

### **3.4.2 Methods**

#### **3.4.2.1 Study design and participants**

A cluster non-randomized controlled trial design was employed for the intervention study.

The participants were 195 healthy primary school-going children aged 8-12 years old from two different Government-owned primary schools, with about 2.6 km apart from each school, in the Ho Municipality of the Volta Region of Ghana. The participants were recruited between May 2018 and July 2018.

#### **3.4.2.2 Study area**

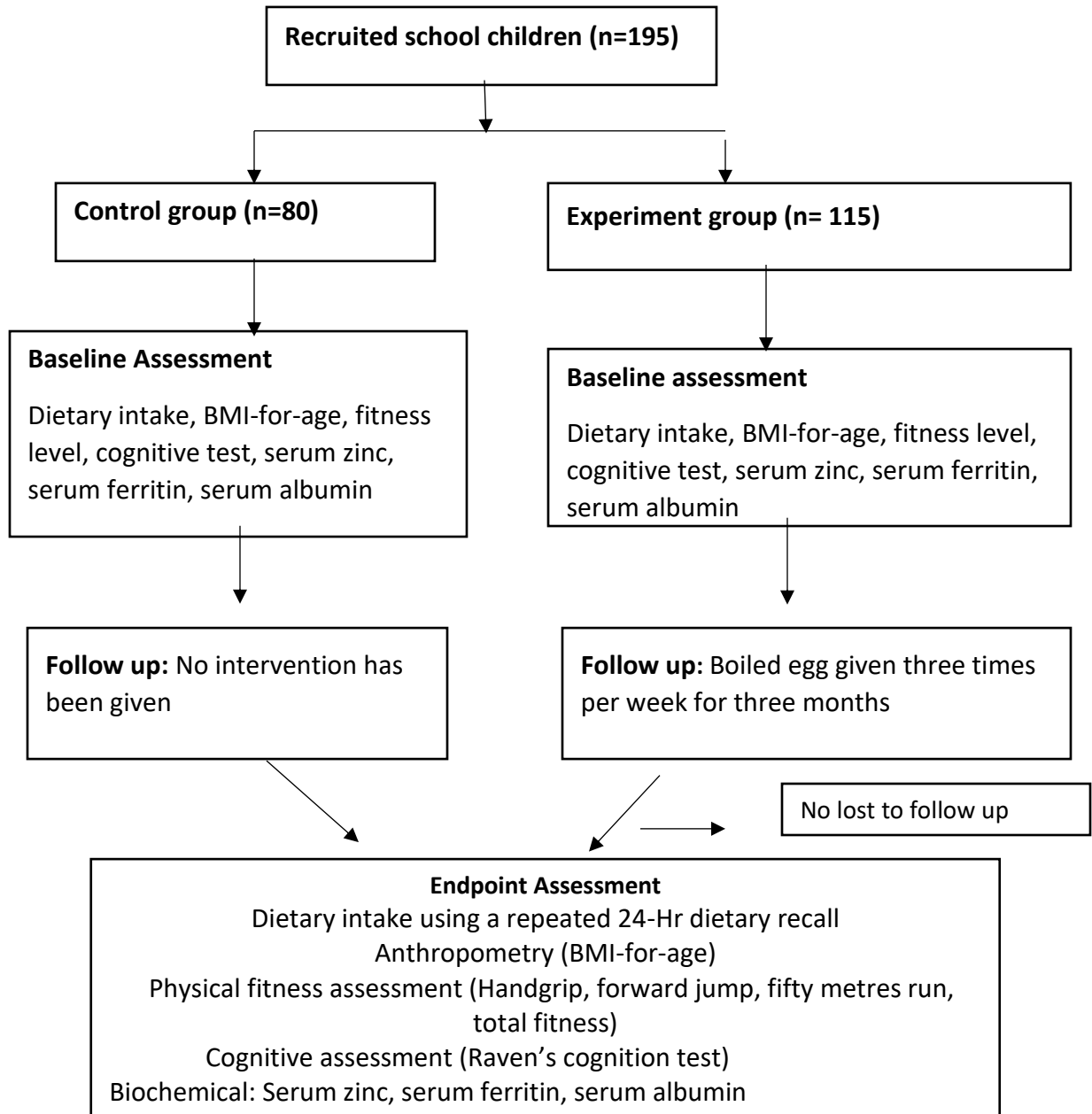
The Municipality of Ho located between latitudes  $6^{\circ} 20' N$  and  $6^{\circ} 55' N$  and  $0^{\circ} 12' E$  and  $0^{\circ} 53' E$  longitudes and covers an area of 2,660 sq km. The municipality shares boundaries with the district of Adaklu-Anyigbe in the south, the district of Hohoe, in the north. The district of South-Dayi in the west, and the Republic of Togo in the east. Ho Municipal is the Volta Region's provincial capital, making it an urban centre with many government schools.

#### **3.4.2.3 Ethical approval**

Approval was obtained from the Committee on Human Research Publications and Ethics of the Kwame Nkrumah University of Science and Technology, and the ethical number is given was (CHRPE/KNUST). Permission was also granted by the Regional and District Offices of the Ghana Education Service and Heads of the Schools. Informed consent for children's participation was obtained from their parents or guardians through written letters. Procedures to be undertaken were communicated to parents at a Parents Teachers Association meeting before data collection.

#### **3.4.2.4 Sample size calculation**

Using the Cochran formula for sample size determination, a minimum sample size of 195 pupils arrived at using a response rate of 15% and an estimated population of 20000 children with a 95% confidence interval at a margin of 5% error.



**Figure 1.** Flow diagram of study recruitment

### 3.4.2.5 Data collection

#### 3.4.2.5.1 Baseline

Baseline data on the nutrition and physical activity knowledge, attitudes, and practices of the school-age children attending the selected schools were assessed with questionnaires. Other

assessments carried out in the baseline survey included dietary intakes using a repeated 24-hour dietary recall, physical fitness levels, cognitive assessment using the Raven's Coloured Progressive Matrices test, anthropometry (BMI-for-age). Blood samples were taken from the participants to determine serum levels of ferritin, zinc, and albumin. Details of the study variables are described in the intervention. The study did not assess whether the children have had lessons on nutrition and physical education in school.

#### **3.4.2.5.2 Intervention**

Selected schools were assigned into two (2) groups; one (1) experiment groups (EG, n=80) and control group (CG- n=115). No intervention was provided to the control group. The intervention group was given three (3) eggs per week for three months. The Eggs were served hardboiled and consumed as a snack during the school days. Baseline variables (dietary intake, physical fitness, cognition test, BMI-for-age) were re-assessed at the end of the intervention. Blood samples of participants were taken at the end of the intervention to determine serum ferritin, zinc, and albumin levels.

#### **3.4.2. 6 Main outcome variables**

##### **3.4.2.6.1 Dietary assessment**

A repeated 24-hour dietary recall was used to collect data on the dietary intake of the school children. Household food models were used to quantify foods eaten by the participants and converted into grams. With the Nutrient Analysis Microsoft Excel Template (the University of Ghana, Department of Food Science and Nutrition, 2010), food products' nutrient compositions were analyzed. The Microsoft Excel Template Nutrient Analysis is a well-known nutrient analysis method in Ghana and was used by Annan *et al.* ( 2019) to report intakes of micronutrients of school children aged 8-12 years. Ho. Macronutrients (energy, carbohydrate, fat, and protein), and micronutrient intake (iron, zinc, vitamin B<sub>6</sub>, folate vitamin B<sub>12</sub>, and vitamin A) was compared with

Dietary Reference Intake (2000 and 2001) by Food and Nutrition Board, Institute of Medicine for recommended dietary allowances (Dietary reference; National Academy of Sciences & Food & Nutrition Board, Institute of Medicine, 2005).

#### **3.4.2.6.2 Anthropometric assessment**

##### **3.4.2.6.2.1 Height**

The height of participants was by positioning the SECA stadiometer in a vertical position. A child barefooted stood straight against the measuring board while looking straight ahead. The heels were maintained together, and the body was positioned so that the shoulder blades, buttocks, and heels were touching the height meter's vertical surface. The feet were laid flat on the floor, although slightly apart with the back straight and the hands freely hanging on the sides. Each participant was asked to stay still while the horizontal headboard was placed lightly but firmly against the head perpendicular to the stadiometer. The child's height was then read to the nearest 0.1cm at the point where the headboard touched the height meter. Two measurements were taken for each child, and the average height was computed as the child's actual height.

##### **3.4.2.6.2.2 Weight**

Weight was taken using an Omron digital weighing scale (BF 508, India), which was calibrated in kilograms and was on the zero marks. The children were weighed in light clothing and without shoes on. Two measurements were recorded to the nearest 0.1kg, and the average was taken as the child's actual weight. The BMI was calculated from the weight and height squared. The WHO Anthroplus software (WHO, 2006), designed to calculate z scores, was used to calculate the participants' BMI-for-age z scores.

##### **3.4.2.6.2.3 Assessment of Physical Fitness**

The following physical fitness components were evaluated: musculoskeletal component (handgrip strength, forward jump or standing broad jump), and motor component (50 meters run) (Ramírez-

Vélez *et al.*, 2015). The fitness assessment was taken twice. Demonstrations and explanations were given before the evaluation began and after that as required. The handgrip was measured using a handgrip dynamometer. Pupils were asked to keep the dynamometer first in their left hands and to squeeze when instructed. The same was done for the right hand. The instrument was set to zero for the next reading, after each reading. The measurement is in kg. Children stood at the starting line to measure forward jump and were instructed to jump forward as far as possible, with both feet off. The distance between the start line and the closer foot's heel was registered. Two trials were recorded to the nearest 1 cm, and the two were recorded for analysis on average. A fifty (50) m run-up, on the other hand, involves running over 50 metres of a single maximum sprint, with the time registered. Starting from a standing position, with one foot in front of the other, stationary (hands may not touch the ground). Behind the start line, the front foot needs to be right. The starter gives the "ready" then "go" instructions until the subject is ready and motionless. The subjects sprinted down to the finish line as soon as possible, 50 m from the start line. Two trials were allowed and recorded the best time. The timing begins with the first move (using a stopwatch) and ends when the chest crosses the finish line. Time taken to cross the finish line for each student was recorded at the nearest 0.1 s.

The physical fitness tests' raw values were converted to standard scores based on age and gender, using Japanese physical fitness performance norms for children (Ramírez-Vélez *et al.* (2015). This standard score design consists of eight physical fitness test scores for each physical fitness test, ranging from one to ten, with one being the lowest and ten the highest. Following the conversions, all the standard scores were added to get a total fitness score, and the fitness level was determined as excellent, very good, average, or poor for each child based on age and gender. However, in this study, the children performed just four of the physical fitness activities.

#### **3.4.2.6.2.4 Cognitive assessment**

The Raven's Coloured Progressive Matrices (RCPM) test was used to assess the schoolchildren's cognition. The cognitive test was performed in a quiet environment, and the test procedure was explained to participants before the test. The test booklets contained three sets of twelve problems (36 Coloured questions), which measures fluid intelligence by problem-solving and abstract reasoning by analogy and has been used extensively as a culturally fair test of intelligence (Raven, 2000). Geometric designs and patterns with a missing piece were increasingly involved in the experiments, and each question has six to eight choices to choose from to fill in the missing piece. Well-trained research assistants performed cognitive assessments.

#### **3.4.2.6.2.5 Biochemical Assessment**

Approximately four (5) mL of the venous blood sample was collected from the participant's median cubital vein in the arm's antecubital fossa into an EDTA vacutainer tube on each sample collection day. Serum was obtained by centrifuging the samples at 2500rpm for 5 minutes, pipetted into Eppendorf tubes, and stored at -20°C until the analysis. The serum was used for three biochemical assays (ferritin, zinc, and albumin); the ferritin assay was done at the Clinical Analysis Laboratory of the Department of Biochemistry and Biotechnology, Kwame Nkrumah University of Science and Technology, while the zinc and albumin assay was done at the laboratory at the Molecular Medicine Department of School of Medicine, KNUST.

WHO recommends serum ferritin in the assessment of iron levels (WHO, 2007). Serum ferritin was determined using Human Ferritin (FE) ELISA Kit (R&D system Inc, USA). The Stop Solution switches the colour from blue to yellow, and a spectrophotometer uses the strength of the colour to measure 450 nm. At the same time, as the samples, the calibration standards were assayed, and the operator developed a typical optical density (O.D.) curve versus FE concentration.

Serum albumin was determined following the Bromocresol Green (BCG) test procedure. The absorbance of the standard and test were measured against the blank at 630nm using a spectrophotometer.

Serum zinc was determined with a zinc reagent manufactured by (Autochemistry analyzer, LE SCIENTIFIC Horizon 850, USA), and analyzed using a Fully automatic biochemical analyzer (Autochemistry analyzer, LE SCIENTIFIC Horizon 850, USA). The optical density using the Spectra Max 190 microplate reader (USA) was measured at 560 nm. All biochemical analyses were done in duplicates.

#### **3.4.2.7 Data analysis**

The IBM Statistical Package for Social Sciences version 25 (SPSS IBM Inc Chicago, USA) was employed for statistical analysis. Absolute and relative frequencies were determined for nutrient intakes. The Kolmogorov-Smirnov normality test was carried out to determine if the data on the continuous variables met the parametric assumptions. For non-comparisons between the test classes, a paired t-test was used to test significance. To compare nutrient intake frequencies, gender, and study groups, a chi-square (Fisher's exact test) cross-tabulation was performed. Changes in nutrient intake, exercise level, BMI-for-age, and biochemical parameters were assessed for significance within each treatment group between baseline and three months of intervention, using a paired t-test. For the continuous variable, data were presented as the mean  $\pm$ SD / SEM. Both experiments were 2-tailed, with p-values < 0.05 being deemed to be significant.

#### **Nutrients composition of the egg**

Table 3.4.1

Nutrient values of a medium-size boiled egg.

<b>Nutrient</b>	<b>Unit</b>	<b>1 large (50.0g)</b>	<b>1Value (100g)</b>
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<b>Proximates</b>			
Energy	Kcal	78	155
Water	g	37.31	74.62
Protein	g	6.29	12.58
Total lipid (fat)	g	5.3	10.61
Carbohydrate by difference	g	0.56	1.12
Fibre, total dietary	g	0	0
Sugars, total	g	0.56	1.12
<b>Minerals</b>			
Calcium, Ca	mg	25	50
Iron, Fe	mg	0.59	1.19
Magnesium, Mg	mg	5	10
Phosphate, P	mg	86	172
Potassium, K	mg	63	126
Sodium, Na	mg	62	124
Zinc, Zn	mg	0.53	1.05
<b>Vitamins</b>			
Vitamin C, total ascorbic acid	mg	0	0
Thiamin	mg	0.033	0.066
Riboflavin	mg	0.275	0.513
Niacin	mg	0.032	0.064
Vitamin B <sub>6</sub>	mg	0.06	0.121
Folate, DFE	µg	22	44
Vitamin B <sub>12</sub>	µg	0.56	1.11
Vitamin A, RAE	µg	74	149
Vitamin A, IU	IU	260	520
Vitamin E (α-tocopherol)	mg	0.52	1.03
Vitamin D (D <sub>2</sub> + D <sub>3</sub> )	µg	1.1	2.2
Vitamin D	IU	44	87
Vitamin K (phylloquinone)	µg	0.1	0.3
<b>Lipids</b>			
SFAs <sup>b</sup>	g	1.633	3.267
MUFAs <sup>b</sup>	g	2.038	4.077
PUFAs <sup>b</sup>	g	0.707	1.414
Trans fatty acids	g	0	0
Cholesterol	mg	186	373

Nutrient values and weights are for an edible portion; <sup>b</sup>SFAs: saturated fatty acids, MUFAs: monounsaturated fatty acids, and PUFAs: polyunsaturated fatty acids.

### **3.4.3 Supplement 1.**

#### **3.4.3.1 Results**

Table 3.4.3 presents the mean differences in nutrient intakes between control and experiment groups. At the end of the intervention, mean intakes of energy ( $1481.3 \pm 68.2$  Kcal versus  $1163.0 \pm 51.4$  Kcal  $p < 0.001$ ), carbohydrate ( $247.2 \pm 12.1$  g versus  $187.3 \pm 9.2$  g,  $p < 0.001$ ) protein ( $45.2 \pm 2.3$  g versus  $36.8 \pm 2.0$  g,  $p = 0.005$ ), iron ( $17.5 \pm 1.0$  mg versus  $13.8 \pm 0.8$  mg,  $p = 0.004$ ), zinc ( $7.0 \pm 0.4$  mg versus  $5.2 \pm 0.3$  mg,  $p < 0.001$ ), folate ( $33.0 \pm 3.6$   $\mu$ g versus  $18.6 \pm 2.5$   $\mu$ g,  $p = 0.001$ ) and vitamin B<sub>6</sub> ( $0.4 \pm 0.0$  mg versus  $0.3 \pm 0.0$  mg,  $p = 0.009$ ) were significantly higher in the experiment group, when compared to the control group. The mean intakes for fat, vitamins A and B<sub>12</sub> were not significantly different between the control and experiment groups ( $p > 0.05$ ).

**Table 3.4.3.** Mean comparison of nutrient intakes between control and experiment groups

Nutrients	Cut-off per day	Control	Experiment	Mean difference (95%CI)	P-value
<b>Energy</b>	1742/2279Kcal <sup>†</sup>				
Baseline		861.7±79.4	1274.2±68.8	412.6 (201.6-623.5)	<0.001
Endpoint		1163.0±51.4	1481.3±68.2	318.3 (152.7-483.8)	<0.001
<b>Carbohydrate</b>					
Baseline	130g <sup>¥</sup>	126.4±9.5	212.3±11.7	86.0 (53.6-118.4)	<0.001
Endpoint		187.3±9.2	247.2±12.1	59.9 (29.7-90.1)	<0.001
<b>Protein</b>	34/52g <sup>¥</sup>				
Baseline		36.7±3.0	43.4±2.9	6.7 (1.8-15.1)	0.119
Endpoint		36.8±2.0	45.2±2.3	8.4 (2.7-14.2)	0.005
<b>Fat</b>					
Baseline		24.2±6.6	28.9±2.2	4.7 (8.7-18.0)	0.488
Endpoint		32.0±2.6	38.2±2.4	6.2 (0.3-12.7)	0.061
<b>Iron</b>	8/10mg <sup>¥</sup>				
Baseline		16.4±1.3	17.7±1.3	1.2 (2.1-4.6)	0.462
Endpoint		13.8±0.8	17.5±1.0	3.7 (2.7-14.2)	0.004
<b>Zinc</b>	5/8mg <sup>¥</sup>				
Baseline		5.3±0.4	7.1±0.5	1.8 (0.5-3.1)	0.008
Endpoint		5.2±0.3	7.0±0.4	1.8 (0.9-2.8)	<0.001
<b>Folate</b>	200/300µg <sup>¥</sup>				
Baseline		5.7±0.8	26.8±4.6	21.2 (12.2-30.1)	<0.001
Endpoint		18.6±2.5	33.0±3.6	14.4 (6.1-22.7)	0.001
<b>Vitamin A</b>	400/600µg <sup>¥</sup>				
Baseline		257.4±58.7	213.6±48.3	-43.8 (-202.3- -114.7)	0.584
Endpoint		319.0±44.4	354.2±53.0	35.3 (95.2-165.7)	0.592
<b>Vitamin B<sub>6</sub></b>	0.6/1.0mg <sup>¥</sup>				
Baseline		0.1±0.0	0.3±0.0	0.2 (0.1-0.3)	<0.001
Endpoint		0.3±0.0	0.4±0.0	0.1 (0.0-0.2)	0.009
<b>Vitamin B<sub>12</sub></b>	1.2/1.8µg <sup>¥</sup>				
Baseline		0.4±0.1	1.1±0.2	0.7 (0.2-1.1)	0.002
Endpoint		1.5±0.2	1.4±0.2	-0.1 (-0.8- -0.5)	0.683

Paired t-test, data are presented as mean SEM (standard error mean), <sup>†</sup>EER, <sup>¥</sup>RDA, P-value is significant at p<0.05.

Table 3.4.4 shows that there were significant mean differences within both the control and experiment groups for the following at the end of the intervention. On average, after three months there were an increase in energy (baseline: 1237.8±71.4Kcal, endpoint: 1472.8±67.9Kcal, mean change= +235.0Kcal, p= 0.017), carbohydrate (baseline: 205.8±12.1g, endpoint: 245.6±12.0g,



Baseline	24.0±4.9			28.3±2.2		
Endpoint	29.8±2.0	5.9 (5.0-16.5)	0.286	38.1±2.4	9.8 (3.0-16.6)	<b>0.005</b>
<b>Iron</b>						
Baseline	16.5±1.0			17.2±1.3		
Endpoint	13.6±0.6	-2.9 (-5.4- -0.4)	<b>0.025</b>	17.4±1.0	0.2 (0.0-3.4)	0.863
<b>Zinc</b>						
Baseline	5.5±0.3			6.9±0.5		
Endpoint	5.0±0.2	-0.5 (-1.4- -0.4)	0.265	7.0±0.4	0.1 (0.0-1.4)	0.884
<b>Folate</b>						
Baseline	6.1±0.6			23.4±3.0		
Endpoint	21.5±2.8	15.4 (9.4-21.4)	<b>&lt;0.001</b>	31.0±3.2	7.6 (1.4-16.5)	0.096
<b>Vitamin A</b>						
Baseline	243.6±51.3			210.2±47.8		
Endpoint	293.5±37.5	49.8 (74.9-174.5)	0.430	352.3±52.4	142.0 (5.2-278.8)	<b>0.042</b>
<b>Vitamin B<sub>6</sub></b>						
Baseline	0.1±0.0			0.3±0.0		
Endpoint	0.3±0.0	0.2 (0.1-0.2)	<b>&lt;0.001</b>	0.4±0.0	0.1 (0.0-0.2)	<b>0.041</b>
<b>Vitamin B<sub>12</sub></b>						
Baseline	0.4±0.1			1.2±0.2		
Endpoint	1.3±0.2	0.9 (0.4-1.3)	<b>&lt;0.001</b>	1.4±0.2	0.2 (0.3-0.7)	0.394

Paired t-test, Data are presented as mean±SEM (standard error mean), the p-value is significant at p<0.05

Table 3.4.2 indicates the baseline demographic, anthropometry, and nutrients inadequacies of participants. The study used 195 participants, of whom 115 were the control group and 80 for the experiment group. The mean age for the control group was 10.7±1.1 years, and the mean age for the test group was 10.3±1.1 years. The experimental group recruited more girls than the control

group. In the control group, more boys than in the experiment group. School children in the experiment group had significantly lower inadequacies for carbohydrate (13.5% versus 59.1%,  $p<0.001$ ), protein (66.2% vs 80.9%,  $p= 0.023$ ), iron (12.3% vs 27.3%,  $p= 0.026$ ), zinc (83.6% vs 68.9%,  $p= 0.018$ ), and vitamin B<sub>12</sub> (72.5% vs 95.2%,  $p= 0.006$ ) compared to control group. The mean weight of participants in the experiment group (38.0±8.3 Kg) was higher than those in the control group (33.6±7.6 Kg,  $p= 0.002$ ). Between the two groups, there was no significant difference in height, BMI, energy, and vitamin A intakes ( $p>0.05$ )

**Table 3.4. 2.** Baseline demographic, anthropometry, and nutrients inadequacies of participants

Variable	Baseline		P-value
	Control, N=115	Experiment, N=80	
<b>Gender, n(%)<sup>‡</sup></b>			
Male	59(51.3)	28(35.0)	<b>0.028</b>
Female	56(48.7)	52(65.0)	
<b>Age (years)<sup>¥</sup></b>	10.7±1.1	10.3±1.1	<b>&lt;0.001</b>
<b>Anthropometry<sup>¥</sup></b>			
Weight Kg	33.6±7.6	38.0±8.3	<b>0.002</b>
Height cm	139.4±15.4	141.6±18.9	0.438
BMI Kg/m <sup>2</sup>	16.9±2.7	17.2±2.8	0.538
<b>Nutrient intakes<sup>‡</sup></b>			
Inadequate Energy	106 (96.4)	69 (93.2)	0.322
Inadequate Carbohydrate	65 (59.1)	10 (13.5)	<b>&lt;0.001</b>
Inadequate Protein	89 (80.9)	49 (66.2)	<b>0.023</b>
Inadequate Iron	30 (27.3)	9 (12.3)	<b>0.026</b>
Inadequate Zinc	92 (83.6)	51 (68.9)	<b>0.018</b>
Inadequate Vitamin B <sub>12</sub>	60 (95.2)	58 (72.5)	<b>0.006</b>
Inadequate Vitamin A	82 (86.3)	70 (95.9)	0.289

<sup>‡</sup>Data are presented as frequency (percentage), <sup>¥</sup>mean±SD (standard deviation), <sup>¥</sup>Paired t-test,

<sup>‡</sup>Fisher's exact P value, P-value is significant at  $P<0.05$

Table 3.4.5 compares mean levels of physical fitness, cognition test, BMI-for-age, and biochemical between control and experiment groups. At the end of the intervention, there were decreases in mean values for fifty metres run and total fitness ( $p=0.004$ ), and there was an increment in serum ferritin, serum zinc, and serum albumin in the experiment group. On the other hand, mean values for fifty meters run, total fitness increased in the control group as well as serum ferritin, serum zinc and serum albumin. On the average, participants in the control group had higher levels in the fifty metres run ( $9.6\pm0.1$  versus  $2.0\pm0.1$ ,  $p<0.001$ ), total fitness ( $14.5\pm0.3$  versus  $8.7\pm0.3$ ,  $p<0.001$ ), serum ferritin ( $89.4\pm4.6\mu\text{g/L}$  versus  $61.2\pm2.0\mu\text{g/L}$ ,  $p<0.001$ ) and serum albumin ( $43.3\pm0.3\text{g/dL}$  versus  $43.1\pm0.5\text{g/dL}$ ,  $p=0.004$ ), when compared to those in the experiment group at the end of the intervention. However, serum zinc ( $145.6\pm9.8\mu\text{g/L}$  versus  $121.5\pm1.0\mu\text{g/L}$ ,  $p<0.001$ ), in the experimental group was higher than the control group at the end of the intervention. The mean BMI-for-age was non-significantly higher in the experiment group ( $-0.02\pm0.1$ ) than in the control group ( $-0.25\pm0.1$ ,  $p=0.274$ ).

**Table 3.4.5.** Comparisons of fitness level, cognition test, BMI-for-age, biochemical between control and experiment group

Variable	Control	Experiment	Mean difference	P-value
<b>Physical Fitness level</b>				
<b>Handgrip</b>				
Baseline	$4.0\pm0.3$	$5.2\pm0.2$	1.2	<b>&lt;0.001</b>
Endpoint	$5.0\pm0.2$	$4.8\pm0.2$	-0.2	0.462
<b>Forward jump</b>				
Baseline	$1.7\pm0.2$	$3.7\pm0.4$	2.3	<b>&lt;0.001</b>
Endpoint	$1.0\pm0.0$	$1.0\pm0.0$	-	-
<b>Fifty metres run</b>				
Baseline	$4.6\pm0.4$	$4.7\pm0.4$	0.1	0.796
Endpoint	$9.6\pm0.1$	$2.0\pm0.1$	-7.6	<b>&lt;0.001</b>
<b>Total fitness</b>				
Baseline	$9.0\pm0.6$	$13.0\pm1.0$	4.0	<b>&lt;0.001</b>
Endpoint	$14.5\pm0.3$	$8.7\pm0.3$	-5.8	<b>&lt;0.001</b>
<b>Total cognition test</b>				

Baseline	14.0±1.0	16.4±1.0	2.4	<b>0.037</b>
Endpoint	20.0±0.8	20.0±0.9	0.1	0.928
<b>Anthropometry</b>				
<b>BMI-for-age</b>				
Baseline	-0.25±0.1	-0.06±0.2	0.17	0.409
Endpoint	-0.25±0.1	-0.02±0.1	0.22	0.274
<b>Biochemical</b>				
<b>Serum ferritin(µg/L)</b>				
Baseline	59.2±2.8	40.8±2.3	-18.3	<b>&lt;0.001</b>
Endpoint	89.4±4.6	61.2±2.0	-36.9	<b>&lt;0.001</b>
<b>Serum zinc (µg/L)</b>				
Baseline	96.9±6.1	75.6±8.2	-21.3	<b>&lt;0.001</b>
Endpoint	121.5±1.0	145.6±9.8	24.1	<b>&lt;0.001</b>
<b>Serum albumin(g/dL)</b>				
Baseline	37.9±1.1	41.1±0.7	3.2	<b>0.004</b>
Endpoint	43.3±0.3	43.1±0.5	-0.2	<b>&lt;0.001</b>

Paired t-test, Data are presented as mean±SEM (standard error mean), the p-value is significant at p<0.05

Table 3.4.6 compares mean levels of fitness, cognition test, BMI-for-age, biochemical within both the control and experiment group. There were a significant increase in mean values for total cognition score (Baseline: 16.5±1.0, endpoint: 19.8±0.9, mean change: +3.3, p= 0.001) and serum ferritin (Baseline: 41.1±2.3µg/L, endpoint: 61.3±2.0µg/L, mean change: +20.1, p<0.001), serum zinc (Baseline: 72.9±8.3µg/L, endpoint: 142.3±9.8µg/L, mean change: +69.4, p<0.001) and serum albumin (Baseline: 40.9±0.7µg/L, endpoint: 43.0±0.5µg/L, mean change: +2.1, p=0.013) in the experiment group at the end of the three months. However, mean values for forward jump (Baseline: 4.0±0.4, endpoint: 1.0±0.0, mean change: -3.0, p<0.001), fifty metres run (Baseline: 4.5±0.4, endpoint: 2.1±0.2, mean change: -2.4, p<0.001) and total fitness (Baseline: 12.8±1.0, endpoint: 8.9±0.3, mean change: -3.9, p<0.001), decreased in the experiment group at the end of the intervention. In the control group, the mean values for handgrip (Baseline: 4.0±0.2, endpoint: 5.0±0.2, mean change: +1.0, p<0.001), fifty metres run (Baseline: 5.8±0.4, endpoint: 9.5±0.2, mean change: +3.7, p<0.001), total fitness (Baseline: 10.2±0.5, endpoint: 14.4±0.3, mean change:



+4.2,  $p<0.001$ ), total cognition scores (Baseline:  $12.8\pm0.8$ , Endpoint:  $17.6\pm0.8$ , mean change: +4.8,  $p<0.001$ ), serum ferritin (Baseline:  $65.1\pm3.1\mu\text{g/L}$ , endpoint:  $83.4\pm3.8\mu\text{g/L}$ , mean change: +18.2,  $p=0.001$ ), serum zinc (Baseline:  $108.7\pm7.2\mu\text{g/L}$ , endpoint:  $123.2\pm0.9\mu\text{g/L}$ , mean change: +14.4,  $p=0.051$ ) and serum albumin (Baseline:  $37.6\pm0.9\text{g/dL}$ , endpoint:  $42.5\pm0.4\text{g/dL}$ , mean change: +4.9,  $p<0.001$ ) increased at the end of three months. The mean forward jump value decreased significantly in the control group at the end of the intervention (Baseline:  $1.5\pm0.0$ , endpoint:  $1.0\pm0.0$ , mean change: -0.5,  $p<0.001$ ).

**Table 3.4.6.** Comparisons of fitness level, cognition test, BMI-for-age, biochemical within both the control and experiment group

Variable	Control	Mean difference	P-value	Experiment	Mean difference	P-value
<b>Physical Fitness</b>						
<b>Handgrip</b>						
Baseline	$4.0\pm0.2$			$5.2\pm0.2$		
Endpoint	$5.0\pm0.2$	1.0	<b>&lt;0.001</b>	$4.9\pm0.2$	-0.3	0.272
<b>Forward jump</b>						
Baseline	$1.5\pm0.0$			$3.8\pm0.4$		
Endpoint	$1.0\pm0.0$	-0.5	<b>&lt;0.001</b>	$1.0\pm0.0$	-3.0	<b>&lt;0.001</b>
<b>Fifty metres run</b>						
Baseline	$5.8\pm0.4$			$4.5\pm0.4$		
Endpoint	$9.5\pm0.2$	3.7	<b>&lt;0.001</b>	$2.1\pm0.2$	-2.4	<b>&lt;0.001</b>
<b>Total fitness</b>						
Baseline	$10.2\pm0.5$			$12.8\pm1.0$		
Endpoint	$14.4\pm0.3$	4.2	<b>&lt;0.001</b>	$8.9\pm0.3$	-3.9	<b>&lt;0.001</b>
<b>Total cognition test</b>						
Baseline	$12.8\pm0.8$			$16.5\pm1.0$		
Endpoint	$17.6\pm0.8$	4.8	<b>&lt;0.001</b>	$19.8\pm0.9$	3.3	<b>0.001</b>
<b>Anthropometry</b>						
<b>BMI-for-age</b>						
Baseline	$-0.24\pm0.1$			$-0.03\pm0.1$		
Endpoint	$-0.17\pm0.1$	0.1	0.180	$-0.02\pm0.1$	0.01	0.894
<b>Biochemical</b>						
<b>Serum ferritin(<math>\mu\text{g/L}</math>)</b>						
Baseline	$65.1\pm3.1$			$41.1\pm2.3$		
Endpoint	$83.4\pm3.8$	18.2	<b>0.001</b>	$61.3\pm2.0$	20.1	<b>&lt;0.001</b>

**Serum zinc( $\mu\text{g/L}$ )**

Baseline	108.7 $\pm$ 7.2			72.9 $\pm$ 8.3		
Endpoint	123.2 $\pm$ 0.9	14.4	<b>0.051</b>	142.3 $\pm$ 9.8	69.4	<b>&lt;0.001</b>

**Serum  
albumin(g/dL)**

Baseline	37.6 $\pm$ 0.9			40.9 $\pm$ 0.7		
Endpoint	42.5 $\pm$ 0.4	4.9	<b>&lt;0.001</b>	43.0 $\pm$ 0.5	2.1	<b>0.013</b>

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Paired t-test, Data are presented as mean $\pm$ SEM (standard error mean), the p-value is significant at p<0.05.