Speculations on pathogenesis of metabolic bone disease in captive polar bears (*Ursus maritimus)* with links to taurine status

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ABSTRACT

A calcium and/or vitamin D3 deficiency can lead to metabolic bone disease. There has been evidence in human pediatric medicine that the amino acid taurine (TAU) might enhance the absorption of vitamin D. Metabolic bone disease in captive polar bears has been historically problematic, and we speculate may be linked to TAU status. Whole blood and plasma TAU content was measured in wild caught (est. 4–5 mo of age; *ii* = 2) and captive (1.3–35 yr; *ii* = 10 individuals from 4 North American zoos) polar bears to determine if dietary differences influenced the concentrations of TAU available for its biologic activities. Plasma TAU (*ii* = 9) in captive bears was significantly lower (99 ± 16 nmol/ml) than measured from the free-ranging bear cubs (237 ± 10 nmol/ml); (t-test; $p < 0.02$). Whole blood TAU concentrations also differed significantly ($p < 0.05$), (253 ± 37 nmol/ml (*ii* = 13) in captive vs. 453 ± 8 nmol/ml (*ii* = 2) for free-ranging bears, respectively. No significant differences in plasma or whole blood TAU concentrations were found with regard to sex or age of the captive animals. TAU concentrations in the wild-caught cubs were monitored over 4 yr in a captive environment, and decreased to levels similar to those reported for other captive polar bears (102 ± 18 and 258 ± 32 nmol/ml for plasma (*ii* = 5) and whole blood (*ii* = 9, respectively). These preliminary results indicate that circulating plasma and whole blood TAU concentrations from wild caught polar bear cubs are higher than considered normal plasma TAU values in domestic carnivores (cats 80–120 nmol/ml, dogs 60–120 nmol/ml), humans (40–100 nmol/ml), or rats (50–95 nmol/ml). The values likely reflect the impact of maritime diets (known to be high in TAU) on free-ranging polar bears, thus likely higher TAU concentrations transferred through maternal

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milk, that may be altered on a captive diet. A current comprehensive nutritional assessment of free-ranging polar bear milk would identify specific nutrient values. This information should improve hand-rearing diets and perhaps minimize MBD in captive polar bears.

KEYWORDS

amino acid, Ursidae, nutrition, protein, taurine

Introduction

Captive born polar bear (*Ursus maritimus)* cubs have a disturbingly high morbidity and even higher mortality rate. The International Species Inventory System (ISIS) records for the past 25 years indicate that more than 50 % of polar bear cubs born in captivity fail to survive beyond age 3 mo. Further, a national survey on fractures in captive polar bears found 20 fractures in 13 animals ranging from 4 wk to > 6 yr of age (Anonymous 1985; Johnston and Cutchins 1985; Engeli 2004, pers. comm; Lin et al. 2005), likely from lack of adequate vitamin D. Seven of these were mother-reared, <3 yr old; all were reported to have fractures suspicious of metabolic bone disease, MBD (Lin et al. 2005). Twenty percent of hand-reared polar bears had evidence of MBD (Anonymous 1985; Thomas, 1986, pers. comm.; Kenny et al. 1999; Silverman, 2001, pers. comm).

MBD is essentially unknown or unreported in polar bear cubs in the wild, raising the possibility that changes in the diet or environment of cubs raised in captivity is a potential underlying factor. When zoo personnel hand-rear polar bear cubs, bears are generally fed a canine milk replacer formula, or may eat a dry commercial omnivore food diet from an early age (Hedberg 2002; Lintzenich et al. 2006).

Nutritional issues may be associated with these health issues. MBD can result from calcium and/or vitamin D deficiency, improper ratios of calcium to phosphorus, or conditions resulting in malabsorption. Absorption of calcium is reduced in the absence of bile salts, which may occur in biliary stasis. If fat absorption is impaired, dietary vitamin D absorption is also reduced, leading to the development of MBD (Fowler 1986). The juvenile form of MBD is called rickets, a disease brought on by nutritional imbalances that can be the result of deficiencies or excesses of several key nutritional elements including calcium, phosphorus, vitamin D_3 and vitamin A (Freedman et al. 1976). Clinical signs in bears are evident by acute lameness caused by fractures. MBD is diagnosed through a combination of clinical signs, radiographic studies, and a thorough dietary evaluation. Blood calcium and phosphorus concentrations tend to

remain normal until severe collapse is imminent (Wallach and Boever 1983). Unfortunately further trauma and injury can result when restraining an animal suspected to have MBD, since the bones can fracture easily. MBD caused by a calcium deficiency or a vitamin D deficiency cause bones to be brittle, soft and weak.

Of note, the polar bear has anatomic and behavioral characteristics that strongly suggest it is a more obligate carnivore than other ursid species. For example, the cranial-dental morphology of polar bears is distinctly different from the more traditional omnivorous bear species, devoid of grinding surfaces (Sacco and Van Valkenburgh 2004). The polar bear is classified a true Arctic marine mammal; it depends entirely on the sea for its existence (Sterling 1988). It displays high digestive efficiencies for its main dietary components of protein and fat, assimilating 84 % percent of the protein and 97% percent of the fat that is ingested, for use as dietary energy (Best 1985).

Taurine (TAU) is an amino acid that is not found in plant-based food, and is a component of essentially all mammalian cells. TAU, an osmolyte, is important in cell volume regulation as a /3-amino acid that is not incorporated into protein synthesis and resides free in the intracellular fluid (Chesney et al. 1998). Additionally TAU is important in preventing eye problems and (eventually) irreversible blindness in felids (Pion et al. 1992). TAU deficiency can cause feline dilated cardiomyopathy, and supplementation can reverse left ventricular systolic dysfunction. TAU has a major biologic role in the conjugation of bile acids. All bile acids must be conjugated to form bile salts, which are important in the formation of mixed micelles that enhance diffusion through the unstirred layer of the small intestine (Hofmann 1999; Zamboni et al. 1993). Carnivores tend to be exclusive TAU-conjugators of cholic acid (Huxtable 1992). TAU-conjugated bile acids are particularly important in triglyceride and fat-soluble vitamin absorption from the gut lumen into the blood stream (Hofmann 1999). TAU-conjugated bile acids aid absorption of vitamin D more efficiently than glycine-conjugates (Hofmann 1999; Zamboni et al. 1993).

The amino acid TAU is sometimes in short supply in human infant formulas (Chesney et al. 1998), and infants fed a TAU-free formula have been reported to have vitamin D deficiency with nutritional rickets (Zamboni et al. 1993). A commercial canine milk formula that is often used in hand rearing carnivores – particularly ursids – along with cow's milk; both contain minimal amounts of TAU (Hedberg et al. 2007; Spitze et al. 2003).

Although specific TAU deficiency has not been identified in captive polar bears, we speculate that low TAU in artificial milk replacers used in hand-rearing may contribute to impaired vitamin D absorption, resulting in increased incidence of MBD in this species. Little information is available concerning TAU metabolism and its effect on infant and adult polar bear nutrition;

inferential data are utilized to describe this relationship until further details are forthcoming.

Methods

Animals. Two free-ranging 3-4 mo old (estimated age) orphaned wild-caught polar bear cubs (male weighing 7.7 kg, female weighing 5.5 kg) were transferred from Alaska to the San Diego Zoo for a 30-day quarantine, with a diet comprising a 1:1 milk formula of Esbilac (PetAg Inc., Hampshire, IL) and Enfamil (Mead-Johnson, Evansville, IN); introduction of solids occurred at 5.5 mo (Simerson 2001). Blood samples were collected after completing 30-day quarantine. Subsequent blood samples were collected opportunistically from the female at 1.3 yr, 2.0 yr, and 2.6 yr. The male was also sampled opportunistically at 2 and 4 years of age. This age information was based on a starting age of 3–4 mo. Plasma (1.0 ml) and whole blood (1.0 ml) were shipped on ice to the San Francisco Zoological Garden, and stored at —70 °C. Additional whole blood (n = 13) and plasma (n = 9) samples were obtained from 5 male and 5 female polar bears ranging from 3 to 35 yr. from animal collections at San Diego Zoo (ZSSD) San Diego, CA, San Francisco Zoological Gardens (SFZG) San Francisco, CA, SeaWorld San Diego (SWSD), San Diego, CA, Detroit Zoological Institute (DZI) Detroit, MI between 2000 and 2005. Samples in cryogenic vials were stored at —70°C at San Francisco Zoological Gardens until transferred frozen to the Amino Acid Laboratory at UC Davis, School of Veterinary Medicine, Davis, CA.

Diet Evaluation. Over the same 5-year period, institutional diet histories were reviewed at three facilities. Three samples of commercial polar bear dry feed (Mazuri Polar Bear Diet, Purina Mills, LLC , St. Louis, Missouri), canned canine diet (Science Diet Canine WD, Hill's Pet Nutrition, Topeka, KS), and a sample of fresh frozen herring were stored at —15°C in air-tight containers until analysis at the Amino Acid Laboratory at UC Davis, School of Veterinary Medicine. Total dietary TAU content was calculated from laboratory results using Zootrition® software (Saint Louis Zoo, St. Louis, MO) based on proportional food intake at the time of blood collections.

Taurine Analysis. On the day of analyses, an equal volume of 0.24 mol 5-sulfosalicylic acid was added to plasma, whole blood, and feedstuffs to precipitate proteins. Whole blood samples were frozen and thawed twice to break the cells and release all TAU before further processing. After centrifugation, the supernatant was prepared with an internal standard and the equivalent of 40 or 20 izl of plasma was injected onto the ion exchange column. The quantity of TAU was determined colorimetrically using ninhydrin for color development

(Model 6300 Amino Acid Analyzer, Beckman Instruments, Palo Alto, CA or Biochrom Ltd, Cambridge UK).

Results

TAU concentrations in plasma and whole blood were obtained from 10 captive individuals, as well as 2 free-ranging polar bear cubs. Plasma TAU in captive bears was significantly lower (99 ± 16 nmol/ml; mean ± SE, $n = 9$) than measured from the wild-caught free-ranging bear cubs (237 ± 10 nmol/ml, $n = 2$); $p < 0.02$.

In whole blood, which permits TAU to leak from platelets and white blood cells, the values also differed significantly between captive (253 ± 37 nmol/ml; $n = 13$) vs. the free-ranging cubs (453 ± 8 nmol/ml; $n = 2$) ($p < 0.05$). No significant differences in plasma or whole blood TAU concentrations were found with regard to sex or age of the captive animals.

The 3 separate diet analyses for TAU concentration in the commercial dry feed resulted in widely varying results: 0.08 % TAU (dry matter (DM) basis) sampled in June 2002, 0.14% DM sampled in February 2004, and 0.39% DM sampled January 2006, as formulation changes occurred in the product. Canned WD canine diet contained 0.05% TAU (DM basis), and the concentration in fresh whole herring was 0.50 % TAU. Diet summaries ($n = 3$) provided estimate ranges of 0.12–0.30% DM TAU in the diet at the time of blood collection.

Discussion

The suggested minimum TAU dietary value suggested by the polar bear nutrition working group is 0.10 % DM (Lintzenich et al. 2006); with the exception of canine milk replacer, feedstuffs analyzed in this study appeared to meet that level. Nonetheless, these preliminary results indicate that plasma TAU concentrations from the free-ranging cubs were higher than those in domestic carnivores (cats 80–120 nmol/ml, dogs 60–120 nmol/ml), humans (40–100 nmol/ml), or rat (50–95 nmol/ml) (Hayes et al. 1975) as well as captive polar bears. TAU is the most abundant amino acid in the milk of pinnipeds (seals and sea lions) (Sarwar et al. 2007) and cetaceans (whales and dolphins) (Walsh and Rogers 1995). Plasma and whole blood TAU of captive killer whales and bottle nose dolphin *Tursiops truncates* are similar to canine and feline species (Walsh and Rogers 1995). The higher blood TAU value in the cubs likely reflects the amino acid pattern transferred through maternal milk, which was the sole source of nutrition during early postnatal development.

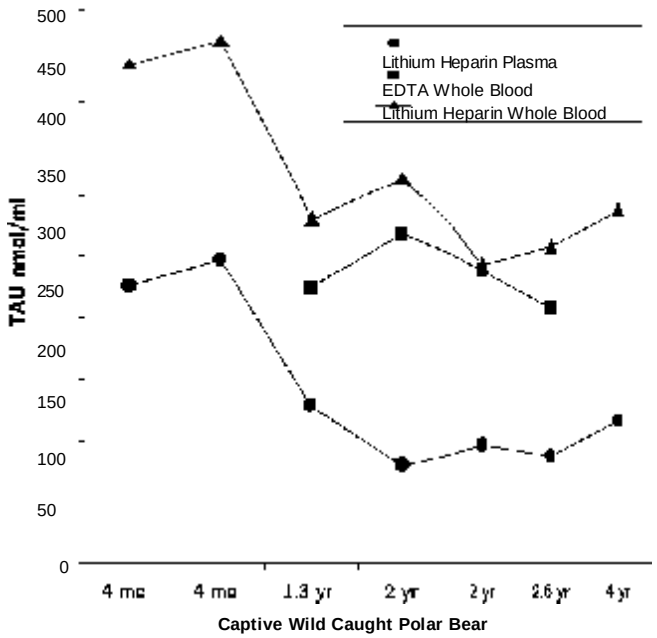

Figure 1: Historical plasma and whole blood TAU values for two wild caught captive polar bear cubs.

Mean values for captive polar bears, overall, were within normal plasma references ranges for cats (80–120 nmol/ml) and dogs (60–120 nmol/ml). Whole blood TAU reference ranges for cats are 300–600 nmol/ml and 200–350 nmol/ml for dogs (www.vetmed.ucdavis.edu/vmb/aal/aal.html). Plasma values can be affected by fasting, whereas whole blood TAU concentrations (in dogs) are most likely a better indicator of overall TAU status (Delaney et al. 2003).

Plasma and whole blood values for the two young cubs slowly declined as they matured and integrated into a captive environment and diet. The decrease in plasma TAU may be the result of a decrease in the intake of the precursor of TAU synthesis, the sulfur amino acids, methionine and cysteine/kg body weight. This may be a result of lower sulfur amino acids in the captive diet but has not been quantified in detail. Additionally, this decrease may occur in animals as they mature since the energy requirement for growth decreases, and food intake is lower per kg body weight for maintenance. There may also be

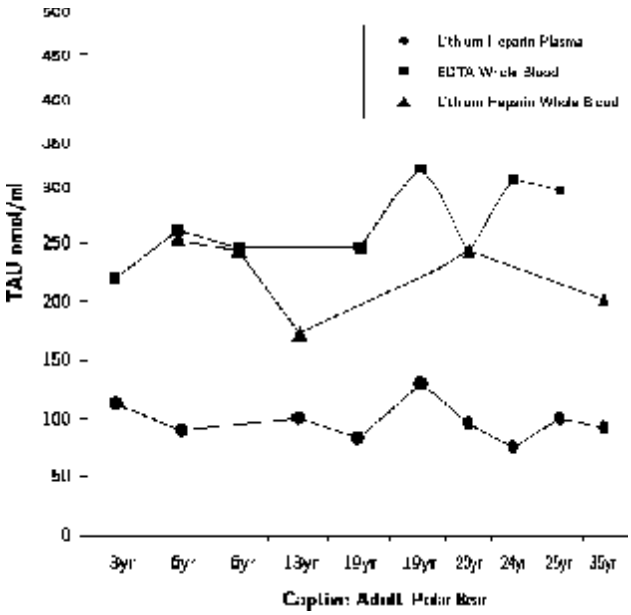

Figure 2: Plasma and whole blood TAU values from ten individual captive polar bears.

less activity in mature animals, resulting in a lower food intake per kg body weight as well.

We speculate that TAU may have a role in the synthesis of the unique bile acids of ursid species – ursodeoxycholic acid (UCDA). UCDA (3oc, 73-dehydroxy-5B-colanic acid) was first identified as a major constituent of dried bile in the Chinese black bear (Beuers et al. 1998) and has been used for centuries in China for liver disorders (Shoda 1927). UCDA has been found effective in the treatment of cholestatic liver disease in man and in the therapy of primary biliary cirrhosis (Hofmann 1999).

Bile acids are needed for cholesterol elimination, stimulation of bile flow, stimulation of biliary phosphalidylcholine secretion and enhancement of lipid absorption as well as other actions. Most membranes are impermeable to conjugated bile acids and thus they can have high intraluminal concentrations. They are also less likely to precipitate in the presence of intraluminal calcium. The two amino acids that are important in conjugation are glycine and TAU.

TAU conjugates of primary and secondary bile acids are always associated with greater lipid absorption (Hofmann 1999). Unless bile acids are present in this micellar form, the fat-soluble vitamins (A, D, E and K) will not undergo absorption and deficiency can occur (Pickett et al. 1990).

We speculate that the TAU conjugation of UCDA may be impaired in polar bear cubs fed puppy milk replacer or dry dog foods because of relatively low TAU content (Aguirre 1978; Hedberg et al. 2007), resulting in poor micellular formation and reduced vitamin D absorption, as has been shown in pre-term human infants (Zamboni et al. 1993). Reduced vitamin D absorption will manifest as vitamin D-deficiency rickets. TAU- sufficient mothers may well have higher milk TAU content compared with captive polar bear females fed a lower-TAU diet, but this has not been specifically determined. It is further speculated that the conjugation of UCDA bile acid with glycine cannot support adequate micellar formation for adequate vitamin D absorption, again shown in human infants fed a TAU- free formula (Zamboni et al. 1993) that resulted in significantly reduced 25 (OH) vitamin D levels. Controlled experiments need to be conducted to confirm these hypotheses.

Conclusions

1. Metabolic bone disease found in polar bears raised in captivity may be linked with multiple nutrient and dietary imbalances. There is evidence of decreased TAU content in captive diets compared with free-ranging bears, which may have implications for absorption and utilization of fat-soluble vitamins even with no direct TAU deficiency.
2. More detailed evaluations of dietary intakes of fat-soluble vitamins and amino acids, correlated with metabolic evaluation of nutritional status of these nutrients in age-matched polar bears raised on wild and captive diets, is recommended to further understand relationships among these nutrients in this species.
3. Additional information could be helpful when comparing diets of captive sows with normal offspring vs. sows that had offspring with MBD.
4. Further studies are also suggested, including complete nutritional assessment of both blood and maternal milk nutrients (fat-soluble vitamins, minerals, fatty acids and amino acids) in polar bears, as well as controlled studies to determine specific dietary requirements of both young and adults.

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