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COMPARISON OF LEAD LEVELS WITH CALCIUM, ZINC AND PHOSPHORUS LEVELS IN HUMAN BLOOD

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Abstract: The purpose of the study was to determine how the levels of calcium, phosphorus and zinc affected the levels of lead in the human blood. The levels of lead, calcium, zinc and phosphorus in human blood of subjects from Nairobi city centre, Nyamira town, Nairobi suburban and Nyamira rural, Kenya are presented in this article. The subjects in Nairobi City Centre had the highest mean blood lead (BPb) level of 29.9 ± 16.91 µg/l, while Nyamira Rural subjects had the lowest mean of 24.20 ± 7.07 µg/l. The mean lead level of the subjects was statistically significant between Nairobi City Centre and Nyamira Rural ($p < 0.01$). The mean calcium level was highest in Nairobi Suburban with a mean of 88.3 ± 26.4 mg/l and lowest in Nyamira Town subjects with a mean of 68.4 ± 26.5 mg/l. The mean zinc level was highest in Nyamira Town subjects with a mean of 1126.2 ± 543.4 µg/l and lowest in Nairobi Suburban subjects with a mean of 806.4 ± 189.9 µg/l. The mean phosphorus level was highest in Nyamira Town subjects with a mean of 36.0 ± 17.4 mg/l while Nyamira Rural subjects had the lowest mean of 26.6 ± 9.7 mg/dl. The mean levels of calcium, zinc and phosphorus for Nairobi City Centre significantly different from those of Nyamira Town, Nairobi Suburban and Nyamira Rural ($p<0.01$, df = 99). There was a negative correlation of the mean levels of lead and calcium, lead and zinc and lead and phosphorus for all the study areas.

Keywords: Lead poisonig, human blood, mitigation

INTRODUCTION

Nutritional factors such as irregular patterns of feeding, high fat intake, marginal calcium ingestion and iron deficiency have been associated with susceptibility to lead (Pb) toxicity (Mahaffey, 1995). Similarly diets low in calcium, zinc, and phosphorus are associated with increased lead absorption and toxicity(Mahaffey,1995; Hernandez-Avila et al. 1996). Foods that are rich in calcium and phosphorus include milk, beans, butter, cheese, beef and eggs while those rich in zinc include millet, sorghum and groundnuts.The presence or deficiency of certain elements in the body is related to absorption and reduction of lead. Low dietary calcium and phosphorous increases the absorption of lead from the gut (Mahaffey, 1995; Wright et al. 2004). Calcium intake has been found to be inversely related to both absorption and retention of lead in infants (Hernandez-Avila et al. 1996). The deficiency of iron was observed to act synergistically with lead in the impairment of haem synthesis (Schell et al. 2004). Lead interferes with copper metabolism which secondarily leads to dysfunction of iron metabolism and haem synthesis (CDC, 2005). Zinc has been recognized as a potent antagonist to lead intoxication (Schell *et al.*,2004) and its administration has been shown to decrease the accumulation of lead in body organs. Therefore, diets rich in zinc reduces severity of lead poisoning by restoring lead - induced biological alteration in urinary and blood parameters in animals. Diets that are deficient in calcium, phosphorus and zinc may therefore result in increased lead absorption (Lagerkvist et al. 1996; Wright et al. 2004).

A recent study by Schell et al. (2004) found significant inverse relationship of the blood lead levels of infants of 6 ±association found between blood lead levels and total dietary intake of vitamin C and iron but not with calcium, phosphorus, zinc or vitamin D. Other studies in humans have indicated an inverse association between blood lead levels and calcium intake (Blake and Mann, 1983; Mahaffey et al. 1995; Sargent et al. 1999). Lead may interfere with calcium mediated cellular processes (Dave et al. 1993). The presence of other micro nutrients besides calcium appears to be a vital factor in the lead absorption from the gastrointestinal tract. For example lead absorption decreases as calcium or phosphorus concentration increases (Blake and Mann, 1983). The reduction of lead absorption and retention were noted with calcium alone and phosphorus alone but calcium was more effective than phosphorus (Blake and Mann, 1983). Dietary calcium and phosphorus were important predictors of blood lead concentrations for children from low income populations (Mahaffey, 1995). Reduction in lead absorptions were also noted in subjects ingesting lead in different foods depending in the calcium, magnesium and phosphorus content of the ingested meal (James et al. 1985). Likewise there were inverse correlation between lead and calcium, vitamin D and milk based food intake (Barany et al. 2005). In many cities people are able to feed on diets rich in calcium, zinc and phosphates which affect absorption and retention of lead while in the rural areas people feed on diets poor in these elements (Mahaffey, 1995).

2 MATERIALS AND METHODS

2.1 Sampling and Sampling Procedures

Four hundred subjects both male and female aged between 18 and 70 years were randomly recruited from four study sites were Nairobi City and Nyamira District. In Nairobi City the Central Business Center assumed to be highly polluted from high vehicular densities, close proximity to industries and other activities and a suburban region with medium level of pollution and residents were expected to be feeding on diets rich in calcium, zinc and phosphorus. In Nyamira District participants from the Nyamira Town as an upcoming town and therefore expected to have medium levels of pollution similar to Nairobi suburban, and a rural region with very few vehicles and no industries nearby were sampled.The subjects from Nyamira District were expected to feed on diets rich in calcium , zinc and phophorus as the foods are cheaply a vailable. The subjects filled a questionnaire and provided blood samples. A questionnaire was used to collect information on frequency of feeding on foods known to be rich in Calcium, Phosphorus and Zinc (Mahaffey, 1995).

The research protocol was approved by the Kenyatta National Hospital Ethics and Research Committee and the relevant Medical Officers in study areas. All participants were explained objectives of the study, counseled on the lead exposure reduction procedures, and their willingness to participate sort. Blood samples (5 ml) was collected from each subject into lead free vacutainer tubes containing 5 drops of EDTA anticoagulant by a qualified laboratory technician. The blood was stored in a cool box and transported to either Nyamira District Hospital or Kenyatta National Hospital for the preservation. Analysis for blood lead was carried out at Kenyatta University, Department of Chemistry laboratory using an AAS andDPASV procedures for Calcium, Zinc and Lead, and UV-Visible spectroscpy for Phosphorus.

2.2. Laboratory Procedures

To 5 ml of whole blood sample 10 ml of concentrated nitric acid added in a beaker and digested slowly below boiling point for 3 hours on a hot plate in a fume chamber. When the volumes had been reduced to about a third, 5 ml of 30 % hydrogen peroxide solution was added, evaporated at the same temperature and then the residues were dissolved in 10 ml of 1 % nitric acid and filtered. The digested blood samples were placed in 10 ml plastic vacutainer tubes which were free from lead and taken to Kenyatta University for analysis. The treatment [by wet digestion] of blood was done at Nyamira District Hospital for samples collected in Nyamira while those collected in Nairobi were treated at Kenyatta National Hospital. Strict precautions were taken when handling the blood samples to minimize HIV infection including disinfecting working area with a 5 % phenol.

The lead,calcium and zinc levels in digested blood samples were determined in triplicate by AAS (Buck Scientific Model 210 VGP) and DPASV (Buck Scientific Model 780 ZPV) procedures, which were validated using calibration, co-efficiency of variation and recovery methods. The levels of phosphorus in each blood sample was determined by UV-Visible Spectroscopy.Freshly prepared standard solutions, together with a blank solution were used to construct the calibration curve and its regression equation used to determine concentration in samples. The relationships between the blood lead levels and the levels of calcium, zinc and phosphorus was determined by correlation coefficient and linear regression. Further linear regression equations were used where applicable to enable prediction or estimation of the blood lead levels. The t-test and r^2 were carried to determine the effect of calcium, phosphorus and zinc on the levels of lead in the human body.

2.3Data analysis

The information from the questionnaire and the blood lead, calcium, zinc and phosphorous measurements were checked, sorted and entered into a computer. Analysis of variance (one way ANOVA) was used to compare the mean metal concentrations among the different study sites. Pearson’s correlation was used to evaluate association between metals in the blood of the study subjects. Statistical significance was described at $P < 0.01$ probability. Data analyses were carried out using a statistical package (SPSS-11.5). Further linear regression equations were used where applicable to enable prediction or estimation of the blood lead, zinc, calcium and phosphorus levels. The relationships between the levels of lead and calcium, zinc and phosphorous in the blood were analyzed by t-test and Pearson’s correlation coefficients.

3.RESULTS

3.1 Method Validation

The regression equation from the calibration curves for lead standards for AAS and DPASV were $Y = 0.123x-0.007$ and $Y = 0.23x+0.047$ respectively and similar results were obtained for zinc and calcium, where Y= absorbance of lead and x = concentration. The intercepts were low, close to zero, suggesting that there was minimum matrix interference. The Spearson's correlation factor, r^2 was 0.998, indicating that 99.8% of absorbance correspond to the concentration and therefore indicate linearity. Six standard solutions of lead and zinc containing 20 µg/dl and six standard solutions of calcium and phosphorus containing 30mg/dl were added to different portions of a blood sample and then analyzed. The co efficiency of variation were 0.403 %, 085 %, 1.413 % and 0.418 % respectively indicating high reproducibility for the elements. The detection limit of AAS was 0.02 µg/l while that of DPASV was 0.01 µg/l while the quantification limits were 80 µg/dl and 93 µg/dl respectivelyforlead and zinc while the detection limit of AAS for calcium was 0.001mg/dl while that for DPASV was 0.0015mg/dl while the quantification limits for the were 76mg/dl and 84mg/dl respectively.

The results of the levels of blood lead, calcium and zinc obtained by the two techniques (Table 1) were compared using the student t-test and the results indicate that there was no significant difference ($p < 0.05$) between the two methods in each study area. A regression curve for DPASV against AAS gave r^2 value of 0.98194, indicating strong correlation between the two methods (Table 1). The regression equation $y = 0.977669x - 0.064545$, (where y = DPASV and x = AAS) gave the slope of 0.977669 which was close to unit and the intercept close to the origin, implying that the two methods gave similar results. The results obtained by AAS technique was however used in this study.

3.2 Diet preferences

The most frequently consumed food substances by the subjects in Nairobi City Centre were milk, groundnuts and eggs with daily percentage consumption of 52 %, 32 % and 28 %, respectively. The most frequently non consumed food substances among Nairobi City Centre study subjects were beef, millet and sorghum with percentages of 30 %, 26 % and 24 %, respectively of the people studied not consuming them. The rest were either weekly or rarely consumers of the food substances.

The food substances which were more frequently consumed by Nyamira Town subjects were milk, eggs and groundnuts with frequency consumption of 72 %, 36 % and 22 %, respectively. The food substances which were mostly non consumed by the study subjects were sorghum, beef and millet with percentages of 20 %, 16 % and 14 %, respectively of the people studied not consuming them.

The food substances which were more frequently consumed by Nairobi Suburban subjects were milk, eggs and groundnuts with daily percentage frequency consumption of 68 %, 22 % and 20 %, respectively. The most non consumed food substances among the study subjects were beef, sorghum and millet with percentages of 14 %, 10 % and 4 %, respectively.

The food substances which were frequently consumed by Nyamira Rural subjects were beans, milk, eggs and millet with daily consumption frequency of 66 %, 50 %, 14 % and 14 %, respectively. The most non consumed food substances among the study subjects were beef, groundnuts and sorghum with percentages of 40 %, 30 % and 28 %, respectively. Most food substances were either consumed weekly or rarely by most of the study subjects recruited. Certain diets of individuals affect the BPb levels depending on the frequency of consumption of these foods. Foods that contain high levels of calcium, zinc and phosphorus normally lower the BPb levels. The relationship between the frequency of consumption of different foods and the BPb levels of the subjects in the four study areas was investigated. Tables 4 - 10 represents the mean values of BPb in different frequency of food consumption.

The individuals who consumed milk daily had the lowest mean BPb level in Nairobi City Centre, Nyamira Town, Nairobi Suburban and Nyamira Rural with means of 24.63 4.65, 22.4 10.7, 23.1 7.83 and 20.6 8.48 µg/l while the rarely consumers in Nairobi City Centre, Nyamira Town and Nairobi Suburban had the highest mean BPb of 30.4 23.07, 38.5 30.5 and 40.3 11.21 µg/l, respectively. These results show an inverse correlation between milk consumption and levels of BPb. The difference between the mean BPb level of rarely and daily milk consumers was statistically significant in Nairobi City Centre, Nyamira Town and Nairobi Suburban ($p < 0.01$).

Studies by Moon *et al*. (2003) and Guxinping (1998) observed inverse correlation between milk consumption and BPb levels among pregnant women and children in Mexico City and Korea, respectively after consuming supplements of milk and calcium. However, Graziano (1990) and Weaver *et al*. (2003) reported a positive association between milk consumption and BPb levels among women with high exposure to lead. Milk is one of the foods rich in calcium and phosphorus. Diets poor in calcium, zinc, phosphorus and iron have been identified to be responsible for the increase of lead absorption and toxicity (Mahaffey, 1995). This meant that consumption of milk which is rich in calcium and phosphorus mitigated the levels of lead in subjects.

The frequency of bean consumption affected the mean BPb levels of the subjects. The subjects who consumed beans daily had the lowest mean BPb levels in Nairobi City Centre, Nyamira Town, NairobiSuburban and Nyamira Rural of 19.1 8.67, 13.4 5.6, 18.9 7.34 and 12.6 6.9 µg/dl while those who consumed them rarely had the highest mean BPb level in Nairobi City Centre, Nyamira Town and Nairobi Suburban of 31.5 26.05, 35.7 34.81 and 31.3 18.46 µg/l, respectively. The difference in mean BPb level between the daily and rarely consumers of beans was statistically significant in Nairobi City Centre, Nyamira Town and Nairobi Suburban ($p < 0.01$).

There was an inverse association between the frequency of bean consumption and mean BPb levels (Table 5). This observation was similar to those of other authors who showed that diets rich in calcium, phosphorus and zinc reduced the absorption and retention of lead in the body (Mahafey, 1995; Roh *et al*., 2000; Moon *et al*., 2003; Jamnicka *et al*., 2007). Beans are rich in calcium and phosphorus and the high frequency of consumption of beans is likely to result in high levels of the elements in the body which reduce absorption and toxicity of lead.

The daily consumer of beef had the lowest mean BPb level in Nairobi City Centre, Nyamira Town and Nairobi Suburban with levels of 23.5 5.31, 15.5 4.4 and 17.4 6.61 μg/dl, respectively. The none consumers had the highest mean BPb levels of 37.2 16.34, 39.3 34.77, 34.3 14.1 and 24.3 5.4 μg/l in Nairobi City Centre, Nyamira Town, Nairobi Suburban and Nyamira Rural subjects, respectively (Table 4.28). The differences between the mean BPb levels of the daily consumers and the non-consumers of beef in Nairobi City Centre, Nyamira Town and Nairobi Suburban were statistically significant ($p < 0.01$).

There was an inversely correlation between the frequency of beef consumption and BPb levels. Diets rich in calcium and phosphorus mitigate the BPb levels in the body (Mahaffey, 1995; Owago, 1999; Moon *et al*., 2003; Coyle *et al*., 2005). Beef is rich in calcium and phosphorus which made the frequency of their consumption to relate inversely with the BPb levels of the subjects.

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The subjects who consumed eggs daily in Nairobi City Centre, Nyamira Town, Nairobi Suburban and Nyamira Rural had the lowest BPb levels of 20.8 7.27, 16.4 8.79, 20.7 8.93 and 16.2 8.70 μg/dl while the rarely consumers had the highest mean BPb levels of 38.81 13.45, 33.25 31.6, 28.82 21.2 and 25.2 9.33 μg/l, respectively. The difference between the mean BPb levels between the daily and rarely consumers of eggs were statistically significant in Nairobi City Centre, Nyamira Town and Nairobi Suburban ($p < 0.01$).

The frequency of eggs consumption associated inversely with the BPb levels as they are rich in calcium and phosphorus and so their consumption supplies the elements to the body (Schell *et al*., 2004). The calcium and phosphorus supplied by consumption of eggs mitigates the levels of BPb in the subjects (Mahaffey, 1995). The subjects in Nyamira Rural who consumed eggs daily had the lowest mean BPb level as compared to the other areas. This was because eggs were cheaply available in the rural area which made the subjects to feed on more eggs mitigating the lead levels more as compared to the other areas.

The subjects who consumed millet daily in Nairobi City Centre, Nyamira Town and Nyamira Rural had the lowest BPb levels of 20.34±6.81, 19.2±4.3 and 17.8±5.65 µg/dl, respectively. The non-consumers of millet had the highest mean BPb levels in Nairobi City Centre, Nyamira Town, Nairobi Suburban and Nyamira Rural with values of 35.75±15.01, 34.8±28.12, and 38.3±16.41 and 25.3±4.89 µg/l, respectively. The difference between the mean BPb levels between the daily and rarely consumers of millet were statistically significant in Nairobi City Centre and Nyamira Town ($p < 0.01$).

The frequency of millet consumption by the subjects associated inversely with the BPb levels. Zinc is a potent antagonizer to lead intoxication and its administration decreases the accumulation of lead in the body organs (Srivastava *et al*., 1984; Moon *et al*., 2003; USCDC, 2004). Zinc reduces severity of lead poisoning by restoring lead induced biological alternation in urinary and blood parameters in animals (Moon *et al*., 2003). The consumption of millet resulted in high levels of zinc in blood (Mahaffey, 1995) and this mitigates the level of lead in the subjects (Table8). The subjects in Nyamira Rural who fed on millet daily had the lowest BPb levels as compared to the other study areas.

The weekly consumers of sorghum had the lowest mean BPb levels in Nairobi City Centre, Nyamira Town, Nairobi Suburban and Nyamira Rural with values of 24.14±10.21, 24.52±11.3, 20.7±7.63 and 18.3±7.8 µg/dl while the non-consumers had the highest mean BPb levels of 34.67±34.21, 40.8±30.4, 34.32±12.1 and 26.83±9.81 µg/l, respectively among subjects (Table 9). The difference in the mean BPb levels between the weekly and the non-consumers of sorghum was statistically significant in Nairobi City Centre, Nyamira Town, Nairobi Suburban and Nyamira Rural ($p < 0.01$).

The frequency of sorghum consumption was inversely associated with BPb levels of the subjects. Sorghum is rich in zinc (Mahaffey, 1995) and high levels of zinc in the body mitigated the BPb levels (Moon *et al*., 2003; Weaver *et al*., 2003). The lowest BPb level was observed among Nyamira Rural daily consumers of sorghum who consumed the light quantity as compared to the other study areas. This was because sorghum was locally grown in Nyamira Rural making the subjects to consume it regularly mitigating their BPb levels.

The subjects who consumed groundnuts daily in Nairobi City Centre, Nyamira Town and Nyamira Rural had the lowest BPb levels of 19.25±9.45, 15.6±4.84, 14.8±8.6 and 10.5±4.31 µg/dl while the non-consumers had the highest mean BPb levels of 39.83 ±28.34, 35.83±19.97, 34.31±9.61 and 28.3±6.89 µg/l, respectively. The difference in the mean BPb levels between the daily and the non-consumers of groundnuts was statistically significant in Nairobi City Centre, Nyamira Town, Nairobi Suburban and Nyamira Rural ($p < 0.01$)

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The frequency of groundnuts consumption correlated inversely with BPb levels of the subjects. Groundnuts have high levels of calcium, phosphorus and zinc and feeding on them resulted to low absorption and retention of lead in the subjects (Zelglar *et al*., 1978; Fewtrell *et al*., 2003; Martin *et al*., 2006). This means that the consumption of groundnuts mitigated the BPb levels in the subjects.

3.3 The levels of the elements in the blood

The levels of lead, zinc, calcium and phosphorus in the blood samples were measured and used to determine the mean of each element in each study area. The determined mean values and ranges are presented in Table 2. The whole blood lead levels for Nairobi city centre ranged from1.0-107µg/l, Nyamira town from 0-117 µg/l, Nairobi Suburban 0-82 µg/l and Nyamira rural 0.8 -40 µg/l with means of 29.9±16.91, 27.69±32.29, 26.12±17.96 and 24.20±7.07 µg/l, respectively. The highest mean BPb level was among Nairobi city centre subjects while the lowest was among Nyamira rural subjects. The median BPb level for Nairobi city centre subjects was 26.0 µg/l, Nyamira town subjects were 29.0 µg/l, Nairobi Suburban was 24 µg/l and Nyamira rural was 21 µg/l. The mean BPb levels of the subjects in the study areas was generally higher than that which was reported in literature for the general population except in Nyamira rural (control). There are is currently no lead level that is safe for infants and young children (CDC, 2005, Schirndig *et al*., 2001). The blood lead of Nairobi city centre residents ranged from 10-107 µg/l, Nyamira town subjects from 0-117 µg/l, Nairobi suburban from 0-82 µg/l and Nyamira rural from 8-40 µg/l. The ranges obtained for each study area are comparable to those obtained from a study of children and adolescents in selected areas of Nairobi and Olkalou, Nyandarua district where the range of 0.4-over 65 µg/dl was reported by Njorenge &UNEP (2005). The higher lead levels could be due to accumulation of lead from fuel emission which could still be in use (Mbaria, 2007). The town residents were exposed to higher lead pollution than the rural dwellers. This means that the majority of the study subjects were at risk.

The whole blood zinc levels for Nairobi City Centre ranged from7-221 µg/dl, Nyamira Town from 20-150 µg/dl, Nairobi Suburban 9-170 µg/dl and Nyamira Rural 53-170 µg/l with means of 96.56±49.03, 80.98±18.18, 112.62±54.34 and 80.64±18.99 µg/dl respectively. The highest mean blood zinc level was among Nairobi Suburban subjects while the lowest was among Nyamira Rural subjects.The whole blood phosphorus levels for Nairobi City Centre ranged from 1.26-5.58 mg/dl, Nyamira Town from 1.40-7.60 mg/dl, Nairobi Suburban 1.68-5.57 mg/dl and Nyamira Rural 1.04-5.60 mg/dl with means of 3.37 ± 0.993, 3.60 ± 1.74, 2.83 ± 0.88 and 2.66 ± 0.97 mg/dl respectively. The highest mean blood phosphorus level was among Nyamira Town subjects while the lowest was among Nyamira Rural subjects. The whole blood calcium levels for Nairobi City Centre ranged from 1.2-13.0 mg/dl, Nyamira Town from 3.64-15.46 mg/dl, Nairobi Suburban 3.4-12.4 mg/dl and Nyamira Rural 2.3-12.2 mg/dl with means of 7.27 ± 3.11, 6.84 ± 2.65, 8.83 ± 2.64 and 6.92 ± 2.63 mg/dl respectively. The highest mean blood calcium level was among Nairobi Suburban subjects while the lowest was among Nyamira town subjects. The t-test values for the differences in the means of blood lead, calcium, zinc and phosphorus levels in the different study areas was determined and presented in Table 2.

The difference between the means of the zinc, calcium and phosphorus for subjects from various sampling stations were statistically significant except for Nyamira town and Nairobi city centre

for phosphorus, Nairobi city centre and Nairobi suburban for calcium, Nairobi city centre and Nyamira rural for calcium and Nyamira town for zinc. The difference between the mean lead was only statistically significant for Nairobi city centre and Nyamira rural ($p < 0.01$; df = 99).
The levels of lead, calcium, zinc and phosphorus determined in the subjects were presented in different range categories of below normal range, normal range and above normal range (Sood, 2006). The frequency percentage distribution of distributions of blood lead levels varied from one study area to another. Seventy four percent of the Nairobi city centre, 52% of Nyamira town, 72 % of Nairobi suburban and 82 % of Nyamira rural subjects had normal BPb levels (0-30µg/l). Twenty four percent of Nairobi City Centre, 14 % of Nyamira town, 16 % of Nairobi suburban and 18 % of Nyamira rural subjects had above normal BPb levels (31-40 µg/l). Two percent of Nairobi city centre, 34 % of Nyamira town and 12 % of Nairobi suburban subjects had excess (above 40 µg/l) BPb levels.28 % of Nairobi city centre, 28 % of Nairobi suburban and 6 % of Nyamira town, 28 % of Nairobi suburban and 4 % of Nyamira rural subjects had blood zinc of below the normal range (below 60 µg/l). Fourty percent of Nairobi city centre, 92 % of Nyamira town, 24 % of Nairobi suburban and 92 % of Nyamira rural subjects had blood zinc level ranging from 60 to 120 µg/dl .The rest of the subjects in study areas had blood lead zinc level above 120 µg/l).

Twenty two percent of Nairobi city centre, 40 % of Nyamira town, 50 % of Nairobi suburban and 60 % of Nyamira rural subjects had blood phosphorus of below normal level (below 2.7 mg/dl). Seventy percent of Nairobi city centre, 16 % of Nyamira town, 42 % of Nairobi suburban and 36 % of Nyamira rural subjects had blood phosphorus within the normal range, 2.71 to 4.5 mg/dl and the rest of the subjects had phosphorus levels of above 4.5 mg/l). Sixty four percent of Nairobi city centre, 76 % of Nyamira town,46 % of Nairobi suburban and 70 % of Nyamira rural subjects had blood calcium below the normal range (below 8.5 mg/dl). Twenty six percent of the Nairobi city centre, 14 % of Nyamira town, 26 % of Nairobi suburban and 22 % of Nyamira rural subjects had blood calcium in the normal range, 8.5-10.4 mg/dl. Ten percent of Nairobi city centre, 10 % of Nyamira town, 28 % of Nairobi suburban and 8 % of Nyamira rural subjects had blood calcium level of above normal range which is above 10.4 mg/dl.

The levels of zinc, calcium and phosphorus in the blood of subjects in the four study areas determined were used to compute the correlation coefficients (r) value for the elements in each study area as presented in table (Table2). The amount of lead in the body correlates inversely to the amount of zinc, calcium and phosphorus in the subjects in the four study areas. The amount of calcium in the body was positively correlated to that of zinc and phosphorus and similar relationship was observed between zinc and phosphorus in all the study areas. This shows that the amount of lead in the body of individual is influenced by the amount of zinc, calcium and phosphorus in the body, and extent of lead exposure.

The binomial analysis (t-test) was performed to determine whether the feeders and non feeders of protective food substances exposed to risk factors statistically affected the BPb level. The results obtained are presented in Table 5.The difference between the mean BPb level of protective feeders and non protective feeders were statistically significant in all areas except for traveling, residence of less than 50 M from the road and working/living near factory for Nyamira Rural subjects ($p < 0.01$) This implies that the frequency of the protective food substances influences the BPb level in human beings. This could be due to calcium, zinc and phosphorus in the food substances which mitigated the levels of lead in the body.

4.Discussion

The amount of lead in the body correlated inversely to the amount of zinc, calcium and phosphorus in the subjects in the four study areas. The amount of calcium in the body was positively correlated to that of zinc and phosphorus and similar relationship was observed between zinc and phosphorus in all the study areas. This shows that the amount of lead in the body of individual is influenced by the amount of zinc, calcium and phosphorus in the body, and extent of lead exposure. The results also revealed that those subjects who had higher levels of Ca, Zn and P (Nairobi suburban residents) in their blood had lower levels of lead. This is because the Ca, Zn and P tend to mitigate the levels of lead in the human body. These elements hinder the absorption and retention of lead in the human body. The subjects who fed on diets poor in those elements were found to have higher BPb levels. It has also been shown that those subjects with high levels of zinc, calcium and phosphorus in their blood had low levels of lead. These results are in agreement with those of Schell et al. (2004) who found significant inverse relationship of the blood lead levels of infants of 6 months of age with their zinc, iron and calcium intake. In another study by Cheng et al. (1998) there was an inverse association between blood lead levels and total dietary intake of vitamin C and iron but not with calcium, phosphorus, zinc or vitamin D. Other studies in humans have indicated an inverse association between blood lead levels and calcium intake (Blake and Mann, 1983; Mahaffey et al. 1986; Sargent et al. 1999).

.The protective efficiency of the food substances on the absorption and retention of lead differed from one study area to another. In some cases the low protection efficiency can be explained by the fact that some food substances such as milk, groundnuts and beef contain a lot of fats. Fats are known to increase the absorption of lead in the body (Mahaffey, 1995; KMOL, 2004). This possibly counteracted the protective effects of the food substance resulting in their low protective ability.

It should be pointed out that there were some cases of very low BPb levels of among the non-consumers of protective foods or high levels of BPb among the frequent feeders of the protective foods in the study areas. This was because not all protective feeding habits were investigated. Another explanation in such cases is that the study subjects had increased calcium, zinc and phosphorus from other food substances other than those studied. There are nutritional conditions such as the irregular food intake besides marginal calcium ingestion and subtle iron deficiency that associated with increased lead absorption (Mahaffey, 1995; Owago, 1999). The investigation could have been further complicated by the complexity of human dietary habit patterns. The frequency consumption of the food substances in the subjects correlated inversely with the mean BPb levels. On the other hand the lower mean BPb levels of the Nyamira Rural subjects could have been due to limited exposure to those factors which exposed them to lead. The study has revealed that those subjects who had higher levels of zinc, calcium and phosphorus in their blood in the four study areas had lower levels of lead . This implies that people living in areas with high lead pollution may have lower lead levels due to feeding on diets rich in zinc, calcium and phosphorus. This may be the reason why the Nairobi Suburban had lower mean blood lead (26.44±17.8 µg/l) than Nyamira Town subjects (28.1±29.3 µg/l), since they were able to feed on diets rich in zinc, calcium and phosphorus. This may have counteracted the environmental lead exposure, making the subjects to have lower lead levels than the subjects from Nyamira Town who despite being exposed to low lead levels had higher levels due to poor feeding habits. The higher levels of zinc, calcium and phosphorus in the diets resulted to low lead absorption and retention in the body (Mahaffey, 1995; Owago, 1999; Moon et al., 2003; Coyle *et al*., 2005; Martin *et al*., 2006).

The difference between the mean BPb level of protective feeders and non protective feeders (Table11) were statistically significant in all areas except for traveling, residence of less than 50 M from the road and working/living near factory for Nyamira Rural subjects ($p < 0.01$) This implies that the frequency of the protective food substances influences the BPb level in human beings. This could be due to calcium, zinc and phosphorus in the food substances which mitigated the levels of lead in the body.

5.Conclusion

The zinc, calcium and phosphorus have been found to mitigate the levels of lead in the human body.Those individuals feed on diets rich in calcium, zinc and phosphorus were found to have low levels of lead. These foods included groundnuts, milk, sorghum, beans, eggs, beef and millet. In view of the adverse effects of lead it is advisable for people to feed on diets rich in calcium, zinc, iron and phosphorus or to take the supplements of these elements toreduce the lead levels in their bodies.

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Competing interests

The authors declare that there are no competing interests with anybody whatsever.

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Table 1: Summary of regression analysis parameters for lead, zinc, calcium and phosphorus calibration curves.

Elements	Line of regression	Range of concentration used	r^2	CV(%)
Lead	Y = 0.0123x-0.007	2 – 80 µg/dl (triplicates)	0.9987	0.403
Zinc	Y = 0.02x+0.0115	5 – 1oo µg/dl (triplicates)	0.9996	1.413
Calcium	Y = 0.01712x-0.0026	0.2 – 9.5 mg/dl (triplicates)	0.9999	0.085
Phosphorus	Y = 0.0468x+0.00	0.05 – 4.0 mg/dl (triplicates)	0.9995	0.418

Y = Absorbance x = Concentration

Table 2: The means and range of the elements in the blood of the subjects in the four study areas.

Element	Nairobi City Centre	Nyamira Town	Nairobi Suburban	Nyamira Rural
Lead (µg/dl)	29.9 ± 16.91 (10-107),p=1.13	27.69 ± 32.29 (0-117)p=0.019	26.12 ± 17.96 (0-82)p=0.008	24.20 ± 7.07 (8-40)p=0.004
Calcium (mg/dl)	7.27 ± 3.11 (1.2-13)p=0.001	6.84 ± 2.65 (3.6415.46),p=0.38	8.83 ± 2.64 (3.4-12.4)p=0.002	6.92 ± 2.63 (2.3-12.2)p=0.001
Zinc (µg/dl)	96.56±49.03 (7-221)p=0.002	80.98 ±18.18 (20-150),p=0.413	112.62± 54.34 (9-170),p=0.531	80.64 ± 18.99 (53-170),p=0.912
Phosphorus (mg/dl)	3.37 ± 0.993 (1.26-5.58)p=0.004	3.60 ± 1.74 (1.40-7.60)p=0.007	2.83 ± 0.88 (1.68-5.57),p=0.83	2.66 ± 0.97 (1.04-5.60),p=1.21

Table 3: The correlation coefficient (r^2) values between various levels of the elements in the blood of the study subjects in the four study areas.

	Pb				Zn				Ca			
	A	B	C	D	A	B	C	D	A	B	C	D
Zn	-0.4	-0.9	-0.6	-0.3	-	-	-	-	-	-	-	-
Ca	-0.7	-0.1	-0.8	-0.4	+0.4	+0.2	+0.1	+0.2	-	-	-	-
P	-0.4	-0.4	-0.7	-0.1	+0.4	+0.3	+0.4	+0.1	+0.3	+0.2	+0.5	+0.3

A – Nairobi City Centre
C – Nairobi Suburban
B – Nyamira Town
D – Nyamira Rural

Table 4: The mean and the range of BPb level (µg/dl) of subjects in various frequencies of milk consumption groups.

Milk consumption	Mean lead concentration (range) (µg/dl)			
	Nairobi City Centre	Nyamira Town	Nairobi Suburban	Nyamira Rural
Daily	24.63 4.65 (10-48)	22.4 10.7 (0-62)	23.1 7.83 (0-42)	20.6 8.48 (8-40)
Once a week	28.36 18.47 (5-74)	26.5 12.95 (10-68)	25.5 10.42 (14-68)	25.2 4.95 (11-33)
Rarely	30.4 23.07 (19-107)	38.5 30.5 (3-117)	40.3 11.21 (34-82)	None

Table 5 : The mean and the range of BPb level (µg/dl) of the subjects in various frequencies of beans consumption groups.

Beans consumption	Mean lead concentration (range) (µg/dl)			
	Nairobi City Centre	Nyamira Town	Nairobi Suburban	Nyamira Rural
Daily	19.1 8.67 (10-45)	13.4 5.6 (11-28)	18.9 7.34 (0-38)	12.6 6.9 (8-40)
Once weekly	27.44 19.41 (13-99)	19.7 19.9 (0-83)	23.6 8.21 (0-42)	25.5 3.84 (12-33)
Rarely	31.5 26.05 (15-107)	35.7 34.81 (2-117)	31.3 18.46 (20-82)	None

Table 6 ; The mean and the range of BPb level (µg/dl) of the subjects in various frequencies of beef consumption groups.

Beef consumption	Mean lead concentration (range) (µg/dl)			
	Nairobi City Centre	Nyamira Town	Nairobi Suburban	Nyamira Rural
Daily	23.5 5.31 (1-23)	15.5 4.4 (0-20)	17.4 6.61 (0-34)	None
Once weekly	29.5 8.54 (10-45)	16.33 6.86 (0-36)	25.4 8.53 (0-32)	20.1 4.51 (12-27)
Rarely	26.9 21.96 (13-107)	22.6 15.53 (10-83)	27.7 19.41 (15-82)	23.4 8.57 (8-38)
None	37.2 16.34 (25-85)	39.3 34.77 (10-117)	34.3 14.1 (24-68)	24.3 5.4 (12-40)

Table 7 : The mean and the range of BPb level (μg/dl) of the subjects in various frequencies of eggs consumption groups.

Eggs consumption	Mean lead concentration (range) (μg/dl)			
	Nairobi City Centre	Nyamira Town	Nairobi Suburban	Nyamira Rural
Daily	20.8 7.27 (10-49)	16.4 8.79 (0-35)	20.7 8.93 (0-42)	16.2 8.70 (12-23)
Once weekly	22.75 21.55 (15-107)	22.9 20.78 (0-83)	22.8 9.09 (10-31)	21.3 7.14 (8-40)
Rarely	38.81 13.45 (30-85)	33.25 31.6 (11-117)	28.82 21.2 (14-82)	25.2 9.33 (12-31)

Table 8 : The mean and the range of BPb level (μg/dl) of the subjects in various frequencies of millet consumption groups.

Millet consumption	Mean lead concentration (range) (μg/dl)			
	Nairobi City Centre	Nyamira Town	Nairobi Suburban	Nyamira Rural
Daily	20.34±6.81 (0-25)	19.2±4.31 (0-21)	None	17.8±5.65 (15-30)
Once weekly	22.92±7.25 (17-35)	26.2±6.17 (10-30)	24.51±8.94 (0-42)	21.14±7.7 (19-40)
Rarely	27.83±21.83 13-107	26.5±19.81 (11-83)	28.57±19.61 (10-82)	22.8±5.46 (8-30)
None	35.75±10.01 (30-85)	34.8±28.12 (20-117)	38.3±16.41 (11-62)	25.3±4.89 (22-36)

Table 9 : The mean and the range of BPb level (μg/dl) of the subjects in various frequencies of sorghum consumption groups.

Sorghum consumption	Mean lead concentration (range) (μg/dl)			
	Nairobi City Centre	Nyamira Town	Nairobi Suburban	Nyamira Rural
Once weekly	24.14±10.21 (10-85)	24.52±11.3 (0-54)	20.7±7.63 (0-46)	18.3±7.8 (8-38)
Rarely	24.76±21.04 (20-107)	26.5±19.8 (1-82)	23.6±14.34 (10-82)	24.75±4.4 (19-31)
None	34.67±19.85 (19-100)	40.8±30.4 (10-117)	34.32±6.84 (48-72)	26.83±5.63 (22-40)

Table 10 : The mean and the range of BPb level (µg/dl) of the subjects in various frequencies of groundnuts consumption groups.

Groundnuts consumption	Mean lead concentration (range) (µg/dl)			
	Nairobi City Centre	Nyamira Town	Nairobi Suburban	Nyamira Rural
Daily	19.25±9.45 (10-78)	15.6±4.84 (0-26)	14.8±8.6 (0-34)	10.5±4.31 (8-22)
Once weekly	22.53±10.68 (16-88)	26.5±7.43 (1-43)	21.3±12.7 (15-47)	23.7±5.31 (10-30)
Rarely	28.8±12-81) (21-81)	33.07±37.3 (1-117)	29.6±18.1 (12-82)	26.3±3.43 (20-30)
None	39.83±28.34 (19-107)	35.83±19.97 (20-85)	34.31±9.61 (31-68)	28.3±6.89 (18-40)

Table 11 : The t-test values for the feeders and non feeders of the protective food substances exposed to risk factors for each study area.

Risk factors	Nairobi City Centre	Nyamira Town	Nairobi Suburban	Nyamira Rural
Traveling	6.38*	4.29*	4.30*	2.30
Smoking	5.28*	4.69*	3.68*	3.61 *
Exposure to cigarette smoke	4.49*	4.38*	3.31*	4.87*
Use of glazed ceramics	7.65*	4.32*	4.46*	3.78*
Residence from the road (M)	5.49*	4.63*	5.30*	-
Working/living near factory	3.62*	3.54*	4.20*	-

Statistically significant ($p < 0.01$) * stands for statistically significant.

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