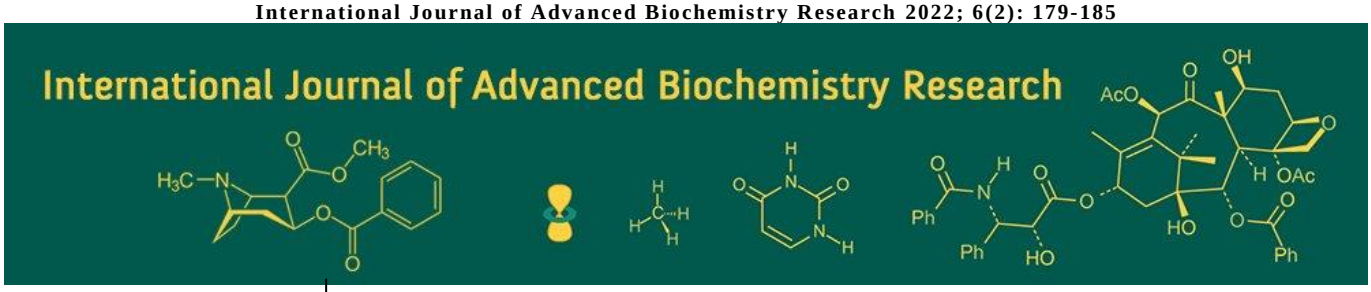

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Mineral composition and marker enzymes status of children on daily multivitamins intake

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Abstract
This study was designed to evaluate the effect of routine intake of multivitamin minerals supplements (MVMS) as children's routine drugs on serum marker enzymes, mineral components and their general wellness. Blood samples were obtained from 40 children within age range of 1 and 5 years old at Ekiti State Teaching Hospital, Ado Ekiti. Serum was obtained from the samples and the level of Potassium (K), Calcium (Ca), Zinc (Zn), Magnesium (Mg), Iron (Fe), Sodium (Na), Alkaline phosphatase (ALP), and Acid phosphatase (ACP) in the serum were determined using spectrophotometry method. Anthropometric parameters such as sex, age, weight, height as well as packed cell volume (PCV) were also determined. All values were expressed as the mean± standard error of mean (mean ±SEM). The significance of the result was calculated using a one-way analysis of variance (ANOVA). Values were considered statistically significant at $P<0.05$. The results showed a significant increase ($P<0.05$) in the children's weight, height and PCV. K, Ca, Mg, Fe, and Na levels were higher in children on routine MVMS than in children who do not. There is no significant difference in the levels of ALP and ACP in both groups of children ($P>0.05$). The results, therefore, suggest that routine intake of MVMS Improves the proper growth and development of children.

Keywords: Vitamins, supplement, micronutrients, children's health

1. Introduction
The importance of children's health in setting the stage for adult health reinforces the need to ensure that children are as healthy as they can be. This is also important in creating and fostering healthy families, and communities to power the workforce of any nation (National Research Council (US), 2004). Adequate nutrition especially for children is essential for proper growth, development, and total well-being. Poor nutrition, however, increases the risk of sickness and has been reported to be directly or indirectly responsible for one-third of the estimated 9.5 million deaths in 2006 in children under 5 years of age (WHO, 2009a) [9]. Inappropriate/inadequate nutrition can also increase the incidence and severity of infections which is the predominant cause of death, especially in Africa (Walson and Berkley, 2018) [14]. Malnourished children are characterized by stunted growth (low height for age), kwashiorkor, marasmus, obesity, increased risk of diarrhea, pneumonia, and reduced efficacy of vaccines, among others. They are also at significant risk of more severe and chronic diseases as well as high mortality and morbidity risk when infected (Walson and Berkley, 2018) [14]. It has also been reported that malnourished children have a longer healing and recovery period from infections, a higher risk of postoperative complications, abnormally low body temperature, and respiratory problems among others (Khan *et al*., 2017) [21]. Evidence existed from national dietary intake surveys reporting deficiencies and inadequacies in micronutrient intake and/or status of children, and that correcting these deficiencies can have tremendous health benefits as it is believed that healthy children are more likely to become healthy adults. (Blumberg *et al*., 2017) [2].
Childhood is the stage in a human's life associated with rapid growth and development. Growth proceeds expeditiously in early life, slowdown in middle childhood, and accelerates at puberty before linear growth ceases. With increasing age, physical and psychomotor maturation also influences activity, body composition, feeding skills, and food choices (Geissler, 2011) [8]. During early childhood and school-age years, children begin to establish habits for eating and exercise that remain for their entire lives. If children are healthy through the consumption of healthy habits, their risk of developing many chronic diseases will be greatly minimized.

Consequently, poor nutrition and physical inactivity during childhood set the stage for health problems in adulthood (Scaglioni *et al*., 2018) [3]. Nutrients in diet should correspond to the need of a growing child for healthy cognitive development (Nilsen *et al*., 2020) [19], quality social interaction, physical development and immunity.

Mothers are advised to breastfeed their children exclusively in the first six months of life. By the time a child is 6 months of age, the need for nutrients and energy begins to exceed what breast milk provides. There is thena need for complementary feeding that is nutritionally adequate and in certain cases, the use of multivitamins, even as breastfeeding is continued for up to 2 years or above, as the mother desires (WHO, 2009a) [9]. Although it is advisable for children to obtain their nutrients from diets, no risk is associated with a parent's desire to give his/her child a standard pediatric vitamin supplement. This is because, diets are most likely to be deficient in nutrients such as Iron, folate, vitamins A, B_6 and C (Haminder *et al*., 2020; Scaglioni *et al*., 2018) [3] especially in a developing country like Nigeria where many parents are unemployed and the poverty level is high.

Multivitamin Multimineral supplements (MVMS) are formulations containing an adequate amount of daily recommended common dietary vitamins and minerals including Vitamins A, C, D, E, B6, B7, B12, and zinc (Dwyer *et al*., 2021) [7]. Varying proportions of these nutrients are found in different commercially available MVMS products nationwide. MVMS are given to children to help them gain weight, improve blood production, boost their immune system, increase their appetite and even make them sleep well. They are taken generally to improve health and well-being, prevent developmental delay, and reduce the risk of chronic diseases (Sesso *et al*., 2022) [10]. Dietary MVMS use is reportedly common among children and adolescents. Approximately one-third of children and adolescents (persons aged ≤19 years) in the United States between 2013 -2014 use a dietary supplement within 30 days and the use varied by demographic characteristics (Stierman *et al*., 2020) [11]. They are also taken by adults with prevalence among women and cancer survivors. About one-third of adults in the US are reportedly on regular use of MVMS (Sesso *et al*., 2022) [10].

The use of dietary MVMS can broadly improve micronutrient intake when they contain at least the micronutrients that are consumed insufficiently or have limited bioavailability from the diet (Blumberg *et al*., 2018) [12]. The common multivitamins product used by participants in this study include Abidec syrup, Orphetal blood tonic, Well-Baby, Astyfers which consists majorly of vitamins A, B complex, C, amino acid, and essential minerals which varies between products

2. Materials and Methods

2.1 Study Design

40 children between the age ranges of 1-5 years were recruited from the Pediatrics unit of Ekiti State Teaching Hospital, Ado-Ekiti and grouped into two: Group A and Group B. Group A consists of 20 children on daily multivitamin supplements since 6 months old. Group B consists of 20 children without daily multivitamin intake except when on treatment for any sickness

2.2 Collection and Preparation of Sample

Blood samples were taken from children in each group into EDTA bottles. Serum was separated from the blood sample by centrifuging the sample in the micro-hematocrit centrifuge at 3000 revolutions per minute for 10 minutes. Serum was collected from the sample using a syringe and needle into the plain bottle.

2.3 Anthropogenic Measurements

The weight of all participants was measured using a weighing scale while height was taken using a standard measuring scale.

2.4 Determination of Potassium

2.4.1 Principle

Potassium tetra phenyl boron reacts with potassium ions in a protein-free alkaline medium to produce a turbid suspension of potassium tetra phenyl boron. The amount of turbidity produced is proportional to the potassium concentration.

2.4.2 Procedure

Pipette into centrifuge tubes		
	Macro	Micro
Sample	100Tl	50Tl
Precipitating Reagent	1000Tl	500Tl

Mix, and centrifuge at high speed for 5-10 minutes.

2.4.3 Potassium Assay

Wavelength	578nm, Hg 578n
Cuvette	1cm light path
Measurement	Against reagent blank
Temperature	+20 - +25^{0}C

2.4.4 Calculation

Potassium concentration

$$\text{Mmol} = \frac{\text{Asample}}{\text{A}_{\text{standard}}} \text{X } 5$$

2.5 Determination of Calcium

2.5.1 Principle

Calcium ions form a violet complex with O-Cresolpthalein Complexone in an alkaline medium.

2.5.2 Procedure

Wavelength	**nm (550-590nm) Hg 578**
Spectrophotometer	570nm
Cuvette	1 cm light path
Measurement	Against reagent blank only one blank required per series
Temperature: 37 °C	20-25 °C /

2.5.3 Calcium Assay

	Reagent Blank	**Standard**	**Sample**
Sample	-	-	-
Distilled water	25µl	-	-
Solution 1	-	25µl	-
Solution 2	0.5ml	0.5ml	0.5ml
Solution 3	0.5ml	0.5ml	0.5ml

Mixed thoroughly and read the absorbance of the sample (A_{sample}) and standard (A_{sample}) against the reagent blank after 5 to 50 minutes.

2.5.4 Calculation

$$\text{Concentration (mmol/l)} = \frac{A_{sample}}{A_{standard}} \text{ X } 2.50$$

$$\text{Concentration (mg/dl)} = \frac{A_{sample}}{A_{standard}} \text{ X } 10.0$$

2.6 Determination of Zinc

2.6.1 Procedure

Zinc present in the sample is chelated by 5-Br-PAPS2-(5-bromo-2-pyridylazo)-5-(N-propyl-N-sulfopropylamino)-phenol in the reagent. The formation of this complex is measured at a wavelength of 560nm.

2.6.2 Zinc Assay

Wavelength	**560 nm (550-570 nm)**		
Incubation Temperature	20/25 °C		
Cuvette	1 cm light path		
Pipette into test tube			
	Blank	Test	Standard
	H_2O	Sample	STD
Supernatant	0.5(0.2)ml	0.5(0.2)ml	0.5(0.2)ml
Working reagent	2.5(1.0)ml	2.5(1.0)m	2.5(1.0)ml

Mix well and incubate for 5 min at 25 °C. Measure the absorbance of the standard ($A_{standard}$) and the sample (A_{sample}) against the reagent blank within 60 minutes.

2.6.3 Calculation

$$\text{Zinc in µmol/l (µg/dl)} = \frac{A_{sample} - A_{blank}}{A_{standard} - A_{blank}} \text{ X standard conc. µmol/l (µg/dl)}$$

2.7 Determination of Magnesium

2.7.1 Principle

Magnesium ion forms a colored complex with xylidyl blue under alkaline conditions. The intensity of the developed color is proportional to the magnesium ion concentration of the sample.

$$Mg^{2+} + \text{xylidyl blue} \xrightarrow[OH^-]{} \text{Magnesium-xylidyl blue complex}$$

2.7.2 Magnesium Assay

Pipette into cuvette	
Blank Standard Sample	
Standard	10µl
Sample	10µl
Working reagent	1ml 1 ml 1 ml

Mix and incubate for 5 minutes then read the absorbance against blank.

2.7.3 Calculation

$$\frac{A_{sample}}{A_{standard}} \text{ x } C_{standard} = C_{sample}$$

A = absorbance, C = concentration.

2.8 Determination of Iron

2.8.1 Principle

At pH = 4.8 and in presence of ascorbic acid, trivalent ion [Fe (III)] dissociated from the transferring becomes reduced to divalent ion [Fe (II)] which forms a red complex with ferrozine. The absorbance read at 570nm is proportional to the ion concentration of the sample.

2.8.2 Procedure

Blank Standard Sample
Working reagent 1000µl 1000µl 1000µl
Distilled water 100µl
Standard 100µl
Sample 100µl

Mix and read absorbance (A) after 5 minutes incubation against reagent blank.

2.8.3 Calculation

$$\frac{A_{sample}}{A_{standard}} \text{ x } C_{standard} = C_{sample}$$

A = absorbance
C = concentration

2.9 Determination of sodium

2.9.1 Principle

Sodium tetra phenyl boron reacts with sodium ions in a protein-free alkaline medium to produce a turbid suspension of sodium tetra phenyl boron. The amount of turbidity produced is proportional to the sodium concentration.

2.9.2 Procedure

Pipette into centrifuge tubes
Macro Micro
Sample 100Tl 50Tl
Precipitating Reagent 1000Tl 500T

Mix, and centrifuge at high speed for 5-10 minutes.

2.9.3 Sodium Assay

Wavelength	578nm, Hg 578nm
Cuvette	1 cm light path
Measurement	Against reagent blank
Temperature	+20 - +25 °C

2.9.4 Calculation

$$\text{Sodium concentration mmol/l} = \frac{A_{sample}}{A_{standard}} \times 5$$

2.10 Determination of Serum Alkaline Phosphatase

Spectrophotometric detection using a fortress kit was used to measure total serum Alkaline Phosphatase (ALP).

2.10.1 Principle

The alkaline phosphatase acts on the AMP-buffered sodium thymolphthalein monophosphate. The addition of an alkaline reagent stops enzyme activity and simultaneously develops a blue chromogen, which is measured at 590nm.

2.10.2 Reagent Composition

R1 (substrate): Sodium Thymolphthalein Monophosphate (3.6 nM), 2-Amino-2-Methyl-1-Propanol Buffer pH10.2 (0.2 M), Magnesium Chloride (1.0 mM); R2 (colour reagent): Sodium Hydroxide (0.09 M), Sodium Carbonate (0.1 M); R4 (standard): 50 U/L.

2.10.3 Procedure

The table below described the procedure for the analysis.

Table 1: Procedure for ALP assay

Wavelength	**Temperature**	**Cuvette**	**Measurement**
590 nm (580-630 nm)	37 ºC	1 cm	Against reagent blank
Test tubes were pipetted into as follows			
	Reagent Blank	Standard / Samples	
R1 Substrate	500 ul	500ul	
Equilibration at 37 ºC for 3 minutes			
DDH_2O	50 ul	-	
Standard / Samples	-	50 ul	

2.10.4 Calculation

Total serum ALT in the sample was calculated using the formula below

$$\frac{\text{Absorbance of Unknown} \times \text{Value of Standard (IU/L)} = \text{Unknown (IU/L)}}{\text{Absorbance of Standard}}$$

2.11 Determination of Serum Acid Phosphatase

Serum acid phosphatase was measured using spectrophotometry method using Fortress kit.

2.11.1 Assay Principle

$$\text{1-naphthyl phosphate} + H_2O \longrightarrow \text{phosphate} + \text{1-naphthol}$$

$$\text{1-naphthol} + \text{Fast Red TR salt} \longrightarrow \text{azo dye}$$

Fast Red TR salt = 4-chloro-2-methyl phenyl diazonium salt

2.11.2 Reagent Composition

The concentration of the reagents used is described in the table below

Table 2: Reagent composition for ACP assay

R1 Buffer	**Citrate buffer**	**75mmol/l**
	Preservative	**0.1% w/v**
R2 Substrate	1-naphthyl phosphate	10mmol/l
	Fast Red TR salt	2.5 mmol/l
R3 Tartrate	Sodium Tartrate	135 mmol/l
R4 Stabilizer	Acetic acid	3.0 mmol/l

2.11.3 Assay Procedure

The table below described the assay procedure for serum ACP analysis.

Wavelength	**Temperature**	**Cuvette**	**Measurement**
Hg 405 nm	37ºC	1 cm light path	Against distilled water
The following were pipetted into test tubes at 37ºC			
		Macro	**Semi-micro**
Sample		200 ul	100 µl
Reagent solution 2(a) or (2b)		2000ul	1000 µl

The constituents of the test tube were thoroughly mixed and incubated for 5 minutes at 37 ºC. The initial absorbance was read, and absorbance was read again after 1, 2, and 3 minutes.

2.11.4 Calculation

$$\text{Total ACP Activity (U/l)} = \frac{\Delta\,\text{Absorbance of solution 2 (a)} \times 743}{\text{Minute}}$$

2.12 Statistical Analysis

All values were expressed as mean, standard error of mean (mean ±SEM). The significance of the result was calculated using a one-way analysis of variance (ANOVA). Values were considered statistically significant at $p<0.05$.

3. Results

Table 3: Results of Anthropometric parameters in children on daily vitamin C and multivitamins and those without.

Group	Age (Years)	Weight (Kg)	Height (cm)	Packed cell volume(g/dl)
A	3.12±0.02	16.2±0.15	109.1±0.04	13.9±0.21
B	3.21±0.03	14.15±0.11	94.1±0.05	9.9±0.19

Table 4: Concentration of Some Mineral Components in Children on Daily Multivitamins (A) compared with Children that Are Not (B)

Mineral components	Group A	Group B
Potassium (K)	3.40±1.0	1.89±1.0
Calcium (Ca)	3.91±1.1	2.98±0.9
Zinc (Zn)	0.01±1.0	0.01±0.9
Magnesium (Mg)	0.59±1.0	0.28±1.0
Iron (Fe)	.02±1.0	0.01±0.9
Sodium (Na)	11.2±1.0	9.6±1.0

Table 5: Concentration of Some Serum Marker Enzymes; Alkaline Phosphatase (ALP) and Acid Phosphatase (ACP) In Children on Daily Vitamin C and Multivitamins and Those Without

Enzymes	Group A	Group B
ALP (u/L)	14.2±1.30	13.99±1.19
ACP (u/L)	9.92±1.39	9.10±1.20

4. Discussion

The influence of MVMs intake on weight, height, and PCV (Table 3) was significant in children on daily intake (16.2 ± 0.15, 102.1±0.04, 13.9±0.21) when compared with children that are not on daily MVMS (14.15±0.11, 94.1±0.05, 9.9 ± 0.10) respectively. The indexes of physical development which describe nutritional wellness in children are age-appropriate weight (underweight), and age-appropriate height (wasting and stunting). Poor physical development is associated with poor nutrition which increases the likelihood of poor school performance, lower productivity as adults, chronic diseases, cardiovascular disease, diabetes, cancer and mental health Saha *et al*., 2022) [35]. Ekwochi *et al*., 2014 [15] observed a prevalence of anemia among the studied under-five children, especially among those of low social economic class. PCV or hematocrit, depending on the method used to determine it, is a measure of the percentage of red blood cells (RBCs) in given whole blood. It is done to determine if there is a decrease (anemia) or an increase (polycythemia) in total RBCs (Chidozie *et al*., 2020) [22]. Iron, vitamins B6, B12, C and copper are important nutrients that greatly influence the production of red blood cells in the body. This means that their deficiency can lead to anemia. Anemia is associated with a decreased capacity of the blood to carry oxygen to tissues resulting in symptoms such as fatigue, reduced physical activities, and shortness of breath, among others. It was reported by the WHO that the age range most vulnerable to anemia includes children under 5 years, especially those under 2 years of age (WHO, 2017) [24].

The level of potassium, calcium, magnesium, iron, and sodium was also higher (3.40±1.0, 3.91±1.1, 0.59±1.0, 0.02±1.0, 11.2±1.0)in children taking MVMS when compared with children who take multivitamins only when on treatment or who do not take at all (1.89±1.0, 2.98±0.9, 0.28±1.0, 0.01±0.9, 9.6±1.0) as shown in Table 4. Weaver, 2013 reported that a proper plasma potassium level is essential for normal heart functioning and potassium ion also take part in the normal functioning of skeletal muscle fibers. Going by the values from the results, the level of potassium in children taking a multivitamin on daily basis from age 6 months is normal and not above the standard level which is (3-3.5mEq/L) because according to He and MacGregor 2008 [4], excessive potassium level leads to hyperkalemia with the value of (6.0mEq/L) resulting in paralysis and cardiac disturbances.

Vitamins and minerals are essential for the development and optimal growth of children (Harinder *et al*., 2020) [36]. Potassium is an important nutrient essential for normal cell function and the maintenance of total body fluid (TBF) volume, acid, and electrolyte balance. WHO recommended an increase in potassium intake from food to control blood pressure in children of at least 90 mmol/day? (WHO, 2012) [17]. The reduced consumption of potassium has been associated with diseases such as hypertension, cardiovascular diseases, and stroke. An appropriate level of K^+ in the body could protect against these diseases although they disproportionately affect adults, they and their risk factors are now being detected more frequently in pediatric populations (WHO, 2012) [17].

Calcium is vital to developing and maintaining bones and teeth, particularly in children. Its intake is paramount in the determination of calcium status and assessing its deficiency. Adequate intake of calcium in childhood is most likely to reduce the risk of rickets, fractures, osteoporosis, and osteomalacia at the peak of life Its level is influenced by vitamin D and sodium (Shertukde *et al*., 2022) [26]. Boozing *et al*. (2018) called for increase in the intake of calcium and urgent specific strategies as a large proportion of the children they studied showed low calcium status. Tytusa *et al*., 2022 [18] observed that children calcium level in children can be increased by supplementation along with modified diet.

Zinc is also an essential mineral needed in minute quantities for the growth and function of the immune cells (B and T cells). It is involved in the synthesis and stabilization of macromolecules throughout the body cells such as proteins, RNA, and DNA. Furthermore, they are involved in gene expression, signal transduction, apoptosis, oxygen transport and anti-oxidative role against free radicals. Its deficiency in children increases the risk of developing infectious diseases such as pneumonia, sepsis, and diarrhea which has been implicated in several deaths across low-income countries (Banupriya *et al*., 2015) [32]. Several reports have shown that Zn supplementation has shown great benefits in protecting children against the incidence of several diseases and reducing the duration and severing of others such as diarrhea (Shimelis *et al*. 2008; Walker and Black 2010) [13, 27], respiratory infections (Hulisz, 2004) [28], Sickle cell anemia (Abdallah *et al*., 1988) [31], malaria (Shankar *et al*., 2000) [20], HIV (Bobat *et al*., 2005) [30] among others.

Magnesium is an important cofactor in several metabolic reactions which also helps maintain the integrity of cell membranes and more generally the healthy functions of the body (Cao *et al*., 2019) [33]. Its deficiency has been observed to occur more often among children with attention deficit hyperactivity disorder (ADHD), than among non-ADHD children. Furthermore, when ADHD children were placed on magnesium supplements, there was a decrease in hyperactivity and an improvement in cognitive functions (El Baza *et al*. 2015) [31]. Sodium is involved in the control of blood, and sodium chloride is the most common form of sodium which is marketed as table salt. Also, magnesium level is also seen to be higher in children on daily multivitamins (0.59±1.0) compared to children who take it only when on treatment (0.28±1.0).

Table 5 shows no significant difference between alkaline phosphatase (ALP) and acid phosphatase (ACP) levels in

children on routine administration of MVMS (14.2±1.30 u/L and 13.99±1.19u/L) and those without (9.92±1.39 and 9.10±1.20) respectively. Farhani (2013) [37] claimed that multivitamins have no direct impact on Liver marker enzymes.

5. Conclusion

From this study, it was shown that children who take multivitamins daily multivitamins than children who only take it when they are on the treatment or do not take at all. MVM is recommended to children especially in developing countries like Nigeria to prevent nutritional deficiencies.

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