Pathophysiological and Physicochemical Basis of Ammonium Urate Stone Formation in Dolphins

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Abbreviations and Acronyms

BUN = blood urea nitrogen

CT = computerized tomography

HOMA-IR = homeostasis model assessment for insulin resistance

NAE = net acid excretion

NH_4U = ammonium urate

pCO_2 = carbon dioxide partial pressure

SI = supersaturation index

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Purpose: Nephrolithiasis is increasingly reported in bottle-nosed dolphins. All cases to date have been ammonium urate nephrolithiasis.

Materials and Methods: A case-control study was performed in dolphins with and without evidence of nephrolithiasis to identify biomarkers and risk factors associated with stone formation in a managed population. Dolphins were sampled in fasting and postprandial states to study the effect of dietary factors on serum and urinary biochemistry. Urine was continuously collected for 6 hours via catheter and divided into 3, 2-hour collections with a bolus fish meal given after completing the first collection. Blood was sampled at the beginning of the fasting period and the end of the postprandial period.

Results: There were no significant differences in serum and urine chemistry or acid-base profiles between dolphins with vs without stones at baseline or postprandially. This suggests that cases and controls represent a continuum of stone risk. On analysis combining cases and controls in a single cohort we noted significant postprandial increases in urinary uric acid, sulfate and net acid excretion accompanied by increased urinary ammonium excretion and a commensurate increase in urine pH. The supersaturation index of ammonium urate increased more than twofold postprandially.

Conclusions: These findings suggest that dolphins are susceptible to ammonium urate nephrolithiasis at least in part because a high dietary load of acid and purines results in a transient but marked increase in the urinary supersaturation of the sparingly soluble ammonium urate salt.

Key Words: kidney, nephrolithiasis, uric acid, risk, bottle-nosed dolphin

In managed bottle-nosed dolphins (Tursiops truncatus) NH_4U nephrolithiasis associated with morbidity was reported but research efforts to evaluate the pathophysiological and physicochemical mechanisms of nephrolithiasis in dolphins have been limited.[1,2] NH_4U nephrolithiasis is rare in humans in the developed world with up to 0.55% of stones containing predominantly NH_4U.[3] Clinical conditions associated with NH_4U nephrolithiasis include diarrheal states due to inflammatory bowel disease, ileostomy and laxative abuse.[4–6] In these conditions the relative abundance of urinary ammonium accompanied by decreased urinary Na and K shifts the balance of urinary urate salts from relatively soluble

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Na^+-urate and K^+-urate toward sparingly soluble NH_4U.[7] The higher prevalence of NH_4U nephrolithiasis in some developing countries today and historically in preindustrial Europe is attributable to a poor cereal based diet combined with inadequate fluid intake as well as recurrent untreated urinary tract infections.[8] However, none of these conditions apply to dolphins with NH_4U nephrolithiasis.[1,2]

To our knowledge no prior study of dolphins specifically investigated the potential role of diet in NH_4U stone risk. We hypothesized that bolus fish meals, naturally high in protein and purines,[9] may predispose dolphins to stone formation through a combination of increased urinary uric acid and physiological amplification of urinary ammonium excretion.[10] Furthermore, we hypothesized that the postprandial urinary milieu may be different in stone forming vs nonstone forming dolphins. To test these hypotheses we studied 8 managed dolphins, including 4 with and 4 without evidence of kidney stones.

METHODS

Study Population

Eight sexually mature bottle-nosed dolphins were selected for study, including 2 male and 2 female stone formers, and 2 male and 2 female body weight matched nonstone formers. Nonstone formers were defined as dolphins with no history of renal azotemia (BUN greater than 59 mg/dl and creatinine greater than 1.8 mg/dl) within the last 10 years and no evidence of nephrolithiasis on ultrasound at the time of the study. Stone formers were defined as dolphins with a history of renal azotemia within the last 10 years and ultrasound evidence of nephrolithiasis at the time of the study. Mean age was 29 years (range 14 to 38) and mean weight was 187 kg (range 155 to 251). All dolphins were housed in ocean enclosures and fed high quality frozen thawed fish according to hazard analysis and critical control points guidelines for food inspection.

All study animals were owned and cared for by the United States Navy Marine Mammal Program. The Secretary of Navy Instruction 3900.41G directs that Navy marine mammals be provided the highest quality care. The Navy Marine Mammal Program is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International and adheres to the national standards of the United States Public Health Service Policy on the Humane Care and Use of Laboratory Animals and the Animal Welfare Act. Study animals were assigned an attending veterinarian specific to the project. Samples were collected under an animal care and use protocol approved by a Navy institutional animal care and use committee and the Navy Bureau of Medicine.

Study Protocol

On the day before sampling study animals were fed the routine diet consisting of a mean of 7.1 kg (range 5.5 to 8.2) of frozen thawed fish (table 1). Dolphins with kidney stones were receiving fresh water orally to prevent renal azotemia. Therefore, fresh water was administered via a small orogastric feeding tube at 1% of body mass to all study animals to eliminate hydration therapy as a confounding variable. After a 12-hour overnight fast all animals underwent light sedation with diazepam (0.12 to 0.20 mg/kg orally) or midazolam (0.06 to 0.08 mg/kg intramuscularly).

Table 1. *Macronutrient composition of bottle-nosed dolphin (Tursiops truncatus) morning meals*

Nutrients	Mean ± SD Total	Mean ± SD/kg Body Wt
Total calories (kcal)	3,253 ± 732	18.1 ± 4.1
Protein (gm)	453 ± 132	2.5 ± 0.9
Fat (gm)	184 ± 43	1.0 ± 0.3
Carbohydrates (gm)	1 ± 1	0

While under sedation, the dolphins were placed in the right lateral recumbent position. The genital slit was flushed with sterile water and surrounding skin was cleaned with alcohol. Urine was collected via catheter under voluntary control. An 8.5Fr 60 cm multipurpose drainage catheter (Cook Medical, Bloomington, Indiana) was used for urinary catheterization of males. For females a 10.2Fr 45 cm multipurpose drainage catheter (Cook Medical) or a 10Fr 90 cm Foley catheter (Mila International, Erlanger, Kentucky) was placed. The bladder was drained and an airtight catheter collection bag was connected to the catheter for continuous urine collection. A baseline blood sample was immediately collected from a periarterial venous rete in the caudal peduncle or a fluke blade using a Vacutainer® needle or a butterfly needle attached to a Vacutainer holder, respectively.

Animals were placed in fleece lined stretchers and suspended in water filled containers for added comfort. Baseline fasting urine was collected from hours 0 to 2. The dolphins were then fed a third of the daily fish ration (range 1.8 to 2.7 kg) consisting of a third herring and two-thirds capelin (table 1). Two 2-hour urine samples were collected postprandially from hours 2 to 4 and 4 to 6. At the completion of each sampling period a urine aliquot was collected in a 1 to 3 cc syringe for pH and pCO_2. The remaining urine was transported to the laboratory for processing on wet ice. A second blood sample was obtained at the end of the 6-hour study and the dolphins were then returned to the ocean enclosures.

Imaging

Renal ultrasound was done in real time B-mode in water or on land. We used Voluson® i and M-Turbo® portable ultrasound units with 2 to 5 MHz variable frequency and curvilinear transducers. The former unit was equipped with Z800 video glasses (eMagin, Bellevue, Washington) and the latter was equipped with Cinemizer video glasses (Carl Zeiss, Oberkochen, Germany). Each kidney was examined for evidence of nephrolithiasis in the dorsal and transverse planes. Nephrolithiasis was defined as hyperechoic foci in the renal parenchyma with distinct acoustic shadows (fig. 1).

Three case dolphins were transported to the Naval Medical Center San Diego CT facility (fig. 1). Sedation was induced with an intramuscular dose of midazolam (0.04