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# Consumption of African Indigenous Vegetables Improves Children's body Fat Free Mass in Machakos County, Kenya

John Wakhanu<sup>1</sup>, Hudson Nyambaka<sup>2</sup>, Judith Kimiywe<sup>3</sup>, Mildred Nawiri<sup>4</sup> and Wilson M. Thagana<sup>5</sup>

<sup>1</sup>Kenyatta University and Department of Education, Presbyterian University of East Africa P.O Box 387-00902, Kikuyu, Kenya

<sup>2</sup>The Department of Chemistry, Kenyatta University, P.O Box 43844, Nairobi, Kenya

<sup>3</sup>The Department of Food, Nutrition and Dietetics, Kenyatta University, P.O Box 43844, Nairobi, Kenya

<sup>4</sup>The Department of Chemistry Kenyatta University, P.O Box 43844, Nairobi, Kenya

<sup>5</sup>The Department of Agriculture, Science and Technology, Kenyatta University-Kitui Campus, P.O Box 43844, Nairobi, Kenya

**Abstract:** School gardens growing African Indigenous Leafy Vegetables (AILVs) (*Amaranthus cruentus* and *Vigna unguiculata*) were established in Kangundo and Kilalani primary schools in Machakos County, Kenya and children aged 6-10 years (Kangundo,  $N = 66$ , Kilalani,  $N = 46$ ) that met the inclusion criteria participated as study subjects. There were two phases, I (13 weeks) and II (12 weeks) with 4 weeks in between to enable crossover of the school as either experimental or control. AILVs were grown in gardens of the experimental school. Study subjects in the experimental group were fed on the AILVs recipe with an accompaniment of a mixture of cooked maize and beans once a day, 5 days a week per phase. The control group fed only on the accompaniment. Body Mass Index (BMI) was determined and a prescribed dose of deuterium oxide was administered and deuterium enrichment determined by Fourier Transform Infrared Spectrometry for % Fat Free Mass (FFM) in children's saliva at baseline and endline. Serum Zn and Fe levels were analyzed by Atomic Absorption Spectroscopy at baseline and endline. Endline analysis in both phase I and II showed the % FFM, mean serum Fe and Zn were significantly higher ( $p < 0.001$ ) only for the experimental group. Food-based intervention through vegetable garden establishments has potential to eradicate malnutrition among school-going children in Kenya. Further, finding by previous studies that DDIM is more accurate in determining nutrition intervention outcomes in children than BMI is supported.

**Key words:** School gardens, body composition, vegetable recipes, malnutrition.

## 1. Introduction

A nutrition intervention ideally results in changes in body composition of individuals. The two-compartment model of body composition divides the body into fat mass (FM) and fat free mass (FFM). FFM includes the mass of bone, muscle, connective tissue, water and organs such as liver, kidney and adrenal glands [22]. FM is also called adipose tissue where fats are stored. The quantities and distribution of body fat and the composition of FFM (lean mass) are parameters used to measure nutrition intervention

[22], making body composition an indicator of both undernutrition and overnutrition [7, 33].

The true relationship between food intake and body composition shows a gap due to unreliable assessment methods. Many studies report nutrition outcomes based on BMI and this underestimates or overestimates nutrition outcomes [38]. Though easier to determine, BMI fails to distinguish FM and FFM in different races of populations and children [23, 32]. For example, an increase in BMI could be due to increase in FM and severe decrease in FFM and thus mislead prediction of health and nutritional outcome, since a decrease in FFM is indicative of poor health outcome [14, 21, 34]. Puberty, gender, age and

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**Corresponding author:** John Wakhanu, PhD candidate, research field: applied analytical chemistry.

ethnicity influence body composition of individuals but BMI does not detect these factors [8]. However, BMI is a useful parameter in predicting health outcomes in adults such as obesity, type II diabetes and eating disorders. The deuterium dilution isotope method (DDIM) was introduced by international atomic energy agency to fill this gap [22]. DDIM, which determines FFM and FM changes in intervention target groups, is accurate, simple to carry out, more reliable and requires very little study subject cooperation hence suitable for children, and is easy to use in field studies. Further, deuterium is non-radioactive isotope hence safe [22, 36].

However, there are no documented studies that have employed DDIM in Kenya to assess the outcome of consuming African indigenous vegetables (AILVs) sourced from school gardens by children. Most of the studies have used DDIM to assess nutrition outcomes of consuming food supplements and fortified foods. Diversified food-based intervention through vegetable garden establishments to eradicate malnutrition was recommended in the 2014 Kenya Demographic Health Survey (KDHS) and Micronutrient Survey's report [24]. The report revealed malnutrition among children aged 5-11 years in Kenya in general, and in Machakos County in particular. An extract of the statistics from the report for Machakos County, a semi-arid area in Kenya, indicates malnutrition manifested as stunting (26.3%), wasting (6.3%), underweight (12.7%), marginal VAD (33.9%), anemia (16.5%), zinc deficiency (82.5%) and iron (9.4%) [24].

AILVs contain high levels of protein, iron, zinc, carotenoids, calcium, anti-oxidants and vitamin C [1, 10, 31]. The use of AILVs sourced from school gardens to eradicate malnutrition comes against the backdrop that school gardens have many potential benefits. School gardens will influence policy development from stakeholders to integrate agri-food systems into solutions to malnutrition, optimize the production of AILVs and increase their accessibility and availability to the vulnerable groups. They will

enhance the participation of the school children together with their parents and provide them an opportunity to access nutrition information on diet diversification [15, 19, 20]. Further, they will increase consumption of fresh vegetables directly sourced from gardens, hence reducing post-harvest loss of nutrients as is the case with market-sourced vegetables, and alleviate the fear of consuming contaminated vegetables suspected to be grown in sewages [26]. With the benefits of establishing and promoting school gardens that grow AILVs on one hand and the advancement in chemical methods of assessment for a nutritive sensitive study on the other, scientific data to support the bridge of the two are presented from the findings of this study.

DDIM measures body composition by determining total body water (TBW) because water in the body is exclusively found within the FFM compartment, hence its estimation enables the determination of the FFM [22]. The body water pool naturally contains a small amount of deuterium ( $^2\text{H}$ ) which represents the natural abundance of  $^2\text{H}$  in body water and is usually close to 0.015% of hydrogen [22]. After collection of a baseline saliva, urine or milk sample, a known quantity of deuterium oxide ( $^2\text{H}_2\text{O}$ ) also known as  $\text{D}_2\text{O}$  (99.8% or 99.9%  $^2\text{H}$ ) is ingested. The dosing is done as per the weights of the study subjects. Children aged 6-10 years with a weight less than 30 kg are given 6-10 g of deuterium [17]. The  $^2\text{H}_2\text{O}$  equilibrates with the body water within a few hours [22]. The amount of deuterium in body water above that naturally present is known as the enrichment and reaches a "plateau" after 3-4 h in the body water after ingestion, by which time deuterium is uniformly distributed in saliva, urine, plasma and milk (for lactating mothers). Consequently body fluid samples can be collected 3 or 4 h after dosing. Participants should not drink water during equilibration period. After equilibration saliva, urine, plasma and milk contain the same concentration of deuterium and any can be used for the determination of the deuterium

enrichment in the body. Saliva is easier to use since its equilibration is faster than urine, plasma and milk. Working with saliva has lower risk of contamination and study subjects can collect saliva without assistance. Fourier Transform Infrared (FTIR) spectrometry has been used to measure deuterium in saliva and measured enrichment reported in  $\text{mg } ^2\text{H}_2\text{O}$  per  $\text{kg H}_2\text{O}$  (ppm) [22, 25].

DDIM was used to validate BMI, physical activity and dietary practices as methods for FM assessment among school children aged 8-11 years in Nairobi, Kenya and the study reported the limitations of BMI [30]. In that study 85.4% of the children found normal by BMI measurement were found obese by DDIM. This finding collaborated with that in a study on BMI verses DDIM for establishing childhood obesity prevalence in eight African countries [12]. In this study the prevalence of excessive fatness by DDIM was three times higher than that by BMI. Similarly the FFM and FM of Senegalese children aged 8-11 years ( $n = 151$ ) was determined using DDIM to validate bioelectric impedance analysis in predicting total body water (TBW) and adiposity among the children [13]. In this study deuterium enrichment in saliva samples of the children was measured using FTIR spectroscopy. The mean ( $\pm$  SD) body weight, height, BMI and height for-age-Z score (HAZ) were  $28.2 \pm 6.5$ ,  $137.2 \pm 7.8$ ,  $-1.34 \pm 1.20$  and  $-0.19 \pm 1.07$ , respectively, with 3 children suffering from stunting ( $\text{HAZ} < -2$  z-score). The mean ( $\pm$  SD) TBW (kg), FFM (kg) and FM (kg) was  $17.2 \pm 2.7$ ,  $22.8 \pm 5.7$  and 4.4. Only 1.9% of the children were obese by BMI but 11% were obese by DDIM. This finding highlighted the limitation of BMI in the determination of body composition in children.

The effects of consumption of AILVs, food supplements and fortified foods on body composition have been highlighted in a number of studies [37]. The FFM of an individual is due to growth of lean tissue as a result of micronutrients like zinc, iron, chromium and vitamins, like beta carotene obtained

from diet. Since vegetables have been demonstrated to contain high levels of these micronutrients, their consumption is likely to have an effect on the FFM of an individual. For example, zinc stimulates appetite and energy intake to enhance FFM [3]. In one study, zinc given to 6-8 months old Peruvian children, who suffered from mild to moderate stunting, increased their FFM by 0.41 kg than those who did not get the zinc supplement, leading to the conclusion that stunted children could be zinc deficient [3]. Zinc plays a critical role in growth and development; it is cofactor for enzymes that control cell division and proliferation. Zinc deficiency impairs the synthesis of DNA, RNA and protein which has implications on the FFM [28]. Iron is also another important cofactor in the synthesis of amino acids and its deficiency has an effect on metabolism of glucose, lipid biosynthesis and amino acid biosynthesis. Iron is required for energy generation and also for DNA and RNA synthesis which in turn affects FFM [39].

A study was done on the efficacy of dried amaranth leaves (*Amaranthus cruentus*), incorporated in fermented maize flour, on vitamin A, iron and zinc status of children in Kajiado County, Kenya [9]. The study reported a significant increase ( $p = 0.044$ ) of zinc from  $9.94 \pm 1.2 \mu\text{mol/L}$  to  $12.78 \pm 1.4 \mu\text{mol/L}$  and iron ( $p = 0.043$ ) from  $10.18 \pm 1.6 \mu\text{mol/L}$  to  $13.11 \pm 1.7 \mu\text{mol/L}$  at endline for the experimental group with no significant change in the control group. The study concluded that consumption of fermented porridge mixed with dry amaranth powder for six months significantly increased the serum iron and zinc of the experimental children. This highlighted the potential of amaranth in eradicating malnutrition in children. In a study on the health outcomes of a subsidized fruit and vegetable program for disadvantaged aboriginal children, the experimental children fed on a diet of fruits and vegetables for a period of 12 months and their iron and hemoglobin levels were analyzed at baseline and endline [6]. The study reported an improvement in hemoglobin and

iron concentrations in children's sera. The hemoglobin count was 12.68 g/dL at baseline and 12.82 g/dL at endline. At baseline the mean iron levels in the aboriginal children were 12.7  $\mu\text{mol/L}$  and at endline were 13.2  $\mu\text{mol/L}$ .

A study on the effect of zinc supplementation on growth and body composition in children with sickle cell disease reported that girls who received zinc supplementation showed a significant increase of FFM by 0.87 kg [4]. The experimental group had an increased linear growth, weight and appetite than the control group who received placebo. The study concluded that zinc deficiency is manifested as poor growth and delayed skeletal and sexual maturation in children. Similarly a group of 20 children who received oral zinc supplementation, mixed with multivitamin, showed significant increase in appetite and significant weight gain in comparison to those who only received oral multivitamin. Increase in weight was associated with FFM increase [35]. The children showed 80% weight increase associated with FFM (lean body tissue) without increase in body fat. The study found out that weight gain of zinc supplemented children was associated with formulation of lean tissue instead of adipose tissue and that zinc improves test equity, hence increased appetite.

In a related study in Senegal on people living with HIV-Aids (PLWA), vegetable soup containing peanut butter and skimmed milk powder fortified with Vitamin-Mineral complex was given to PLWA [2]. This recipe was rich in  $\beta$ -carotene, zinc, iron, calcium and magnesium. The control group was not fed on the recipe. The results showed that consumption of the recipe for 3 months by experimental groups showed a more significant increase in body weight, FFM, FM, and haemoglobin than in the control groups. FFM increased by 11.8% while FM by 10.7%. The increase in FFM in PLWA was attributable to vitamins and minerals in the recipe.

An intervention study in which pre-pubertal,

non-zinc deficient (8-9 years old) children were divided into experimental (31) and control (31) was done [29]. The former received zinc supplementation while the latter were given placebo in three months. The experimental group showed significant increase in soft tissue, mainly FFM than the control group [29]. The % FFM increase ranged from  $80.03 \pm 4.64$  to  $81.13 \pm 4.35$  and % FM ranged from  $18.87 \pm 4.35$  to  $19.97 \pm 4.80$ .

Studies on the effect of the combination of iron, zinc and vitamins on FFM have also been done. Weight, length and micronutrient status were determined in a study on the effect of a combination of zinc, iron and vitamin A on 800 infants aged 3 months in rural East Lombok, West Nusa Tenggara for a period of 6 months [18]. Zinc alone disadvantaged the haemoglobin and iron status of the subjects. Both zinc and iron combination improved both the zinc and the iron status while zinc, iron and vitamin A realized the highest increase in vitamin A and Haemoglobin. The height of the study subjects increased by 1.1-1.5 cm more than those with placebo. The study concluded that zinc intake can only have a positive effect on FFM if low haemoglobin, iron status and Vitamin A are also addressed and corrected. Similar findings were obtained in an investigation on the efficacy of combined iron and zinc supplementation on micronutrient status and growth in 915 Vietnamese infants [5]. The infants were divided into 4 groups: Group 1 received 10 mg of iron daily for six months, group 2 received 10 mg of zinc, group 3 received 10 mg iron plus 10 mg zinc and group 4 received a placebo. Weight gain was higher in the zinc group while serum ferritin and haemoglobin was higher in the iron and iron plus zinc groups (22.6 g/L and 20.6 g/L for haemoglobin; 36.0 and 24.8 mg/L for serum ferritin) and lower in placebo groups (haemoglobin: 6.4 and 9.8 g/L serum ferritin -18.2 and -16.9 mg/L).

The effectiveness of food supplements in increasing FFM in children with moderate acute malnutrition in

Burkina Faso was investigated [17]. To assess FFM, a dose of 6 g of deuterium oxide (D<sub>2</sub>O) (99.8%) diluted in 5 g of mineral water was given orally after collection of pre-dose saliva samples. Post-dose saliva samples were collected after 3 h equilibration period and D<sub>2</sub>O abundance was measured by FTIR. Baseline mean weight was 6.91 kg and baseline mean percentage FFM was 83.5%. At endline the investigation revealed a weight increase of 0.90 kg and FFM increase of 93.5%. The study concluded that children with malnutrition when nutritionally rehabilitated with zinc, iron, and vitamin A put on predominately FFM. In another study, it was demonstrated supplementary food increased FMM and BMI in Haitian school-aged children [27] after FM increased  $0.73 \pm 0.34$  kg compared to the control who never received the supplement.

## 2. Materials and Method

### 2.1 Chemicals, Reagents and Operational Parameters for Analytical Instruments

Deuterium oxide liquid was for determination of total body water. The electronic balance (Shimadzu Corporation Japan AT × 224, max. 220g, min. 10 mg with a readability of 0.1 mg), accurate to 0.0001 g, was used for weight measurements. Sterile non-toxic, non-pyrogenic, revival healthcare syringes were used for blood sampling. Centrifuge (Hettich Zentrifugen EBA 20, D-78532 Tuttingen-Germany) set at 2,500 rpm at room temperature for 10 minutes was used to centrifuge blood. Fourier transform infra-red spectrometer (IR Tracer-100 FTIR SHIMADZU) was used to analyze deuterium in saliva samples. The FTIR instrument was set with absorbance as the measurement mode, apodization was square triangle, number of scans was 32, resolution was 2.0 and range was minimum 2,300 cm<sup>-1</sup> and maximum 2,900 cm<sup>-1</sup>. Healthcare weighing machine (Salter scale model 2006) and UNICEF scale were used to measure weight and height of study children respectively. Concentrated sulphuric acid and hydrogen peroxide

were used for extraction of iron and zinc from the AILV recipe. Ferric nitrate and zinc nitrate were used to prepare standard for calibration. Atomic absorption spectrophotometer (Buck Scientific, model 210 VGP) was used for analysis of iron and zinc in the AILV recipe. Whatman No. 42 filter papers into 100 mL volumetric flask and distilled water were used for preparation of standards.

### 2.2 Study Design

This was a 13-week food intervention study conducted in Kangundo and Kilalani primary schools in Machakos County, Kenya in the year 2018. The study was reviewed and approved by the National Ethical Review Committee at Kenyatta University and permit was obtained from the National Commission for Science, Technology and Innovation (NACOSTI). The study involved children aged 6-10 years who were fed on a recipe of *Amaranthus cruentus* and *Vigna unguiculata*. Kangundo and Kilalani primary schools were purposively selected from 67 public primary schools within the study area based on their accessibility, number of children, accessibility to water for irrigation, land for school garden and an ongoing lunch programme. There were two phases, I (13 weeks) and II (12 weeks) with 4 weeks in between to enable crossover of the school as either experimental or control. In Phase I, study children, from Kangundo primary school were assigned to the experimental group while those in Kilalani primary were assigned to the control group. In Phase II, the roles of the schools were interchanged. In Phase I, there were 76 study children in the experimental group and 49 for the control group for a sample size of  $n = 125$  [11]. Following drop outs, there were 66 and 46 study subjects in the experimental and control groups respectively in Phase I.

### 2.3 Establishment of School Garden

Green amaranth was planted on a quarter of an acre while cowpea was planted on a half of an acre at the

experimental school. Couch grass and other weeds were eliminated by spraying with Roundup herbicide. Furrows were made and 4 kg of diammonium phosphate (DAP) fertilizer was mixed with soil in the furrows prior to planting cowpea seeds (type M66) sourced from Kenya Agricultural Research Institute in Katumani, Machakos County. Green amaranth seeds were broadcasted on top of the prepared soil and covered with maize stovers (mulching) to provide humus and retain moisture required for germination. Green amaranth was top dressed with calcium ammonium nitrate (CAN) two weeks after germination and a dose of superglo was applied to make them leafier and develop more biomass. The vegetables were ready for harvest and use as recipe for the intervention 21 days after germination.

#### 2.4 Preparation of Vegetable Recipe

During both phases, the experimental school was supplied with vegetable preparation and cooking accessories which included fuel, tomatoes, onions, cooking oil and salt. The harvested *Amaranthus cruentus* and *Vigna unguiculata* vegetables were cleaned under clean running water and whole leaves were chopped into small pieces and mixed in the ratio of 1:1 (weight/weight). The mixture was cooked as per the local community procedures of boiling the vegetables before frying with oil and adding tomatoes and onions. Each study subject in the experimental group consumed an average of 80 g (wet weight) of the recipe with an accompaniment of a mixture of cooked maize and beans, once a day, 5 days a week, per phase. The control group fed only on the accompaniment. Parents/caregivers were allowed in school during all procedures including sample collection, deworming and feeding. Prior, meetings were held between the researcher, parents and pupils to explain the study purpose, procedures and roles during the study. For inclusion, consent was sorted from parents or guardians. Children who had been ill or hospitalized two weeks prior to the study were

excluded.

#### 2.5 Anthropometric Measurement, Blood and Saliva Sampling

The weight and height of the study subjects were taken by a qualified nutritionist. The weight and height indices were converted to Z-scores and BMI to classify the nutrition status of the target population. Age was obtained from the class teacher's records that had been verified using the child's health card, birth certificate or baptismal card. The study subjects were then dewormed using an anthelmintic drug (albendazole syrup, 10 mL of 400 mg/child). Approximately 5 mL of blood for both the experimental and control subjects was collected at baseline and endline during both phases I and II through venipuncture of an antecubital vein (peripheral vein) by trained personnel from the Ministry of Health (Kangundo Hospital). The sample was dispensed into trace-element free tubes and transported on ice packs in a cool box to Kangundo hospital laboratory for centrifugation to obtain serum. The separated serum was transferred into clearly labeled cryo tubes and transported on ice packs in a cool box to Kenyatta University laboratory for refrigeration at  $-80^{\circ}\text{C}$ . Each sample was used for the analysis of both Fe and Zn.

Deuterium oxide ( $\text{D}_2\text{O}$ ) liquid (99.8%) was diluted with tap water. A 1:5 dilution of the  $\text{D}_2\text{O}$  was prepared by adding 800 g (800 mL) of tap water to 200 g (180 mL, density of  $\text{D}_2\text{O}$  is 1.105 g/mL) of  $\text{D}_2\text{O}$ . The weight of both the  $\text{D}_2\text{O}$  and the added tap water was recorded to 0.01 g. The study subjects consumed 30 mL of the diluted  $\text{D}_2\text{O}$  liquid and were starved with minimal motions for 3 h to allow the  $\text{D}_2\text{O}$  to equilibrate with body water, and also minimize water loss through sweating. A pre- and post-dose saliva collection was done by qualified medical staff according to IAEA standard operating procedures by placing two cotton balls in the mouth of the study subjects for 2 min. The cotton balls were sodden with

saliva and transferred to a clean 20 mL syringe. The saliva was squeezed out of the cotton balls into another clean sterile vial using a syringe plunger and the vial tightly sealed and labelled. Collected samples were kept in cool boxes and transported to Kenyatta University laboratory and kept in the freezer at -80 °C. Post-dose saliva was sampled 3 h after administration of the D<sub>2</sub>O dose in a similar manner.

#### *2.6 Laboratory Procedures for Analysis of Deuterium Zinc and Iron*

To prepare 1,000 ppm of the calibration standard, 2 g of deuterium oxide was diluted to 1 L with water and transferred to a clean, dry glass bottle and stored in a cool place. A similar volume of water was kept for use as a blank to measure the background spectrum. Working standards of 0 ppm, 100 ppm, 200 ppm, 400 ppm, 600 ppm, 800 ppm, 1,000 ppm, 1,500 ppm and 2,000 ppm were prepared by pipetting 5 mL, 10 mL, 20 mL, 30 mL, 40 mL, 50 mL and 100 mL of 2,000 ppm of D<sub>2</sub>O stock solution and then diluted with water in 100 mL volumetric flask. Exactly 1 mL of each of the working standards was introduced to the FTIR cell using a 1 mL syringe and scanned. The peak area of the working standards was plotted against the concentration of the respective working standard to obtain a standard calibration curve.

The vials with saliva samples were allowed to thaw for 1 h and centrifuged for 15 min at 1,000 rpm to remove bubbles. The samples were then introduced into the instrument and the peak areas determined from which the concentration of deuterium oxide was obtained by regression analysis. To obtain the body FFM, the TBW of study subjects was determined by dividing the gravimetrically determined mass of the dose of deuterium water consumed in mg by the respective reading of the FTIR instrument in ppm (mg/kg).

To extract iron and zinc, serum samples were left out to thaw for 10 min and 1,000 µL of the serum was transferred to 50 mL beakers. Exactly 20 mL of

distilled water was added to the samples followed by 8 mL of concentrated sulphuric acid and 2 mL of hydrogen peroxide. The samples were then placed on a hot plate at 150 °C for 10 min and thereafter filtered through whatman filter paper grade one. The filtrate was transferred into 50 mL volumetric flask then topped up to the mark with distilled water and transferred into 60 mL storage containers for AAS analysis for iron and zinc.

To analyze iron in the serum samples a standard iron stock solution of 1,000 ppm was prepared by weighing 1.083 g of ferric nitrate in a small beaker and dissolving it in 50 mL of distilled water. The resulting solution was transferred into a 250 mL volumetric flask and topped to the mark using distilled water. A working standard of 100 ppm was prepared from the stock solution by serial dilution. Working standards of 0 ppm, 4 ppm, 6 ppm and 8 ppm were then prepared by pipetting 0 mL, 4 mL, 6 mL and 8 mL of the 100 ppm standard solution respectively into 100 mL volumetric flask and topped up to 100 mL using distilled water. The standards were aspirated into the AAS instrument to obtain their absorbance. A standard calibration curve was obtained by plotting absorbance versus the respective concentration of the standards to give the regression equation. The extracted samples were then also aspirated into the AAS instrument to obtain their absorbance. The concentrations of the samples were obtained by inserting the absorbance into the regression equation ( $Y = 0.138X - 0.0100$ ), where  $Y$  is the absorbance and  $X$  is the concentration of iron in the sample.

To analyze zinc in the serum samples, a standard zinc stock solution of 1,000 ppm was prepared by accurately weighing 0.724 g of zinc nitrate in a small beaker and dissolving it in 50 mL of distilled water. The resulting solution was transferred into a 250 mL volumetric flask and topped to the mark using distilled water. Working standards were prepared as per procedure for iron above. The concentrations of the samples were obtained by inserting the absorbance



into the regression equation ( $Y = 0.270X - 0.003$ ).

Statistical data analysis was performed using Statistical Package for Social Sciences (SPSS) version 21 software. Data were presented in form of tables. Independent *t*-test was used to compare the mean FFM, Zn and Fe between the experimental groups and control groups at baseline and endline in both phases I and II. Paired *t*-test was used to compare the percentage mean FM and FFM at baseline and endline for the experimental school and the control school in both phases I and II.

### 3. Results and Discussion

Table 1 shows levels of iron and zinc in the serum of the study children in both groups for both phases I and II. In phase I independent *t*-test showed no significant difference at baseline in the mean serum levels of Zn and Fe between the experimental group and control group ( $p > 0.05$ ). However, at endline the mean levels of Zn and Fe in the serum of the experimental group were significantly higher than for the control group ( $p < 0.001$ ). This is attributable to the consumption of the AILV recipe by the experimental group. Paired *t*-test showed that the mean levels of Zn and Fe in the serum of experimental group after feeding on the recipe for 13 weeks significantly increased ( $p < 0.001$ ) but no significance difference for the control group after the same period of time ( $p > 0.05$ ) for the two micronutrients.

Findings in phase II support the view that phase I results were not a coincidence but were due to the consumption of the vegetable recipe by the experimental group. The experimental group in phase II had been the control in phase I and at baseline had lower mean levels of Zn and Fe compared to the control in phase II which had been experimental in phase I. On intervention for 12 weeks in phase II the serum mean levels of Zn and Fe significantly increased ( $p < 0.001$ ) but not for control group in the same period ( $p > 0.05$ ). These results are comparable to findings by Ref. [9] in a study on the efficacy of

dried amaranth leaves (*Amaranthus cruentus*), incorporated in fermented maize flour consumption on vitamin A, iron and zinc of children in Kajiado County, Kenya. There was a significant increase in the serum iron and zinc at endline for the experimental group. The experimental group's serum iron mean contents at baseline and endline were  $10.18 \pm 1.6$   $\mu\text{mol/L}$  and  $13.11 \pm 1.7$   $\mu\text{mol/L}$  respectively while zinc mean contents at baseline and endline were  $9.94 \pm 1.2$   $\mu\text{mol/L}$  and  $12.78 \pm 1.4$   $\mu\text{mol/L}$  respectively.

The iron levels reported in the present study are slightly more than those by Ref. [9]. However, the mean zinc levels in a study by Ref. [9] are slightly higher than in the present study. These correlate with findings by Black and co-workers [6] who reported baseline mean iron levels in the aboriginal children of  $12.7$   $\mu\text{mol/L}$  but at endline were  $13.2$   $\mu\text{mol/L}$ .

The significant increase in the mean serum Zn and Fe levels of the experimental groups after consumption of the vegetable recipe in the present study correlated with a significant increase in mean % FFM and FM in the same experimental groups, suggesting an effect of Zn and Fe on the body composition of the study children. Zinc stimulates appetite and energy intake to enhance FFM [3] which plays a critical role in growth and development; and is cofactor for enzymes that control cell division and proliferation. Zinc deficiency impairs the synthesis of deoxyribonucleic acid, ribonucleic acid and protein which affect FFM [28]. Iron is also a cofactor and is required for energy generation and also for deoxyribonucleic acid and ribonucleic acid synthesis which in turn affect FFM [39].

Table 2 presents the body composition (mean FFM and FM) and mean BMI of the phases I and II control and experimental groups. In phase I the statistical comparison revealed no significant difference in the mean BMI for both control and experimental groups at baseline ( $p = 0.156$ ) and endline ( $p = 0.540$ ). The mean percentage FFM and FM at baseline for the children from the two study groups did not differ

significantly ( $p = 0.826$ ) but differed significantly at endline ( $p < 0.001$ ). The significant increase in %FFM, %FM, FFM and FM at endline in the experimental group can be attributed to the consumption of the vegetable recipe by the experimental group. Increase in FFM implies growth of soft tissue as a result of increased cell division due to the nutrients supplied by the AILVs consumed. The phase I baseline mean percentage FFM for the experimental group at baseline was  $77.50770 \pm 4.95$  and increased to  $80.41966 \pm 4.90$  at endline. The phase I mean percentage FFM for the control group was  $77.70074 \pm 3.89$  and at endline it was  $77.53346 \pm 3.82$  attributable to non-consumption of the AIVL recipe.

These results are comparable to findings by a similar study in which the experimental group received zinc supplementation while the control was given placebo for three months [29]. There was significantly higher increase in soft tissue and mainly FFM in the experimental group than in the control group. The mean percentage FFM increase ranged from  $80.03 \pm 4.64$  to  $81.13 \pm 4.35$  and mean percentage FM ranged from  $18.87 \pm 4.35$  to  $19.97 \pm 4.80$ . In the present study from baseline to endline the mean FFM for the phase I experimental group increased by 1.23 kg. This concurred with findings by Refs. [16] and [27] in a study that sought out the effectiveness of food supplements on the FFM of children with acute malnutrition. They reported a baseline mean weight of 6.91 kg and baseline mean percentage FFM of 83.5%. At endline there was a weight increase of 0.90 kg and FFM increase of 93.5%, implying children with moderate acute malnutrition when nutritionally rehabilitated with micronutrients put on predominately FFM. In the present study the increase of FFM by 1.23 kg in the experimental group is comparable to the FFM increase by 0.87 kg reported by Ref. [4], where girls received zinc supplements. Study subjects in the experimental group increased in linear growth and weight and had

increased appetite and food consumption compared to control group who received placebo. This collaborated with findings by Refs. [2, 35] where increase in weight was associated with FFM (lean body tissue) increase. BMI measurement in the present study indicated that consumption of AILV recipe had no significant effect on the nutritional status of both the experimental and control group at endline. However, DDIM showed increase in FFM in experimental group, thus bringing into perspective the limitations of BMI over DDIM as a measure of intervention outcome.

This is consistent with findings by Ndung'u [30] and Diouf and co-workers [12]. Ndung'u [30] used DDIM to validate obesity measurement by BMI and demonstrated that 85.4% of the children found to be normal by BMI measurement turned to be obese by DDIM measurement [30]. In a study on BMI verses DDIM for establishing childhood obesity prevalence in eight African countries it was reported that the prevalence of excessive fatness by DDIM was three times higher than that by BMI. Similarly the FFM and FM of Senegalese children aged 8-11 year were determined using DDIM to validate bioelectric impedance analysis in predicting total body water (TBW) and adiposity [13]. Deuterium enrichment in saliva samples of the children was measured using FTIR spectroscopy. According to BMI measurement 1.9% of the children had obesity but by DDIM measurement 11% suffered obesity. This finding highlighted the limitation of BMI in the determination of body composition in children [12, 30].

The significant increase in % FFM and FM in phase I experimental group was not coincidental for in phase II the experimental group that had been control in phase I had significant increase in % FFM and FM at endline on consumption of the AILV recipe (Table 2 phase II). The endline mean percentage FFM of phase II experimental group was significantly higher than the baseline ( $p < 0.001$ ). There was no significant difference between the endline and baseline mean percentage FFM ( $p = 0.692$ ) for the phase II control

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**Table 1 Phase I and phase II serum constituents (Zn and Fe) levels of Kilalani and Kangundo schools.**

Phase 1								
Serum constituents	Experimental (N = 66)			Control (N = 46)			Baseline <i>p</i> -values	Endline <i>p</i> -values
	Baseline	Endline	<i>p</i> -value	Baseline	Endline	<i>p</i> -value	Expt. vs. Control	Expt. vs. Control
Fe (µg/L)	11.644 ± 1.81	14.239 ± 1.80	< 0.001	11.634 ± 0.23	11.874 ± 0.21	0.097	0.978	< 0.001
Zn (mg/L)	0.582 ± 0.15	0.712 ± 0.15	< 0.001	0.577 ± 0.10	0.584 ± 0.11	0.706	0.843	< 0.001
Phase 2								
Serum constituents	Experimental (N = 46)			Control (N = 66)			Baseline <i>p</i> -values	Endline <i>p</i> -values
	Baseline	Endline	<i>p</i> -value	Baseline	Endline	<i>p</i> -value	Expt. vs. Control	Expt. vs. Control
Fe (µg/L)	11.874 ± 1.45	14.320 ± 1.71	< 0.001	14.239 ± 1.80	14.235 ± 1.80	0.090	< 0.001	0.802
Zn (mg/L)	0.583 ± 0.11	0.719 ± 0.15	< 0.001	0.712 ± 0.15	0.708 ± 0.14	0.064	< 0.001	0.698

Independent and paired *t*-tests, 95% confidence level, *p* = 0.05.

**Table 2 Phases I and II body FFM, FM and BMI of the experimental and control groups.**

Phase I								
	Experimental (N = 66)			Control (N = 46)			Baseline <i>p</i> -value	Endline <i>p</i> -value
	Baseline	Endline	<i>p</i> -value	Baseline	Endline	<i>p</i> -value	Expt. vs. Control	Expt. vs. Control
BMI (kg/m <sup>3</sup> )	15.112 ± 1.40	15.126 ± 1.45	0.861	14.747 ± 1.22	14.968 ± 1.16	0.177	0.156	0.540
FFM (kg)	18.866 ± 2.64	20.097 ± 2.80	< 0.001	18.340 ± 2.27	19.001 ± 2.29	0.074	0.275	0.003
FFM (%)	77.508 ± 4.95	80.420 ± 4.90	< 0.001	77.701 ± 3.89	77.533 ± 3.82	0.64	0.826	0.001
FM (kg)	5.488 ± 1.41	4.909 ± 1.47	< 0.001	5.291 ± 1.21	5.553 ± 1.36	0.076	0.443	0.041
FM (%)	22.492 ± 4.95	19.580 ± 4.90	< 0.001	22.299 ± 3.89	22.467 ± 3.82	0.64	0.826	0.001
Phase II								
	Experimental (N = 46)			Control (N = 66)			Baseline <i>p</i> -value	Endline <i>p</i> -value
	Baseline	Endline	<i>p</i> -value	Baseline	Endline	<i>p</i> -value	Expt. vs. Control	Expt. vs. Control
BMI (kg/m <sup>3</sup> )	14.968 ± 1.16	14.887 ± 1.91	0.802	15.126 ± 1.45	15.136 ± 1.42	0.863	0.540	0.431
FFM (kg)	18.597 ± 2.19	19.916 ± 2.95	0.002	20.097 ± 2.78	20.097 ± 2.79	0.977	0.003	0.743
FFM (%)	77.533 ± 3.82	81.058 ± 4.64	< 0.001	80.420 ± 4.90	80.320 ± 5.10	0.692	0.001	0.437
FM (kg)	5.460 ± 1.26	4.636 ± 1.23	< 0.001	4.909 ± 1.47	4.927 ± 1.44	0.809	0.041	0.267
FM (%)	22.467 ± 3.82	18.942 ± 4.64	< 0.001	19.580 ± 4.90	19.712 ± 5.12	0.603	0.001	0.418

Independent and paired *t* tests, 95% CL, *p* = 0.05.

group. The mean percentage FFM for phase II experimental group at baseline was 77.533 ± 3.82 and at endline was 81.058 ± 4.64 while for the phase II control group it was 80.420 ± 4.90 at baseline and 80.320 ± 5.10 at endline. The control group in phase II compared to experimental group in phase II had elevated mean percentage FFM at baseline from phase I treatment which was not given to the experimental group I.

#### 4. Conclusion

The finding from previous studies that Deuterium

Dilution Isotope Method is more accurate than BMI in determining nutrition intervention outcomes in children is supported in the present study. Further, food-based intervention through vegetable garden establishments as recommended by KDHS and Micronutrient Survey's report [24] is supported to alleviate malnutrition among school-going children in Kenya and beyond. Policy makers in government should encourage school garden establishments and lunch programs in primary schools that incorporate garden-sourced AILVs to fight malnutrition among school-going children, especially in arid and semi-arid

areas.

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