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Influence of a Nigerian-like Diet on Calcium, Phosphate and Alkaline Phosphatase Levels in the Plasma and Bone of Cadmium Exposed Rats

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The purpose of this study was to assess the role of a wholly compounded Nigerian diet on some indices of bone metabolism in oral cadmium toxicity. Nine weeks old Wistar albino rats were exposed to 100 ppm cadmium in their drinking water and fed a Nigerian-like diet (high in carbohydrate and fibre but low in protein) for sixteen weeks. The study reveals that the Nigerian-like Diet (NLD) caused less accumulation of cadmium in both the epiphysis and metaphysis of femur bones compared with the control diet. Plasma cadmium and phosphate levels did not change significantly with diet and cadmium exposure. Plasma alkaline phosphatase activity was enhanced in rats fed with the NLD and cadmium exposure reduced the enzyme activity. The exposure to cadmium compared with the control significantly ($p < 0.05$) reduced calcium and phosphate in both the epiphysis and metaphysis of the femur of rats, which was further enhanced by the NLD. The NLD increased alkaline phosphatase activity in both sections of the bone studied, though cadmium reduced the enzyme activity. The study shows that the NLD predispose rats to the debilitating effect of cadmium on bones by improving calcium and phosphate loss which promote bone resorption.

Key words: Bone, cadmium, calcium, phosphate, Nigerian diet

INTRODUCTION

Cadmium is an environmental pollutant and is toxic to a number of organs. Chronic exposure to cadmium causes kidney and bone damage. With the manifestations of the itai-itai disease in Japan, considerable attention has been drawn to the effects of cadmium on bone metabolism; as characteristics of the disease are osteomalacia, osteoporosis and renal tubular lesions (Horiguchi *et al.*, 1994; 1996; Alfvén *et al.*, 2002).

Diet composition and the nutritional status of the individual are some of the important factors associated with the severity of oral cadmium toxicity (Anderson *et al.*, 1992; Reeves *et al.*, 2001). Diets containing high fiber content have been reported to decrease absorption of cadmium (Berglund *et al.*, 1994; Lind *et al.*, 1998). Also, the protein content of the diet had earlier been shown to affect cadmium toxicity in rats (Revis, 1981; Tewari *et al.*, 1986). Protein consumption has an anabolic effect on cell growth and may also increase bone-formation (Lafage-Proust *et al.*, 1999). However there is controversy as to the contribution of dietary proteins to bone mineral density. Some studies show that protein intake positively correlates with bone mineral density (Lau *et al.*, 1998). Other studies show no correlation between high protein intake and calcium metabolism, bone composition, or bone resorption (Creedon, 2000).

Bone metabolism is a complicated homeostatic process involving calcium, vitamin D, collagen, the bone cells, the thyroid and parathyroid gland (Kjellström, 1992). Oral cadmium has been reported to cause negative calcium balance in rats and administration with a low protein diet led to reduction in bone calcium level (Itokawa *et al.*, 1973). Alkaline phosphatase is richly produced by osteoblasts and is also involved in bone metabolism (Nakamura *et al.*, 1988). Toxicity to the bone may lead to osteoporosis and increased alkaline phosphatase (Krook and Minor, 1998).

Increasing industrial activities in Southern Nigeria is thought to be linked with increase cadmium in the environment (Egborge, 1994) where the population subsists on diets that are low in protein (fish protein) but high in carbohydrate and fiber. Experimental studies examining the effect of diet on some aspects of bone metabolism in cadmium toxicity is still undergoing investigation. However, little reports exist on the effect of wholly compounded diet on bone metabolism in cadmium toxicity and even fewer still have been reported using wholly compounded Nigerian-like Diet (NLD). This underscores the need to investigate the role that a typical

Nigerian diet plays in bone metabolism in cadmium toxicity. This article, therefore reports the effect of a wholly compounded NLD on the levels of calcium, phosphate and alkaline phosphatase in the plasma and bone of rats orally exposed to cadmium.

MATERIALS AND METHODS

Experimental design: Nine weeks old male albino rats (Wistar strain) with an average weight of 100.00 g were housed individually in stainless steel cages with wire mesh floor to prevent coprophagy. The animals were assigned to four groups with ten animals in each group such that the weight difference between the groups was less than 0.2 g. Of the four groups of rats, two were maintained on a control diet (COD) while the other two groups of animals were fed on a Nigeria-like diet (NLD) patterned after those of Eriyamremu *et al.* (1995). The NLD is low in fish protein and high in carbohydrate and fiber and served as the test diet. The composition of both diets is shown in Table 1. One group of rats maintained on each diet was given deionized water while the other group of rats was given 100 ppm cadmium ion (as CdSO₄) in drinking water which is equivalent to 100 mg Cd kg⁻¹. A preliminary investigation in our laboratory had shown that this dose of cadmium ion was tolerated by the rats with quantifiable tissue biochemical changes without fatality.

The animals were given the food and water *ad libitum*. They were acclimatized with their respective diet and water for one week before the commencement of the study, which lasted for sixteen weeks. During this period, water consumption, food intake and dry fecal output were measured daily, while weight gain was recorded weekly. All these animal treatment were carried out in accordance with the principles of laboratory animal care of the NIN guide for Laboratory Animal Welfare as contained in the NIN guide for grants and contracts. At the end of the study period, the animals were fasted for 18 h and sacrificed after chloroform anesthesia.

Table 1: Percentage composition of the diets

Dietary component	Diet type	
	Control diet	Nigerian-like diet
Fish (defatted)	-	14.00
Casein	19.00	-
Sucrose	15.00	-
Garri	-	65.00
Corn starch	50.65	-
Fiber	-	10.65
Cellulose	5.00	-
Salt mix	4.00	4.00
Vitamin mix	1.00	1.00
Palm oil	5.00	5.00
Methionine	0.35	0.35
Sulphur content	0.25	0.16

Note: Garri is a cassava based meal commonly consumed in Nigeria and contributed the fiber in the Nigerian-like diet

Collection and treatment of samples: While under chloroform anesthesia, the left common carotid artery was exposed and cannulated. Blood sample was obtained from each rat via the cannula and transferred to heparinized tube. Plasma was obtained by centrifugation of the blood at 3000 g for 10 min and subsequently used for analysis of calcium and alkaline phosphatase activity. The epiphysis (0.5 cm immediately after the cartilage) and metaphysis (0.5 cm immediately after the epiphysis) were recovered from the femur bones of each rat and washed several times with ice-cold physiological saline solution in a glass wash to remove marrow. The epiphysis and metaphysis were then immediately used for biochemical analysis. Weighed portions of the epiphysis and metaphysis were digested with 20 mL HNO₃-HClO₄ mixture (4:1) at 100°C. The digests were made up to 100 mL with deionized water and used for the analysis of cadmium, calcium and phosphate contents in these bone sections. Weighed portions of the epiphysis and metaphysis were homogenized in ice-cold normal saline solution, centrifuged at 4000 g and the residues were recovered for alkaline phosphatase assay.

Biochemical analysis: The assay for calcium was carried out using O-cresolphthalein dye binding method (Henry and Dryer, 1963), phosphate was analyzed by the method of Fiske and Subbarow (1925). Alkaline phosphatase activity was estimated by the method of Annino and Giese (1976) and was expressed in μ mole p-nitrophenol produced/min/mg protein. The protein content of the samples was determined by the method of Lowry *et al.* (1951).

Cadmium analysis: Bone cadmium was analyzed with an atomic absorption spectrophotometer (Varian AA, 1475).

Statistical analysis: The values are reported as Mean \pm SEM. Statistical difference was determined using ANOVA and differences in the means were tested by Duncan's multiple range test (Sokal and Rohlf, 1969).

RESULTS

The study shows a statistically significant decrease in the weight gain of rats exposed to 100 ppm cadmium ion in the drinking water of rats compared with those not given cadmium (Table 2). A similar trend was observed in the quantity of water consumed by the rats. In the rats fed the Nigerian-like diet, there was a significant decrease in weight gain and feed efficiency, but the fecal output of the rats was significantly increased. Like the typical Nigerian diet, oral cadmium exposure to rats significantly decreased feed efficiency and significantly increased fecal output. This study shows that cadmium as well as the Nigerian-like diet decreased weight gain and feed efficiency and increased fecal output.

Statistical evaluation of the data reveal that cadmium was significantly ($p < 0.05$) accumulated in the bone sections studied in the cadmium treated rats as compared with those given deionized water (Table 3). Higher cadmium was however recorded in the epiphysis relative to the metaphysis. In the rats fed with the Nigerian-like diet and exposed to cadmium there was a significantly ($p < 0.05$) reduced cadmium accumulation in both the epiphysis and metaphysis compared with those fed the

Table 2: Weight gain, feed consumption, water intake and dry fecal output of rats in the experimental groups

Parameter	Experimental groups			
	Control diet	Control diet and cadmium	Nigerian-like diet	Nigerian-like diet and cadmium
Weight gain (g/day/rat)	1.69 \pm 0.21 ^a	0.96 \pm 0.05 ^b	1.01 \pm 0.14 ^b	0.62 \pm 0.06 ^c
Feed consumption (g/day/rat)	13.08 \pm 1.14 ^{ab}	11.04 \pm 1.23 ^{ab}	15.42 \pm 1.02 ^a	13.81 \pm 1.81 ^{ab}
Water intake (mL/day/rat)	43.50 \pm 3.50 ^a	34.61 \pm 2.00 ^b	49.06 \pm 3.41 ^a	36.02 \pm 2.10 ^b
Feed efficiency (g/body wt/g feed) \times 10	1.29 \pm 0.02 ^a	0.87 \pm 0.02 ^b	0.65 \pm 0.02 ^c	0.47 \pm 0.02 ^d
Dry fecal output (g/day/rat)	1.24 \pm 0.03 ^a	1.70 \pm 0.03 ^b	2.06 \pm 0.02 ^c	2.62 \pm 0.02 ^d

Means of the same row followed by different letters differ significantly ($p < 0.05$). Values are means \pm SEM, n = 10

Table 3: Cadmium concentration in the bones of the experimental rats

Parameter	Experimental groups			
	Control diet	Control diet and cadmium	Nigerian-like diet	Nigerian-like diet and cadmium
Cadmium concentration				
Epiphysis (μ g g ⁻¹) \times 10 ⁻²	0.9 \pm 0.2 ^a	287.0 \pm 3.1 ^b	1.1 \pm 0.3 ^a	202.6 \pm 4.2 ^c
Metaphysis (μ g g ⁻¹) \times 10 ⁻²	0.8 \pm 0.3 ^a	264.0 \pm 3.4 ^b	0.9 \pm 0.3 ^a	199.4 \pm 4.7 ^c

Means of the same row followed by different letters differ significantly ($p < 0.05$). Values are means \pm SEM, n = 10

Table 4: Plasma calcium, phosphate and alkaline phosphatase of rats in the experimental groups

Parameter	Experimental groups			
	Control diet	Control diet and cadmium	Nigerian-like diet	Nigerian-like diet and cadmium
Plasma calcium (mg dL ⁻¹)	13.50±1.10 ^a	11.90±2.80 ^a	12.70±1.80 ^a	11.60±2.50 ^a
Plasma phosphate (mg dL ⁻¹)	2.60±0.20 ^a	2.80±0.30 ^a	2.63±0.20 ^a	2.72±0.30 ^a
Plasma alkaline phosphatase	1.60±0.20 ^a	0.80±0.30 ^b	2.30±0.20 ^c	1.80±0.30 ^a

Values are means±SEM and n = 10, Means of the same row followed by the different letters differ significantly (p<0.05), Alkaline phosphatase activity is expressed as μ moles p-nitrophenol/min/mg protein

Table 5: Bone calcium, phosphate and alkaline phosphatase of rats in the experimental groups

Parameter	Experimental groups			
	Control diet	Control diet and cadmium	Nigerian-like diet	Nigerian-like diet and cadmium
Bone calcium (mg g ⁻¹ wet wt.)				
Epiphysis	354.49±11.20 ^a	311.70±13.91 ^b	321.34±13.03 ^b	270.55±14.17 ^c
Metaphysis	390.21±12.05 ^a	346.70±14.60 ^b	356.42±12.63 ^b	311.41±14.67 ^c
Bone phosphate (mg g ⁻¹ wet wt.)				
Epiphysis	238.14±13.20 ^a	193.60±15.40 ^b	191.34±14.14 ^b	159.33±10.17 ^c
Metaphysis	260.46±13.05 ^a	225.20±14.90 ^b	217.42±12.46 ^b	168.75±12.67 ^c
Bone alkaline phosphatase				
Epiphysis	34.90±2.50 ^a	28.80±3.30 ^b	45.20±3.10 ^c	36.70±4.60 ^a
Metaphysis	43.30±2.45 ^a	35.80±3.42 ^b	52.20±3.20 ^c	44.40±4.56 ^a

Values are means±SEM and n = 10, Means of the same row followed by the different letters differ significantly (p<0.05), Alkaline phosphatase activity is expressed as μmoles p-nitrophenol/min/mg protein

control diet and exposed to cadmium. The study thus suggests that the Nigerian-like diet cause less accumulation of cadmium in the bones of rats.

There was no statistically significant change (p>0.05) in the levels of plasma calcium and phosphate in all the experimental groups (Table 4). The plasma alkaline phosphatase activity in rats fed the Nigerian-like diet was significantly (p<0.05) increased compared with the rats fed with the control diet. Cadmium treatment led to a significantly reduced plasma alkaline phosphatase activity. This study shows that while the Nigerian-like diet increases plasma alkaline phosphatase activity in rats, cadmium exposure decreases it.

Cadmium treatment significantly (p<0.05) decreased calcium and phosphate concentrations in both the epiphysis and metaphysis of femur bones of rats (Table 5). Feeding rats with the Nigerian-like diet significantly decreased calcium and phosphate concentrations in both sections of the bones studied to levels that are statistically similar to those observed in rats fed the control diet and cadmium. When the rats fed with the Nigerian-like diet were exposed to cadmium the bone calcium and phosphate levels were further significantly decreased. However feeding rats with the Nigerian-like diet significantly increased bone alkaline phosphatase activity, but cadmium treatment significantly decreased the activity of this enzyme in the bone sections studied. This study reveals that bone calcium, phosphate and alkaline phosphatase levels are responsive to diet type and cadmium treatment.

DISCUSSION

The present study was to investigate the relationship between a wholly compounded Nigerian-like diet (NLD) and some aspects of bone metabolism in cadmium toxicity. The reduction in weight gain of rats exposed to cadmium and/or a low protein, high fibre diet observed in this study corroborates those of earlier studies (Horiguchi *et al.*, 1996; Tewari *et al.*, 1986; Asagba *et al.*, 2002). Weight gain is partly dependent on availability and absorption of nutrients. Recent studies have shown that cadmium interferes with nutrient digestion and absorption (Elsenhans *et al.*, 1999). It is not surprising therefore that a combination of the NLD and cadmium further reduced weight gain.

Earlier reports show that a high carbohydrate and low protein diet can lead to increase in fecal bulk (Burkitt, 1984). The NLD may have contributed to the observed increase in fecal output. The increased fecal bulk observed with cadmium administration may not be unconnected to its effect on nutrient digestion and absorption.

After absorption of cadmium, it is delivered to the liver by endogenous intestinal metallothionein. It is assumed that hepatic cadmium-metallothionein then gradually redistributes the metal to the other organs (Elsenhans *et al.*, 1997). The less marked accumulation of cadmium in the bones of rats fed the NLD and oral cadmium (Table 2) could be due to decrease metallothionein production occasioned by low availability of dietary proteins. It may also relate to available sulphur as metallothionein has been shown to contain about 30% cysteine, a sulphur containing amino acid (Taniguchi and

Cherian, 1990). The NLD contains less sulphur (Table 1), so there would be reduced metallothionein production in the rats fed this diet and thus reduced ability to handle cadmium. Dietary fibre has been shown to have inhibitory effect on gastrointestinal absorption of cadmium (Berglund *et al.*, 1994). Hence the high fiber content of the NLD (Table 2) may also have contributed to the decreased accumulation of cadmium in the bone.

Increased plasma alkaline phosphatase activity has been associated with alteration in liver and bone metabolism. Among the bone diseases, elevated serum alkaline phosphatase activity is encountered in improved action of the osteoblastic cells as they try to rebuild bone that is been resorbed by the uncontrolled activity of osteoclasts (Moss and Henderson, 1999). In this study there was observed increase in plasma alkaline phosphatase activity in rats fed the NLD as compared to control (Table 3), which may be an indication of alteration in bone metabolism. This position is strengthened when the observed results in the bone (Table 4) is taken into account.

Protein intake correlates positively with bone mineral density (Lau *et al.*, 1998). There appears to be increase in bone turnover with low protein intake and this may be associated with improved bone alkaline phosphatase activity which eventually translates to increased plasma alkaline phosphatase level. Thus the low protein content of the NLD may have resulted in the observed increase in plasma alkaline phosphatase activity in the animals maintained with the diet. The observed decrease in the activity of plasma alkaline phosphatase of cadmium treated rats (Table 3) may be due to inhibition of this enzyme. Alkaline phosphatase is a metalloenzyme containing zinc as an integral and essential part of its molecule. Cadmium can inhibit enzymes associated with zinc by competing with this element and displacing it from the enzyme (Suzuki *et al.*, 1989).

The metabolism of calcium and phosphate is intimately linked. There are homeostatic mechanisms which are principally directed towards maintaining plasma calcium and phosphate concentrations (Endres and Rude, 1999). These mechanisms include improved absorption of the minerals from the intestine, resorption from the kidney and demineralization of the bones. The similar plasma calcium and phosphate levels in rats of all the experimental groups (Table 3) may be accounted for by the observed increase in bone mineral loss (Table 4).

This study consistently showed a decrease in bone calcium and phosphate with cadmium exposure (Table 4). This corroborates the results of earlier studies (WHO, 1992; Gur *et al.*, 1995; Carlsson and Lundhohn, 1996). Cadmium has been shown to cause increased osteoclast

activity (Carlson and Lundhohn, 1996) which may lead to unusual bone proliferation and irregular arrangement of cartilage cells and other pathological changes which results in the development of bone lesions (Iguchi and Sano, 1982). These changes may favour bone demineralization which may account for the observed reduction in calcium and phosphate levels in the bone. Also, a point worth noting is that the toxic effects of cadmium on renal function have been suggested as a causal factor of bone pathology in itai-itai disease, which is characterized mainly by renal dysfunction and osteomalacia (Ohta *et al.*, 2000). Cadmium induced kidney damage interferes with the renal hydroxylation of 25-hydroxycholecalciferol to 1, 25-dihydroxycholecalciferol (Kjellström, 1992), the active form of vitamin D which is responsible for intestinal calcium absorption and promotes bone mineralization (Kjellström, 1992).

Bone is formed primarily from collagen upon which hydroxyapatite is layered. A low protein diet as is with the NLD would affect collagen synthesis and therefore bone formation. This may in part account for the observed decrease in bone calcium and phosphate in the rats fed the NLD (Table 4). Besides, one of the mechanisms of intestinal absorption of calcium is by binding to protein carrier molecules (Popovtzer *et al.*, 1992). The intestinal absorption of calcium would therefore be reduced in rats fed a diet low in protein such as the Nigerian-like diet. It is not surprising that these observed changes in bone calcium and phosphate upon administration of NLD and/or cadmium did not result in changes in these ions in the plasma (Table 3) as these bone changes would have helped in maintaining the homeostatic levels of the ions.

The decrease in calcium level in bone can be used as a marker of bone resorption (Endres and Rude, 1999). Thus the observed decrease in bone calcium concentration in cadmium treated rats may be an indication of cadmium induced bone resorption, a process that was aided by the NLD. It should be reiterated that the NLD is not only low in protein but also low in sulphur containing amino acids (Table 1). Glutathione and metallothionein both of which are rich in sulphur amino acids have been shown to play a crucial role in the detoxification of cadmium in the bones (Klaassen and Liu, 1998). It is therefore conceivable that the effect of glutathione and metallothionein would be higher in rats fed the control diet relative to those fed the Nigerian-like diet.

The epiphysis (cartilaginous end of the bone) appears to be more susceptible to cadmium induced calcium and phosphate loss than the metaphysis (the ossified portion of the epiphysis). This may not be

surprising in view of the fact that the epiphysis contains younger bone cells which may be more susceptible than the older ones of the metaphysis.

Alkaline phosphatase is richly produced by osteoblasts in the bone and is involved in the transport of phosphate across cell membranes and calcification of bone matrix (Nakamura *et al.*, 1988). Increased action of the osteoblastic cells occur as they try to rebuild bone that is being resorbed by the uncontrolled activity of osteoclasts (Endres and Rude, 1999) The increased bone alkaline phosphatase activity in the NLD fed rats (Table 4) may indicate increased action of osteoblastic cells as an adaptive feature to bone resorption occasioned by the low protein content of the diet. This observed increase in the bone alkaline phosphatase may have accounted for the increased plasma alkaline phosphatase activity in the rats (Table 3). As earlier noted, cadmium inhibits alkaline phosphatase activity by displacing zinc which is essential for the enzyme activity. This may account for the decrease in bone alkaline phosphatase activity of the cadmium treated rats and could have led to the observed decrease in bone calcium and phosphate levels (Table 4).

In conclusion, this study demonstrates that exposure to Nigerian-like diet rendered rats more susceptible to cadmium induced alteration in bone metabolism.

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