

Assessment of Nutrition Intervention Impact on Children in Machakos, Kenya by Deuterium Dilution Isotope Method

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How to cite this paper: John Wakhanu. (2023) Assessment of Nutrition Intervention Impact on Children in Machakos, Kenya by Deuterium Dilution Isotope Method. *International Journal of Food Science and Agriculture*, 7(1), 56-65. DOI: 10.26855/ijfsa.2023.03.009

Received: December 28, 2022

Accepted: January 26, 2023

Published: February 28, 2023

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Abstract

Deuterium Dilution Isotope method (DDIM) was used to assess body composition changes among school-going children in a nutrition intervention with Amaranthus cruentus and Vigna unguiculata, African indigenous leafy vegetables (AILVs), grown in Kangundo and Kilalani primary school gardens in Machakos County, Kenya. It was an experimental cross-over design in two phases, I (13 weeks) and II (12 weeks), with 4 weeks between phases then experimental and control groups for phase II interchanged. In phase I children aged 6-10 years, who met the inclusion criteria, were grouped as experimental (Kangundo, N=66) and control (Kilalani, N=46). The experimental group fed on a recipe of AILVs with an accompaniment of a mixture of maize and beans once a day, 5 days a week per phase while the control group fed only on the accompaniment. A pretested structured questionnaire was administered to parents/caregivers of the children to gather data on demographic and socio-economic characteristics, the methods of preparation and consumption patterns of vegetables, and morbidity patterns to establish the nutritional status of the study children before intervention. Saliva, before and after administration of deuterium oxide, was analyzed at baseline and endline by Fourier Transform Infrared Spectroscopy (FTIR) to assess body composition changes and compare with Body Mass Index (BMI) changes. Methods of assessment were validated and a 24 hour dietary recall was used to monitor food intakes away from school. Findings indicated high morbidity and low nutrition status in the study subjects. Poverty and poor consumption of AILVs were reported. As opposed to BMI, DDIM showed a significant improvement in the body composition of experimental groups at endline ($p < 0.001$) during both intervention phases. The higher potential of DDIM over BMI in the assessment of nutrition intervention impact is highlighted in this study.

Keywords

Body composition, DDIM, nutrition intervention, Vegetables

1. Introduction

The Kenya Demographic Health Survey (KDHS) and Micronutrient Survey's report of 2014 presented high prevalence of malnutrition in Kenya particularly in semi-arid counties such as Machakos in Kenya [1, 2]. Some global interventions such as food supplementation and food fortification have proved costly and unsustainable. On the contrary, the role of African indigenous leafy vegetables (AILVs) in fighting malnutrition in vulnerable populations has been demonstrated by a number of studies. In this regard, their promotion for cultivation as well as consumption is called for. Kenya has over 210 locally available AILV varieties although their availability is challenged by seasonality, poor ac-

cessibility, bioavailability of micronutrients, and negative perception that lead to their low consumption [3, 4, 5]. However, AILVs availability and consumption can be increased by cultivating them in school gardens. Further, school gardens will enhance the participation of the school children together with their parents and provide them an opportunity to access nutrition information on diet diversification. In view of the advantages of school gardens, primary schools in semi-arid areas are targeted for vegetable garden establishment, because they bring together pupils prone to malnutrition, making these schools best placed to achieve homogeneity and control of the intervention studies.

1.1 Assessment of nutrition interventions

A nutrition intervention should ideally result in changes in body composition of target population. 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 [6, 7]. 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 outcomes [7, 8, 9]. However, the true relationship between food intake and body composition shows a gap due to unreliable assessment methods.

Common methods of measuring nutrition intervention impact on the bodies of individuals include Body Mass Index (BMI), Skinfold Thickness, Waist circumference, Bioelectric Impedance Analysis, Dual Energy X-ray Absorptiometry, Magnetic Resonance Imaging, Densitometry and Total Body Electrical Conductivity [7]. Many studies report nutrition outcomes based on BMI and this underestimate or overestimate nutrition outcomes [10]. Though easier to determine, BMI fails to distinguish FM and FFM in different races of populations and children [11, 12, 13, 14]. The DDIM was identified by the IAEA in 2010 to address this gap. This method determines FFM and FM changes during intervention, and is safe, accurate, simple to carry out, more reliable and suitable for children [6, 15]. It 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 [6]. The body water pool naturally contains a small amount of deuterium (^2H) which represents the natural abundance of 2H in body water. When a known quantity of deuterium oxide ($2\text{H}_2\text{O}$) (99.8 or 99.9 % ^2H) is ingested it equilibrates with the body water within a few hours [6]. The enrichment of deuterium in body water reaches a 'plateau' after 3-4 hours and is uniformly distributed in saliva, urine, plasma and milk (for lactating mothers). Any of these body fluids can be used for the determination of the deuterium enrichment in the body hence TBW, however, saliva is easier to use since its equilibration is faster than the rest. Further, working with saliva has relatively lower risk of contamination and study subjects can collect saliva on their own [6]. Participants shouldn't drink water and should minimize motion during equilibration period. Saliva can be collected 3 or 4 hours after dosing and its deuterium concentration analyzed by Fourier Transform Infrared (FTIR) spectrometry. The deuterium concentration is reported in mg $2\text{H}_2\text{O}$ per kg H_2O (ppm) [6].

Apart from [16] there is no evidence that DDIM has been used in Kenya to measure intervention outcomes despite the discussed advantages. Ref. [16] used DDIM to validate BMI, physical activity and dietary practices as methods for FM assessment among school children aged 8-11 years in Nairobi, Kenya and reported BMI limitations. In that study 85.4% of the children found normal by BMI measurement were found obese by DDIM. This corroborated findings by [17] in a study on BMI verses DDIM for establishing childhood obesity prevalence in eight African countries. In that study, the prevalence of excessive fatness by DDIM was three times higher than that by BMI. Ref. [17] also determined the FFM and FM of Senegalese children aged 8-11 years ($n=151$) using DDIM to validate bioelectric impedance analysis in predicting Total body water (TBW) and adiposity among the children. The 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 ± 6.5 , 137.2 ± 7.8 , -1.34 ± 1.20 and -0.19 ± 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 ± 2.7 , 22.8 ± 5.7 and 4.4. Only 1.9% of the children were obese by BMI but 11% were obese by DDIM. This highlighted the limitation of BMI in the determination of body composition in children and generated interest in DDIM as a relatively more reliable chemical method. With the benefits of establishing and promoting school gardens on one hand, and the advancement in chemical methods of assessment for a nutrition intervention on the other, scientific data is presented from the findings of this study.

1.2 Effects of food micronutrients on body composition

The effects of consumption of AILVs, food supplements and fortified foods on body composition have been documented in a number of studies [8, 19, 20]. The changes of free fat mass (FFM) of an individual are due to growth of lean tissue as a result of micronutrients like zinc, iron, chromium and pro-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. The exact mechanism of how zinc influences growth is unknown, however, 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 didn't 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 deoxyribonucleic acid (DNA), ribonucleic acid (RNA) and protein which has implications on the FFM [21]. 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 [22].

Another study was carried out on the effect of zinc supplementation on growth and body composition in children with sickle cell disease [23]. The study reported that girls who received zinc supplementation were shown to significantly increase FFM by 0.87 kg. Study subjects in the experimental group had increased linear growth and weight, increased appetite and food consumption more than in the control group who received placebo. The study concluded that zinc intake increased linear growth, weight, appetite, and food consumption in children. Further, zinc deficiency is manifested as poor growth and delayed skeletal and sexual maturation common in children [23]. Similarly a group of 20 children who received oral zinc supplementation, 20mg/day 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 [24]. The children showed 80% weight increase associated with FFM (lean body tissue) without increase in body fat. The study reported 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 [24].

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 [25]. Vitamins and minerals present in this recipe were Vitamin A (rich in β -carotene), zinc, iron, calcium and magnesium among others. 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 significant increase in body weight, FFM, FM, and Hemoglobin than in the control groups. FFM increased by 11.8% while FM by 10.7% [25]. The increase in FFM in PLWA was attributable to vitamins and minerals in the recipe.

Ref. [26] did an intervention study in which pre-pubertal, non-zinc deficient (8-9 years old) children were divided into two groups: experimental (31) and control (31). Of these 62 children examined, 32 were boys and 30 were girls. The experimental group received zinc supplementation while the control was given placebo in a three months intervention. The experimental group showed significant increase in soft tissue, mainly FFM than the control group [26]. The % FFM increase ranged from 80.03 ± 4.64 to 81.13 ± 4.35 and % FM ranged from 18.87 ± 4.35 to 19.97 ± 4.80 .

Studies on the effect of the combination of iron, zinc and vitamins on FFM have also been done. For example, [27] carried out a study on the effect of a combination of zinc, iron and vitamin A on 800 infants aged 3 months in rural East Lumbok, West Nusa Tenggara for a period of 6 months. Weight, length and micronutrient status were determined. Zinc alone disadvantaged the hemoglobin 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 hemoglobin. The height of the study subjects increased by 1.1- 1.5cm more than those with placebo. The study concluded that zinc intake can only have a positive effect on FFM if low hemoglobin, Iron status and Vitamin A are also addressed and corrected [27].

2. Materials and Methods

This was a 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 NACOSTI/P/15/3659/5730 obtained from the National Commission for Science, Technology and Innovation (NACOSTI). The study adopted an experimental cross-over design that involved two phases, I (13 weeks) and II (12 weeks), with 4 weeks between the phases to enable wash out. The role of study subjects as experimental group was interchanged during phase II to become the control group and vice versa for the control group. *Amaranthus cruentus* and *Vigna unguiculata* (AILVs) were grown in school gardens of Kangundo and Kilalani primary, Machakos. In phase I study subjects (children aged 6-10 years) who met the inclusion criteria were grouped as experimental (Kangundo, N=66) and control (Kilalani, N=46). The study children were dewormed prior to the feeding on the recipe. Parents/caregivers signed consent forms to allow their children to participate in the study. Prior to any field procedures, meetings were conducted in each school for parents/caregivers, agricultural officers, health officials, head teachers and class teachers to explain the study purpose, procedures (including saliva sampling by qualified laboratory medical staff). Parents/caregivers were allowed in school during all procedures including sample collection, deworming and feeding. The experimental group fed on a recipe of *Vigna unguiculata* and *Amaranthus cruentus* with an accompaniment of a mixture of cooked maize and bean seeds once a day, 5 days a week per phase while the control group fed only on the accompaniment. Other food intakes of the study children were also monitored by 24 hour dietary recall. The end line

effect of the vegetable recipe on body FFM and FM was determined by DDIM and saliva analyzed by FTIR.

2.1 Sampling and sample size.

In Phase I, there were 76 study subjects in the experimental group and 49 for the control group for a sample size of $n=125$ (including 10% of the calculated sample) that was determined using equation 1 [28].

$$n = \frac{Z^2 pq}{e^2} \quad (1)$$

where

Z = confidence limits of the survey results. For 95% confidence level, $Z=1.96$

P = proportion of the population with the attribute of interest (the prevalence of malnutrition among children aged 5-11 years in Machakos County is 8.1% weight for age) =0.081.

q = (1-p) the proportion of population without the attribute of interest =0.919

e = desired precision of the estimate (5%) =0.05.

Following drop outs, there were 66 and 46 study subjects in the experimental and control groups respectively in phase I.

2.2 Establishment of school gardens

Amaranthus cruentus was planted on a quarter of an acre while *Vigna unguiculata* was planted on a half an acre at the experimental school. Couch grass and other weeds were eliminated by spraying with Roundup herbicide. Furrows were made and 4.0 kg of Diammonium Phosphate (DAP) fertilizer was applied in the furrows prior to planting cowpea Seeds (type M66) sourced from Kenya Agricultural Research Institute in Katumani, Kangundo sub-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.

2.3 Collection of baseline information

A pretested structured questionnaire was administered to parents/caregivers of the children to gather data on demographic and socio-economic characteristics, baseline data on the methods of preparation and consumption patterns of vegetables and morbidity patterns (respondents recalled the incidences of child illness for the previous two weeks, types of illness and their frequency). The weight and height of the study subjects was taken by a qualified nutritionist using a healthcare weighing machine (Salter scale model 2006) and UNICEF scale respectively. Weight measurement, taken to 0.1 g accuracy was done in triplicates and mean weight calculated. 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 teachers records that had been verified using the child's health card, birth certificate or baptismal card. The study subject was then dewormed using an anthelmintic drug (albendazole syrup, 10 mls of 400 mg/child).

2.4 Dose preparation and saliva sampling

The dose preparation and sampling procedures were performed according to International Atomic Energy Agency (IAEA) standard operating procedures [6]. Deuterium oxide (D_2O) liquid (99.8%) was diluted with tap water by adding 800g (800ml) of tap water to 200g (180ml, density of D_2O is 1.105g/ml) of D_2O . The weight of both the D_2O and the added tap water was recorded to 0.01g. The study subjects consumed 30 ml. of the diluted D_2O liquid and were starved and with minimal motions for three hours to allow the D_2O to equilibrate with body water, and minimize water loss through sweating. A pre-dose and post dose (after consumption of Deuterium oxide (D_2O) liquid/water) saliva collection was done by qualified medical staff by placing two cotton balls in the mouth of the study subjects for 2 minutes. Post-dose saliva was sampled 3 hours after administration of the D_2O dose in a similar manner. 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 $-80^\circ C$. The procedure of saliva sampling was repeated after the intervention period of 13 weeks for subjects in both the experimental and control groups during phase II.

2.5 Preparation of vegetable recipe

In each of the schools, two assistants were recruited and trained on vegetable recipe preparation and serving, working in collaboration with the school cooks. 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 (wt./wt.) and cooked by saut éng method. Each study subject in the experimental group consumed on average 100g (wet weight) of the recipe of *Amaranthus cruentus* and *Vigna unguiculata* expected to meet Recommended Daily Allowance (RDA) for children. The vegetable recipe was served together with a mixture of cooked beans and maize (being part of the school feeding programme) once a day for 5 days a week, for 13 weeks.

2.6 Materials and reagents

Fourier Transform Infra-red Spectrometer (IR Tracer-100 FTIR SHIMADZU). The FTIR instrument set with absorbance as the measurement mode, apodization was square triangle, number of scans was 32, resolution was 2.0 and range was minimum 2300 cm^{-1} and maximum 2900 cm^{-1} . The electronic balance (Shimadzu Corporation Japan AT x 224, max.220g, min. 10 mg with a readability of 0.1mg), accurate to 0.0001g, was used for weight measurements of chemicals and reagents, cool boxes, 20 ml syringes, sterile vials, dry glass bottles with a polytetrafluoroethylene (PTFE) lined screw caps, volumetric flasks, cotton wool and deuterium oxide liquid.

2.7 Calibration of deuterium oxide

Calibration and quantification of deuterium oxide (D_2O) was done according to the IAEA (2010) procedures. To prepare 2000 ppm of the calibration standard, 2g of D_2O was diluted to 1L with drinking water and transferred to a clean, dry glass bottle with a polytetrafluoroethylene (PTFE) lined screw cap and stored in a cool place. A similar volume of drinking water was kept for use as a blank to measure the background spectrum. Weighing of the water and the D_2O was done using an electronic balance. Working standards of 0 ppm, 100 ppm, 200 ppm, 400 ppm, 600 ppm, 800 ppm, 1000 ppm, 1500 ppm and 2000 ppm were prepared by pipetting 5 mL, 10 mL, 20 mL, 30 mL, 40 mL, 50 mL and 100 mL of 2000 ppm of D_2O stock solution and then diluting with water in 100mL volumetric flask. The FTIR instrument was switched on and initialized to obtain background spectrum. Exactly 1mL of each of the working standards was introduced to the FTIR cell using a 1mL 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 and its regression equation (Figure 1).

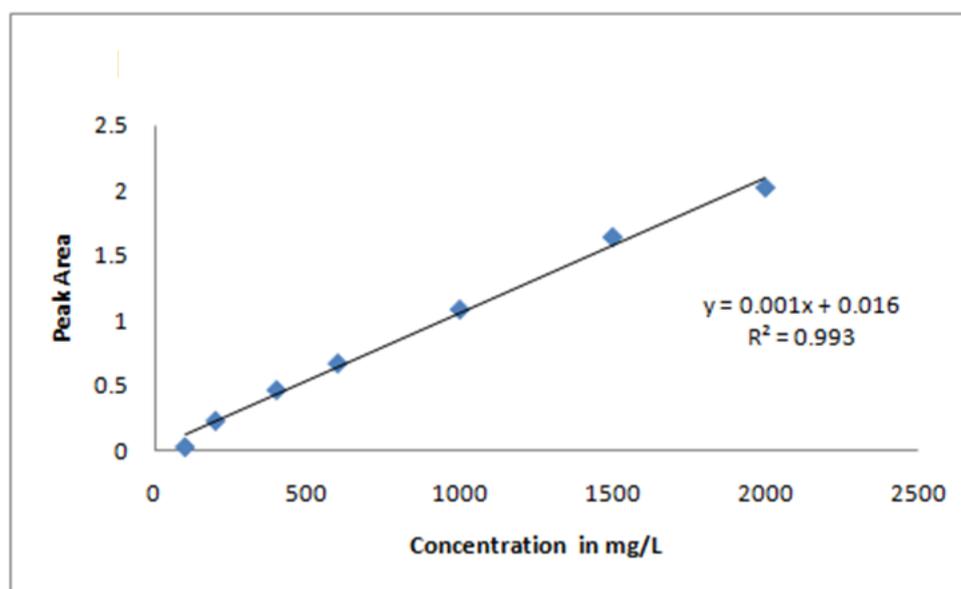


Figure 1. Deuterium oxide standard calibration curve.

2.8 Method validation

Linearity test of concentration and limit of detection for DDIM were also done. The linearity domain was checked from the standard calibration curve. The linearity of the calibration curve is given by $y = mx - c$ equation, where the calculated blank sample absorbance is given by the intercept c and the method sensitivity is given by the slope and the degree of linear relation between the signal and concentration is shown by the correlation coefficient R^2 . Limit of detection (LOD) were calculated using equation 2 [29] using the determined absorbance values for 10 replicates of the blank

solution, then transformed into concentration values in order to be compared with the data obtained from the calibration curve.

$$\text{LOD} = \bar{X}_{\text{blank}} + 3S_{\text{blank}} \quad (2)$$

The percentage recovery of the analyte from saliva samples was determined to verify the accuracy of the method. Precision of the method was determined by calculating the relative standard deviation (RSD) of repeated measurements of the test sample. The results of these method validations are presented in Table 1.

Table 1. Method validation results

Percentage recovery of the analyte					
Method	Analyte	Cx (ppm)	Cadd (ppm)	Cs (ppm)	% Recovery
FTIR	FFM	406.36	100	507.4	101.0
Relative standard deviation (RSD) of repeated measurements of FFM					
Parameter	Mean n=3	SD	%RSD		
FFM	18.84	0.24	1.27		
Linearity test of concentration and limit of detection(LOD)					
Analyte	LOD	Regression equation		R ²	
Deuterium oxide	4776.89	Y = 0.001X + 0.016		0.993	

2.9 Quantification of deuterium in saliva samples

The vials with saliva samples were allowed to thaw for one hour before centrifugation for 15 minutes at 1000 rpm to remove bubbles. The samples were then introduced into the FTIR instrument and the peak areas determined from which the concentration of deuterium oxide was obtained by regression analysis. To obtain the body free fat mass (FFM), the Total body water 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). The TBW of the study subject was then used to work out the subject's FFM [6].

2.10 Data analysis

Statistical data analysis was performed using Statistical Package for Social Sciences (SPSS) version 21 software. Independent t-test was used to compare the mean FFM, between the experimental groups and control groups at baseline and endline in both phases I and II while paired t-test was used to compare the percentage means at baseline and endline in each group. All significance levels were determined at 95% confidence level and p=0.05.

3. Results and analysis

3.1 Method validation results

The percentage recovery was 101.0%, indicating that the method of analysis used was accurate [29]. The relative standard deviation was 1.27% which agrees with work done by [30] that showed that the relative standard deviation of less than 3% is sufficiently precise. The R² value was 0.993, meaning that the instrumental response to concentration was 99.3%. The R² values being closer to 100 % indicate that the established calibration curve (Figure 1) is linear over the respective range of the concentration of the working standards that were used thus obeys Beer-Lambert's law.

3.2 Baseline demographic and socio-economic characteristics of parents/caregivers

In terms of gender, there were more female than male in both the experimental and control groups thus with over 70.8 % female respondents in each case. The majority (over 50%) of the respondents were aged between 31-50 years. Findings indicated that most respondents fell in the bracket of being self-employed or were unemployed for the experimental group while in the control group the distribution across the three categories that described their occupation was fairly uniform. However, irrespective of their category of occupation, more than 60% of the respondent had earning of less than ksh 4000 per month, this pointing well the extent of poverty levels. It was noted that despite the school feeding programme, where every parent/caregiver contributed 4kg of maize and 1kg of beans per month per pupil some parents could not afford.

3.3 Food sources and consumption of indigenous vegetables

A list of common vegetables consumed in Kenya was presented to gather information on their preferences. These included cabbages, spinach and kale which are exotic among the indigenous ones cowpea, amaranth, jute mallow, nightshade and spider plant. Although the exotic ones were more preferred the study was inclined to AILVs [31]. This was not unique since AILV have been reported to be underutilized amidst the challenges of their availability [19].

Cowpea and amaranth were found to have higher preference among the indigenous vegetables (range 15-20%) and were therefore used to prepare the vegetable recipe in this study. The study investigated on the most common method of preparation of the vegetables among three common ones boiling, steaming and Saut éng. Saut éng of vegetables was the most preferred method of cooking. Notably, over 90% of respondents in the experimental group preferred this method against about 50% in the control group. The study scope did not establish the immediate reason to this though factors such as income, education level, and socio-economic factors are attributed to such trends [19]. The preference on saut éng gave basis of using the same method in the recipe preparation in this study. The method holds an advantage that it releases nutrients and makes them more bioavailable. Findings indicated that among the possible sources of vegetables that include market sources, donated and grown in home gardens most of the respondents in the experimental group sourced their vegetables from home gardens (67.4%) against most of the respondents in the control group who purchased vegetables (69.6%). Vicinity to the local market among other unestablished factors such as the socio-economic factors would be attributed to these findings [19].

3.4 Morbidity patterns

A majority of the study subjects in the experimental group were reported to have anemia (63.2%) unlike those in the control group (25%). Anemia is common among pre-school children and is closely related to chronic micronutrient deficiencies. The lower percentage in the control group though can be attributed to the socio-economic status was still alarming. These findings indicated high morbidity in the study subjects.

3.5 Body composition

Table 2 shows the levels and percentage of body FFM, FM and BMI of the experimental and control groups in phase I and II. All the experimental groups showed significant improvements in the mean FFM and not for BMI in both phases I and II. In both phases, subjects in both control and experimental groups had similar indices of BMI ($p = 0.156$ and 0.540). On the other hand, subjects in both control and experimental groups had different indices of FFM (0.275 and 0.003) and FM (0.443 and 0.041) at baseline entities. The effect of intervention is projected from changes in the indices at endline. At endline, FFM increased as FM decreased as would be attributed to a positive impact of the intervention for the experimental group which consumed the vegetable recipe. The body's FFM is inversely proportional to FM, the constant of proportionality being total body mass. Hence, the percentage FFM for the control group which was 77.70074 ± 3.89 at baseline would be explained to decrease to 77.53346 ± 3.82 at endline.

The changes in the experimental study subjects were significant for FFM ($p=0.002$) and FM ($p<0.001$) unlike for BMI ($p=0.802$). While the BMI showed the intervention had no significant effect on the nutritional status of both the experimental and control group, the converse was true using the DDIM. The body composition changes in the present study were attributed to the consumption of the AIVs and these corroborated findings of similar studies done earlier [3, 18, 19, 20, 22, 23, 24, 27, 28]. Increase in FFM implies growth of soft tissue as a result of increased cell division due to the nutrients supplied by the AILVs consumed [21].

The findings affirm the assertion that BMI underestimates body composition and also fails to distinguish FM and FFM [10, 13, 14]. These findings also highlight the limitation of BMI in the determination of body composition in children and are consistent with those by [16] and [17]. Ref. [16] used DDIM to validate obesity measurement by BMI and demonstrated that a high percentage of the children found to be normal by BMI turned to be obese by DDIM. Ref. [17] in a similar study demonstrated that excessive fatness by DDIM was three times higher than that by BMI.

The present study supports nutritive intervention to address malnutrition since all the experimental groups showed significant improvements in body compositions. Though the present study was limited by the general inability to control other food intakes away from school, a 24 hour dietary recall was used to monitor micronutrient and energy intakes. These food intakes by the study children at home may have contributed to the body composition assessed. However, results obtained in phase II showed the same trend observed in phase I, despite this limitation, leading to the conclusion that the positive nutrition impact most likely was due to the consumption of the vegetable recipe. It was also difficult to explain why the control group's mean FM and FFM did not reduce significantly at endline in phase II, since they had stopped feeding on the vegetable recipe at school. Perhaps the 4 weeks in between phase I and II was not long enough to allow significant changes in body composition. Additionally foods eaten away from school may have played a role in this observation.

Table 2. Levels and percentage of body FFM, FM and BMI of the experimental and control groups in phase I and II

	Phase I								
	Experimental (n=66)			Control (n=46)			Baseline P- value	Endline P- value	
	Baseline	Endline	P- value	Baseline	Endline	P-value	Expt. Vs Control	Expt. Vs Control	
BMI(Kg/m ²)	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	0.640	0.826	0.001	
FM (kg)	5.488 ± 1.41	4.927 ± 1.47	<0.001	5.291 ± 1.21	5.553 ± 1.36	0.076	0.443	0.041	
FM (%)	22.492 ± 4.95	19.712 ± 5.12	<0.001	22.299 ± 3.89	22.467 ± 3.82	0.640	0.826	0.001	
	Phase II								
	Experimental (n=46)			Control (n= 66)			Baseline P- value	Endline P- value	
	Baseline	Endline	P- value	Baseline	Endline	P-value	Expt. Vs Control	Expt. Vs Control	
BMI(Kg/m ²)	14.868 ± 1.09	14.887 ± 1.91	0.802	15.138 ± 1.25	15.136 ± 1.42	0.863	0.530	0.431	
FFM (Kg)	18.597 ± 2.19	19.916 ± 2.95	0.002	20.107 ± 2.75	20.097 ± 2.79	0.977	0.004	0.743	
FFM (%)	77.224 ± 2.34	81.058 ± 4.64	<0.001	80.400 ± 4.05	80.320 ± 5.10	0.692	0.001	0.437	
FM (kg)	5.460 ± 1.26	4.636 ± 1.23	<0.001	4.916 ± 1.47	4.909 ± 1.44	0.809	0.042	0.267	
FM (%)	22.776 ± 2.34	18.942 ± 4.64	<0.001	19.683 ± 5.02	19.580 ± 4.90	0.603	0.001	0.418	

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

4. Conclusion

While the end line body composition of experimental groups as indicated by the DDIM measurement of FFM and FM significantly improved ($p < 0.001$) during both intervention phases, there was no significant improvement ($p > 0.05$) by BMI. This empirical data highlights the sensitivity of DDIM over BMI as a nutrition intervention assessment method. There was a positive micronutrient impact, as shown by significantly higher levels of FFM in the experimental group that consumed the garden sourced selected AILVs as compared to the control group at end line during both phase I and II. Since DDIM, unlike BMI, captured significant differences in the baseline and endline body composition of the experimental groups, DDIM is recommended for use in the determination of nutrition intervention outcomes among children in field studies.

5. Acknowledgements

I wish to thank the following key persons and organizations for their involvement and support during the study. Prof. Hudson N. Nyambaka, Prof Judith Kimiywe, Dr. Mildred P. Nawiri and Dr. Wilson Thagana offered guidance at every stage in the study. NACOSTI and Kenyatta University Ethics and Review Committee gave the permits to carry out this study, and the office of the Vice Chancellor, Kenyatta University, for partly funding this research through the Vice Chancellors Grant. The staff from Kangundo sub county hospital, Machakos County, Dr. Robert Kilonzo, Mr. Bonface Gichuhi (data officer), laboratory technicians, Mr. Emmanuel Musyoka, Mr. Julius Mutiso, and the nutritionist, Ms. Ngina Onesmus, for saliva sampling from the study subjects and Mr. Peter Munguti (Sub county Agricultural Officer) in the supervision for the establishment of the vegetable gardens, and the feeding of the study subjects. I am indebted to the County Director of Education and County Director of the Ministry of Health, Environment and Emergency Services, Machakos County for embracing the research in their area of jurisdiction. Special thanks to Mr. Eliud Mutisya and Mr. Francis Kioko, the head teachers of Kangundo and Kilalani primary schools respectively, the parents, class teachers, teachers, school cooks of the two schools and Anthony Maina for facilitating transport during the field work. John Gachoya and Jane Mburu (Kenyatta University, Chemistry Laboratory) were of great assistance in saliva sample analysis for deuterium using FTIR while Felix Ondiek and Dennis Osoro assisted with data analyses.

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