NISEB JOURNAL Vol. 1, No. 2 , April 30, 2001
Printed in Nigeria

1595-6938/2001 $12.00 + 0.00

NISEB 1314/020

Bone metabolism in occupational lead toxicity: Implications for polluted environments in developing countries

J. I. Anetor*, O. O. Babalola**; T O Alonge*** and F. A. A. Adeniyi*

*Department of Chemical Pathology, College of Medicine, University of Ibadan, Ibadan, Nigeria
**Department of Environmental Toxicology, University of Agriculture, Abeokuta, Nigeria
***Orthopaedic Unit, Department of Surgery, College of Medicine, University of Ibadan, Ibadan, Nigeria

(Received July 31, 2000)

ABSTRACT: Bone, the largest repository of lead (Pb) in the body, is not metabolically inert. Bone thus responds to environmental toxins and pollution. Bone metabolism was examined in 86 all male lead workers drawn from various lead-based occupations, and 51 apparently healthy subjects who had never been occupationally exposed to Pb. The mean age of the Pb workers was 36.0 ± 0.03 years (± SEM) while that of the control population was 36.6 ± 1.2 years (± SEM).

Blood lead concetration was significantly highe in the exposed than in unexposed subjects ($p < 0.001$). The biomarker of environmental exposure, uric acid, was also significantly higher in Pb workers than in controls ($p < 0.01$). Additionally, uric acid was positively correlated with blood lead (PbB) level ($r=0.24$; $p< 0.026$), serum zinc level was also significantly higher in the exposed than unexposed subjects ($p < 0.001$).

In contrast, total and ionized levels of the major bone mineral, calcium, were lower in exposed than unexposed subjects ($p < 0.01$ and $p < 0.001$) respectively. Calcium also appeared in the multiple regression analysis model, indicating important association between lead and calcium. In addition, 19% of exposed subjects demonstrated calcium level below 2 mmol/l (8 mg/dl) while only 4% of unexposed subjects demonstrated similar calcium levels. Magnesium, inorganic phosphate, total protein and sub-fractions total and bone specific alkaline phosphatase (BAP) were statistically similar in exposed and unexposed subjects ($p > 0.05$) in all cases.

Occupational lead toxicity and exposure to increasingly polluted environments impair bone mineralization which may lead to increased incidence of bone disease (such as osteomyelitis and osteoporosis) in human and animal populations. This is already a big health problem in the developed countries. This is frequently ignored in current environmental health concern in developing countries.

Key Words: Lead toxicity; Bone metabolism; Bone mineralization; Blood lead; Rickets; Environmental pollution.

Introduction

Lead is a prime environmental pollutant and occupational toxin (Goyer, 1971; Rainey, 1993). Over 95% of the adult body burden of lead is in bone (Anon, 1991; O'Flaherty, 1992). About three decades ago, it was considered that the average adult harbours about 150 to 400 mg of body lead and has blood levels

which average 25 µg/dl (Goyer, 1971). With increasing environmental pollution arising from increasing industrialization which entails more consumption of lead and lead products including use of gasoline with high lead content (Sofoluwe, 1977; Ayoola, 1979; Okoye, 1994) this level is likely to rise. Many variables in the absorption, storage and excretion of lead modify the blood levels and therefore the toxicity and severity of lead poisoning.

Contrary to common beliefs, bone is an active tissue which undergoes constant remodelling through the opposing forces of bone formation and resorption and these are controlled by a complex array of growth factors and cytokines (Scott, 1995).

When considering lead toxicity and its associated environmental impact, it is often forgotten that the highest reserve of lead, i.e. the bone, is not inert and that there is a dynamic interplay between the reserve and the commonly measured blood lead level. Therefore, our understanding of bone metabolism needs to be improved in considering lead poisoning. This study was, therefore, designed to fill this gap inknowledge which currently exists in Nigeria and most developing countries where the problem of heavy metal pollution is paralleling industrial development (Last, 1987; Anon, 1996; Attah, 2000).

Materials and Methods

Subjects

The subject population comprised eighty-six (86) workers. They were all drawn from various lead-based occupations such as battery charging, house painting, welding, auto-mechanics; plumbing and panel-beating work.

The mean age of the lead workers was 36.0 (± 0.03 SEM; range 21 – 66) years and they were all adjudged to be clinically healthy based on a medical and social questionnaire which was administered. Most of these lead workers had low level of education and had little knowledge of the risk of continued exposure to lead.

The control population comprised fifty-one (51) workers who had never been occupationally exposed to lead. Their mean age was 36.6 (± 1.2 SEM; range 22 – 58) years. Informed consent was obtained from all the subjects selected and the ethical committee of the University of Ibadan College of Medicine approved the conduct of the study.

Collection of Blood Samples

About 15 ml of blood was obtained from each subject using disposable, pyrogen-free, plastic syringe (Becton-Dickinson, Dublin). The blood was dispensed into lead free heparin tubes (Becton-Dickinson, Canada) and anticoagulant free tubes for lead and other biochemical measurements respectively. Serum samples that were not immediately required were stored at 20^{o}C.

Determination of Biochemical Parameters

Whole blood lead concentration was determined by atomic absorption spectrophotometry using the modified method described by Hessel (1968). The AGW AES model 200A flame atomic absorption spectrophotometer (Analysengerate GmBH, Germany) was employed. Total blood serum calcium was determined by standard methods employing O-cresol phthalein complexone (Baginski *et al.*, 1973). Serum ionized calcium was computed by the modified method of Beeler and Catrou (1983).

Inorganic phosphate was measured by the method of Fiske and Subbarow (1925). Uric acid was determined by the method of Fossati *et al.* (1980). Serum zinc level was determined by the method of Smith *et al.* (1989). Total protein and albumin were determined by standard methods using Biuret and bromocresol purple respectively.

Total and bone isoenzymes of alkaline phosphatase were determined by the native and the heat inactivation methods of Fitzgerald *et al.* (1969). Magnesium was determined using a commercial magnesium kit (Sigma Diagnostics, St. Louis, MO, USA).

Statistical Analysis

Statistical analysis included Student's two-tailed t-test for unpaired data, single and multiple regression analysis. The dispersion of data is given by standard error of the mean (SEM).

Results

The results obtained from exposed and unexposed subjects are shown in Tables 1 and 2. The blood lead concentration in occupationally exposed subjects was significantly higher than in unexposed subjects ($P < 0.001$, Table 2). Serum uric acid level was also significantly higher in exposed subjects than in unexposed individuals ($P < 0.001$). In addition, there was also significant positive correlation between serum uric acid level and blood lead level ($r = 0.24$, $P < 0.026$).

In contrast, total serum and ionised calcium levels were significantly lower in occupationally exposed than in unexposed subjects ($P < 0.01$ and $P < 0.001$, respectively) (Table 1). Additionally, 19% of exposed subjects had calcium levels below 2 mmol/l (8 mg/dl) while only 4% of unexposed subjects exhibited similar levels.

The srul levels of inorganic phosphate, magnesium , total protein and albumin were all statistically similar in exposed and unexposed subjects. Similarly, the biochemical markers of osteoblastic disorder, total and bone isoenzyme of alkaline phosphatase in exposed and unexposed populations also remained statistically similar ($P > 0.05$) respectively.

Table 1: Indices of bone metabolism in exposed and unexposed subjects.

Biochemical Parameters	Exposed	Unexposed	t	P
Total calcium (mmol/l)	2.2 ± 0.02	2.31 ± 0.02	2.6	$P < 0.01$
Ionized calcium (mmol/l)	0.87 ± 0.001	0.99 ± 0.001	6.67	$P < 0.001$
Inorganic phosphate (mmol/l)	1.18 ± 0.003	1.12 ± 0.029	1.5	$P > 0.05$
Total alkaline phosphatase (ALP) (I.U./l)	32 ± 1.48	33 ± 1.71	0.5	$P > 0.05$
Bone isoenzyme of ALP (I.U./l)	27 ± 1.17	24 ± 1.67	0.5	$P > 0.05$
Total protein (g/l)	82 ± 5.6	75 ± 1.60	1.05	$P > 0.05$
Albumin (g/l)	44 ± 0.4	44 ± 0.5	0.5	$P > 0.05$
Percent of subjects with total calcium below 2 mmol/l	19	4	–	–

Values represent the mean ± SEM

Discussion

Lead in bone is of interest for two principal reasons. Bone is the largest repository of the body lead burden. Secondly, it is feared that lead may have effect on bone metabolism.

The significantly elevated lead level in occupationally exposed subjects is consistent with previous reports in similar populations (Gibbon *et al.*; 1968; Mason *et al.*, 1990; Gennet *et al.*; 1992; Kim *et al.*, 1995). the level of blood lead in occupationally exposed subjects falls within the range currently clasified as severe lead poisoning (WHO, 1980; US Department of Labour, 1990; Adeniyi and Anetor, 1999). The blood Pb level for unexposed subjects also gives cause for concern. It also reflects and confirms significant

environmental pollution earlier reported by Okoye (1994). This has also been extensively commented on in a previous report (Adeniyi and Anetor, 1999).

Table 2: Blood lead, serum magnesium, uric acid and zinc levels in exposed and unexposed subjects.

Biochemical Parameters	Exposed	Unexposed	t	P
Blood lead (µmol/l)	2.72 ± 0.05	1.47 ± 0.07	18.9	P < 0.001
Zinc (µmol/l)	112 ± 6.11	85 ± 2.65	4.06	P < 0.001
Magnesium (mmol/l)	0.90 ± 0.05	0.85 ± 0.06	0.66	P >0.05
Uric acid (mmol/l)	311 ± 16.6	204 ± 11.3	5.28	P < 0.001

Values represent the mean ± SEM

The significantly elevated level of blood lead implies heavy lead burden in the bone, since over 95% of the adult body burden of lead is in the bone (O'Flaherty, 1992; Goyer, 1993). Information on the tissue turnover and redistribution of absorbed lead (biokinetics) for adults is non-existent in this environment. The data of Gulson *et al*. (1995) have shed new light on the use of blood lead level for this purpose. Recognition that the predominant source of lead in blood was tissue stored rather than the contemporaneous environment should greatly modify recommendations on the application of blood lead to monitor occupational and environmental interactions. Another practical implication of these data is that current blood lead level reflects both current and past (long term) exposure.

Alkaline phosphatase activity, specifically the bone isoenzyme that was employed in part to assess bone metabolism, was similar in exposed and unexposed subjects. The bone isoenzyme of ALP found in plasma is a measure of osteoblastic activity. Thus, it may be suggested that in occupational exposure to lead there is no significant osteoblastic activity. This is partly consistent with the observation of Klaasen and Rozeman (1991) that deposition and storage of toxicants in bone may not be detrimental.

Thecause of the significantly decreased total and ionized calcium levels may be attributed to impaired vitamin D metabolism. Vitamin D is the major stimulus needed for calcium absorption. The physiologically active form of the vitamin, 1,25-dihydroxycholecalciferol (1,25-DHCC) which results from final hydroxylation of 25 hydroxycholecalciferol in the proximal tubules of the kidney (after the initial hydroxylation at the 25 position in the liver) is required for adequate calcium absorption. This activation process is inhibited by lead, since the kidney is one of the critical organs affected by lead and the portion of the proximal convoluted tubule (PCT) involved in vitamin D activation is particularly susceptible to the toxic effect of lead (Goyer and Rhyme, 1973a,b; Quaterman, 1986).

Thus, the most likely explanation for the decrease in calcium level is impairment of vitamin D metabolism by lead. The decreased calcium levels suggest generalised defects in bone mineralization. This implies biochemical or metabolic bone disese which encompasses osteoporosis and osteomalacia (adult rickets).

The effect of lead on renal tubular function is well recognise. Damaged tubules apparently cause retention of uric acid, which is now considered to be responsible for elevated uric acid level (Ball and Sorensen, 1969). The significantly raised uric acid level which was also positively correlated to blood lead level appears to corroborate proximal tubular damage which impaired the metabolism of 1,25 DHCC resulting in lower calcium level. This is why uric acid is a useful biological marker in environmental health research (Goldstein *et al.*, 1987). Gitteman *et al.* (1994) have also reported that uric acid may be a consistent and reliable biomarker of significant exposure to elad.

The similarity in the total protein and albumin levels in this study excludes alteration in protein metabolism as possible contributors to the decreased calcium level. The depressed ionized calcium level may suggest that lead probably has an inhibitory effect on the parathyroid gland. Ionized calcium is metabolically the fraction which exerts a feedback mechanism on the parathyroid gland. Lead probably alters this mechanism thus abolishing the expected homeostatic response of this endocrine gland to restore

ionized calcium to its normal status. This may be peculiar to bone metabolism in excessive occupational or environmental exposure.

The level of magnesium and inorganic phosphate did not differ between exposed and unexposed subjects. It is not clear why these minerals which are also intimately involved in bone metabolism are unchanged. Both minerals are predominantly intracellular. Fifty percent of magnesium is found in bone and marked alteration in body's magnesium metabolism can therefore occur with little or no detectable change in serum magnesium concentration (Smith *et al*., 1998). Thus, the non-significant difference in this mineral constituent of bone may not exclude disordered magnesium metabolism.

Phosphate absorption is also regulated by 1,25-DHCC, thus decreased levels would have been expected, but exposure to Pb causes damage to proximal tubular cells of the kidney (Goyer and Rhyne, 1973a,b; Goyer *et al*., 1989; Goyer, 1993; Landrigon, 1991), leading to retention of phosphate thus obliterating the expected decrease in this bone mineral. Classically, in kidney pathology, decreased serum calcium level is accompanied by elevation in serum phosphate (Smith *et al*., 1998). Thus, the findings in the present study appear to be in agreement with this classical pattern.

Bone consists of osteoid, a collagenous organic matrix on which is deposited complex inorganic hydrated calcium salts called hydroxyapatite. Bone formation (osteogenesis) requires osteoid synthesis and adequate calcium and phosphate for the laying of hydroxyapatite. Bone provides an important reservoir of calcium and phosphate and, to a lesser degree, magnesium. Alteration in these minerals imply disordered metabolism leading to decreased mineralization and reduction in bone density, a principal feature of osteoporosis (a disease due to brittle bones).

Alkaline phosphatase, the zinc-dependent enzyme secreted by bone-laying cells (osteoblasts) is essential to the process of osteogenesis due to the releasing of inorganic phosphate from pyrophosphate. The significantly elevated zinc level in the exposed population excludes zinc deficiency as a factor in the inadequate bioavailability of phosphate for bone minralization. Decrease in zinc level accounted for the reduced activity of alkaline phosphatase in the study of Guldager *et al*. (1996).

In occupational lead toxicity, therefore, owing to the impairment of calcium and phosphate absorption, bone mineralization is impaired which may lead to defective bone formation that may in turn lead to osteomalacia (soft and deformed bone) and decreased bone density that may result in brittle bones (osteoporosis). This may subsequently give rise to increase in the incidence of fractures.

This pattern is also operative in polluted environments as we have it today in Nigeria and most developing countries (Last, 1987; Okoye, 1994; Adeniyi and Anetor, 1999). Indeed, the reports of Staessen *et al*. (1991, 1992) are instructive. A study in a general population showed that chronic exposure to a polluted environment caused by another environmental pollutant, cadmium, impairs calcium metabolism. Impairment of calcium metabolism ultimately leads to abnormal bone metabolism that may lead to bone disease in human and animal populations with serious economic implications for the latter.

The second study by Staessen *et al*. (1992) revealed impairment of kidney function with increasing blood lead concentration in the general population, a situation comparable to that in Nigeria (Okoye, 1994; Adeniyi and Anetor, 1999). This reflects serious environmental pollution with lead. This can lead to kidney disease, induced bone disorder (renal osteodystrophy), resulting in a painful softening of the bone (osteomalacia). We are now in a period of epidemiological transition in developing countries associated with increased environmental pollution and attendant chronic and non-communicable/metabolic diseases of which osteoporosis is a prime candidate.

The increasing importance of bone metabolism in lead toxicity is indicated by the development of a non-invasive technique, X-ray fluorescence of living bone (Wieloposkoy *et al*., 1983; Sommervaile *et al*., 1985). Additionally, the stability of lead in bone has led to numerous studies of collection of fossil bones from cemeteries and human collections in efforts to re-evaluate the role of environmental lead in historical cultures (Zoomies, 1997). This implies that bone metabolism in occupational and environmental lead exposure will assume a greater significance in our continuing environmental contamination of the global environment.

It is, however, often forgotten that observations in human populations also affect animals particularly the livestock industry. Thus, bone metabolism is also of importance to the agricultural sector. The study of chronic debilitating disease in horses by Schimitt *et al*. (1971) in which increased environmental lead exposure was incriminated serves to illustrate how cooperation among various sectors of the community can investigate and solve a complex environmental pollution problem. The horses presented with the following signs and symptoms, loss of weight, generalised skeletal weakness, stiffness of the joints and harsh dry coats. Later, they became archbacked, listless and emaciated (Cachetic).

The maintenance of proper skeletal structure is of importance for animal and human health and well being which, as is evident from the study of Schimitt *et al*. (1971), can be distorted in occupational lead toxicity and environmental pollution.

The economic and social implications of inadequate skeletal development and attendant disease have recently been pointed out by Scott (1995). In the elderly, environmental pollution may exacerbate the abnormal bone metabolism normally associated with ageing. The population of ageing subjects is also increasing in Africa and many other developing countries with improvement in economic indices (Unwin and Albert, 2000). The skeleton also provides important calcium reserve during lactation (breast feeding) if this reserve is impaired owing to environmental pollution, rickets (soft and deformed bones) may result. Rickets, a predominantly nutritional disease, has recently been reported to be common in developing countries (Thatehen *et al*.; 1999; Bishop, 1999). The contribution of environmental pollution to this nutritional and metabolic disease may yet be unknown to the scientific and medical communities. Im addition, lead may be mobilised during normal bone homeostasis which may put the developing brain of children in developing countries at risk of lead-induced brain disorders (neurotoxic effects) such as behavioural abnormalities and reduction in intelligence quotient (I.Q.) levels elegantly enunciated by Needleman (1990) and Rodier (1995).

A clear consequence of occupational lead toxicity or a polluted environment is impaired bone metabolism, specifically defective mineralization arising from decreased calcium and phosphate absorption owing to lead-induced impairment of 1,25-DHC. This may lead to bone disease in human and animal populations, with serious implications for the livestock industry.

The health of children may also be affected. Impaired bone metabolism in occupational lead toxicity and increased environmental pollution probably contributes to the incidence of osteoporosis already a major concern in the more industrialised economies which may also spread to developing countries with progressive industrrialization.

References

Adeniyi, F. A. A. and Anetor, J. I. (1999) Lead-poisoning in two distant states of Nigeria: An indication of the real size of the problem. Afr. J. Med. Sci. 28, 107 – 112.

Anon (1996) lead in bone: A blight on the future. Africa health 18, 7.

Arah, R. O. (1985) Lead-free gasoline in Nigeria by the year 2000. Proc. 4th International Seminar on Petroleum Industry and Nigerian Environment. Federal Ministry of Works and Housing/Nigerian National Petroleum Corporation (NNPC), Kaduna, Nigeria. pp. 339 – 346.

Attah, E. (2000) Cancer in Africa: Environmental factors. Africa Health 22, 10 – 11.

Baginski, E. S.; marie, E. S.; Clark, W. L.; Zak, B. (1973) Calcium in biological fluids. Clin. Chim. Acta 46, 49.

Ball, G. V. and Sorensen, L. B. (1969) Pathogenesis of hyperuricaemia in Saturmine gout. N. Engl. J. Med. 280, 1199 – 1202.

Beeler, F. B. and Catron, P. G. (1983) Interpretations in Clinical Chemistry: A Textbook Approach to Chemical Pathology, 2nd Edition, American Society of Clinical Pathologists Press, p. 37.

Bishop, N. (1999) Rickets Today – Children still need milk and sunshine. N. Engl. J. Med. 341, 602 – 604.

Farkas, W. R.; Stanawitz, T. and Schneider, M. (1978) Saturnine gout: Lead induced formation of guanine crystals. Science 199, 786 – 787.

Fiske, C. H. and Subarrow, Y. (1925) The colorimetric determination of phosphorus. J. Biol. Chem. 16, 375.

Fizgerald, M. X. M.; fennelly, J. J. and McGeeney, K. (1969) the value of differential alkaline phosphatase thermostability in clinical diagnosis. Am. J. Clin. Pathol. 51, 194.

Flavus, M. J. (1996) Primer on the metabolic bone disease and disorders of mineral metabolism, 3rd Edition. Lippincott-Raven, New York.

Fossati, P.; Prescipe, L. and Berti, G. (1980) Use of 3,5-dichloro-2-hydroxybenzene sulfonic acid 14-aminophenazone chromogenic system indirect enzymatic asay of uric acid in serum and urine. Clin. Chem. 26, 227 – 230.

Fullmer, C. S. 91997) Lead cadmium interactions: Involvement of 1,25-dihydroxy vitamin D. Environ. Res. 72, 45 – 55.

Gennert, J. P.; Buchet, J. P.; Roels, H.; ghyselen, P.; ceuleman, E. and Lauweys, R. (1992) Fertility of male workers exposed to cadmium, lead or magnesium. Am. Epidemiol. 135, 1208 – 1219.

Gibson, S. C. M.; Mackenzie, J. C. and Gldberg, A. (1968) The diagnosis of industrial lead poisoning. Brit. J. Ind. Med. 25, 40 – 51.

Gittleman, J. L.; Enelgan, M. M.; Shaw, J.; Willekk, and Seligman, P. J. (1994) Lead poisoning among battery reclamation workers in Alabama. J. Occup. Med. 36, 526 – 532.

Goldstein, B.l Gibson, J.; Henderson, R. *et al*. (1968) Biological markers in environmental health research. environ. Hlth. Perspect. 74, 3 – 9.
Goyer, R. A. (1971) Lead toxicity: A problem in environmental pathology. Amer, J. Path. 64, 167.
Goyer, R. A. (1993) Lead toxicity: Current concerns. Environ. Hlth. Perspect. 100, 177 – 187.
Goyer, R. A. and Rhyne, B. C. (1973a) Pathological effects of lead. Int. Rev. exp. Pathol. 12, 1 – 77.
Goyer, R. A. and Rhyne, B. C. (1973b) Pathological effects of lead. In: International Review of Experimental Pathology. Richier, G. W.; Epstein, M. A. (eds.). Academic Press, New York, Vol. 12, p. 39.
Goyer, R. A.; Weinberg, C. R.; Victery, W. M. and Miller, C. R. (1989) Lead induced nephrotoxicity: Kideny calcium as indicator of tubular injury. In: Lead-Indicator of Toxicity. Back, R. and Lock, J. (Eds.). Plenum Publishing, London, pp. 11 – 20.
Gulson, B. L.; Mahaffey, K. R.; Mizon, K. J.; Norseh, M. J.; Cameron, M. A. and Vimpari, T. (1995) Contribution of tissue lead to blood lead in adult female subjects based on stable lead isotope methods. J. Lab. Clin. Med. 125, 703 – 712.
Hessel, D. N. 91968) A simple and rapid quantitative determination of lead in blood. Atom. Absorpt. Newslett. 17, 50 – 55.
Kim, Y.; Nadara, K.; Ohmori, S.; Lee, B. K.; Mivra, H. and ueda, A. (1995) Evaluation of lead exposure in workers at a lead-acid battery factory in Korea: With focus on activity of erythrocyte pyrimidine 5-nucleotidase. Occup. Environ. Med. 52, 484 – 488.
Klaasen, C. O. and Rozman, K. (1999) Absorption, distribution and excretion of toxicants. In: Cassarett and Dovill's Toxicology: The Basic Science of Poison. Amdur, M. O.; Doull, J and Klaasen, C. O. (Eds.), 4th edition, Pergamon Press, New York. p. 66.
Landrigan, D. J. (1991) Strategies for epidemiological studies of lead in bone in occupationally exposed populations. Environ. Hlth. Perspect. 91, 81 – 86.
Last, J. M. (1987) Environmental Health. In: Public and Human Ecology, Appleton and Lange, Norwalk, Connecticut, pp. 131 – 187.
Long, G. J. and Rosen, J. F. (1994) Lead perturbs 1,25-dihydroxy vitamin D3 modulation of intracellular calcium metabolism in clonal osteoblastic (RoS/7/2.8) cell. Life Sci. 54, 1395 – 1402.
Mahaffey, S. R.; Cappa, S. G.; Gladen, B. C. and Fowler, B. A. (1981) Concurrent exposure to lead, cadmium and arsenic: Effects on toxicity and tissue metal concentrations in the rat. J. Lab. Clin. Med. 98, 463 – 481.
Marshall, W. J. 91988) Calcium, phosphate, magnesium and bone. Clinical chemistry: Illustrated textbook. Gower Medical Publishing, London, pp. 190 – 194.
Moon, J. (1994) The role of vitamin D in toxic metal absorption: A review. J. Ann. Coll. Nutr. 13, 559 – 569.
Needleman, H. L. (1990) The long term effects of exposure to low levels of lead in childhood. N. Engl. J. Med. 232, 83 – 88.
Norman, A. W. 91974) 1,25-Dihydroxy vitamin D3: A kidney produced steroid hormone essential to calcium homeostasis. Am. J. Med. 57, 21 – 27.
O'Flaherty, E. J. (1992) Modelling bone mineral metabolism with special reference to calcium and lead. Neurotoxicology 13, 789 0 798.
Okoye, C. O. B. (1994) Lead and other metals in dried fish from Nigerian markets. Bull. Environ. Contam. Toxicol. 52, 825 – 832.
Osibanjo, O. and Ajayi, S. O. (1989) Trace metal analysis of petroleum products by flame atomic absorption spectrometry. Nig. J. Nat. Sci. 4, 33 – 40.
Rainey, P. M. (1992) Lead – A heavy burden for small children. In: Frontiers in Therapeutic Drug Monitoring and Clinical Toxicology. American Association for Clinical Chemistry, Washington, D.C., pp. 37 – 39.
Rodier, P. M. 91995) Developing as a target of toxicity. Environ Hlth. Perspect. 103, 73 – 76.
Rosen, J. F.; Chesney, R. W.; Hamstra, N. R.; Delula, H. F. and Maahffey, K. R. 91980) Reduction in 1,25-dihydroxy vitamin D in children with increased lead absorption. N. Engl. J. Med. 302, 1128.
Sargent, J. D. (1994) The role of nutrition on the prevention of lead poisoning in children. Pediatr. Ann. 23, 636 – 642.
Schimit, N.; Delvin, E. L.; Larsen, A. A.; McCausland, D. V. M. and Suville, J. M. (1971) Lead poisoning in horses: An environmental health hazard. Arch. Environ. Health. 23, 185 – 195.
Scott, D. (1995) Controlling bone metabolism. The Rowett research Institute Annual Report. Buckburn Aberdeen, p. 96.
Silbergeld, E. K.; Sauk, J.; Somerman, M.; Todd, A. and Van Buren, J. (1993) Lead in bone: Storage site, exposure source and target. Neurotoxicology 14, 225 – 236.
Silbergeld, E. K. 91991) Lead in bone: Implications for toxicology during pregnancy and lactation. Environ. Hlth. Perspect. 91, 63 – 70.
Smith, A. F.; Beckett, G. J.; Walker, S. W. and Rae, P. W. H. Lecture Notes on Clinical Biochemistry, 6th Edition, Blackwell Science, Oxford.
Sofoluwe, G. O. (1977) Industrialization and health. Nig. Med. J. June, 103 – 104.
Sommervaile, C. J.; Chettle, D. R. and Scotte, M. C. (1985) *In vivo* measurement of lead in bone using X-fluorescence. Phys. Med. Biol. 30, 929 – 943.

Staesen, J.; Amery, A.; Bernard, A.; Braux, P. *et al*. (1991) Effect of exposure to cadmium on calcium metabolism: A population study. Brit. J. Ind. Med. 48, 710 – 714.

Staesen, J. A.; Lauweys, R. R.; Buchet, J. P.; Bulpit, C. J.; Tondia, D. *et al*. (1992) Impairment of renal function with increasing blood lead concentrations in the general (U.S.) population. N. Engl. J. Med. 327, 151 – 156.

Thatcher, T. D. *et al*. (1999) A comparison of calcium, vitamin D or both for nutritional rickets in Nigerian children. N. Engl. J. Med. 341, 563 – 568.

United States Department of Labor (1990) Ocupational Safety and Health Administration (OSHA) 29 Code of Federal regulations 1910.1025 Lead. Washington, D.C., United States Printing Press.

Unwin, A. (2000) Primary Healthcare Intervention for Non-communicable Diseases. Africa Health 22, 12 – 14.

Wasserman, R. H. and Fullmer, C. S. (1995) Vitamin D and Intestinal Calcium Transport: Facts, Speculations and Hypothesis. J. Nutr. 125, 1971S – 1979S.

World Health Organization (1980) Recommended Health Base Limits in Occupational Exposure to Heavy Metals. Tech. Rep. Series 647, p. 116. WHO, Geneva.Wielopolsky, L.; Rosen, J. F.; Slatkin, P. N.; Vartsky, D.; Ellis, N. J. and Cohn, S. N. (1983) Feasibility of non-invasive analysis of lead in the human tibia by soft X-ray fluorescence. Med. Phys. 10, 248 – 251.

Zonies, D. (1997) Lead disease and social deviance in a 19th century prison: X-ray fluorescence analysis of skeleton lead burden as an indicator of health status. M.S. Thesis, Department of Anthropology, University of Pennsylvania.