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CORRELATIONS BETWEEN THE LEVELS OF ELEMENTS IN THE BLOOD AND SOME PHYSIOLOGICAL / BIOCHEMICAL PARAMETERS IN FEMALE ROADSIDE WORKERS IN ILE-IFE, NIGERIA

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ABSTRACT

This work determined the levels of As, Cd, Cu, Hg, Pb, Se and Zn in the blood of sixty-two Nigerian women roadside workers in Ile-Ife using Inductively-Coupled Plasma Mass Spectrometry and Direct Mercury Analyzer and investigated the toxic metal correlation with some physiological / biochemical parameters. Body fat was estimated using bioelectrical impedance and isotope dilution methods. The results showed that arsenate correlated ($p \leq 0.05$) with total cholesterol (TC) and plasma creatinine (P.Cr) in underweight subjects, P.Cr in obese subjects and with creatinine clearance and urinary creatinine in all the subjects. Cd correlated with low density lipid–cholesterol (LDL-C) and TC in underweight, waist and waist/hip ratio in overweight and packed cell volume in obese subjects while Hg had a high correlation factor with LDL-C in overweight and triglycerides and TC in obese subjects. Pb correlated highly with age and total body water in obese subjects. These indicated that interactions between trace elements and the physiological / biochemical parameters are influenced by the nutritional status of the individual subjects and possibly provide a basis for determining the effects of these toxic elements on the exposed subjects.

Keywords: Correlation, Blood Trace Elements, Nutritional Status

INTRODUCTION

One of the global problems confronting mankind is exposure to environmental pollutants/chemicals, most especially toxic elements, resulting in negative impacts on the quality of human life (FDA/EPA, 2004). Vehicular exhaust, amongst others, is believed to account for more than 80% of this pollution in some urban centers in Nigeria (Obioh *et al.*, 1993). Apart from fossil fuel combustion, studies have shown that pollution by heavy metals such as Cd, Pb, Fe, Ni, Co, Mo, As and Zn are associated with the wear and tear in tires, brake pads, lead weights for wheel balancing, corrosion of bushings etc. of automobiles (Muschack, 1990; Viklander, 1998; USEPA, 1997). The levels of these pollutants or toxic elements such Pb in air (Agbo, 1997), roadside topsoil (Fakayode, 2003; Ogunsola and Akeredolu, 1994) or run-off water sediments from paved roads (Adekunle *et al.* 2009) have been found to vary according to traffic volume and distance from high ways. It has been established that blood samples from traffic wardens contain these pollutants depending on total length of exposure to these damaging heavy metal pollutants.

In developing countries such as Nigeria, women of low socio-economic status are particularly exposed to vehicular elemental pollution by virtue of extensive street-related activities such as street trading, which is associated with poverty since most cannot afford regular shops in designated centres. These women, more often than not, are of child bearing age and often go into their trading post with infants and pre-school children. According to Antham *et al* (2006), continual exposure can affect the lungs of growing children and may aggravate or complicate medical conditions in elderly. Furthermore, some of these women become pregnant from time to time. The exposure to these pollutants has been found to affect the fetus (ASTDR, 1999). For instance, maternal blood crosses the placenta and put the fetus at risk. Increasing evidence indicates that maternal lead can adversely affect fetus viability as well as fetal and early childhood development (ATSDR, 1999). This makes environmental exposure in women of child-bearing age more precarious because such women can become a

source of exposure to fetuses and infants through placental exchange and during lactation.

There is a growing national and international interest in the probable effects of exposure to pollutants through road side economic activities and its relationship with the nutritional status of subjects thus exposed. Vahter (2007) provided evidence of the association between nutrients (1-carbon metabolism) and arsenic toxicity in Bangladeshi women while the relationship between heavy metals (especially blood Pb levels and urine Hg concentrations) and blood pressure and / or cholesterol level has been studied in Korean children by Kim *et al* (2005). In the latter study by Ching *et al* (2018), no significant correlation was obtained between blood Pb level and blood pressure or cholesterol while the urine Hg level was positively associated with the serum cholesterol thus becoming a risk factor of myocardial infarction and coronary/cardiovascular diseases .Urinary creatinine has also been found as a strong predictor of total urinary arsenic as dimethyl arsenic acid in adults (Basu *et al.*, 2011).

In previous publications, we had reported on the anthropometric predictive equations for percentage body fat in Nigerian women using some of the anthropometric results now presented (Oketayo and Ojo, 2011) while another paper reported the evaluation of the nutritional status using body fat, physiological and biochemical parameters in the same set of Nigerian women (Ojo *et al.*, 2011).

This present report compared two methods of body fat estimation and considered the possible correlations between the level of selected toxic metals in the blood and some physiological/biochemical parameters of nutritional importance in Nigerian women. This is with a view to finding links between environmental exposure to exogenous chemicals and adverse health effects. The study provides the levels of these elements as well as nutritional status of the subjects and determines the relationship as possible diagnostic tools for predicting their impacts.

MATERIAL AND METHODS

Subject description

Sixty-two Nigerian women (aged between 18 and 52 years) with known exposure to vehicular elemental pollution were purposively selected. An initial population of 300 was surveyed but 81 subjects were selected; some dropped out and only 63 subjects completed the study. Selected subjects were non-pregnant, non-lactating and non-smoking; they were either traders having their shops and stalls close to busy bus stops, petrol station attendants or traffic wardens. Ethical clearance was obtained from the Ethics and Research Committee of the Obafemi Awolowo University Teaching Hospital Complex before the commencement of the work. Written consent was also obtained from each subject before her recruitment into the study. Physical and medical examinations were conducted and a detailed questionnaire was administered to each subject.

Sample Collection

Anthropometry

Anthropometry measurements were made in accordance with WHO (2003) guidelines. Subjects' heights were measured to the nearest 0.1 cm while the body weight was measured to the nearest 0.1 kg. Body mass index (BMI) was calculated as weight (kg) divided by the square of height (W / H^2). The subjects were then stratified into Underweight (BMI ≤ 18.5), Normal (BMI > 18.5 but ≤ 25), Overweight (BMI > 25 but ≤ 30), and Obese (BMI > 30) groups using NIH / WHO (2004) definitions.

Skinfold thickness such as biceps, triceps, scapular, subscapular, suprailiac and abdominal was determined using Langre calipers while forearm, neck, wrist, waist and hip circumference was measured with a standard tape rule.

Biochemical Parameters

Body fat was determined using Stable Isotope Dilution (SID) techniques (IAEA, 2009). The Bioelectrical Impedance Analytical (BIA) technique, which involves the use of BF- 350 Tanita body composition Analyzer (Tanita, 1999) to compute body fat from the subject's impedance, gender, height, fitness level, age and weight. The second method - an isotope dilution

method - involved the ingestion of D_2O and the determination of deuterium in saliva before (baseline) and in post-dose after its equilibration (IAEA 2009). The saliva samples were kept in a freezer at -20 $^{\circ}$C until determination of deuterium enrichment by Fourier Transform Infrared (FTIR) Spectrometry.

Blood samples: Ten (10) ml of venous blood was collected from each subject through ante-cubical venipuncture (using a 10 ml syringe and a 21G needle) and the blood was discharged slowly without the needle into two separate 5 ml heparinized bottles.

Packed cell volume was determined using a 41A2502 capillary tubes plain-glass heamatocrit reader after spinning with haematocrit centrifuge (Hawksley). The plasma for each specimen was separated, using a disposable Pasteur pipette, into a clean aliquot bottle and stored frozen at -20 $^{\circ}$C. Blood samples for elemental analysis were thawed, lyophilized and sterilized with between 20 and 30 KGy of gamma irradiation using Co-60 from the Gamma cell 220 irradiator. The wet-to-dry mass ratio of each sample was thereafter calculated. The systolic and diastolic blood pressures (BP1 & BP2, respectively) were measured by qualified medical personnel using a calibrated manual sphygmomanometer.

Urine Samples: Spot urine samples were collected from the subjects into sterile universal bottles, kept under ice storage and frozen at – 20 $^{\circ}$C until analyzed for urinary creatinine.
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Analytical procedure
Laboratory Determination of Biochemical Parameters
Total cholesterol and HDL-cholesterol in the plasma were assayed by enzymatic methods using RANDOX kits. LDL-cholesterol value was obtained using Friedwald formula:
LDL-C = Total Cholesterol – HDL-C – Triglyceride / 2.22
In vitro determination of triglycerides in plasma was determined by the enzymatic method using RANDOX kits.
Creatinine levels in both plasma and urine were determined by optical absorbance measurement at 500 nm. Creatinine clearance was calculated using the expression:

$$Cr.Cl\,(ml\,/\,min) = \frac{(140 - age[yr]) \times (wt[kg])}{72 \times serumCr[mg\,/\,dl]} \times 90\%$$

Uric acid in urine was determined by the enzymatic colorimetric method [RANDOX, kit]. Precision controls at low, normal, high as well as accuracy control using the RANDOX brand were included in all batch analyses.

Elemental Analysis of Blood Samples
The levels of lead, arsenic, selenium, cadmium, copper and zinc in the blood samples were determined using Inductively Coupled Plasma Spectrophotometer (ICP-MS). Total Mercury in blood was determined using Direct Mercury Analyzer (DMA) .
2.5   Statistical analysis
Pearson's product moment correlation was determined among all the parameters using Excel software, and the significance of the correlations were determined using the table of critical values for Pearson r provided by the University of New England, Armidale, Australia. The correlations were determined based on the values of physiological and biochemical parameters reported in Ojo *et al.* (2011)

RESULTS AND DISCUSSION
The descriptive statistics of the subjects' body fat, BMI, skin-fold measurement for the chin, bicep, tricep, scapular, sub-scapular, abdominal, suprailiac and circumference of the neck, forearm, wrist, abdomen, waist, hip and waist to hip ratio are presented in table 1.

Table 1: Body Fat (%) and Anthropometric Measurements of all Subjects and Stratified according to WHO Classification of Weight by BMI

Parameters	All (n=62)		Underweight (n=10) BMI< 18.5 kg/m²		Normal (n=33) BMI>18.5-≤ 25 kg/m²		Overweight (n=12) BMI>25-≤ 30 kg/m²		Obese (n=7) BMI >30 kg/m²	
	Range	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range	Mean±SD
Weight (kg)	35.3-105.8	60.1±14.2	35.3-54.4	44.2±5.3	42.7-76.9	54.8±7.1	56.3-76.4	67.9±4.9	75.1-105.8	11.7±89.8
Height (m)	1.5-1.8	1.6±0.7	1.5-1.7	1.6±0.7	1.5-1.8	1.6±0.7	1.5-1.7	1.6±0.6	1.6-1.7	1.6±0.5
BMI (kg/m²)	15.7 - 41.2	23.4±4.9	15.7-18.5	17.7±0.9	18.6-24.5	21.3±1.8	25.2-28.5	26.7±1.1	30.7-41.2	33.9±4.3
TBW(%BW)	33.3-65.8	52.9±7.6	51.1-64.7	57.3±3.9	42.6-65.8	54.0±6.8	37.8-65.7	52.7±8.6	33.3-50.8	43.6±5.7
% Body fat	10.1-54.6	27.7±10.7	11.7-30.1	21.2±5.4	10.2-41.8	26.2±9.3	10.9-48.4	27.9±11.7	30.6-54.6	41.8±8.4
Fat mass² (%BW)	6.3-47.4	27.0±10.3	6.3-22.4	13.2±4.6	8.9-36.8	23.8±4.6	29.6-41.2	34.6±3.0	36.0-47.4	43.9±4.4
Chin (mm)	6.0 - 20.0	10.0 ± 2.0	8.0 - 11.0	9.0 ± 1.0	6.0 - 13.0	10.0 ± 1.0	9.0 - 12.0	11.0 ± 4.0	10.0 - 20.0	13.0 ± 3.0
Bicep (mm)	4.0 - 40.0	14.0 ± 7.0	5.0 - 11.0	9.0 ± 3.0	4.0 - 23.0	12.0 ± 5.0	9.0 - 26.0	18.0 ± 5.0	12.0 - 40.0	26.0 ± 10.0
Tricep (mm)	4.0- 42.0	19.0 ± 8.0	7.0 - 24.0	12.0 ± 5.0	4.0 - 29.0	17.0 ± 6.0	14.0- 29.0	21.0 ± 5.0	16.0 - 42.0	31.0± 9.0
Scapular (mm)	7.0 - 41.0	16.0 ± 7.0	7.0 - 15.0	10.0 ± 2.0	7.0 - 21.0	13.0 ± 4.0	15.0- 25.0	18.0 ± 3.0	16.0 - 41.0	30.0 ± 8.0
Subscapular (mm)	7.0 - 45.0	17.0 ± 8.0	7.0 - 15.0	11.0 ± 3.0	7.0 - 21.0	14.0 ± 5.0	12.0 - 33.0	21.0 ± 5.0	9.0 - 45.0	32.0 ± 8.0
Abdominal SF (mm)	7.0- 49.0	19.0 ± 7.0	8.0- 20.0	14.0 ± 4.0	7.0- 36.0	17.0 ± 6.0	15.0 – 27.0	21.0 ± 4.0	22.0 - 49.0	31.0 ± 10.0
Suprailiac (mm)	5.0- 44.0	14.0 ± 8.0	6.0-19.0	10 .0 ± 4.0	5.0- 23.0	12.0 ± 5.0	9.0 – 25.0	14.0 ± 5.0	16.0 - 44.0	30.0 ± 11.0
Neck (cm)	13.0- 39.0	31.4 ± 3.4	13.0 - 29.9	27.2± 5.1	28.0 -33.6	30.9 ± 1.6	31.0 - 31.2	32.9 ± 1.4	33.0 - 39.0	35.9 ± 2.4
Forearm (cm)	20.5- 39.5	26.8 ± 4.1	20.5 –39.5	22.1 ± 1.3	21.8 -29.8	25.4 ± 2.0	25.6 –32.4	29.6 ± 2.1	29.0 -39.5	34.1 ± 3.9
Wrist (cm)	13.0 -21.0	15.4 ± 1.3	13.0 – 21.0	14.0 ± 0.6	13.0 -16.5	15.0 ± 0.8	14.5 -17.4	16.0 ± 0.7	15.0 - 21.0	17.5 ± 1.9
Abdominal CF (cm)	60.0 -116.0	79.0 ± 13.5	60.0 -116.0	69.4 ± 12.2	60.0 - 90.0	73.5 ± 7.6	75.0 - 100.9	85.5 ± 7.5	85.0 -116.0	104.1 ± 10.0
Waist (cm)	63.6 -113.5	82.1± 11.3	63.6 -114.0	70.4 ± 6.2	66.1 - 92.0	78.2 ± 6.5	82.0 - 99.8	88.9 ± 5.6	86.0 - 114.0	102 ±10.9
Hip (cm)	78.2 -123.0	96.3 ± 10.7	78.2 - 123.0	83.5 ± 4.0	83.5 -105.1	92.6 ± 6.1	97.3 – 113.0	104.3 ± 3.9	108.5 - 123	114.8 ± 6.2
Waist/Hip	0.75 - 0.99	0.85 ± 0.53	0.75 - 0.93	0.84 ± 0.06	0.76 - 0.94	0.85 ± 0.46	0.78 - 0.95	0.85 ± 0.46	0.79 - 0.99	0.89 ± 0.08

Note: ¹= Isotope dilution,   ²= BIA   SF =Skinfolds   CF= Circumferences

The BMI result indicated that 16.1% of the subjects were underweight, 53% were normal, 19.3% were overweight while 11.3% were obese using the WHO classification. The percentage body fat of the subjects estimated by isotope dilution ranged from 10.1 to 54.6. There was an excellent correlation ($r^2 = 0.9999$) between the measured D_2O enrichment by FTIR and the calculated D_2O in the saliva. Thus, the isotope dilution procedure is validated for the calculation of body water and subsequently the calculation of body fat. Results for % body fat (6.3 to 47.4) obtained by the bio-impedance technique correlated significantly ($r = 0.517$ at 0.01 level) with those obtained by the stable isotope method, indicating that either method could be used for body fat estimation, especially if the Bioelectrical Impedance Analysis method could be standardized regularly. The bio-impedance method is however simpler to operate once the equipment is available and would be preferred in a third world country with limited infrastructural facilities for research. As indicated earlier (Oketayo and Ojo 2011), there were significant correlations between BMI or body fat and each of the hip, forearm, abdominal and wrist circumferences such that the circumference measurements could be used to predict nutritional status and body fat in Nigerian women. Two certified whole blood materials were analyzed using the same digestion and analytical procedures used for freeze dried blood samples from our subjects. The result presented in table 2 indicated a very good agreement between the laboratory results and the certified values for the seven elements determined in this study.

Table 2: Results obtained for Reference Materials used for Quality Control

Element	PT-WB1:Lyophilised Whol e Human Blood Sample		L1-Seronom (Norway) Trace Elements Whole Blood Sample	
	Certified value	Laboratory value	Certified value	Laboratory value
	Mean ± SD			
Pb (ng/g)	159.0 ± 16.0	160.0 ± 13.0	23.0 ± 6.0	31.0 ± 5.0
Cd (ng/g)	3.3 ± 0.9	2.6 ± 0.3	0.7 ± 0.3	0.4 ± 0.1
As (ng/g)	8.5 ± 0.7	8.3 ± 0.9	1.8 ± 0.4	2.2 ± 0.4
Se (μg/g)	0.32 ± 0.04	0.36 ± 0.03	0.078 ± 0.005	0.079 ± 0.002
Cu (μg/g)	2.05 ± 0.25	1.79 ± 0.10	0.564 ± 0.033	0.533 ± 0.006
Zn (μg/g)	33.9 ± 4.2	33.7 ± 2.0	5.5 ± 0.3	5.2 ± 0.15
Hg (μg/g)	7.8 ± 0.9	6.73 ± 1.40	-	-

Table 3: Level of Heavy Metals in the Subjects' Blood and WHO limit

	As	Cd	Hg	Pb	Se	Cu	Zn
		ng /ml				μg / ml	
All Subjects (No)	62	62	61	61	62	62	62
Max.	13.0	0.7	13.5	150.9	1.1	2.3	10.7
Min.	0.8	0.04	1.5	24.6	0.2	0.8	3.6
Mean	3.9	0.2	4.1	68.3	0.4	1.1	5.9
Std dev.	2.8	0.2	2.5	26.2	0.2	0.3	1.3
Underweight (No)	10	10	10	10	10	10	10
Max.	6.0	0.5	3.9	131.2	0.5	2.3	9.5
Min.	1.4	0.04	1.7	39.6	0.2	0.9	3.6
Mean	3.0	0.3	2.8	70.9	0.4	1.3	5.8
Std dev.	1.3	0.2	0.66	27.3	0.1	0.6	1.7
Normal (No)	31	31	30	30	31	31	31
Max.	13.0	0.7	13.5	131.6	0.6	1.3	10.7
Min.	1.2	0.04	1.5	30.8	0.2	0.9	4.1
Mean	4.9	0.2	4.9	66.7	0.4	1.0	5.9
Std dev.	3.4	0.2	3.2	21.6	0.1	0.1	1.2
Overweight (No)	14	14	14	14	14	14	14
Max.	8.6	0.6	5.6	150.9	1.1	1.5	8.5
Min.	1.4	0.1	2.2	24.6	0.2	0.9	4.0
Mean	3.1	0.2	3.5	70.2	0.5	1.1	6.0
Std dev.	1.9	0.1	1.1	36.2	0.3	0.2	1.4
Obese (No)	7	7	7	7	7	7	7
Max.	4.0	0.3	4.7	111.7	0.7	1.3	8.4
Min.	0.8	0.1	2.4	35.5	0.3	0.8	4.3
Mean	2.6	0.2	3.6	63.1	0.4	1.1	6.2
Std dev.	1.2	0.1	0.9	24.6	0.1	0.2	1.3
WHO Blood concentration limit	<10.0	<0.3	<5.0	<10.0	<0.2	-	-

Table 3 contained the metal content of the subjects' blood samples. Four percent (4%), 11%, 19.3% and 98.4% of the subjects had their blood arsenic, lead, mercury and selenium levels, respectively in excess of the WHO recommended values while others were within. Heavy metals such as mercury, plutonium, and lead are toxic metals that have no known vital or beneficial effect on organisms. Their accumulation over time in the bodies of animals can cause serious illness (Reena *et al.*, 2011). Mercury in the mother's body can be passed to the fetus, where it moves into fetal brain. Once mercury enters the human body, it can remain for months (NRC, 2000).

The choice of tissues to be used as an index for intake levels of heavy metals in the body depends on the expected exposure pattern, the rate of contamination and the metal species involved under steady state conditions. When lead (Pb) enters the body, it travels through the blood to the soft tissues such as brain, liver and kidneys. In adults, most lead taken into the body is excreted, with a small amount stored in bones and teeth, where it can accumulate with repeated exposure (ATSDR, 2000; EPA, 2000b). For heavy metals like Pb and Hg, hair and nails are regarded as the most suitable indicators of intake levels. Age, health status, geographical location and sample preparation affect the uptake of these metals by

hair (Norman, 1970). Although, Chen *et al* (2007)'s finding support the hypothesis that dietary selenium intake may reduce the incident of arsenic-related premalignant skin lesions among population exposed to arsenic through drinking water. Values of the various physiological and biochemical parameters have been previously published and discussed (Ojo *et al.*, 2011).The Pearson's correlation among these important parameters of nutritional importance, anthropometric variables and levels of the seven heavy metals in subjects' blood is presented in table 4.

Table 4: Significant Correlations ($p < 0.05$) between the Subjects' Elemental Blood levels and Parameters of Nutritional Status

Elements	Nutritional status / Subjects				
	All (n = 62)	Underweight (n = 10)	Normal (n = 31)	Overweight (n = 14)	Obese (n = 7)
As	TC (0.27), P.Cr (0.28), Cr.Cl (-0.26), U.Cr (-0.36).	Forearm CF (-0.73), TC (0.64), P.Cr (0.71), Cr.Cl (-0.71), TC / HDL-C (0.64)	BP1 (0.36), U.Cr (-0.51)	None	P.Cr (0.77), Cr.Cl (-0.79)
Cd	Body fat (-0.25), Abdominal CF (-0.29), Cr.Cl (-0.31)	LDL-C (0.64), TC (0.63)	None	Waist CF (-0.59), Waist / Hip (-0.74)	PCV (-0.84)
Cu	Neck CF (-0.31)	U.Cr (-0.67)	Scapular SF (-0.50), Waist CF (-0.38), Waist / Hip (-0.39)	Abdominal SF (-0.56)	None
Hg	BP1(0.26), TG (-0.29)	None	Age (0.36), Height (0.36), Weight (0.38), Body fat (0.39)	LDL-C (0.65)	TG (0.77), TC (0.57)
Pb	PCV (0.29), Cr.Cl (-0.27), LDL-C / HDL-C (0.38)	Wrist CF (0.67)	Height (-0.38), Weight (-0.43), Hip (-0.38), Abdominal CF (-0.40)	Scapular SF (0.75), HDL-C/LDL-C (0.54)	Age (0.79), TBW (0.83), FFM (0.83)
Se	BP2 (0.26)	None	None	BP1 (0.59), BP2 (0.61), Biceps SF (-0.72)	None
Zn	PCV (0.31), TG (0.33), UA (0.24)	UA (0.64)	BP2 (0.39), PCV (0.44)	Waist CF (-0.70), Waist / Hip (-0.72), TG (0.56)	None

Value in parenthesis = Correlation coefficient legend should also explain all the abbreviations

Arsenic (As): The element correlated positively with blood Hg in all the nutritional status groups, except in the obese. Arsenic correlated positively with plasma creatinine and negatively with creatinine clearance in both underweight and obese groups (Table 4). Arsenic acid, furthermore, correlated positively with systolic blood pressure and negatively with urinary creatinine in normal subjects alone; positively with the ratio TC/HDL-C and TC in underweight group alone. In agreement with the present observation, Basu *et al* (2011) had earlier observed a strong positive correlation between urinary creatinine and arsenic acid. There was no significant correlation whatsoever between blood As and any of the biomarkers in the overweight group.

Cadmium: Blood Cadmium positively correlated with blood selenium in normal subjects, with arsenic in underweight group and with Zinc in the overweight group. The correlation between these heavy metals might indicate the same or similar anthropogenic source of the metals. There was no correlation with any biomarker in the obese group. Blood cadmium did not correlate with any of the biochemical parameters in normal and overweight groups. However, it correlated positively with TC and LDL-C in underweight subjects and negatively with PCV in obese subjects.

Copper: The liver is the main storage organ of copper as a constituent of some metallothioneins.

It occurs in various complex forms loosely bound to albumin, amino acids and to an alpha 2-globulin, ceruloplasmin. Blood levels of this essential element, Cu, did not have any significant correlation with any other element or with any biomarker in any of the nutritional status groups. The only exception was the significant negative correlation with urinary creatinine in the underweight group.

Mercury: Blood mercury correlated with blood As in all the nutritional status groups, except in obese subjects. This again may indicate a common anthropogenic source of these two heavy metals. There was no significant correlation with any biomarker in the underweight group but a positive correlation with body fat in normal group alone; with TC and LDL-C in overweight group alone and with triglycerides in obese group alone. Correlation between urinary Hg level and serum cholesterol had been reported earlier by Kim *et al.*, (2005). The correlation pattern between blood Hg and body fat in underweight subjects, though not significant, was actually inverse of that observed in the normal subjects as indicated in figure 1.

Lead: There was also no correlation between blood Pb and nutritional parameters in underweight and normal subjects. However in the overweight group, blood Pb correlated positively with the HDL-C / LDL-C ratio and scapular skinfolds (Sf) and the obese group in which age, total body water and fat free mass correlated positively with blood Pb. This may indicate bioaccumulation of Pb. Kim *et al.*, (2005) did not observe any correlation between Pb and blood pressure or cholesterol and did not investigate the HDL-C / LDL-C ratio. This would therefore be the first indication that overweight and obese people may be subject to ill effects of exposure to this metal than normal subjects.

Selenium: Also, the only significant correlation with the biomarkers was the positive correlation with both systolic and diastolic blood pressure – observed only in the overweight group. Selenium is however an essential element, necessary in very minute quantities by man for its antioxidant activities as well its being a vital component of some antioxidant enzymes such as glutathione peroxidase.

Zinc: Zn is stored in form of metallothioneins and proteins which are the neural growth factor (NGF), gustin of the saliva and cold precipitable protein of the plasma. It is believed to possess anti-oxidant properties, which protect against premature aging of the skin and muscles of the body. It has also been discovered to be very effective in accelerating wound healing. Also for the biochemical markers, blood Zn correlated with plasma creatinine and diastolic blood pressure (BP2) only in normal subjects; with uric acid in underweight only; and with triglycerides only in overweight group. Zinc can be found in man as a coenzyme of antioxidants and metabolic enzymes.

Table 5a: Significant (p<0.05) Correlations between Age / BMI; Some Biomarkers and Anthropometric Parameters in Nigerian Women

PARA-METER	ELEMENTAL	BIOCHEMICAL	ANTHROPOMETRY
Age	Normal: Hg (+) Underweight: None Overweight: None Obese: Pb (+) All: None	Normals: BMI, BF, BP1, BP2 (+) Underweight: UA(-) Overweight:HDL-C/LDL-C, TC/HDL-C, LDL-C, U.Cr (+) Obese:TC/HDL-C (+), BP2 (+) All:%BF, BP1, BP2	Normals:Wt, Ht (+) Underweight: None Overweight: None Obese: None All: ALL Parameters except wrist cm, chin sf, and height
BMI	Normal: None Underweight:None Overweight:None Obese:None All:None	Normals: %BF (+) Underweight:LDL-C/HDL-C, TC/HDL-C, P.Cr (+),:%BF (+) Overweight: TG (+),:%BF (+) Obese:None All:%BF, Cr.Cl, BP1, BP2	Normal: Age, Wt, 3 sf, 4cf (+) Underweight:Wt (+) Overweight: Ht (-) Obese:Wt, Chin sf, Wrist (+) All (+): ALL Parameters, except Waist/Hip

Note: sf = Skinfolds cf = Circumferences

Table 5b: Numerical Values for some Correlations of Elements with Biomarkers showing Influence of Nutritional Status

	Normal Subjects n = 31	Underweight Subjects n = 10	Overweight Subjects n = 14	Obese Subjects n = 7	All subjects n = 62
As vs P.Cr	0.202 (NS)	0.712* (0.02)	0.2446 (NS)	0.774* (0.02)	0.277* (0.05)
Hg vs %BF*	0.386* (0.05)	- 0.245 (NS)	0.193 (NS)	0.301 (NS)	0.155 (NS)
Pbvs LDL/HDL	0.159 (NS)	0.564 (0.01)	0.539* (0.05)	0.438 (NS)	0.377** (0.01)
Zn vs UA*	0.152 (NS)	0.638* (0.05)	0.24 (NS)	0.192 (NS)	0.239 (0.01)

Note: Values in brackets are the levels of significance * = Significant at 0.05 level ** = significant at 0.01 level

Table 4 is a summary of the correlation pattern between each of the seven elements (column 1) and other elements (column 2), the various biochemical markers (column 3), and anthropometric parameters (column 4) - for the four different Nutritional Status Groups, as well as for all the subjects combined. Similar data are presented in table 5a for correlations involving age, BMI,—biochemical markers and anthropometric parameters. The parameters with significant correlations are indicated in front of the group where such correlation is found. Positive Correlations are indicated with the "+" while negative correlations are shown with the "-" symbol. Where there is no correlation, this is indicated by "None". Tables 5b also contain other interesting correlations involving all the subjects taken together as a group. It is observed that blood arsenic levels correlated positively with total cholesterol (TC) in underweight as well as creatinine levels in both urine and plasma for the obese (Table 5); while blood mercury levels correlated negatively with TG (n = 62, r = 0.273 and -0.285 respectively). Cadmium concentration in subjects' blood (n = 62) also correlated negatively with body fat (r = 0.252) and creatinine clearance (r = -0.309). Similarly, at p< 0.05, blood lead level (BLL) correlated positively with PCV (r = 0.292) and LDL-C / HDL-C (r = 0.379) but negatively with Cr.Cl (r = -0.267). In the obese subjects alone, positive significant correlation was found between BLL and age (r = 0.792) as well as the length of exposure.

In the subjects with normal BMI, the blood mercury content correlated positively with age, weight, height and body fat (p < 0.05, r values between 0.364 and 0.761). This is an indication that any alteration in the levels of these metals in blood could directly or indirectly influence the values of some of the physiological and biochemical parameters of nutritional importance in the subjects observed

CONCLUSION
It has been established in this work that the concentrations of As, Pb, Hg and Se (out of the seven elements determined) in the whole blood of some subjects were above the WHO limits. Clear relationships have also been observed between the levels of these metals and some physiological/biochemical parameters of nutritional importance as well as anthropometry, generally and on the basis of their nutritional status. This suggests that interactions between trace elements and these important physiological/ biochemical parameters are influenced by the nutritional status of the individual subjects. This may also provide a basis for determining the effects of these toxic elements on the exposed subjects.

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