. Pathophysiology of uric acid nephrolithiasis. Endocrin Metab Clin N Amer **31:**895–914.
23. **Ngo TC, Assimos DG.** 2007. Uric acid nephrolithiasis: recent progress and future directions. Rev Urol **9:**17–27.
24. **Nicar MJ, Skurla C, Sakhaee K, Pak CY.** 1983. Low urinary citrate excretion in nephrolithiasis. Urology **21:**8–14.
25. **Nowacek DP.** 2002. Sequential foraging behavior of bottlenose dolphins, *Tursiops truncatus*, in Sarasota Bay, FL. Behaviour **139:**1125–1145.
26. **Office of Laboratory Animal Welfare.** [Internet]. 2002. Public health service policy on humane care and use of laboratory animals. [Cited 20 Sep 2009]. Available at http://grants.nih.gov/grants/olaw/references/phspol.htm.
27. **Pajor AM.** 1995. Sequence and functional characterization of a renal sodium–dicarboxylate cotransporter. J Biol Chem **270:**5779–5785.
28. **Pichette V, Bonnardeaux A, Cardinal J, Houde M, Nolin L, Boucher A, Ouimet D.** 1997. Ammonium acid urate crystal formation in adult North American stone-formers. Am J Kidney Dis **30:**237–242.
29. **Ridgway SH.** 1972. Mammals of the sea: biology and medicine, p 690–747. Springfield (IL): Charles C Thomas.
30. **Rudman D, Dedonis JL, Fountain MT, Chandler JB, Gerron GG, Fleming GA, Kutner MH.** 1980. Hypocitraturia in patients with gastrointestinal malabsorption. N Engl J Med **303:**657–661.
31. **Rudman D, Kutner MH, Redd SC, Waters WC, Gerron GG, Bleier J.** 1982. Hypocitraturia in calcium nephrolithiasis. J Clin Endocrinol Metab **55:**1052–1057.
32. **Sakhaee K, Adams-Huet B, Moe OW, Pak CYC.** 2002. Pathophysiologic basis for normouircosuric uric acid nephrolithiasis. Kidney Int **62:**971–979.
33. **Sakhaee K, Nicar M, Hill K, Pak CY.** 1983. Contrasting effects of potassium citrate and sodium citrate therapies on urinary chemistries and crystallization of stone-forming salts. Kidney Int **24:**348–352.
34. **Skoog D, West D.** 1976. Fundamentals of analytical chemistry, 3rd ed, p 731. Chicago (IL): Holt, Rinehart, and Winston.
35. **Soble JJ, Hamilton BD, Streem SB.** 1999. Ammonium acid urate calculi: a reevaluation of risk factors. J Urol **161:**869–873.
36. **Stroud RK.** 1979. Nephrolithiasis in a harbor seal. J Am Vet Med Assoc **175:**924–925.
37. **Taylor EN, Sampfer MJ, Curhan GC.** 2005. Diabetes mellitus and the risk of nephrolithiasis. Kidney Int **68:**1230–1235.
38. **Venn-Watson SK, Ridgway SH.** 2007. Big brains and blood glucose: common ground for diabetes mellitus in humans and healthy dolphins. Comp Med **57:**390–395.
39. **Venn-Watson SK, Smith CR, Dold C, Ridgway SH.** 2008. Use of a serum-based glomerular filtration rate prediction equation to assess renal function by age, sex, fasting, and health status in bottlenose dolphins (*Tursiops truncatus*). Marine Mammal Science **24:**71–80.
40. **Venn-Watson SK, Smith CR, Johnson S, Daniels R, Townsend F.** 2010. Clinical relevance of urate nephrolithiasis in bottlenose dolphins (*Tursiops truncatus*). Dis Aquat Organ **89:**167–177.
41. **Wells RS, Rhinehart HL, Hansen LJ, Sweeney JC, Townsend FI, Stone R, Casper D, Scott MD, Hohn AA, Rowles TK.** 2004. Bottlenose dolphins as marine ecosystem sentinels: developing a health monitoring system. EcoHealth **1:**246–254.
42. **Welshman SG, McGeown MG.** 1976. Urinary citrate excretion in stone-formers and normal controls. Br J Urol **48:**7–11.
43. **Whalley NA, Meyers AM, Martine M, Margolius LP.** 1996. Long-term effects of potassium citrate therapy on the formation of new stones in groups of recurrent stone formers with hypocitraturia. Br J Urol **78:**10–14.