Short Term Effect of Lipid-Based Nutritional Supplement on Caloric Intake, Appetite, Glucose, and Insulin Levels in Moderately Malnourished Children

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Abstract: Background: Malnutrition is a major health problem specially effecting children under the age of 5 years. Malnutrition has serious outcomes such as suppressed immune system, delayed and stunted growth, slow wound healing etc. Objective: To look into the impact of a lipid-based nutritional supplement on caloric intake, appetite, glucose and insulin levels of school going children of age 5-10 years. Methods: In this single blind randomized controlled trial, 38 children with BMI (Z score between -2 and -3SDS) were randomly allocated to Lipid based nutritional supplements & Placebo groups. They were given supplements (535kcal) /Placebo (92kcal). They were served ad libitum buffet meals (breakfast and lunch). The appetite responses were measured by marking the Likert scale questionnaire at 0, 30, 60, 120, 150, 180, 210 and 240 minutes. The total caloric intake was calculated for the ad libitum buffet breakfast and lunch. The insulin and glucose levels were measured using COBAS C3 analyzer. Result: On the trial day, there was no significant difference in total caloric intake between lipid based nutritional supplements LNS (766.32±72.4 kcal) and Placebo (806.31±11.5 kcal). Appetite was measured using a Likert scale, but no significant differences were found between the two groups. There was also no significant difference between glucose in the LNS vs. PLACEBO, i.e. (929.13±97.111.7) mg/dL and insulin (1.6±0.97 vs. 2.09±1.15) µU/mL in both the groups. Conclusion: In mildly underweight children the Lipid based nutritional supplements reduces the caloric intake from the regular habitual diet, which extends to the lunch time. This suppression of caloric intake might be related to the changes in appetite.

Keywords: Caloric intake, Lipid based nutritional supplements, Placebo, Appetite.

Received: October 29, 2022
Accepted: January 12, 2023
DOI: 10.46568/bios.v4i2.101

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Introduction
Malnutrition is a serious primary public health issue in many developing countries. Millions of children with poor eating habits and low socio-economic status are annually affected by different kinds of malnutrition [1]. Protein-energy malnutrition (PEM) is the most prevalent pathological condition. However, children suffered from moderate acute malnutrition (MAM) have a higher death rate and are more likely to develop infectious illnesses and have delayed physical and cognitive development [2].Worldwide, out of every twelve persons, one must be affected from malnutrition. The majority of the deaths are mostly seen in children from low and middle-income countries (LMICs), with an estimated 32 percent (178 million) of children suffering from MAM.
(a weight-for-age z-score (WAZ) of less than -3) [3]. Whereas, three South Asian countries, including India, Bangladesh, and Pakistan, have a high incidence of such children, with 33.03% being underweight, 11.52% wasted and 53.38% suffered from stunting [4]. Malnutrition is a global cause, responsible for the acclimatization of an individual’s health. Different research studies proposed that malnourished children's appetite and satiety are generally quite low, therefore, the alternate of taking food is lipid based nutritional supplements (LNS) or ready to use food (RTUF) [5]. When compared to well-nourished children, undernourished children have a greater likelihood of morbidity and mortality. The risk of death increases with increased severity of malnutrition [6]. If malnutrition is not treated properly or efficiently, it may lead to delayed recovery from the diseases, higher complication rates; hence poor quality life of the patients resulting into more expensive healthcare and rehabilitation needs [1, 5, 7].

Malnourished children must be provided with nourishment, according to WHO recommendations, which is high in important nutritional contents such as proteins vitamin and minerals to ensure rapid recovery and weight gain [8]. For this reason, a variety of solid, RTUFs, have been introduced that have good nutritional characteristics, low cost, resistance to contamination by bacteria, long shelf life, no refrigeration needed, and highly palatable [9]. According to a South Korean study, the overall blood level of macro- and micronutrients was higher in the nutritional supplement group versus the non-nutritional supplement group [10]. Another study found that using dietary supplements improved the quality and recovery of lives of malnourished people [11]. Furthermore, some research on underweight females found that taking these dietary supplements increased energy consumption, which decreased the overall impact of such nutritional supplements. However, underweight females and children may have different relationships between hunger and calorie intake after nutritional supplement delivery [12]. Poor children should be provided a protein-and energy-rich meal supplemented with essential vitamins and minerals during the rehabilitation period to encourage rapid weight gain, in accordance with WHO recommendations for the management of malnutrition [12]. It does not require any additional processing before feeding, and its thickness is appropriate for newborns and children [13, 14]. The blood insulin and glucose are important regulators for satiety and hunger; therefore, changes in the levels of glucose and insulin may help to determine the appetite changes of the patients before and after supplementation [15]. Use of LNS may affect the energy intake, appetite and change the insulin and glucose concentration in moderately underweight children with BMI Z-score between -2 to -3. This short-term (5-day) trial was conducted to investigate the effect of LNS on caloric intake and appetite of underweight school-aged kids, as well as changes in blood glucose and insulin levels before and afterwards supplementation.

**Methodology:**
This single-blinded randomized control trial, starting for September, 2017 and ended in April 2018, included 38 healthy children who were underweight, 5 to 10 years old and were recruited from the 17 government primary schools and 3 orphanages of Hayatabad, Peshawar. The sample size was calculated by the help of MINITAB version 30. It was calculated that nineteen subjects were sufficient to detect a difference of round 500 KJ of energy intake with 80% power at level of 5% to detect a difference of round two third of the standard deviation between the supplement and the placebo for any measure. This sample size calculation was based on the differences energy intake from article by Fatima et al.[12]

Children of aged 5-10 years & moderately underweight having BMI Z-score between -2 and -3 were included in the study while those children who were allergic to supplements or certain food or with eating disorders or who were previously taking any supplements or medication and had gastrointestinal tract surgery or disorder were not included in the study. Ethical approvals were obtained from Advanced Study and Research Board (AS&RB) and Ethical Committee of Khyber
After confirmation of age of the participants, from their parents and respected school administration, their weight and height was measured using digital weight scale (Beurer GS 200 Allium) and Portable stadiometer Seca Leicester 214. BMI was calculated using the formula
\[ \text{BMI} = \frac{\text{Weight}}{\text{height}^2} \text{(kg/m}^2) \]
Mid upper arm circumference (of the non-dominant arm) of each participant was measured to the nearest 0.1cm with non-elastic, “Shakir’s measuring tape”. For the bicep measurement, the anterior midline of the upper arm, 1 cm above the cross mark used for the triceps was used to clip the calliper and measured the reading. For measuring the triceps of a participant, posterior midline of the upper arm was first located and a cross mark was marked at that point. Then central part of the skin between acromion and olecranon was measured.

After taking measurements, the data obtained was then analyzed for SDS values using “LMS growth”, computerized software used to express the child growth status in form of SD scores and is designed for use with growth references based on British 1990 growth reference data using the LMS growth [16]. Children with BMI-Z scores within -2 and -3 were initially chosen to participate in the clinical trial. After obtaining their parents’ consent, a proper health questionnaire was given to the parents so that children with any allergy to supplement gastrointestinal disorder, surgery, or any other auto immune illnesses could be excluded from the study. Parents were then provided with the information sheets.

The participants were invited to the clinical trial room at KMU’s Institute of Basic Medical Sciences (IBMS) in Peshawar, along with their parents for the main trial day. Anthropometric measurements included height, weight, mid upper arm circumference (MUAC), waist circumference, and skin fold thickness were measured. Along with these measurements, their fasting blood samples were also obtained. After blood sampling the participants were asked to mark the Likert scale (appetite questionnaires) which were used to measure their satiety, hunger, desire to eat and fullness. The LNS Achaa Mum (535 Kcal/day) or PLACEBO (92kcal/day) (Table 1) was subsequently given to the participants. The participants were randomly divided into two groups; supplement (LNS) group and PLACEBO group using Computer Randomizer software (version 30) to eliminate the selection bias.

In our study Likert scale questionnaires were used to study the attitude of moderately underweight children towards hunger, satiety, appetite, fullness and desire to eat. [17, 18]. Likert scale questionnaires consisting of 5 point is an effective scale which describes attitude towards a specific behaviour. Likert scales were marked at 30 and 60 minutes after the intake of LNS or PLACEBO. Then \textit{ad libitum} buffet breakfast was provided to children of their choice at the interval of 90 minutes. The Likert scales were again marked at 120, 150 and 180 minutes after the intake of LNS or PLACEBO. Then the \textit{ad libitum} lunch was served at 240 minutes. Windiet® software 2005 was then used by three researchers to measure the accuracy of the caloric intake of participants during breakfast and lunch. Approximately 2ml blood was drawn from anti cubital vein by an expert lab technician. The samples were then centrifuged at 3000rpm for 10 minutes in the biochemistry laboratory of Khyber Medical University Peshawar. The layer of plasma was carefully separated using a micropipette into a labelled blue screw top disposable tube. The samples were then stored at -40 degree C for further analysis of insulin & glucose by Roche Cobas C3 system.

**Statistical Analysis**

The obtained data was than evaluated using MINITAB® version 17. The Anderson Darling Test was used to verify that the data was normal. Comparison between the groups was done by using t-test. The results obtained with P-value less than 0.05 were significant.
Table 1: Nutritional value of Lipid based nutritional supplements and PLACEBO

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

Results
The socio demographic status of the participants showed that most of the fathers were illiterate (4(21%) in LNS and 4(22.2%) in PLACEBO), while (6(31.1%) in LNS and 2(11.1%) in PLACEBO) were educated up to the level of primary and only (1(5.5%) in PLACEBO) was educated up to graduation level. All the participants had an average of 4-9 family members and 4-6 siblings.

The baseline anthropometric measurements of the participants calculated by applying t-test are illustrated in (Table 2)

Table 2: Baseline anthropometric measurements of the study participants

<table>
<thead>
<tr>
<th>Factors</th>
<th>LNS</th>
<th>PLACEBO</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>17.68 ± 2.78</td>
<td>19.47 ± 3.11</td>
<td>0.10</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>116.4 ± 8.82</td>
<td>122.1 ± 10.13</td>
<td>0.10</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>12.97 ± 0.34</td>
<td>12.94 ± 0.32</td>
<td>0.86</td>
</tr>
<tr>
<td>Age(yrs)</td>
<td>6.94 ± 1.31</td>
<td>7.21 ± 1.75</td>
<td>0.59</td>
</tr>
<tr>
<td>MUAC (cm)</td>
<td>14.76 ± 0.94</td>
<td>15.17 ± 0.82</td>
<td>0.17</td>
</tr>
<tr>
<td>Biceps fold(mm)</td>
<td>3.92 ± 1.14</td>
<td>3.65 ± 1.00</td>
<td>0.47</td>
</tr>
<tr>
<td>Triceps fold(mm)</td>
<td>6.18 ± 1.27</td>
<td>6.21 ± 1.40</td>
<td>0.90</td>
</tr>
<tr>
<td>Sub-scapula (mm)</td>
<td>4.58 ± 0.66</td>
<td>4.65 ± 0.80</td>
<td>0.79</td>
</tr>
<tr>
<td>Mid abdomen (mm)</td>
<td>4.49 ± 1.49</td>
<td>4.77 ± 1.23</td>
<td>0.63</td>
</tr>
<tr>
<td>Waist/Hip</td>
<td>0.86 ± 0.04</td>
<td>0.86 ± 0.07</td>
<td>0.98</td>
</tr>
</tbody>
</table>

Significant difference (*P<0.05)

No significant difference was seen between the baseline values of age (years): LNS vs. PLACEBO, (6.88 ± 1.29 vs. 7.28 ± 1.65 P=0.44), weight(kg): LNS vs. PLACEBO, (17.46 ± 2.83kg, vs. 19.48 ± 2.92kg, P= 0.05), Height(cm): LNS vs. PLACEBO, (115.70 ± 9.01, vs. 122.3 ± 9.54, P=0.05) in both the groups. Also no significant differences were observed in other factors such as BMI, MUAC, biceps, triceps and waist to hip ratio between the two groups.

The comparison of total caloric intake of both the groups calculated by t-test is shown in (Table 3).

Table 3: Caloric intake during different meals on trial day

<table>
<thead>
<tr>
<th>Meal</th>
<th>Calories</th>
<th>LNS</th>
<th>PLACEBO</th>
</tr>
</thead>
</table>

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Significant difference (*P<0.05, **P<0.01, ***P<0.001)
the total caloric intake during ad libitum buffet meals (breakfast and lunch) was combined, a significant difference was observed between the two groups (P=0.003), as the caloric intake of PLACEBO (776.4 ± 300.8 kcal) group was higher than LNS (492.7±185.7 kcal) group. However, the combined caloric intake of the LNS (766.3±272.4 kcal), PLACEBO (806 ± 311.5 kcal), ad
libitum breakfast & lunch of both the groups showed no significant difference (P=0.695). The results obtained by comparison of Likert scale questionnaires between two groups are shown in (Tables 4). The results show that no significant difference is observed between the two groups at 0, 60, 150, 180 and 210 minutes. However, a significant change is observed in satiety and fullness at 120 minutes that is soon after the breakfast in both the groups. This shows that the participants of both the groups were feeling full and their satiety was decreased. Therefore, it is noted that the changes in both groups were same.

Table 4: Likert scale for appetite responses (hunger, satiety, appetite and fullness)
Relationship between glucose and insulin levels on main trial day and day 5 is shown in (Table 5).

**Discussion**

Studies are available which suggests that ready to use food and oral nutritional supplement can improve the nutritional status of underweight children who are at risk of malnutrition [12]. On the trial day, the calorie intake during breakfast for LNS was significantly decreased. This drop in caloric intake was caused by the LNS group's consumption of a high caloric lipid-based dietary supplement, (535kcal) before the breakfast. Previous studies are available which suggested that immediate suppression in caloric intake was seen during the meal after the intake of nutritional supplement [12, 19]. A study on underweight females found that after taking a high energy supplement, calorie consumption decreased after breakfast [19]. Another study, done by Megan P Hume et al., found that after taking the supplement, calorie intake was lowered during the buffet meal. [20]. In our study on the main trial day, suppression in caloric intake was also noted during the lunch in LNS group, which is in contradiction with a study conducted on females in 2015, in which no suppression in caloric intake was observed during lunch (it shows that energy suppression was prolonged till lunch in underweight children) [19, 21]. More mechanistic techniques are needed to investigate the mechanism involved in caloric intake restriction following lipid-based nutritional supplementation in moderately underweight children, which may differ from underweight females.

**Table 5:** Comparison on fasting insulin and glucose concentration on day 1 and day 5

<table>
<thead>
<tr>
<th>Glucose (mg/dL)</th>
<th>Day 1</th>
<th>Day 5</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LNS (Mean± S.D)</strong></td>
<td><strong>PLACEBO (Mean± S.D)</strong></td>
<td><strong>P-value</strong></td>
</tr>
<tr>
<td><strong>Glucose (mg/dL)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>91.2±10.79</td>
<td>96.9±10.05</td>
<td>0.13</td>
</tr>
<tr>
<td><strong>Insulin (µU/mL)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.86 ± 1.21</td>
<td>2.75± 1.61</td>
<td>0.09</td>
</tr>
</tbody>
</table>

No significant difference is observed in glucose and insulin level on day 1 and day 5 in both the groups. Also when a combined comparison of both days was made between glucose and insulin in both the groups, no significant difference was observed.
Moreover, on the main trial day, it was observed that the sum of caloric intake from *ad libitum* buffet meals (breakfast, lunch and LNS / PLACEBO) showed no significant difference between LNS and PLACEBO group. While a study conducted on adult men suggested that overall caloric intake was increased in intervention group when compared to controls [22]. Blood glucose is an important regulator of satiety and hunger [23]. In our study, we had observed that the difference between fasting glucose levels of LNS and PLACEBO group were not significant on main trial day and 5 days after supplementation. A study conducted on men suggested that low blood glucose level was observed after the test meal due to presence of different nutrients, causing reduction in caloric intake [24].

Insulin is a short-term appetite regulator [25]. In our study, no significant changes in fasting blood insulin concentration were observed after 5 days supplementation in moderately underweight children. A study conducted on older men and women documented that the plasma insulin level was increased after the intake of supplement [22]. The difference between the responses of LNS and PLACEBO group was not significant. In this study, we did not find Likert scale an effective scale for the assessment of nutritional status of moderately underweight children. A research conducted in 2016 suggests the use of visual analogue scale rather than Likert scale as the children are more receptive towards visual analogue scale [25].

The participants included in our studies belonged to low socio-economic background, having limited resources and accessibility to healthy and nutritious fortified food or supplements. Low socio-economic background is a major factor causing undernourishment in children at younger age specifically belonging to underdeveloped areas [26]. Several other studies also documented that children belonging to poor backgrounds are likely to be malnourished [18, 27]. Another study conducted by Ahmed Shoukry Rashad *et al.* suggested that economic growth is an effective factor which can combat malnutrition [25]. Along with the nutritional supplements to the participants, Nutritional counseling was also given to the participants and their parents that might help to change their dietary habits and move towards a healthy lifestyle. A study has suggested that nutritional counseling has positive impact on nutritional status of people [28]. Similarly, in our study it was observed that parents and children were more receptive towards nutritional counseling [29]. Nutritional dietary counseling programs should be arranged, addressing parents and children about the health issues and giving recommendations about balanced healthy diet. Along with counseling, proper nutritional health sessions should be provided to the teachers of all schools by expert health professionals to raise awareness among them. Monthly health check-ups should be arranged in schools, so that a small or initial health issue of the children can be reported and cured on time.

**Recommendations**

Further studies are required to check the impact of LNS on caloric intake and appetite regulatory hormones. Studies on mechanism for caloric intake suppression by LNS are also required. Dietary counseling programs should be arranged in schools to motivate children towards healthy nutritional diet. Parents and teachers should be provided with health education which can help their children to move towards healthy eating habits. Further multi-sectorial approaches and community based trial are needed for underweight school going children to combat the issue of malnutrition.

**Conclusion:**

LNS are responsible for suppression of caloric intake but have no effect on the overall caloric intake and appetite of moderately underweight children. Insulin and glucose concentrations are also not affected by the intake of LNS for 5 days.
ETHICS APPROVAL AND CONSENT TO PARTICIPATE
Not applicable.

HUMAN AND ANIMAL RIGHTS
No animals were used in this study. The study on humans was conducted in accordance with the ethical rules of the Helsinki Declaration and Good Clinical Practice.

CONSENT FOR PUBLICATION
Not applicable.

AVAILABILITY OF DATA AND MATERIALS
None.

FUNDING
Funded by Khyber Medical University, Peshawar.

CONFLICT OF INTEREST
The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS
None.

REFERENCES

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Annexure IV: Data Collection Sheet

Effect of Nutritional Supplement on Appetite, Energy Intake and Lipid Profile of Mild to Moderate Underweight Children

<table>
<thead>
<tr>
<th>Participant No:</th>
<th>Date of birth:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date of Assessment:</td>
<td>Age:</td>
</tr>
</tbody>
</table>

History of Children:

Sex: Male ☐ Female ☐ No of People: 

Socioeconomic Status:

Low Income ☐ Low Middle ☐ Middle Income ☐ Higher Income ☐

Education of Parents:

Matric ☐ Intermediate ☐ Undergraduate ☐ Post-Graduate ☐

Personal health:

Eating disorder: Y/N Allergic to supplements: Y/N

Gastro intestinal tract surgery Y/N Any food allergy: Y/N

Gastro intestinal tract disorder: Y/N Family history of CVD: Y/N

Previously taking any supplements: Y/N Auto immune diseases Y/N

Body Measurements:

Height: ...............  
Weight: ...............  
Skin fold measurement: (Biceps, triceps, mid-abdomen and sub-scapular measurements) 
Mid upper arm Circumference: ...............  
Head Circumference: ...............  
Waist to hip ratio: ...............  


Annexure VI: Likert Scale Appetite Questionnaire

Please answer the following questions by marking through the points ( ) for each question. Regard the end of each line as indicating the most extreme sensation you have ever felt and mark how you feel NOW.

1. How hungry do you feel (now)?

   I am not hungry
   Quite full
   Neither
   Quite hungry
   Never been hungrier

2. How satisfied do you feel (now)?

   Not satisfied
   Quite hungry
   Neither
   Quite full
   Can't eat another bite

3. How full do you feel (now)?

   Not at all full
   Quite hungry
   Neither
   Quite full
   Totally full

4. How much do you think you can eat (now)?

   A lot
   Quite full
   Neither
   Quite hungry
   Nothing at all

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5. How strong is your desire to eat (now)?

Not at all  Quite full  Neither  Quite hungry  Very

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Subject Number; 

Annexure I: CONSENT FORM

Effect of High Energy Nutritional Supplements on appetite and Energy Intake of Malnourished Children

Meera Tanveer, M.Phil Scholar, IBMS, Khyber Medical University, Peshawar

Please Initial BOX

1. I confirm that I have read and understood the information sheet dated............................................. for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.

2. I understand that my child’s participation is voluntary and that he/she is free to withdraw at any time without giving any reason, my medical care or legal rights being affected.

3. I understand that data from my child’s medical files, only that part which is relevant to this study, will be looked at by the researchers of this study and perhaps by the ethics committee of Khyber Medical University for auditing purposes. I give permission for these individuals to have access to my his/her records.

4. If my child decides to withdraw from the study, I am happy for the researchers to retain and use any collected data and samples.

5. I agree to my child’s blood samples being stored and used for further analysis, as new techniques become available.

7. I allow my child to take part in the above study.

Name of Participant ___________________________ Date ___________________________ Signature ___________________________

Name of person taking consent ___________________________ Date ___________________________ Signature ___________________________

I copy for participant; 1 copy for researcher site file; 1 copy (original) to be kept with case record form
Subject Number: 

Annexure III: ASSENT FORM FOR CHILDREN

Effect of High Energy Nutritional Supplements on appetite and Energy Intake of Malnourished Children

Meera Tanveer, M.Phil Scholar, IBMS, Khyber Medical University, Peshawar Carefully read the following and mark the appropriate answer.

Have you read or has anybody made you understood about this research? Y/N
Do you know that you can leave this research anytime? Y/N
Would you like to visit our metabolic suit? Y/N
Do you agree to give your blood samples? Y/N
Do you allow your blood samples to be stored for further research? Y/N
Do you agree to participate in this research? Y/N

Name of Participant ___________________________ Date __________ Signature __________

Name of Researcher ___________________________ Date __________ Signature __________

1 copy for participant; 1 copy for researcher site file; 1 copy (original) to be kept with case record form

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Annexure III: ASSENT FORM FOR CHILDREN

**Effect of High Energy Nutritional Supplements on appetite and Energy Intake of Malnourished Children**

Meera Tanveer, M.Phil Scholar, IBMS, Khyber Medical University, Peshawar Carefully read the following and mark the appropriate answer.

<table>
<thead>
<tr>
<th>Question</th>
<th>Y/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Have you read or has anybody made you understood about this research?</td>
<td></td>
</tr>
<tr>
<td>Do you know that you can leave this research anytime?</td>
<td></td>
</tr>
<tr>
<td>Would you like to visit our metabolic suit?</td>
<td></td>
</tr>
<tr>
<td>Do you agree to give your blood samples?</td>
<td></td>
</tr>
<tr>
<td>Do you allow your blood samples to be stored for further research?</td>
<td></td>
</tr>
<tr>
<td>Do you agree to participate in this research?</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Name of Participant</th>
<th>Date</th>
<th>Signature</th>
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<tr>
<td></td>
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<table>
<thead>
<tr>
<th>Name of Researcher</th>
<th>Date</th>
<th>Signature</th>
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<td></td>
<td></td>
<td></td>
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</table>

1 copy for participant; 1 copy for researcher site file; 1 copy (original) to be kept with case record form