AFRICAN JOURNAL OF MEDICINE
and medical sciences

VOLUME 31, NUMBER 2 JUNE 2002

EDITOR:
B. O. OSOTIMEHIN

ASSISTANT EDITOR:
A. O. UWAIFO

ISSN 1116 — 4077

Afr. J. Med. med. Sci (2002) **31**, 131-135

Trace element status and early physical growth of exclusively Breastfed normal and asphyxiated Nigerian babies.

OO Adeodu, OA Jegede and GA Oyedeji
Department of Paediatrics and Child Health, Obafemi Awolowo University, Ile-Ife, Nigeria

Summary
Serial anthropometry and assay of plasma iron (Fe), zinc (Zn) and copper (Cu) were done on 72 babies with birth asphyxia during the first six months of life to assess the pattern of their physical growth and trace element status, respectively. Eighty-seven non-asphyxiated babies served as controls. The mothers of babies in both groups also had their plasma assayed for Fe, Zn and Cu immediately following delivery. Asphyxiated babies were lighter than non-asphyxiated controls during the first two months of life and they also had smaller mean occipito-frontal circumference at birth. While Fe, Zn and Cu status was generally comparable in both groups, the newborn Cu concentrations were very low compared with earlier reports. No baby showed any overt signs of trace element deficiency.

Keywords: *Trace elements, physical growth, babies, asphyxia*

Résumé
Pour évaluer la manière de la croissance physique et le statue d'oligo-élément respectivement, 72 bébés ayant l'asphyxie d'accouchement pendant leurs premiers six mois de vie ont été passés à l'anthropométrie en série et l'essai de plasma du Fer (Fe), du zinc (Zn) et du cuivre (CU).Quatre-Vingt sept bébés non-asphyxié ont servi de groupe de contrôle. Les mères des enfants dans les deux groupes ont passé un essai de Fe, Zn et du Cu juste après l'accouchement. Les poids des bébés asphyxiés étaient plus légers que ceux dans le groupe de contrôle dans leur deux premiers mois de vie. Ils avaient aussi un circonférence d'occiput-frontal de moyenne inférieur à la naissance. Tandis qu' il y avait une comparaison dans le statues de Fe, Zn, et Cu des deux groupes, les nouveau-nés ayant des concentrations Cu étaient très bas par contre à ceux qui étaient préalablement signalés. Il n'y pas d'évidence de carence d'oligo-éléments chez tous les bébés.

Introduction

Trace elements play an important role in nutrition, growth and development of humans [1,2]. Deficiencies of the essential trace elements may result in embryonal and foetal pathologies in animals and have been associated with different disease states in man [1,3,4] Iron (Fe), zinc (Zn) and copper (Cu) are perhaps the most important trace elements, particularly with regard to the functions they perform and their relative abundance in the human body. For example, Fe is a constituent part of the haemoglobin and myoglobin molecules and a co-factor to a large number of essential enzymes [5]. Similarly, Zn is a co-factor to more than seventy enzymes and it has regulatory roles in signal transduction pathways and gene transcription systems [5,6,7]. The trace element Cu is a component of a number of copper metallo-enzymes, including mitochondrial cytochrome oxidase and superoxide dismutase that are important in metabolism and oxidation-reduction reactions [4,6,8,9]

Fe, Zn and Cu deficiencies have all been associated with growth failure – physical, sexual and probably mental [3,4,10,11,12,13,14]. Low birth weight (LBW) is reportedly associated with deficiencies of these trace elements which are transferred actively to foetal tissues from maternal stores mainly in the third trimester of pregnancy [5]. Rapid anabolic processes such as the physical growth occurring during infancy [6] may constitute a great demand for trace elements [14] An infant's growth may therefore be retarded should he be deficient in the essential trace elements at birth or have sub-optimal replenishment in his feeds. With the exception of selenium (Se), the daily intakes of the essential trace elements of exclusively breast-fed infants are considerably lower than dietary recommendations [17]. However, the higher bio-availability of breast milk trace elements compared to cow milk formulas makes up for the relative deficiency [10,18]. Though human breast milk provides the essential trace elements required by the normal term baby until weaning, considerable changes occur in the concentrations of these micronutrients during the course of lactation [18,19]. For example, the concentration of Cu and Zn decrease during the course of lactation while that of cobalt generally increases [17].

The trace element status of the infant is therefore dependent on a host of factors which include maternal trace element status, gestational age at delivery, the type of milk feeds given, substances that modify trace element absorption in the infant like binding proteins [10,20] and a variety of other factors that may alter compartmentation of trace elements in the infant like chronic illness, interleukin - 1 and corticosteroids which are associated with catabolism of tissues [14]. The assessment of trace element status is therefore difficult considering the many factors that influence it. A single measurement of plasma trace element concentrations is clearly unreliable. While the most satisfactory method of assessment is the response to supplementation, the use of serial determinations of trace element concentrations, taken and interpreted in the context of the clinical situation can give fairly reliable data [14].

Tissue catabolism can cause mobilization, re-distribution and urinary loss of appreciable amounts of trace elements [14]. The risk of certain trace element deficiencies is apparently aggravated by catabolic states. For example, Richards *et al* [21] demonstrated that the brains of hypoxic chick embryos contained more Zn, Cu and Fe than normoxic controls. This may indicate increased trace element consumption as a selective tissue adaptation to hypoxia and may consequently influence both total body and plasma trace element concentrations in such embryos. It is unclear what effects birth asphyxia may have on trace element status in the human infant or whether newborns successfully resuscitated following moderate to severe birth asphyxia are disadvantaged with respect to trace element status compared to non-asphyxiated newborns.

It is however known that asphyxia constitutes a catabolic stress to the body. We hypothesized that the stress of asphyxia may cause consumption of trace elements in the human newborn and may negatively affect physical growth compared with non-asphyxiated babies. Therefore, the primary objective of this study was to use the serial determination of plasma Fe, Zn and Cu concentrations to assess trace element status of asphyxiated and non-asphyxiated babies at birth and during the first six months of exclusive breastfeeding so as to determine the effect of the temporary stress of asphyxia on trace element

Correspondence: Dr. O.O. Adeodu, Department of Paediatrics and Child Health, Obafemi Awolowo, Ile-Ife, Nigeria.

concentrations of the babies. The relationship, if any, between maternal and the newborn's trace element concentrations at birth and the pattern of physical growth of the babies were also to be determined during the period.

Methodology

One hundred and eighty one women with singleton pregnancies were consecutively recruited into study after giving informed consent. A total of 159 products of these pregnancies were however studied. Twenty-two babies were excluded from the study on account of perinatal death, severe illness, the receipt of transfusion with blood or blood products, placement on formular feeds or iron supplementation or because they were lost to follow-up at the consultant out-patient clinic during the period of study. The 159 babies were delivered vaginally or by caesarean section at the Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife, and they comprised 72 with moderate or severe birth asphyxia and 87 non-asphyxiated and apparently healthy newborns. The Apgar score at one minute was used to determine the severity of asphyxia and the need for and approach to resuscitation. Babies with scores 4 to 6 were considered moderately asphyxiated and those with scores 3 or less severely asphyxiated.

The indices used for the measurement of early physical growth were length, occipito-frontal circumference (OFC) and weight. Length was measured (in cm) with the infant naked and placed in a supine position, legs stretched straight out, on a small measuring couch with a fixed head-end and an adjustable foot-end. The OFC was measured (in cm) with an inelastic tape whose accuracy had been checked by placing it against a wooden measure. Weight was measured using a basinet weighing scale in which the infant was positioned supine after adjustment of the dial to zero point. The accuracy of the machine was checked from time to time by measuring the weights of standard orthopaedic iron weights. The average of two readings was taken as the correct measurement for each variable per sitting in all babies. These anthropometric recordings were made for each infant at birth on the hospital ward and at the second, fourth and sixth post-natal months at the consultant out-patient clinic.

Following successful resuscitation using standard techniques, 3 ml of peripheral venous blood was collected from each baby into heparinised bottles using broken 23 gauge needles. Maternal blood samples were collected at the same sitting. Plasma separation was done by centrifugation within 2 hours of blood collection and the plasma samples were stored frozen in 1 ml plastic insulin syringes at $-20^{\circ}C$ pending analysis for Fe, Zn and Cu concentrations. The procedure of blood sampling, plasma separation and storage was repeated for each baby at post-natal clinic visits at ages 2, 4 and 6 months.

Assays of plasma Fe, Zn and Cu were done on all samples at the Chemistry Department of the Ladoke Akintola University of Technology, Ogbomoso, using an Atomic Absorption Spectrophotometer [AAS] – Model 210 VGP, Buck Scientific Incorporation, Norwalk, Connecticut, USA, and standard procedure [22]. All tubes used for sample analysis were washed with nitric acid and rinsed with de-ionised water to prevent contamination from extraneous sources of trace elements. The plasma samples were prepared for trace element analysis by brief thawing and dilution (1 : 200) with de-ionised water which was also used for quality control. The AAS has a stand-by analysis mode of operation that is completed in less than one minute with the trace element concentrations displayed in ug/ dl in the liquid crystal display section. To obtain the actual concentrations of the trace elements the displayed concentrations were multiplied by the dilution factor of 200 and recorded.

Statistics

The mean and standard deviation(SD) were used as a measure of central tendency and dispersion of the numerical data, respectively. A basic assumption was made that the concentration of trace elements, like most human variables, is normally distributed in the population. This informed the selection of the sample sizes used in the study in conformity with the Central Limit Theorem [23] which accepts a sample size of at least thirty as representative of a large sample when considering variables that are normally distributed in the population. The Student's t-test was the inferential statistical test employed for comparison of the sample means of the two groups. Any difference was considered statistically significant if the p-value was less than 0.05.

Results

Ninety-two (57.8 %) male and 67 (42.2 %) female babies completed the study. The mean gestational age of the babies at delivery was 37.7 +/- 2.9 weeks. There was no significant difference between the mean gestational age at delivery of the 72 asphyxiated (37.3 +/- 3.3 weeks) and the 87 non-asphyxiated (38.1 +/- 2.4 weeks) babies. Twenty-seven (37.5 %) of the asphyxiated babies were delivered by mothers with serious complications of pregnancy, e.g,. ante-partum haemorrhage, hypertension, pre-eclampsia and eclampsia. Similarly, 33.3 % of the non-asphyxiated babies were products of eventful pregnancies. Sixty-six babies were of low birth weight, defined as birth weights less than 2.5 kg. The evolution of the babies' Apgar scores during the first five minutes of life is shown in Table 1. Only 2 of the 19 babies who were severely asphyxiated remained so at five minutes. However, all the babies were successfully resuscitated before age 10 minutes.

Table 1: Apgar scores in asphyxiated infants at 1 and 5 minutes of age

Apgar score	Number of babies 1 minute	5 minutes
0-3	19	2
4-6	53	47
7-10	87	110
Total	159	159

Eighty percent of mothers of recruited babies were booked. The mean age of all the mothers was 28.6 +/- 5.2 years. Majority (66.8 %) of them were within the age range 21 to 30 years. There was no statistically significant difference between the mean age of mothers of asphyxiated (28.8 +/- 5.2 years) and that of non-asphyxiated (28.4 +/- 5.2 years) babies. Similarly, the mothers of asphyxiated babies did not differ significantly from those of non-asphyxiated babies with regard to parity (2.5 +/- 1.7 and 2.5 +/- 1.5), height (154.7 +/- 15.3 cm and 150.4 +/- 32.5 cm) or weight (65.0 +/- 9 1 kg and 59.3 +/- 12.4 kg), respectively.

Maternal trace element concentrations at delivery

The mean plasma Fe, Zn and Cu concentrations for mothers of asphyxiated babies were 46.8 +/- 39.3 ug/dl, 45.2 +/- 49.9 ug/dl and 134.0 +/- 108.7 ug/dl, respectively. Corresponding values for mothers of non-asphyxiated babies were 41.8 +/- 31.2 ug/dl, 37.8 +/- 27.0 ug/dl and 162.5 +/- 106.6 ug/dl, respectively. The differences were not statistically significant.

Trace element concentrations of babies at delivery and in the first six months of life

Table 2 shows mean plasma Fe concentrations of asphyxiated and non-asphyxiated babies at birth and in the 2nd, 4th and 6th months of life. The mean values were slightly lower at each stage in the asphyxiated babies but the differences were not statistically significant. There was a wide variation in the mean plasma Fe concentrations of the babies during the period as evidenced by the large standard deviations. In both groups, the mean plasma Fe concentrations fell steadily from birth to age six months.

Table 2: Mean plasma Fe concentrations (ug/dl) of asphyxia ted and non-asphyxiated babies in relationship to age.

Age	Mean plasma Fe +/- SD Asphyxiated	Non-asphyxiated	p value
Birth	86.5 +/- 53.0	90.6 +/- 52.0	0.64
2 months	62.2 +/- 50.2	69.7 +/- 48.4	0.34
4 months	47.8 +/- 17.6	49.3 +/- 50.6	0.85
6 months	36.3 +/- 39.4	37.2 +/- 41.4	0.89

Tables 3 and 4 show the corresponding mean plasma Zn and Cu concentrations, respectively, for asphyxiated and non-asphyxiated babies. Mean plasma Zn values were lower in asphyxiated than non-asphyxiated babies although the differences between mean values were not statistically significant. Mean Zn concentrations fell in both groups from birth to age six months. Mean Cu concentrations of non-asphyxiated babies showed a gradual rise from birth to age six months while in asphyxiated babies there was an initial fall in the first four months followed by a rise such that their sixth month mean Cu concentration was comparable to that of non-asphyxiated controls. The differences noticed in the trends were however not statistically significant.

Table 3: Mean plasma Zn concentrations (ug/dl) of asphyxia ted and non-asphyxiated babies in relationship to age.

Age	Mean plasma Fe +/- SD Asphyxiated	Non-asphyxiated	p value
Birth	94.1 +/- 45.6	95.7 +/- 39.0	0.82
2 months	68.8 +/- 48.5	72.8 +/- 43.0	0.59
4 months	49.9 +/- 41.7	52.8 +/- 46.6	0.41
6 months	37.9 +/- 38.4	39.3 +/- 39.6	0.82

Table 4: Mean plasma Cu concentrations (ug/dl) of asphyxia ted and non-asphyxiated babies in relationship to age.

Age	Mean plasma Fe +/- SD Asphyxiated	Non-asphyxiated	p value
Birth	21.2 +/- 17.6	18.7 +/- 15.7	0.35
2 months	18.5 +/- 15.7	21.3 +/- 18.4	0.31
4 months	18.5 +/- 19.4	21.9 +/- 21.3	0.31
6 months	23.7 +/- 28.1	23.7 +/- 26.2	1.0

Mean mother : newborn trace element ratios at delivery

The mean mother : newborn Fe ratio at birth was 0.42 +/- 0.14 for asphyxiated babies and 0.44 +/- 0.12 for non-asphyxiated babies. Corresponding ratios for Zn were 0.45 +/ 0.10 and 0.41 +/- 0.12 and for Cu 7.0 +/- 2.9 and 8.1 +/- 2.5, respectively. The differences were not statistically significant.

Anthropometry

Table 5 compares the anthropometric data of the asphyxiated and non-asphyxiated babies from birth to six months of age. The mean birth weight of asphyxiated babies was significantly (2.6 +/- 0.9 kg) lower than that of non-asphyxiated (2. 8 +/- 0.6 kg) controls. Asphyxiated babies remained significantly lighter than non-asphyxiated ones for the first two months of life. Regarding occipito-frontal circumference (OFC), only at birth was there a statistically significant difference between the mean OFC of asphyxiated (31.1 +/- 7.5 cm) and non-asphyxiated (33.1 +/- 2.1 cm) babies. At no time during the period of study was there a significant difference between the mean lengths of asphyxiated and non-asphyxiated babies.

Table 5: Anthropometric data of asphyxiated and non-asphyxiated babies in relationship to age

Age (mo)	Mean weight +/- SD (kg)			Mean OFC +/- SD (cm)			Mean Length +/- SD (cm)		
	Asp	N-Asp	p*	Asp	N-Asp	p*	Asp	N-Asp	p*
Birth	2.6 (0.9)	2.8 (0.6)	0.05	31.1 (7.5)	33.1 (2.1)	0.03	42.1 (10.5)	44.3 (3.9)	0.10
2 month	3.1 (2.0)	3.7 (1.8)	0.05	32.8 (9.9)	35.1 (8.9)	0.22	48.3 (11.1)	51.7 (8.8)	0.29
4 month	4.3 (1.7)	4.4 (1.7)	0.81	33.8 (9.1)	35.6 (8.7)	0.91	53.4 (7.5)	55.2 (6.7)	0.69
6 months	6.2 (0.3)	6.6 (0.3)	1.0	40.4 (10.5)	42.7 10.7	0.10	62.5 (0.6)	63.4 (0.5)	0.86

SD in parentheses
OFC = Occipitofrontal circumstance
ASP = Asphyxiated babies
N-ASP = Non-asphyxiated babies
P = P value*

Discussion

In this study, plasma concentrations were used to assess trace element status. This method has its limitations particularly in respect of its inability to correctly reflect the dynamics of trace element status [10,14,20]. However, since plasma trace element concentrations were serially assessed at different ages in patients and controls, fairly reliable inferences may be made [14].

Mean plasma trace element concentrations of babies vary from one place to another [4,8,24,25,26]. For example, Bogden *et al* [25] found the mean plasma concentrations of Fe, Zn and Cu in cord blood of their patients to be 134.0 +/- 8.0, 103 +/- 4.0 , and 51.0 +/- 4.0 ug/dl, respectively. The mean Fe and Cu concentrations at birth for patients and controls in this study are significantly lower than and the Zn concentrations comparable to Bogden's results. The range of physiologic or normal trace element concentrations for adults and children in any given culture probably depends on the evolution of peculiar trace element homeostasis based on dietary exposure [27]. In this regard, trace element status of breasted babies may be a reflection of the adequacy or otherwise of trace elements in mothers' diet as well as the result of the materno-foetal bio-availability of these elements. In this study, Fe and Zn plasma concentrations were higher and Cu concentration lower in both groups of babies at birth in comparison with maternal values. Earlier reports from

Nigeria by Atinmo *et al* [28] and Ette and Ibeziako [29] had shown for Zn and Cu a similar relationship. Airede's work [30] on Nigerian full term newborns showed a triphasic pattern of evolution of Zn concentrations during the first six months of life. In contrast, our results indicate that Zn concentrations fell steadily throughout the period in both asphyxiated and non-asphyxiated babies. Since no newborn or infant in this study showed overt signs of Fe, Zn or Cu deficiency, the mean concentrations of the trace elements determined for mothers and babies in this study may lie within the normal range for the populations.

Mean plasma Fe, Zn and Cu concentrations of babies asphyxiated at birth did not significantly differ from those of non-asphyxiated controls anytime during the first six months of life. It would appear therefore that moderate or severe asphyxia, from which full recovery occurs, does not significantly affect trace element status of exclusively breastfed babies. Furthermore, the immediate post-delivery trace element status of mothers of asphyxiated babies was comparable to that of mothers of non-asphyxiated controls. Birth asphyxia in the babies studied was therefore probably due to inherent factors in the baby or to maternal factors other than trace element insufficiency.

The different patterns of the mean trace element concentrations of the asphyxiated and non-asphyxiated babies during the first six months of life are interesting. Mean Fe and Zn concentrations fell from birth till age six months in both groups. Mean Cu concentrations rose during the period in non-asphyxiated babies while in asphyxiated babies there was an initial fall during the first four months of life with a rise thereafter. The fall in the mean Fe and Zn concentrations is not unexpected. The iron content of breastmilk, the babies' sole nutrient source, is small and normally its Zn concentration falls as lactation progresses [3,7,10,31,32]. However, because of the excellent bio-availability of these two trace element in breastmilk, deficiency states do not supervene in healthy exclusively breastfed infants despite the demands of normal growth. Plasma Cu concentration, unlike Fe and Zn normally increases with age [33]. It is unclear why there was an initial fall in mean plasma Cu in the first four months of life in asphyxiated babies. Perhaps this was due to increased tissue uptake of Cu from plasma triggered off by the acute phase response to the stress of asphyxia [34].

The relationship between maternal and newborn trace element concentrations in this study is similar to the findings of Atinmo *et al* [28] and Ette and Ibeziako [29] except in respect of Cu. They found that newborn plasma Cu was about one-third maternal values. Bogden *et al* [25] had earlier shown that cord plasma Cu was approximately one-fourth of that in maternal plasma. In this study however, the mean plasma Cu concentrations of the babies at birth was only about one-eighth maternal values. The reason for the different mother : newborn plasma Cu ratio is unclear but the finding may be a normal variant may explained by differing maternal genetic or nutritional factors, the degree of stress to baby during delivery, the gestational age at delivery and the adequacy of the babies' liver store of Cu. Mean plasma Cu concentrations throughout the first six postnatal months were appreciably lower in the two groups of babies studied than earlier reports in the literature which range from 50.0 to 90.0 ug/dl [8,25,35]. This finding does not confirm Cu deficiency, but may suggest that these groups of Nigerian babies were at risk.

With regard to growth pattern during the first six postnatal months, the patients and controls were generally comparable but differed only in two respects. First, asphyxiated babies were lighter than non-asphyxiated ones during the first two months of life. Secondly, they also had significantly smaller mean occipito-frontal circumferences than non-asphyxiated controls. These differences are probably explainable by obstetric factors.

In conclusion, the physical differences noted at birth between the asphyxiated and non-asphyxiated babies was temporary. The trace element status of both groups was comparable. No baby showed overt signs of trace element deficiency. Mean newborn Cu concentrations were however much lower in the babies studied compared with reports on newborns by earlier workers. Larger studies would be desirable if reference values of trace element concentrations are to be determined for the Nigerian infant population.

References

1. Avtsyn AP. An insufficiency of essential trace elements and its manifestations in pathology. (Eng. Translation). Acta Pathol 1990; 52(3): 3-8.
2. Gibson RS. Assessment of trace element status in humans. Prog Food Nutr Sci 1989; 13(2): 67-111.
3. Shaw JCL. Trace elements in the foetus and young infant I. Zinc. Am J Dis Child 1979; 133: 1260-1267.
4. Shaw JCL. Trace elements in the foetus and young infant II. Copper, manganese, selenium and chromium. Am J Dis Child 1980; 134: 74-81.
5. Woodruff CW. Iron deficiency in infancy and childhood. Ped Clin North Am 1977; 24(1): 85-94.
6. Walravens PA. Nutritional importance of copper and zinc in neonates and infants. Clin Chem 1980; 26(2); 185-189.
7. Aggett PJ. Zinc. Annales Nestle 1994; 52(3): 94-106.
8. MacMaster D, Lappin TRJ, Halliday HL et al. Serum copper and zinc in the preterm infant. A longitudinal study of the first year of life. Biol Neonate 1983; 44: 108-113.
9. Kruse-Jarres JD. Rare essential trace elements. Annales Nestle 1994; 52(3); 138-141.
10. Oski FA and Stockman JA. Anaemia due to inadequate iron sources or poor utilization. Ped Clin North Am 1980; 27(2): 237-251.
11. Lozoff B, Brittenham GM, Viteri FE et al. Developmental deficits in iron deficient infants: effects of age and severity of iron lack. J Pediatr 1982; 101(6): 948-951.
12. Sivasubramanian KN and Henkin RI. Behavioural and dermatological changes and low serum zinc and copper concentrations in two premature infants after parenteral alimentation. J Pediatr 1978; 93(5): 847-851.
13. Walravens PA, Van Doornick WJ and Hambidge KM. Metals and metal function. J Pediatr 1978; 93(3): 535-538.
14. Hurley LS. Trace elements in prenatal and neonatal development: Zinc and Manganese. In: Chandra RK (ed). Trace elements in nutrition of children. Nestle Nutrition Workshop Series 1985; Volume 8: 121-135.
15. Lockitch G. Perinatal and paediatric nutrition. Clin Lab Med 1993; 13(2); 387-406.
16. Saha K, Kaur P, Srivastava G et al. A six month follow-up of growth, morbidity and functional immunity in low birth weight neonates with special reference to intrauterine growth retardation in small for

gestational age infants. J Trop Pediatr 1983; 29: 278-282.

17. Krachler M, Li FS, Rossipal E and Irgolic KJ. Changes in the concentrations of trace elements in human milk during lactation. J Trace Elements Med & Biol 1998; 12(3): 159-176.

18. Lonnerdal B. Effects of milk and milk components on calcium, magnesium and trace element absorption during infancy. Physiological Reviews 1997; 77(3): 643-669.

19. Vuori E and Kuitunen P. The concentrations of copper and zinc in human milk. Acta Pediatr Scan 1978; 68: 112-115.

20. BatesCJ and Prentice A. Breastmilk as a source of vitamins, essential minerals and trace elements. Pharmacology and Therapeutics 1994; 62(1-2); 193-220.

21. Richards MP, Stock MP and Meltalfe J. Effects of brief hypoxia and hyperoxia on tissue trace elements in the developing chick embryo. Magnes Trace Elem 1992; 10(5-6): 305-320.

22. Corney A. Atomic absorption spectroscopy. In: Atomic and Laser Spectroscopy. Oxford University Press London. Pages 310-313.

23. Spiegel MR. Theory and problems of statistics in SI units. Schaum,s Outline Series. Mcgraw-Hill Book Company, New York, page 142.

24. Krebs NF and Hambidge KM. Zinc requirements and zinc intakes of breastfed infants. Am J Clin Nutr 1986.43: 288-292.

25. Bogden JD, Thind IS, Kemp FW *et al.* Plasma concentrations of calcium, chromium, copper, iron, magnesium and zinc in maternal and cord blood and their relationship to low birth weight. J Lab Clin Med 1978; 92(3): 455-460.

26. Butte NF and Calloway DH. Proteins, vitamin A, carotene, folatin, ferritin and zinc in Navajo maternal and cord blood. Biol Neonate 1982; 4: 273-278.

27. Okonofua Fe, Amole FA, Emofurieta WO et al. Zinc and copper concentrations in plasma of pregnant women in Nigeria. Int J Gynaecol Obstet 1989; 29: 19-23.

28. Atinmo T, Mbofung C and Osinusi BO. Relationship of zinc and copper concentrations in maternal and cord blood and birth weight. Int J Gynaecol Obstet 1980; 18(6): 452-454.

29. Ette A and Ibeziako PA. Plasma zinc and copper concentrations in pregnant Nigerian women and newborn. Afr J Med Med Sci 1985 Mar-Jun; 14(1-2): 99-103.

30. Airede AI. Zinc levels in the Nigerian full-term newborn from birth to six months. East Afr Med J 1997 Apr; 74(4): 221-3.

31. Hambidge KM. The role of zinc and other trace metals in paediatric nutrition and health. Ped Clin North Am 1977; 24(1): 95-106.

32. Nyazema NZ, Mahomva O and Andifasi W. The levels of zinc in breastmilk of urban African women in Zimbabwe. Afr J Med Sci 1989; 18(2): 159-162.

33. Krachler M, Rossipal E and Micetic-Turk D. Concentrations of trace elements in sera of newborns, young infants and adults. Biol Trace Elem Res 1999; 68(2): 121-135.

34. Shenkin A. Trace elements and inflammatory response: implications for nutritional support. Nutrition 1995; 11(1 suppl): 100-105.

35. Van der Elst CW, Dempster WS, Woods DL *et al.* Serum zinc and copper in thin mothers, their breastmilk and their infants. J Trop Pediatr 1986; 32: 111-114.