Effect of Nutritional Supplements on Energy Intake, Appetite, Lipid Profile and Micronutrient Status of Moderate Underweight Children

Study Protocols

1. Written ethical approval will be acquired from respective orphanages and schools after approval from the research ethics committee of KMU and the ASRB. Permission will be asked from principals, teachers and parents of children.

2. Main researcher will identify potential participants from the respective orphanages and schools. Aims and objectives of study will be explained to both, the parents and children.

3. Parents of the participants will be asked to sign a consent form and written informed assent will be taken from the children who will be participating in the study.

4. The participants will be asked to come to the clinical trial room of Khyber Medical University, Peshawar in fasted state in the morning. A health questionnaire will be duly filled by the main researcher. Children with any chronic disease or those taking supplements, medication or are allergic to supplements will be excluded as well as those with surgery and gastro intestinal tract disorder.

5. The preliminary anthropometric measurements at baseline will be obtained which will include weight, height and skin fold measurements i.e. biceps, triceps and sub-scapular thickness.

6. Data on multiple pass 24 hours dietary recalls will be collected thrice for three continuous days including a weekend (Sunday). The data will be collected before recruitment, after recruitment and then after fifteen days of trial.

7. The participants will be allocated randomly to the intervention and placebo (nutritional counseling) groups by using “Computer Randomizer”, computerized software.

8. On the first day of experimental trial, anthropometric measurements and the baseline blood samples in a fasted state will be obtained in the morning (Fig. 1). The data on appetite will also be obtained in fasted state. Then the supplement (Acha Mum by WFP; Table 1) /placebo will be provided. Further appetite questionnaire will be marked at time interval of 30 and 60 minutes. An *ad libitum* buffet breakfast and *ad libitum* buffet lunch will be presented at 90 and 240 minutes after providing supplementation respectively. Again at 120, 150, 180 and 210 minutes, appetite questionnaires will be marked after the supplementation. Three researchers will measure the energy intake during the breakfast and lunch to enhance the accuracy of the results. “Windiet® software” will be used to calculate the total caloric intake.

9. The participants will be given supplements/placebo till 30^{th} day. On thirty-first day, anthropometric measurements and fasting blood samples will be collected again from both groups. And the whole procedure will be repeated as explained above (point 8).

10. Weight will be measured using digital weight scale, height by height measuring scale and skin fold measurements will be taken using Holtain caliper.

11. Mid upper arm circumference, head circumference and waist to hip ratio will be measured using flexible measuring tape.
12. Insulin and glucose levels will be checked by "ELISA kit" and "automated Cobas C3 analyzer" respectively.
13. Lipid profile will also be analyzed using Cobas C3 analyzer.
14. Hemoglobin (Hb) levels will be checked by using hematology analyzer.
15. Status of Iron (Fe), zinc (Zn) and copper (Cu) in plasma will be analyzed by atomic absorption spectrometry (AAS).
16. Vitamin D and Calcium (Ca) levels will be checked using a Cobas 8000 Modular Analyzer.

Fig. 1:

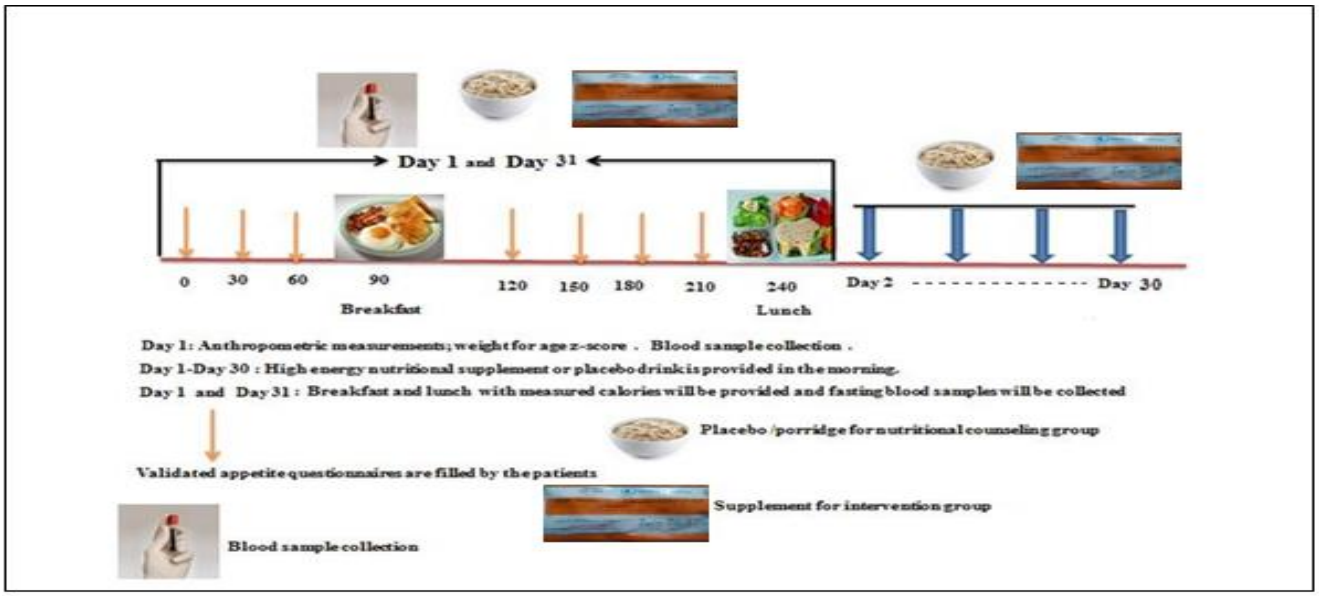

Table 1: Acha Mum composition

	RTSF (100g)
Energy (kcal)	510-560
Macronutrients	
Proteins (g)	11-16
Fats (g)	26-36
Minerals (mg)	
Zinc	11
Iron	10
Phosphorus	450
Magnesium	150
Copper	1.4
Potassium	900
Calcium	535
Vit D	15 mcg

Statistical Analysis

- The data will be organized in Microsoft excel 2010.
- Data will be analysed using MINITAB® version 17 and LMS Growth (WAZ score).
- Normality of the data will be checked using Anderson Darling test of normality.
- Data will be expressed as Mean and SD or median and IQR depending on the distribution of data.
- Comparison between the study groups will be done using two sample T-Test or Mann-Whitney U test.
- The total area under the curve for appetite measurement versus time curve (AUC) will be used as summary measures for the pre-breakfast and post-breakfast response.
- Comparison of lipid profile will also be done using two sample T-Test