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Case Report

Hypervitaminosis D in Guinea Pigs with α-Mannosidosis

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A colony of guinea pigs (*n* = 9) with α-mannosidosis was fed a pelleted commercial laboratory guinea pig diet. Over 2 mo, all 9 guinea pigs unexpectedly showed anorexia and weight loss (11.7% to 30.0% of baseline weight), and 3 animals demonstrated transient polyuria and polydipsia. Blood chemistry panels in these 3 guinea pigs revealed high-normal total calcium, high-normal phosphate, and high ALP. Urine specific gravity was dilute (1.003, 1.009, 1.013) in the 3 animals tested. Postmortem examination of 7 animals that were euthanized after failing to respond to supportive care revealed renal interstitial fibrosis with tubular mineralization, soft tissue mineralization in multiple organs, hepatic lipidosis, and pneumonia. Analysis of the pelleted diet revealed that it had been formulated with a vitamin D3 content of more than 150 times the normal concentration. Ionized calcium and 25-hydroxyvitamin D values were both high in serum saved from 2 euthanized animals, confirming the diagnosis of hypervitaminosis D. This report discusses the clinical signs, blood chemistry results, and gross and histologic findings of hypervitaminosis D in a colony of guinea pigs. When unexpected signs occur colony-wide, dietary differentials should be investigated at an early time point.

Abbreviation: HD, hypervitaminosis D.

Vitamin D is one of the main regulators of calcium homeostasis.[10,35] Although intake of sufficient vitamin D is crucial to many normal processes within the body, toxicity through excess intake can result in anorexia, dehydration, hypercalcemia, metastatic calcification, renal failure, and urolithiasis.[6,12,14,27,37] Humans can acquire hypervitaminosis D (HD) by chronic voluntary use of excessive vitamin D supplements.[32,34] However, most reported cases of HD in animals occur after ingestion of toxic plants[26] or a nonfood substance, such as rodent bait[21] or antipsoriasis ointment.[12,27,36] Laboratory animals have limited access to such toxins but are at risk for accidental overdose from contamination of pelleted diets. In these cases, entire colonies of animals may be exposed, risking extinction of unique genotypes.

Diagnosis of HD usually is prompted by consistent clinical signs and concomitant serum hypercalcemia.[30] However, diagnosis may be complicated in guinea pigs. Clinical signs are often vague.[28] Documentation of hypercalcemia may be difficult, especially because serum or blood total calcium may not parallel ionized calcium.[28] Although total blood calcium is measured usually, assessing ionized calcium is more important because it is the major active form in the body. Ionized calcium levels typically are about half of total calcium levels, but this ratio may vary with disease state.[28] Ill guinea pigs may not tolerate venipuncture or the collection of sufficient blood for evaluation of ionized calcium and vitamin D levels by commercial laboratories, and normal ranges for these values are not well established. However, as we show here, various other clinical findings should increase suspicion of HD.

This report describes the clinical signs, whole blood and serum calcium results, and gross and histologic findings of HD in a unique colony of guinea pigs carrying a genetic mutation for α-mannosidosis. HD in this colony was caused by contamination during manufacture of a pelleted laboratory guinea pig diet. The associated disease was further complicated by hepatic lipidosis and pneumonia. The resulting clinical signs prompted euthanasia of most of the colony, leaving only 2 living guinea pigs that carry the allele for α-mannosidosis. We reported our guinea pig morbidity to the manufacturer of the pelleted diet, who subsequently identified numerous diets with excessive vitamin D.[23]

Case Report

Clinical presentation. The colony of guinea pigs consisted of 9 animals (designated A through I; male, 4; female, 5; age at onset, 7.3 to 20.5 mo). The guinea pigs were housed on an IACUC-approved breeding protocol at the University of Pennsylvania. They were cared for according to the *Guide for the Care and Use of Laboratory Animals*[13] and the Animal Welfare Act[1] and Regulations.[2] Eight of the animals were socially housed in nonfiltered, solid-bottom cages with wire-bar tops on direct-contact shredded paper bedding. One male animal (I) was housed individually when not being bred, because of behavioral incompatibility with other male guinea pigs. The animals were fed an unautoclaved laboratory guinea pig pelleted diet (Guinea Pig Diet 5025, LabDiet, Richmond, IN), irradiated timothy hay, and baby carrots or fruit pieces weekly.

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The colony was derived in Australia from a small number of guinea pigs born with a spontaneous autosomal recessive form of α-mannosidosis due to a missense mutation of the gene coding for lysosomal α-mannosidase.[3,20] The colony was established from 2 breeding pairs of carrier animals imported 3 y prior to the HD diagnosis. All 9 animals had been born inhouse. The research laboratory staff had confirmed by PCR analysis of blood or skin tissue that 7 animals were heterozygous for the missense gene; α-mannosidosis heterozygotes are phenotypically normal. Two animals had not been genotyped.

In June 2012, guinea pig body weights were noted to have decreased by 11.7% to 30.0% of baseline (the April body weight). Partial to complete anorexia was present. On exam, animals were alert and active, with normal rectal temperature. However, all were judged to have excessively thin body condition scores. The fact that all animals in the colony seemed to be affected pointed toward either nutritional or infectious causes. On May 2, the pelleted diet lot had been changed to a lot manufactured on April 17, thereby prompting us to request that the manufacturer analyze the diet for nutritional abnormalities and toxins.

In addition to the anorexia and weight loss seen in all 9 animals, three female guinea pigs (animals A, B, and C) were reported to have polydipsia and polyuria. Differential diagnoses included diabetes mellitus or insipidus, pyometra, pyelonephritis, hyperadrenocorticism, and electrolyte changes such as hypercalcemia or severe hypokalemia. Lithium-heparinized blood (catalog no. 365971, Microtainer, Becton Dickenson, Franklin Lakes, NJ) was obtained from each of these 3 animals for a CBC and chemistry panel. According to the reference range established inhouse for the analyzer (model VS2, VetScan, Abaxis, Union City, CA), calcium was normal in all 3 guinea pigs tested, albeit high normal in 2 (Table 1). Similarly, phosphate was high normal in all 3 animals, which also had elevated ALP levels, a finding most consistent with hepatic lipidosis resulting from anorexia. Two guinea pigs (B and C) were housed for less than 8 h without bedding so that a free-catch urine sample could be obtained from the cage pan. The results of urine dipstick analysis of both samples were normal. Specific gravity was hyposthenuric (1.003, animal C) to isosthenuric (1.009, animal B). A primary diagnosis was not reached based on these findings, and the reported polyuria resolved without treatment.

Shortly after this, 3 guinea pigs (animals A, C, and D) developed increased expiratory effort with slightly decreased lung sounds. Differential diagnoses included infectious or aspiration pneumonia and cardiac or skeletal myopathy resulting from cachexia, infection, toxin, or nutritional deficiency. Guinea pig D was chosen to undergo diagnostic testing and necropsy in an attempt to reach a definitive diagnosis and provide effective treatment for the remaining guinea pigs. After sedation with 10 mg ketamine and 0.5 mg midazolam subcutaneously, the animal was anesthetized with isoflurane via face mask. Abdominal and thoracic radiographs appeared normal except for a diffuse mild bronchial pattern in all lung fields. A tracheal wash was done by instilling 2 mL of sterile 0.9% saline into a 3-French silicone feeding tube introduced via laryngoscopy into the trachea. Returned fluid was submitted for aerobic culture, yielding a heavy growth of *Klebsiella* sensitive to enrofloxacin. A pharyngeal swab submitted for *Bordetella* PCR analysis was negative. A urine sample obtained by cystocentesis was normal on urinalysis, although the sample was dilute (specific gravity, 1.013). Serum was submitted to a commercial laboratory for a serum chemistry panel and ascorbic acid level. Serum calcium was normal. ALP was high, which in an anorexic animal was likely due to hepatic lipidosis. Serum ascorbic acid level was high also, ruling out hypovitaminosis C as a cause of clinical signs. While anesthetized, the guinea pig was euthanized with pentobarbital-based euthanasia solution (780 mg IP), and the carcass was submitted for necropsy.

All 8 remaining guinea pigs were placed on enrofloxacin (10 mg/kg PO once daily; Baytril, Bayer Animal Health, Shawnee, KS) for 11 d. The increased respiratory effort noted in animals A and C resolved within 2 d of instituting enrofloxacin. Guinea pigs were weighed daily, and those with daily weight loss were treated with 100 mg vitamin C oral solution once daily and syringe-fed 15 to 30 mL/kg of a combination of timothy-hay–based herbivore recovery diet (Critical Care, Oxbow Hay Animal Health, Murdock, NE) and rice baby cereal once to twice daily. In addition, all animals were provided with irradiated timothy hay ad libitum.

In late June, due to continued anorexia, 4 animals (B, C, E, and F) were euthanized (780 mg IP pentobarbital-based euthanasia solution) after profound sedation with 20 mg ketamine and 0.1 mg dexmedetomidine (Dexdomitor, Zoetis, Madison, NJ) subcutaneously. Their carcasses were submitted for gross necropsy.

In early July, the manufacturer of the pelleted diet notified us that the lot that had been fed and that prompted our request for analysis was being recalled due to excessive vitamin D. On telephone follow-up (July 7) with the company, one of the authors (JAJ) was informed that the diet contained cholecalciferol at 505 IU/g feed; the normal cholecalciferol level for this feed is 3.1 IU/g. The remaining 4 guinea pigs (A, G, H, and I) were sedated (0.1 mg acepromazine and 0.2 mg butorphanol SC) to obtain heparinized blood samples for a chemistry panel and ionized calcium levels. The inhouse analyzer was used for the chemistry panels, whereas a handheld analyzer (i-STAT, Abbott, Princeton, NJ) was used for ionized calcium. All 4 guinea pigs continued to have elevated blood ALP levels suggestive of hepatic lipidosis (Table 1). Blood total calcium and phosphate were normal in all guinea pigs. The handheld blood analyzer used has no reference range for ionized calcium in guinea pigs, but blood ionized calcium was slightly higher than was expected in all 4 guinea pigs.

Each guinea pig was given 10 mL normal saline SC in an attempt to acutely decrease calcium levels while ensuring normal hydration. Although the guinea pigs tolerated oral feeding and medication well, they became very vocal and appeared distressed with injections. Therefore, we decided that injectable therapy may be too aggressive given the potential for injection-related stress to worsen hepatic lipidosis. Glucocorticoid therapy was considered too risky due to the hepatic lipidosis and recent *Klebsiella* infections. Furosemide treatment was not used because of the potential to worsen suspected kidney disease. Oral phosphate binders were considered but not used, because the total calcium and phosphate concentrations were normal in all 4 guinea pigs.

After body weights continued to decline over the next 2 d, ranitidine (2 mg PO) was begun once daily to reduce gastric acidity and improve gastrointestinal motility. At the same time, 2 additional guinea pigs were euthanized (animals A and G with 780 mg and 975 mg IP pentobarbital-based solution, respectively) after sedation with 10 mg ketamine and 0.025 mg dexmedetomidine SC. Blood from both animals was obtained via cardiocentesis and allowed to clot. Serum was collected and then frozen for 1 d at –20 °C until it could be sent to a commercial laboratory for measure-

Table 1. Pertinent blood chemistry results from guinea pigs fed a diet containing about 150-fold excess cholecalciferol.

	Date of sampling	Total calcium (mg/dL)	Creatinine (mg/dL)	ALP (U/L)	Phosphate (mg/dL)	Blood iCa[a] (mmol/L)	Serum iCa[b] (mmol/L)	25(OH)-Vitamin D (nmol/L)
Reference range		8.0–15.5	0.5–2.2	15-45	4.2–8.5	No range established	1.30–1.60	No range established
Animal								
A	June 6	11.2	0.9	62	6.2			
	July 6	11.0	0.7	90	4.7	1.42		
	July 9						1.74	445
B	June 6	13.2	0.9	104	7.1			
C	June 6	12.8	0.9	96	7.1			
D[c]	June 26	11.4	0.9	160	7.5			
G	July 6	11.7	1.0	73	6.7	1.43		
	July 9						1.76	541
H	July 6	11.4	0.6	69	4.3	1.59		
I	July 6	11.9	0.8	154	7.3	1.43		
	July 25	12.2	0.4	99	3.4			
	October 11	12.3	0.6	41	3.2			
Control 1	July 9							93
Control 2	July 9						1.59	93

[a]Ionized calcium measured by using an in-clinic handheld analyzer.
[b]Ionized calcium measured by using ion-selective electrode.
[c]Serum chemistry rather than blood chemistry was obtained from animal D. Because the laboratory has not established reference ranges for this species, results were evaluated by using the inhouse reference ranges.

ment of serum 25-hydroxyvitamin D via radioimmunoassay and for measurement of ionized calcium via ion-selective electrode. Serum ionized calcium was increased in both animals (Table 1). For comparison, serum was obtained from 2 unrelated guinea pigs fed unaffected diet lots. The laboratory did not have enough information to establish normal vitamin D levels for guinea pigs, but the levels from the 2 affected guinea pigs were clearly higher than were the levels from the unaffected guinea pigs. The combination of high serum ionized calcium with high 25-hydroxyvitamin D confirmed the diagnosis of HD.

The 2 remaining colony guinea pigs clinically recovered but required more than 3 mo of supplemental feeding. Blood obtained from animal I in late July and in October showed decreases in both ALP and phosphate levels (Table 1) compared with the previous values. Several milliliters of blood are required for assessment of 25-hydroxyvitamin D, and the remaining 2 guinea pigs are too valuable to risk unnecessary general anesthesia for venipuncture. Blood will be obtained for serum ionized calcium and vitamin D levels when both animals next require anesthesia for other reasons.

Gross necropsy findings. Seven guinea pigs were submitted for gross postmortem examination. The kidneys of all 7 guinea pigs were bilaterally pale with a granular, pitted surface (Figure 1 A), and pale streaks extending from the cortex through the medulla on cut surface. The lungs of animals D and G were extensively dark red and consolidated with multiple pale foci (diameter, 2 to 3 mm). Guinea pig D's heart exhibited abundant white to tan streaks of pallor replacing 90% of the normal myocardium (Figure 1 B), and the gastric mucosa contained a focally extensive erosion.

Histologic findings. Tissues were collected in 10% buffered formalin saline for histopathologic studies. For 4 of the 7 guinea pigs, the kidneys were the only organ submitted for microscopic examination (B, C, E, and F), whereas all organs were examined microscopically in the remaining 3 guinea pigs (A, D, and G). Microscopically, there was soft tissue mineralization of several organs, including kidney (all 7 guinea pigs), myocardium (D), lung (D and G), and stomach (D and G). The extensive soft tissue calcification observed in these guinea pigs is consistent with previous reports of vitamin D toxicity in other species.[15,19,24]

Affecting approximately 90% of the myocardium of guinea pig D, there was abundant mineralization within and replacing normal myocardium (Figures 1 C and D), with degeneration and necrosis of the mineralized fibers. The affected foci also had a moderate amount of fibrosis admixed with a moderate lymphohistiocytic infiltrate. Occasionally blood vessel walls were mineralized.

The kidneys of all 7 guinea pigs had an irregular, multifocally depressed capsular surface with abundant, multifocal, interstitial fibrosis admixed with a moderate lymphoplasmacytic infiltrate (Figure 1 E). The tubular lumen and tubular basement membranes contained deeply basophilic, granular, mineralized multifocal deposits. The tubular epithelium was hypereosinophilic and degenerative multifocally, often sloughed within the tubular lumen. The glomeruli were largely unaffected.

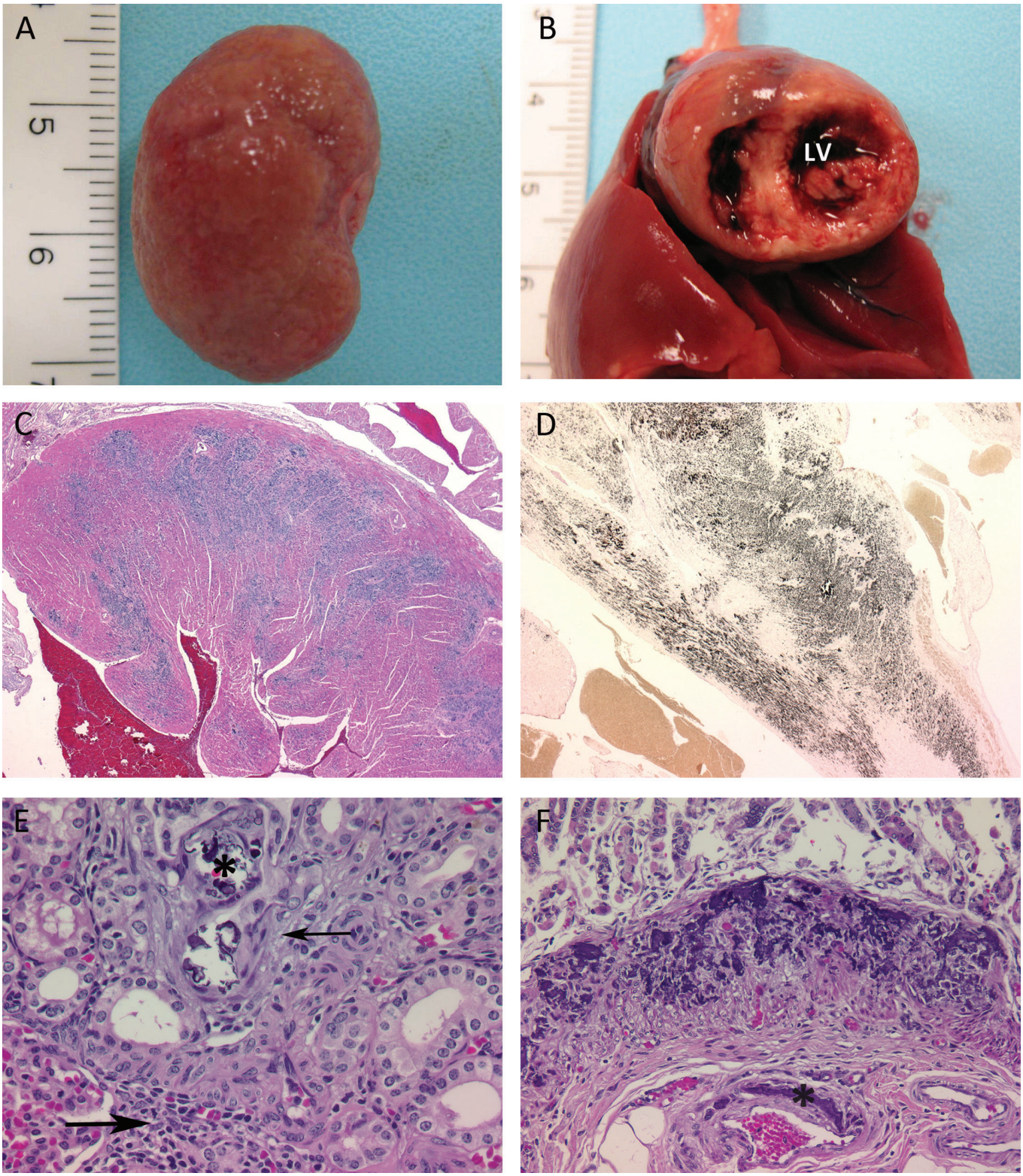

Figure 1. Gross and microscopic images of guinea pigs fed a diet containing about 150-fold excess cholecalciferol. (A) The kidney is pale, with a granular, pitted surface. (B) Cross section of the heart shows white to tan streaks of calcification. LV, lumen of the left ventricle. (C, D) Myocardium is replaced by extensive granular mineralization, which stains (C) deeply basophilic with hematoxylin and eosin and (D) black on Von Kossa stain. Magnification, 2×. (E) Kidney demonstrates lymphoplasmacytic interstitial inflammation (large arrow) and fibrosis (small arrow), and mineralization of tubular epithelial cells (asterisk). Hematoxylin and eosin stain; magnification, 40×. (F) Stomach has extensive mineralization of a blood vessel wall (asterisk) and the muscularis externa. Hematoxylin and eosin stain; magnification, 20×.

The stomach (guinea pigs D and G) showed mineralization of the muscularis externa, muscularis mucosa, mucosa, and occasional blood vessel walls (Figure 1 F). Guinea pig D had a focally extensive gastric mucosal erosion. The lung (animals D and G) contained multifocal granular mineralized deposits within the peribronchiolar smooth muscle. In addition, animals A, D, and G had pneumonia characterized by a moderate lymphohistiocytic interstitial infiltrate with a moderate neutrophilic infiltrate in the terminal bronchioles and alveoli. These 3 animals also had mild to moderate hepatocellular vacuolation, consistent with hepatic lipidosis.

Discussion

Here we report a guinea pig colony that developed HD due to overdose of vitamin D in a pelleted laboratory animal diet. The resulting anorexia led to hepatic lipidosis. Internal organs were commonly mineralized, but despite this abnormality, the guinea pigs tested did not have high total blood calcium levels according to the reference range for the analyzer used at the time of diagnosis. Rather, high-normal total blood calcium ('high normocalcemia') was present, along with high-normal blood phosphate values. This combination of findings should prompt consideration of HD. Although anorexia and weight loss were the most common signs, 3 guinea pigs showed transient polyuria and polydipsia, which we realized in retrospect may have constituted an important diagnostic clue. Very dilute urine (less than 1.007) can be found in normal animals, but in a polyuric animal, it is characteristic of a limited number of disorders, one of which is HD.[28]

Cholecalciferol (vitamin D3) is a fat-soluble vitamin with a long half-life (approximately 2 mo in humans).[17] The half-life in guinea pigs has not been specifically reported. The liver converts cholecalciferol to 25-hydroxyvitamin D, which the kidney converts to 1,25-dihydroxyvitamin D (calcitriol).[10] Calcitriol causes increased absorption of dietary calcium and phosphorus in the small intestine. In concert with parathyroid hormone, calcitriol increases osteoclastic absorption of calcium and phosphorus from bone and distal renal tubular reabsorption of calcium and phosphorus from urine.[28] Because calcitriol increases blood phosphorus as well as calcium, the presence of normal to increased blood phosphate in a hypercalcemic patient should prompt strong consideration of vitamin D toxicity. Normal to elevated blood phosphorus can compound the adverse effects of increased calcium, because the product is responsible for tissue mineralization.[33]

In blood, calcium is free (ionized), bound to proteins, or complexed with other molecules. Ionized calcium is usually about one half of total serum calcium and is the major active form of calcium. Intracellular ionized calcium is tightly maintained at a very low level, because small increases can lead to cell dysfunction and death.[28] Ionized calcium should be measured to assess the body's calcium status. This analysis presents a problem when very small animals, such as guinea pigs, are the patients. Handheld analyzers can be used to measure ionized calcium on small amounts of blood, but measurements of whole-blood ionized calcium by these instruments may be lower than ionized calcium in serum, especially in hypercalcemic patients.[8,16] This situation may explain why the blood ionized calcium values that were measured in 2 of the guinea pigs were lower than were the serum ionized calcium levels measured via ion-selective electrode at a later time point.

The blood total calcium levels of all animals, except guinea pig D, were determined on an inhouse analyzer. The advantage of this analyzer is that it requires only 0.2 mL of lithium heparinized blood. Our inhouse reference range for guinea pigs includes a total calcium level (8.0 to 15.5 mg/dL) whose high-end value exceeds that of most published reference ranges for guinea pigs.[9,25] Such wide reference ranges, although reflective of the species as a whole, may not be appropriate for individual populations. However, even if the more typical upper-end value of about 12 to 12.5 mg/dL had been applied, in only 2 instances (guinea pigs B and C on June 15) would the total calcium level have been interpreted as high. In all other instances, the total calcium levels would have been interpreted as high normal. Therefore, our findings demonstrate that, as in humans,[30] a normal total calcium level cannot be used to rule out vitamin D toxicity in guinea pigs—either hypercalcemia or high normocalcemia may be present.

Guinea pigs' vitamin D nutritional requirement is not known, but it is estimated to be about 0.025 mg/kg diet (1000 IU/kg diet).[22] Experimental vitamin D deficiency[7,29] and overdose[4] have both been reported. The cholecalciferol level in the diet fed to these guinea pigs should have been 3.1 IU/g. Therefore, 505 IU/g represents a dose that is more than 150 times the normal amount. According to the feed manufacturer's recommendations, each guinea pig should eat 25 to 40 g of pelleted food daily. A guinea pig eating this amount of the misformulated feed would have a daily vitamin D intake of 12,625 to 20,200 IU. Although there are no prior published reports of nonexperimental HD in guinea pigs, this amount of vitamin D would be expected to cause toxicity in other species.[18,19]

In humans, clinical toxicity is unlikely when the 25-hydroxyvitamin D serum level is less than 375 nmol/L.[14] The level found in the guinea pigs reported here exceeded this threshold when measured in July and might have been higher if measured in June, when presumably the guinea pigs were still eating some of the pelleted diet. Although the serum level of 25-hydroxyvitamin D is used as a marker of toxicity and in itself contributes to toxicity,[28] many other metabolites also increase. The level of serum total calcitriol may remain within normal limits, particularly later in the disease.[14]

Signs of HD and hypercalcemia include anorexia, lethargy, polyuria and polydipsia, and renal disease. Cardiac arrhythmias and seizures may be seen, especially when hypercalcemia is severe or sudden. Increased gastrin and gastric hydrochloric acid can result in gastric erosion, as seen here in animal D. Polyuria in early or mild hypercalcemia may be associated with hyposthenuria that results from reduced antidiuretic hormone efficacy and reduced tubular sodium absorption.[28] One sample of the 3 we evaluated was hyposthenuric, whereas the other 2 were isosthenuric. Isosthenuria is expected when disease is more advanced, after hypercalcemia has damaged the kidneys. Hypercalcemia causes direct toxic effects on renal cells, as well as ischemic renal disease due to chronic renal vasoconstriction. Dehydration, inflammation, fibrosis, and tubular obstruction worsen the damage.[28] All of the 7 euthanized guinea pigs had substantial renal lesions observed at postmortem examination, although none were azotemic.

This guinea pig colony is the only one in the world known to carry the gene for the lysosomal storage disease α-mannosidosis. The foundation breeding pairs were imported from the Adelaide Children's Hospital, Australia, which then closed down their colony. Because guinea pigs with α-mannosidosis are considered good models for affected humans,[3] the investigator had intended to breed these animals and use the offspring to test potential therapies. Only 2 guinea pig carriers remain, both males, and their lifespan may be limited by the effects of HD on their organs. Although guinea pig ovarian cryopreservation has been reported recently,[38] cryopreservation of guinea pig gametes and embryos is considered difficult.[5] Successful activation of motile guinea pig sperm after cryopreservation has not been reported. Given these constraints, the loss of these 2 remaining α-mannosidosis carriers will be considerable for the researcher.

This study was limited by the difficulty of obtaining an adequate volume of blood for determination of ionized calcium and vitamin D levels from living guinea pigs and by the lack of accepted reference ranges for these values in this species. However, the clinical and pathologic findings in these guinea pigs confirm the diagnosis.

Older guinea pigs have been reported to have a high incidence of metastatic or dystrophic mineralization of soft tissues, including heart and kidney. Although an etiology has not been determined, it may be associated with a dietary deficiency (magnesium) or excess (calcium or phosphorus).[11] Vitamin D levels in affected guinea pigs have not been reported.

Contamination of pelleted animal diets with excessive vitamin D has been reported previously,[18,31] but to our knowledge, HD due to ingestion of laboratory animal diets and nonexperimental vitamin D toxicity in guinea pigs have not. HD should be a differential diagnosis in laboratory animals with anorexia or polyuria–polydipsia and concomitant normal to high levels of blood or serum phosphate and calcium. Feeding a contaminated diet to laboratory animals can cause disease in many animals in a colony and have severe effects on research. Therefore, members of the laboratory animal medicine field should consider the potential for illness resulting from commercially available pelleted food, particularly when many members of an animal colony show unexpected signs. Early diagnosis and reporting may reduce animal morbidity and mortality resulting from such dietary errors.

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