ORIGINAL ARTICLE

EFFICACY OF MULTIPLE MICRONUTRIENTS SUPPLEMENTATION ON ENERGY INTAKE, CALCIUM AND VITAMIN D LEVELS IN UNDERWEIGHT CHILDREN

Ikhlaq Ahmad[1], Sadia Fatima[1], Muhammad Waqas Luqman[2], Rubina Nazli[1], Syed Hamid Habib[1], Inayat Shah[1]

[1]Institute of Basic Medical Sciences Peshawar, [2]KMU Institute of Medical Sciences Kohat, Khyber Medical University, Peshawar-Pakistan

Background: Under-nutrition is a major public health problem worldwide. Several studies have documented the effects of vitamin D and calcium supplements in healthy children and in children with bone abnormalities but the effects of multiple micronutrients supplementation in underweight children is limited in literature. **Methods:** In this randomized controlled trial 38 participants were recruited and divided into two groups of 19 subjects. On the first day of experimental trial, in fasting state 5cc blood samples were collected from all subjects followed by their anthropometric measurements, and serum levels of calcium and vitamin D. All subjects of one group were given Lipid-based nutritional supplement (LNS) and whereas the other group was given the placebo. After one month of compliance the same measurements were repeated and compared. **Results:** After one month of supplementation mid-upper arm circumference (p=0.005), weight (p<0.001), height (p=0.000), and BMI-Z score (p=0.002) the energy intake (p<0.001), were significantly increased in LNS as compared to Placebo. However, no improvement was observed in the plasma vitamin D (p=0.44) and calcium levels (p=0.46) of underweight children in both groups. **Conclusion:** Multi micronutrient supplementations are effective in improving anthropometry in the short duration of time. They also improve the energy intake of underweight children. However, no improvement is observed in levels of vitamin D and calcium in LNS group even after a one-month use.

Keywords: Vitamin D; Vitamin D deficiency; Lipid-Based Supplement; Placebo

Citation: Ahmad I, Fatima S, Luqman MW, Nazli R, Habib SH, Shah I. Efficacy of multiple micronutrients supplementation on energy intake, calcium and vitamin d levels in underweight children. J Ayub Med Coll Abbottabad 2022;34(3 Suppl 1):593–601.

DOI: 10.55519/JAMC-03-S1-9296

INTRODUCTION

Malnutrition one of the leading health problems and is referred to as "silent emergency".[1] In malnutrition the individual's ability to sustain normal body functions, such as growth, fertility, lactation etc., are reduced.[2,3] About 200 million school going children are found to be underweight and have lower height for age (stunted); the number is feared to increase to 1 billion by 2020.[4] Malnutrition is on rise in and 50% of the children here are assessed as malnourished and contribute to about 13 million deaths of infants and children annually in these developing countries.[5]

Countering malnutrition has been a dilemma for many years and one of the quick solutions is administrations of micronutrients. Vitamins and minerals fall into the category of micronutrients and are required in a very small amount and have a key role in normal body functions.[6]

Vitamin D, an essential fat-soluble vitamin, is one of these micronutrients that is necessary for the body in adequate amount to attain genetic growth potential between the children, but uptil now definition of "adequate" is not well established.[7] It is important for maintenance of calcium haemostasis, and for preventing bone fractures, its deficiency is also related to other diseases such as diabetes, infectious diseases and metabolic disorders.[8] Generally, vitamin D is divided into two active forms vitamin D_2 (Ergocalciferol) which is synthesized by plant and vitamin D_3 (Cholecalciferol) which is synthesized by animals in the body. Vitamin D_3 is important to humans and prepare in the skin. It is gained exogenously from the dietary sources and supplements or can be synthesized endogenously by the body. Therefore, the main risk factors of vitamin D insufficiency includes lack of sun exposure, insufficient dietary consumption and malabsorption disorders for-example Crohn's disease and celiac disease.[9] Moreover, its deficiency can lead to growth retardation, skeleton abnormalities and increased risk of fracture later on in life.[10]

Vitamin D deficiency (VDD) is prevalent globally among children and acknowledged to be a major public health issue and several studies on teenagers and infants in low and middle income states, show that VDD reaching from 28–62%.[11] Moreover, because of lack of information from various countries regarding VDD, prevalence still remains uncertain, it is documented that nearly one billion people all over the world have low vitamin D levels in all age groups.[8] VDD isn't just restricted to population living in the

South Asian countries e.g. India and Pakistan[12, 13] but immigrants of South Asian counties living in UK, Denmark and Norway also have low VD levels.[14]

Calcium is a vital nutrient for maintenance and growth of healthy bones.[15] It is also required for the various functions of the cells. Insufficient intake of calcium results in osteoporosis which is accountable for the fractures and also related with high danger of the non-skeletal damages which results in hypertension[16], colorectal cancer[17], and type 2 diabetes etc.[18] Calcium is stored in high quantity in the human body. It is estimated that more than 99% of the calcium is stored in teeth and bones. Moreover, about less than 1% of calcium is found in extracellular serum calcium. When a healthy adult devour calcium only 30% of it is absorbed by the body, as several factors effects the rate of absorption of calcium for-example during pregnancy calcium absorption increases because of growing fetus.[18]

The use of vitamin D and calcium supplements, to counter their deficiency, is very common practice. But do these supplements really have an influence on improving the levels of both vitamin D and calcium in serum in underweight children? To find the answers this study has been carried out to find the effect of micronutrients supplements on the levels of calcium and vitamin D in underweight children. A study as such has never been performed in our area and is limited in other literature as well.

MATERIAL AND METHODS

The current study was a single-blind randomized control trial, conducted in the Clinical trial room (CTR) after approval from the Advance Studies and Research Board (ASRB) of the Institute of Basic Medical Sciences (IBMS), Khyber Medical University (KMU) Peshawar, Pakistan. This trial was registered in ISRCTN registry under/ ISTRCN14718521 and took 8 months in completion from September 2017 to April 2018. In this randomized controlled trial 38 participants were recruited through convenient sampling technique and randomly divided into two groups; each comprised 19 subjects following the inclusion criteria. Moderately underweight children with (BMI-Z score between -2 and -3) aged between 5–10 years were included. Children having eating complaints, allergies to supplements, Gastrointestinal tract infection, gastrointestinal tract surgery, or were taking any other supplements were excluded from the study.

After getting a proper informed consents and assents from parents, children, aged of 5–10 years, were selected from orphanages, government primary schools for boys and girls in Hayatabad, Peshawar, Pakistan. Study was conducted to find out the effect of Lipid-based nutritional supplement (LNS) on concentrations of VD and calcium and energy intake in moderately underweight children. Body mass index -Z (BMI-Z) between -2 and -3. BMI-Z scores were determined using LMS Growth Software.[19] The Sample collection method is depicted in figure-1. The 9 points hedonic scale from “like extremely” to “dislike extremely” was used to check the taste of served foods.

On day first, the children presented to the KMU CTR in fasting state. All baseline anthropometric measurements and 5ml blood samples (in heparin tubes) were obtained using standard protocol. The children were later provided with either LNS or Placebo and were allowed to finish it in half an hour; after which an ad *libitum* buffet breakfast was also served to the children. Then 4 hours later, after the supplementation an *ad libitum* buffet lunch was served. Participants also filled a proforma regarding the taste of both LNS and Placebo. LNS/Placebo sachets were provided to parents of children along with directions on how to use them for next one month. They were also asked to keep the record of empty sachets and ensure compliance.

Table 1: Composition of LNS (Achaa mum) and placebo as supplementary food

	LNS 100g/day	PLACEBO 100g/day
Energy (kcal)	535	92
Macronutrients		
Proteins (g)	14	5.54
Carbohydrates (g)	22	14.2
Fats (g)	30	0.58
Minerals		
Zinc (mg)	11	-
Iron (mg)	10	0.22
Potassium (mg)	900	-
Copper (mg)	1.4	-
Calcium (mg)	535	0.16
Vitamin D (mcg)	15	-
Vitamin C (mg)	-	0.02

One month later (Day 31) the participants presented to CTR again, in fasting state. Anthropometric measurements and 5ml blood samples were repeated according to standard protocol; and participants were given LNS/Placebo, *ad libitum* buffet breakfasts and lunches as was given on day 1.

The participants’ energy intake (EI) was determined from the contents of *ad libitum* buffet breakfasts and lunches through Windiet-Software (version 2005).

After this vitamin D and calcium levels were analyzed using COBASe411 and COBAS 111 respectively. For the quantitative determination of total vitamin D (25-OH) in human serum and plasma we used Roche Diagnostic electrochemiluminescence (ECL) which is a competitive protein binding assay. This is used to help the measurement of VD (25-OH).

The collected data was analyzed by using MINITAB® Version 17 and LMS growth (WAZ score). Normality of the data was checked by using the Anderson Darling test of normality. Age, height, weight, MUAC and skinfolds were expressed as Mean and SD. Socio-economic data were analyzed using the Chi-square test. Comparison of energy intake, VD and calcium between the study groups was analyzed by using two samples T-test.

RESULTS

In the current study, differences in energy intake, anthropometric measurements as well as changes in VD (25-OH Cholecalciferol) and calcium levels were assessed in 38, mild to moderate underweight children between age 5-10 years with BMI Z score between -2 SDS and -3 SDS. The socioeconomic data of both groups LNS/Placebo shows that all participants of this study belong to a low socioeconomic family, according to NNS 2011 with a maximum range of monthly income of (5,000 to 35,000 RS) (Table-2).

The 9 points hedonic scale from "like extremely" to "dislike extremely" was used to check the taste of severed foods. (Table-3)

The baseline anthropometric measurements of the participants show no significant difference in both LNS and Placebo group. (Table-4) After one month of supplementation a significant improvement in the height, weight, BMI Z score, Mid-upper arm circumference (MUAC) and triceps was observed in LNS group as compared to Placebo (Table-5). Hence in Placebo group waist to hip ratio (WHR) was significantly improved. The data on multiple pass recalls 3 days before the trial in both LNS/Placebo no significant difference was observed. Moreover, three days after trial the total EI and macronutrient significantly increased in LNS group as compared to Placebo group. Likewise, after 15 days of trial the EI again significantly increased in LNS group as compared to Placebo group, while carbohydrate in Placebo group 3 days after trial and after 15 days of trial was improved as compared to LNS group. (Figure-2, Table-6)

No significant increase was observed in total EI. Likewise, a significant improvement was observed in macronutrients in term of protein (p=0.006) and carbohydrate (p=0.002) in both groups respectively. However, during the breakfast, the total EI was significantly decreased in the LNS group as compared to Placebo group. (Table-7)

On the first day of experimental trial EI was measured after providing the LNS/Placebo before the breakfast to the participants in their respective groups. During the breakfast, the total EI was significantly decreased in the LNS group as compared to Placebo group. Similarly, the EI during the lunch was lowered in LNS group (Table-7). After one month of supplementation on the second day of experimental trial the total EI and macronutrients were significantly increased in LNS groups when only supplement was given. However, during the breakfast no significant improvement was observed in LNS group. (Table-8)

The plasma levels of VD and calcium on day 1 and day 31 were measured in both groups LNS/Placebo. After one month of supplementation the calcium levels in LNS group [p=0.465] and in Placebo group [p=0.375] which shows that there was no change observed in the calcium levels in both groups. Similarly, there was no significant increase observed in VD levels in the LNS group while in Placebo group the VD levels significantly decreased [p=0.003]. (Table-9)

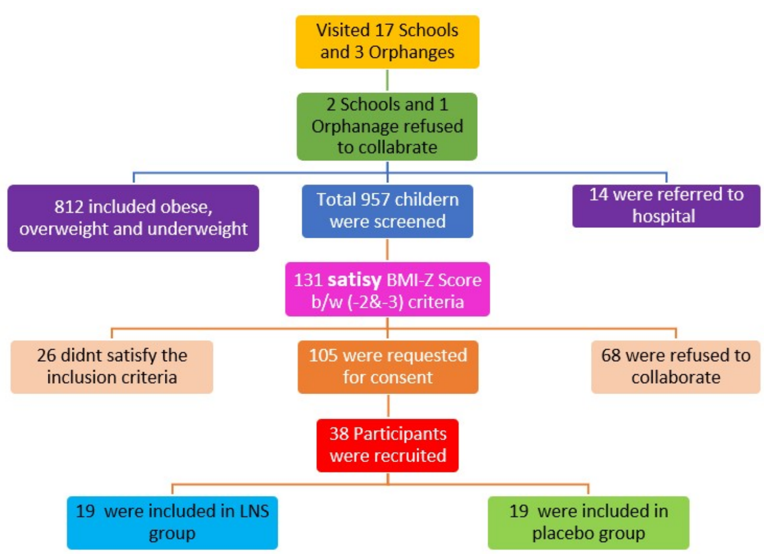

Figure-1: Diagrammatically representation of study samples according to CONSORT guidelines

Table-2: Socioeconomic data of participants

	LNS (n=19) Count (%)	*p*-value	PLACEBO (n=19) Count (%)	*p*-value
Professional Status of Father				
Skilled	16 (84.22)	1.000	16 (84.22)	1.000
Unskilled	3 (15.79)		3 (15.79)	
Father Education				
Illiterate	3 (15.79)	0.426	5 (26.32)	0.331
Primary	5 (26.32)		2 (10.53)	
Middle	2 (10.53)		5 (26.32)	
SSC	2 (10.53)		4 (21.05)	
HSSC	4 (21.05)		2 (10.53)	
Graduate	3 (15.79)		1 (5.26)	
Postgraduate	0 (0)		0 (0)	
Professional Status of Mother				
Skilled	3 (15.79)	1.000	5 (26.32)	1.000
Unskilled	16 (84.22)		14 (73.68)	
Mother Education				
Illiterate	9 (47.37)	0.109	12 (63.16)	0.666
Primary	3 (15.79)		3 (15.79)	
Middle	3 (15.79)		4 (21.05)	
SSC	4 (21.05)		0 (14.29)	
HSSC	0 (0)		0 (0)	
Graduate	0 (0)		0 (0)	
Postgraduate	0 (0)		0 (0)	
Total Family Members				
0-3	0 (0)	1.000	0 (0)	0.990
4—6	9 (47.37)		8 (42.11)	
7—9	7 (36.84)		6 (31.57)	
10—12	3 (15.79)		3 (15.79)	
>12	0 (0)		2 (7.14)	
No of Sibling				
0-3	15 (78.94)	0.999	15 (78.94)	0.995
4—6	4 (21.05)		4 (21.05)	
7—9	0 (0)		0 (0)	
10—12	0 (0)		0 (0)	
>12	0 (0)		0 (0)	
Household				
Separate	14 (78.57)	1.000	15 (78.94)	1.000
Joint	5 (26.32)		4 (21.05)	
House type				
Bungalow	0 (0)	1.000	0 (0)	1.000
Apartment	3 (15.79)		3 (15.79)	
Town house	16 (84.22)		13 (68.42)	
Village house	0 (0)		3 (7.14)	
House structure				
Pakka	13 (68.42)	0.9698	13 (68.42)	0.922
Kacha	4 (21.05)		2 (10.53)	
Semi Pakka	2 (10.53)		4 (21.05)	
Others	0 (0)		0 (0)	
House status				
Rented	7 (36.84)	1.000	9 (47.37)	0.993
Self	11 (57.89)		10 (52.63)	
Employer/Govt	0 (0)		0 (0)	
Rent payment				
Self	13 (68.42)	0.997	11 (57.89)	0.968
Govt	5 (26.32)		7 (36.84)	
Other	1 (5.26)		1 (5.26)	
No of Kitchen				
0-3	19 (100)	1.000	19 (100)	1.000
No of Rooms				
0-3	13 (68.42)	1.000	12 (63.16)	0.995
4—6	5 (26.32)		7 (36.84)	
7—9	1 (5.26)		0 (0)	
No of Bathrooms				
0-3	13 (68.42)	1.000	12 (63.16)	1.000
4—6	5 (26.32)		7 (36.84)	
7—9	0 (0)		0 (0)	
Residence Location				
Rural	0 (0)	1.000	1 (5.26)	1.000
Urban	19 (100)		18 (94.73)	
Water supply				
Bore water	3 (15.79)	1.000	3 (15.79)	1.000
Community tape water	16 (84.22)		16 (84.22)	
Monthly wages (Rs)				
5000- 15000	0 (0)	0.998	1 (5.26)	0.953
16000-25000	5 (26.32)		4 (21.05)	
26000-35000	14 (73.68)		14 (73.68)	

Table-3: Hedonic scale of participants

Hedonic scale	LNS (n=19)		PLACEBO (n=19)	
Like extremely	3 (15.79)	0.844	2 (10.53)	0.635
Like very much	4 (21.05)		6 (31.57)	
Like moderately	5 (26.32)		2 (7.14)	
Like slightly	5 (26.32)		3 (15.79)	
Neither like nor dislike	0 (0)		4 (21.05)	
Dislike slightly	2 (10.53)		2 (10.53)	
Dislike moderately	0 (0)		0 (0)	
Dislike very much	0 (0)		0 (0)	
Dislike extremely	0 (0)		0 (0)	

Table-4: Baseline anthropometric measurement on day 1 of the trial

Variables	LNS (n=19) (Mean±S.D)	PLACEBO (n=19) (Mean±S.D)	*p*-value
Age (years)	6.944±1.39	7.06±1.71	0.840
Height (cm)	115.08±8.61	120.58±9.60	0.122
Weight (kg)	17.22±2.65	19.11±3.08	0.094
BMI (kg/m^2)	12.93±0.35	13.01±0.26	0.500
MUAC (cm)	14.74±1.00	15.18±0.81	0.216
Biceps (mm)	4.02±1.09	3.6±1.02	0.299
Triceps (mm)	6.22±1.31	6.38±1.37	0.759
Mid abdomen (mm)	4.57±1.58	4.69±1.26	0.823
Sub-scapula (mm)	4.64±0.68	4.66±0.81	0.960
Waist/Hip (cm)	0.85±0.04	0.86±0.07	0.728

Table-5: Changes in anthropometric measurement after 30 days' supplementation

Variables	Before LNS (Mean±S.D)	After LNS (Mean±S.D)	*p*-value	Before PLACEBO (Mean±S.D)	After PLACEBO (Mean±S.D)	*p*-value
Height (cm)	115.08±8.61	115.8±8.54	0.002	120.58±9.60	120.64±9.36	0.773
Weight (kg)	17.22±2.65	17.89±3.01	0.000	19.11±3.08	19.24±3.56	0.585
BMI (kg/m^2)	12.93±0.35	13.24±0.48	0.001	13.01±0.26	13.10±0.72	0.634
MUAC (cm)	14.74±1.00	15.12±0.87	0.001	15.18±0.81	15.44±0.97	0.186
Biceps (mm)	4.02±1.09	3.85±1.14	0.374	3.6±1.02	3.6±1.21	1
Triceps(mm)	6.22±1.31	6.63±1.53	0.051	6.38 ±1.37	6.31±1.94	0.747
Mid abdomen (mm)	4.57±1.58	4.84±1.66	0.195	4.69±1.26	4.81±1.44	0.401
Sub scapular (mm)	4.64±0.68	4.72±0.79	0.318	4.66±0.81	4.69±0.86	0.689
Waist to hip ratio (cm)	0.85±0.04	0.84±0.03	0.192	0.86±0.07	0.82±0.05	0.029

Table-6: Comparison between energy intake (EI) and macronutrients in LNS/PLACEBO group through Windiet

	LNS (n=19)	PLACEBO (n=19)	*p*-value
Three Days Before Trial			
Energy (kcal)	650.1±139.76	635.5±140.48	0.785
Proteins (g)	20.0±3.16	20.4±5.30	0.797
Fats (g)	26.2±8.15	24.8±8.34	0.735
CHO (g)	88.4±17.82	82.5±25.15	0.48
Vitamin D (IU)	0.806±0.332	1.216±1.115	0.5384
Calcium (mg/dl)	113.3±25.69	194.6±21.50	5.57823E -07
Three Days After Trial			
Energy (kcal)	1041.0±140.57	696.5±164.72	0.000***
Proteins (g)	31.5±6.29	23.6±5.88	0.002**
Fats (g)	49.0±9.3	24.7±9.13	0.000***
CHO (g)	95.8±13.85	97.8±20.89	0.77
Vitamin D	9.82±8.172	0.056±0.051	0.000***
Calcium	650±93.74	219.33±91.34	0.917
Three Days After 15 Days of Trial			
Energy (kcal)	1011.2±178.38	742.3±156.0	0.000***
Proteins (g)	30.1±7.19	24.0±4.87	0.014*
Fats (g)	45.2±8.63	45.2±8.140	0.000***
CHO (g)	102.7±26.56	105.1±21.87	0.800
Vitamin D	14.47±0.242	0.18±0.07	0.000***
Calcium	716±152.6	206.33±34.55	0.000***

Table-7: Comparison between total energy intake & macronutrients on first day of trial

			LNS (n=19) (Mean±S.D)	PLACEBO (n=19) (Mean±S.D)	*p*-value
Supplement	Energy (kcal)	Day 1	267.64±167.55	32.64±21.28	0.000***
Breakfast			289.5±114.56	464.86±242.24	0.021
Lunch			214.14±110.02	306.79±135.12	0.05
Breakfast+ Lunch			503.71±158.97	771.57±303.66	0.007
Breakfast +Lunch+ Supplement			771.36±276.51	804.21±316.84	0.772
	Vitamin D (IU)		8.6035±5.091	1.070±0.963	0.757
	Calcium (mg/dl)		413±197.27	245.28 ±107.963	0.976

Table-8: Comparison between energy intake & macronutrients after one month of supplementation on second main trial day

Day 31			LNS (n=19) (Mean±S.D)	PLACEBO (n=19) (Mean±S.D)	*p*-value
Supplement	Energy (kcal)	DAY 31	252.21±141.22	34.14±26.16	0.000***
Breakfast			313.86±132.53	439.14±225.22	0.084
Lunch			340.43±264.43	356.14±156.94	0.849
Breakfast + Lunch			648.86±322.94	796±317.43	0.234
Breakfast +Lunch+ Supplement			901±347.59	830.14±320.46	0.565
	Vitamin D (IU)		8.067±3.191	1.0614±0.691	0.438
	Calcium (mg/dl)		468.714±176.60	253.357±124.23	0.855

Table-9: Comparison between Vitamin D & Calcium levels in LNS & PLACEBO groups

Calcium and Vitamin D						
	LNS (n=19)			PLACEBO (n=19)		
	Day 1	Day 31	*p*-value	Day 1	Day 31	*p*-value
	Mean±SD	Mean±SD		Mean±SD	Mean±SD	
Calcium (mg/dl)	9.14 ±0.41	8.81±1.74	0.4651	8.90 ±0.36	8.67 ±0.85	0.375
Vitamin D (IU)	19.47 ±12.79	20.61 ±9.47	0.4458	12.97±4.87	11.42±4.72	0.003

DISCUSSION

Undernutrition a common public health problem worldwide, especially in low and middle income countries that usually occurs due to low socioeconomic status, insufficient energy, proteins, and vitamins intake.[20] It is one of the foremost causes of the death of children under the age of 5 years and the most common reason of the decline in the health and life of children. It not only results in stunting, increase in the sustainability to disease but also impact on learning ability, inadequacy, and incapability to attain skills.[21] Therefore, urgent implementation of intervention needed to reduce the occurrence and outcomes of undernutrition.

In this study the effect of LNS was compared with low calorie placebo for one month in underweight children having aged between 5 to 10 years with BMI score -2 SDS and -3 SDS to check that how LNS improve the energy intake, VD and calcium levels in these children.

Moreover, the plasma levels of VD were measured in both the LNS and Placebo groups. At baseline ninety-four percent of the study population have low VD levels in both the LNS and Placebo group based on the National Nutritional Survey (NNS) 2011.[22] According to NNS 2011 at national level the prevalence of VDD among the children was 40.0%. In our study 94.5% of the children were VD deficient which were higher as compared to other studies as we have conducted the study on moderate underweight children having age between 5–10 years. However, a study conducted in India on children aged between 6–18 years also documented that 92.3% of children were VD deficient as per serum 25 (OH) D levels of <20 ng/ml.[23]

Our results revealed that after four weeks of supplementation there was no change in the levels of VD in LNS group and Placebo groups. Several studies have been conducted on children, toddlers and healthy school children showing an improvement in VD levels after supplementation[24] while in some of the studies no improvement was observed.[25, 26] A double randomized control trial conducted on healthy children aged between 1–3 years after twenty weeks it was observed that regular consumption of micronutrient fortified Young Child Formula (YCF) increased ferritin and VD status in European children rather than non fortified cow milk (0.02 mg Fe per100 mL; no VD).[24] Likewise, another double-blind randomized control trial conducted in Dunedin New Zealand on 225 healthy toddlers aged between 12–20 months found effective changes in vitamin D levels.[27] However, in our study no improvement in VD level was observed as our study was of short duration (one month only). Future prolonged randomized control trials are required to observe the effect of LNS on VD and calcium deficient participants.

Similar to our clinical trial results some of the studies have observed no improvement in VD status after supplementation.[25, 26] In one of the studies conducted on eight children having age between 5–18 years suffering from severe burns to improve the VDD as inability of burns skin to produce VD. After 6 months it was observed that the provision of multivitamin tablets contains 400 IU of VD_2 fails to improve VD levels in these children.[28]

Along with plasma VD levels we also measured plasma levels of calcium in both LNS/Placebo groups. At the baseline the calcium levels were in normal range both in LNS and Placebo groups. After the four week of supplementation no change in calcium levels were observed in both the groups. Our results are in contradiction to other studies in which calcium levels were significantly increased in supplement group after the supplementation.[29] Similarly, in one of the study two experiments were performed in which calcium absorption was analysed at different VD levels. In which 500 mg oral calcium was provided to two groups. One with pre-treated VD in which participants have 86.5 nmol/l VD level while the other have 50.2 nmol/l VD levels. So it was observed that calcium absorption was reduced at the level of 50.2 nmol/l as compared to the pre-treated participants having 86.5 nmol/l VD level.[30] The above study is accordance with our results as calcium absorption is considered to be reduced under conditions of VD deficiency. As far as our study participants were VD deficient due to which calcium was not absorbed and the calcium levels remained unchanged. Up to our knowledge none of the previous study conducted has checked the effect of multi nutritional supplements on calcium levels in moderately underweight children.

Similarly, in a community based randomized control trial on 138 children having age between 2–5 years, after 12 months' VD supplementation along with calcium improved the growth of children and serum levels of VD, calcium and alkaline phosphate in group 1 as compared to group 2 which was provided calcium alone.[29] However, in our study no change in calcium levels were observed as our study was of short duration (four weeks only), moreover, our participants were VD deficient due which calcium absorption is reduced.

Furthermore, the energy intake of all the participants was measured in the clinical trial room at the baseline and after 1 month of supplementation. We observed that on the first day of experimental trial the energy intake in LNS group was lower as compared to Placebo group during the breakfast. Some previous studies have observed the suppression of EI during the breakfast after intake of supplements.[31, 32] In one of the studies conducted on underweight females it was observed that EI was significantly decreased during the breakfast after the provision of high energy nutritional supplement.[31]

After one month of supplementation on the 2nd day of experimental trial the overall energy intake during supplement, breakfast and lunch was significantly higher and no suppression of energy EI was observed in LNS group as compared to Placebo group. Thus, it indicates that children can accommodate, if LNS was provided on daily basis and the suppression of EI was reduced from the regular habitual diet. The results matched with other studies, however, it's worth mentioning that the children were provided with the same kind of food at same time and at same place using same utensils served in same manner on both trials to avoid bias.[31]

In our study the data obtained on multiple pass recalls it shows that three days after the main trial the total EI, macronutrients, VD and calcium levels were significantly increased in LNS group (p<0.001) as compared to Placebo group. Our results are similar to previous studies which have documented an improvement in total caloric intake of the participants after the provision of supplements.[33] Likewise, in another study a significant increase in EI was observed in malnourished participants due to provision of oral nutrition supplementation (ONS).[34] Moreover, in one of the study (intake of ONS) significantly increased the micronutrient and the habitual dietary intake of malnourished patients.[33] Our results are in accordance with the studies available in the literature. However, the methods of measurement of EI in earlier trials were different.

In the current study after one month of supplementation a significant improvement was observed in anthropometric measures which includes height, weight, BMI Z score, MUAC and triceps in LNS group as compared to Placebo. This increased in the nutrition outcomes was expected to LNS feeding which was also observed in some previous studies.[35-37] A study conducted on malnourished children suffering from gastrointestinal infection a significant improvement was observed in the weight gain after three months in supplement group as compared to Placebo group.[35]

Likewise, in another randomized control trial (RCT) which was carried out in Mali on MAM children a significant increase was observed in weight gain after the provision of LNS. Similarly, several trials conducted previously shows significant improvement in the BMI as well as in MUAC in malnourished children due to the use of LNS.

CONCLUSION

Multi micronutrient supplementations are effective in improving anthropometry in the short duration of time. It also improves the energy intake of underweight children regardless of the prolonged suppression, but the overall improvement is less as compared to expected. Moreover, no improvement is observed in levels of vitamin D and calcium in LNS group even after a one-month use.

Further prolonged period studies are needed in underweight children to check the effect of multi micronutrient supplements on Ca and VD levels, EI and anthropometric measurements. Moreover, awareness regarding dietary adequacy and the supplement use is necessary among the parents and children. Large community-based trials should be conducted on schools going children to handle the issue of malnutrition. To provide educational and health benefits to vulnerable children and to diminish short term hunger in undernourished children school feeding programs should be started.

AUTHORS' CONTRIBUTION

SF conceived, designed and did statistical analysis, data interpretation and editing of manuscript. IA did data collection, statistical analysis and manuscript writing. SHH, MWL did critical review, data interpretation and editing, final approval of manuscript. IS and RN did review and final approval of manuscript.

Funding

The project is partially funded by Office of Research Innovation and Commercialization (ORIC), Khyber Medical University, Peshawar.

Competing Interest None to declare

REFERENCES

1. Ahsan T, Rahman M, Ramatullah M, Ahsan M, Khan MNI, Islam SN. Multiple micronutrient (zinc, magnesium) therapy to severe malnourished children: effect on growth catch up and clinical recovery. Eur Sci J 2013;9(30):103–15.
2. Ingutia RA, Islam KZ, Hossain MM, Ingutia R. Analysis of the relation between rural poverty, malnutrition and hunger, and the interlinks with agricultural production growth and productivity growth with special reference to Mozambique and Bangladesh. 2009.
3. Park SE, Kim S, Ouma C, Loha M, Wierzba TF, Beck NS. Community management of acute malnutrition in the developing world. Pediatr Gastroenterol Hepatol Nutr 2012;15(4):210–9.
4. Srivastava A, Mahmood SE, Srivastava PM, Shrotriya VP, Kumar B. Nutritional status of school-age children-A scenario of urban slums in India. Arch Public Health 2012;70(1):8.
5. Rahman A, Chowdhury S. Determinants of chronic malnutrition among preschool children in Bangladesh. J Biosoc Sci 2007;39(2):161–73.
6. Gong W, Liu A, Yao Y, Ma Y, Ding C, Song C, *et al*. Nutrient supplement use among the Chinese population: a cross-sectional study of the 2010–2012 China nutrition and health surveillance. Nutrients 2018;10(11):1733.
7. Rovner AJ, Stallings VA, Schall JI, Leonard MB, Zemel BS. Vitamin D insufficiency in children, adolescents, and young adults with cystic fibrosis despite routine oral supplementation. Am J Clin Nutr 2007;86(6):1694–9.
8. Holick MF, Chen TC. Vitamin D deficiency: A worldwide problem with health consequences. Am J Clin Nutr 2008;87(4):1080S–6.
9. Dedeoglu M, Garip Y, Bodur H. Osteomalacia in Crohn's disease. Arch Osteoporos 2014;9:177.
10. Holick MF. Resurrection of vitamin D deficiency and rickets. J Clin Invest 2006;116(8):2062–72.
11. Camargo CA, Ganmaa D, Frazier AL, Kirchberg FF, Stuart JJ, Kleinman K, *et al*. Randomized trial of vitamin D supplementation and risk of acute respiratory infection in Mongolia. Pediatrics 2012;130(3):e561–7.
12. Atiq M, Suria A, Nizami S, Ahmed I. Vitamin D status of breastfed Pakistani infants. Acta paediatr 1998;87(7):737–40.
13. Harinarayan C. Prevalence of vitamin D insufficiency in postmenopausal south Indian women. Osteoporos Int 2005;16(4):397–402.
14. Roy DK, Berry JL, Pye SR, Adams JE, Swarbrick CM, King Y, *et al*. Vitamin D status and bone mass in UK South Asian women. Bone 2007;40(1):200–4.
15. Ross AC. The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. J Clin Endocrinol Metab 2011;96(1):53–8.
16. Nicklas TA, Qu H, Hughes SO, He M, Wagner SE, Foushee HR, *et al*. Self-perceived lactose intolerance results in lower intakes of calcium and dairy foods and is associated with hypertension and diabetes in adults. Am J Clin Nutr 2011;94(1):191–8.
17. Larsson SC, Bergkvist L, Rutegård J, Giovannucci E, Wolk A. Calcium and dairy food intakes are inversely associated with colorectal cancer risk in the Cohort of Swedish Men. Am J Clin Nutr 2006;83(3):667–73.
18. O'Connor LM, Lentjes MA, Luben RN, Khaw KT, Wareham NJ, Forouhi NG. Dietary dairy product intake and incident type 2 diabetes: a prospective study using dietary data from a 7-day food diary. Diabetologia 2014;57(5):909–17.
19. Institute of Medicine (US) Standing Committee on the Scientific Evaluation of Dietary Reference Intakes. Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride. Washington (DC): National Academies Press (US); 1997.
20. Mei Z, Yip R, Grummer-Strawn LM, Trowbridge FL. Development of a research child growth reference and its comparison with the current international growth reference. Arch Pediatr Adolesc Med 1998;152(5):471–9.
21. Hajhir M. Diseases Induced by Malnutrition. Tehran: Andisheh Farda. 2004.
22. WHO. Stakeholders meeting on maternal interventions vigilance: safety monitoring and surveillance in vaccine and other research settings: Domaine de Penthes, Geneva, Switzerland, 20-21 November 2017. World Health Organization, 2018.
23. Bhutta ZA, Soofi SB, Zaidi SSH, Habib A. Pakistan National Nutrition Survey, 2011.
24. Kapil U, Pandey RM, Goswami R, Sharma B, Sharma N, Ramakrishnan L, *et al*. Prevalence of Vitamin D deficiency and associated risk factors among children residing at high altitude in Shimla district, Himachal Pradesh, India. Indian J Endocrinol Metab 2017;21(1):178–83.
25. Akkermans MD, Eussen SR, van der Horst-Graat JM, Van Elburg RM, van Goudoever JB, Brus F. A micronutrient-fortified young-child formula improves the iron and vitamin

D status of healthy young European children: a randomized, double-blind controlled trial. Am J Clin Nutr 2017;105(2):391–9.
26. Rousseau AF, Damas P, Ledoux D, Cavalier E. Effect of cholecalciferol recommended daily allowances on vitamin D status and fibroblast growth factor-23: an observational study in acute burn patients. Burns 2014;40(5):865–70.
27. Rich-Edwards JW, Ganmaa D, Kleinman K, Sumberzul N, Holick MF, Lkhagvasuren T, *et al*. Randomized trial of fortified milk and supplements to raise 25-hydroxyvitamin D concentrations in schoolchildren in Mongolia. Am J Clin Nutr 2011;94(2):578–84.
28. Klein GL, Herndon DN, Chen TC, Kulp G, Holick MF. Standard multivitamin supplementation does not improve vitamin D insufficiency after burns. J Bone Miner Metab 2009;27(4):502–6.
29. Reddy V, Sivakumar B. Magnesium-dependent vitamin-D-resistant rickets. Lancet 1974;1(7864):963–5.
30. Heaney RP, Dowell MS, Hale CA, Bendich A. Calcium absorption varies within the reference range for serum 25-hydroxyvitamin D. J Am Coll Nutr 2003;22(2):142–6.
31. Fatima S, Gerasimidis K, Wright C, Tsiountsioura M, Arvanitidou EI, Malkova D. Response of appetite and potential appetite regulators following intake of high energy nutritional supplements. Appetite 2015;95:36–43.
32. Fatima S, Gerasimidis K, Wright C, Malkova D. Impact of High Energy Nutritional Supplement Drink consumed for five consecutive days on cardio metabolic risk factors in underweight females. Proc Nutr Soc 2015;74(OCE1).
33. Parsons EL, Stratton RJ, Cawood AL, Smith TR, Elia M. Oral nutritional supplements in a randomised trial are more effective than dietary advice at improving quality of life in malnourished care home residents. Clin Nutr 2017;36(1):134–42.
34. Huynh D, Devitt A, Paule C, Reddy B, Marathe P, Hegazi R, *et al*. Effects of oral nutritional supplementation in the management of malnutrition in hospital and post-hospital discharged patients in I ndia: a randomised, open-label, controlled trial. J Hum Nutr Diet 2015;28(4):331–43.
35. Norman K, Kirchner H, Freudenreich M, Ockenga J, Lochs H, Pirlich M. Three month intervention with protein and energy rich supplements improve muscle function and quality of life in malnourished patients with non-neoplastic gastrointestinal disease—a randomized controlled trial. Clin Nutr 2008;27(1):48–56.
36. Ackatia-Armah RS, McDonald CM, Doumbia S, Erhardt JG, Hamer DH, Brown KH. Malian children with moderate acute malnutrition who are treated with lipid-based dietary supplements have greater weight gains and recovery rates than those treated with locally produced cereal-legume products: a community-based, cluster-randomized trial. Am J Clin Nutr 2015;101(3):632–45.
37. Nackers F, Broillet F, Oumarou D, Djibo A, Gaboulaud V, Guerin PJ, *et al*. Effectiveness of ready-to-use therapeutic food compared to a corn/soy-blend-based pre-mix for the treatment of childhood moderate acute malnutrition in Niger. J Trop Pediatr 2010;56(6):407–13.

Submitted: March 11, 2021	*Revised: May 7, 2021*	*Accepted: May 13, 2021*

Address for Correspondence:

Sadia Fatima, Khyber Medical University Institute of Basic Medical Sciences (KMU-IBMS), Peshawar-Pakistan
Cell: +92 331 581 2345
Email: drsadiafatima@gmail.com