

# Effectiveness of Leucine Supplementation in the Management of Moderate Wasting in Children

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**Abbreviations** Degrees Celsius;  
ANOVA: Analysis of Variance; CM: Centimetres; FAO: The Food and Agriculture Organization of the United Nations; G: Grams; KG: Kilograms; MG: Milligrams; MG/KG/DAY: Milligrams per Kilogram Body-weight per Day; ML: Millilitre; MM: Millimetres; MUAC: Mid Upper Arm Circumference; UNICEF: United Nations Children Fund

## Abstract

**Study background:** Wasting in children is a serious form of malnutrition where the affected child has a low weight-for-height. While the severe form has published guidelines for its treatment, moderate wasting has no standardized treatment protocol and consequently, common feeds used to treat it have limited effectiveness.

When leucine, an amino acid, is administered in a large dose in catabolic conditions it functions as a nutraceutical that accelerates muscle protein synthesis. The objective of this study was therefore to establish the effectiveness of leucine supplementation in treating moderate wasting in children.

**Methods:** A double blind placebo controlled trial that involved 144 children aged 6-24 months with moderate wasting was conducted at Mbagathi Hospital (Kenya) between 6<sup>th</sup> March-23<sup>rd</sup> June, 2017. Study children were randomly assigned to the treatment group (n = 72) or the control group (n = 72). Children in the treatment group and control group were administered with the leucine supplement and placebo respectively every day for 28 days. Baseline weight and height measurements were taken then measured weekly and at the end of the study. Differences between study groups were analyzed using analysis of variance and Chi-square. The significance level was set at <0.05.

**Results:** A significant difference (p = 0.00) was observed between the treatment group 67 (93.05 %) and control group 43 (40.28 %) in the proportion of children who recovered from moderate wasting. There was also a significant difference in the mean weight gained between the two study groups (p = 0.02).

**Conclusion:** Leucine supplementation in moderate wasting results in a significantly greater weight gain than any of the current treatment methods and is therefore effective in the treatment of moderate wasting.

## Introduction

Wasting in children is a form of malnutrition that manifests in the weight for height z-score being below minus two standard deviations from the median of the reference population. It is classified as either; moderate wasting, when the weight for height z-score is greater than or equal to minus 3 but less than minus 2 ( $-3 \leq \text{z-score} < -2$ ), or severe wasting where the z-score is less than minus 3 (z-score  $< -3$ ) [1]. Statistical estimates indicate that moderate wasting affects more than 33 million children globally [2]. According to United Nations Children Fund (UNICEF) and The Food and Agriculture Organization of the United Nations (FAO) September 15, 2017 report, 370,000 children in Kenya suffer from wasting, 80.4% of whom have moderate wasting. This figure reflects an increase of 37,000 children from the February 2017 figure of 363,000 children [3].

Wasting is brought about by an acute failure to receive sufficient nutrition. This could be related to inadequate nutrient intake or a recent illness that inhibits nutrient intake and utilization or that accelerates nutrient losses especially through diarrhoea and postprandial emesis. Wasting in children has been shown to impair the immune system and therefore it increases the risk of death from infectious diseases. In addition, episodes of wasting undermine children's physical as well as mental growth resulting in impaired motor and cognitive development. This compromises a child's learning ability and ultimately their school performance [4]. To improve child survival, it is then important to invest in interventions that prevent child wasting and those that ensure timely recovery from wasting.

Treatment guidelines for severe wasting have been defined and published by the World Health Organization [2]. These comprise the use of measures to correct fluid and electrolyte imbalances and measures to replenish calories, proteins and micronutrients through the use of Ready-to-use therapeutic food [5]. If a child responds positively to this treatment they progress to moderate wasting but there is no standardized treatment protocol despite ongoing research and consultation [6,7].

In the absence of a standard protocol, practices in the treatment of moderate wasting as well as outcomes from these practices vary in different health care facilities [6]. The results of two separate studies on the effectiveness of the two commonly used foods in the treatment of moderate wasting-corn-soy blend and lipid-based nutrient supplements - show no significant difference in the outcomes between the treatment groups and the control groups [8]. In addition, lipid-based nutrient supplements do not reduce the risk of advancement to severe wasting and/or mortality as they also induce more vomiting [9]. The existing treatment methods of moderate wasting therefore have limited effectiveness and are less efficient, which compromises the quality of care in the treatment of wasting. Quality in healthcare can only exist when health services for individuals and populations increase the likelihood of attainment of the desired health outcomes and are based on current research and professional knowledge [10]. This therefore creates the need to conduct research on an effective treatment of moderate wasting to fill the existent gap in knowledge and management of moderate wasting.

Previous studies have provided adequate evidence to suggest that leucine, a branched chain amino acid, has nutraceutical properties that accelerate muscle protein synthesis and maintenance of muscle mass in catabolic conditions associated with food restriction such as malnutrition [11]. A nutraceutical refers to a nutrient or nutritional component that occurs naturally and may have been purified and/or concentrated that is used to improve health through prevention and/or treatment of disease(s) [12].

Leucine functions as a nutraceutical through activation of a compound in muscles called mammalian target of rapamycin which when activated, accelerates protein synthesis and growth. Larger doses of leucine-than those typically found in high-quality protein food sources-have been shown to be significantly more effective at stimulating muscle protein responses [13]. Leucine, primarily being an amino acid, must be sufficient in the body so as to first satisfy all structural roles before it can engage in signalling and metabolic roles. The capacity of leucine to function as a nutraceutical is based on sufficient intracellular concentration [14].

The main objective of this study was to therefore establish the effectiveness of leucine supplementation in correcting moderate wasting in children aged 6-24 months.

## Materials and Methods

### Study Design and study site

A randomized double blind placebo controlled trial was conducted at Mbagathi Hospital in Nairobi County, Kenya to demonstrate the effectiveness of leucine supplementation in moderate wasting between 6<sup>th</sup> March-23<sup>rd</sup> June, 2017. This hospital was purposively selected for the study because it has a large and functional outpatient therapeutic and supplementary feeding programme with a total of 807 children enrolled as of May 2016 based on the Outpatient Therapeutic Program Health Facility Record of 2016. The catchment area for children in this hospital is Nairobi County and this therefore sufficed as the catchment area for the study.

### Sample size determination

A sample size of 72 child-caregiver dyads per group (144 in total) was computed based on the expected difference in the primary

outcome, that is, change in weight among those provided with either leucine supplement or placebo. This expected difference was based on the assumed mean change in weight of  $+ 0.5 \pm 0.15$  g among study children receiving leucine supplement and  $+ 0.27 \pm 0.15$  g among those receiving the placebo. This gave the trial 80% power and a type 1 error of no more than 5% to detect a difference of  $\geq 0.23$  g in the mean weight gain between the treatment group and control group.

### Sampling procedure

Children aged between 6-24 months attending Mbagathi Hospital who had a weight-for-height z-score of less than -2 but greater than or equal to -3 were referred to the study before being discharged. After obtaining written consent from the child caregivers, the children were screened to determine their eligibility for enrolment to the study.

Children who had chronic conditions and those who had participated in other clinical trials in the preceding three months were excluded from the study. This information was documented on the study case report form which thus formed the basis of inclusion or exclusion of study children. On enrolment, the study children were randomly assigned to either the treatment group or the control group. Both study participants and project team were blinded on the assignment.

### Randomization procedure

Randomization allocation sequence was computer generated by an independent statistician (who was not involved in the study) to ensure allocation concealment. Based on the sample size, 144 unique study codes were generated and fed into a computer program that randomly allocated 72 codes to the treatment group and 72 codes to the control group. Every nth study participant recruited to the study received the next available study code and by default was allocated to the respective study group in which the code was assigned to.

### Ethical Considerations

Ethical consent was sought and obtained from the Kenyatta National Hospital Ethics Review Committee (P 519/7/2016) and the Mbagathi Hospital Research Committee (17715/2017). Mothers/caregivers were introduced to the study and informed on the objectives, possible benefits as well as risks of the study. Each mother/caregiver was given a chance to ask questions related to the study and have them answered satisfactorily. Afterwards they were required to provide written consent prior to enrolment of the study child and this was captured on the consent form. Participation in the study was purely voluntary and mothers/caregivers were only financially compensated for their transport to and from the clinic.

On completion of this study the control group was accorded similar interventions as the treatment group and therefore there were no discriminative ethical issues.

### Demographics and nutrition status data collection

Demographic characteristics for all the study children were collected through face-to-face interviews with the child's caregiver/mother using a semi-structured questionnaire then baseline anthropometric measurements were taken. This was done by measuring the weight (kg) using a Salter Bathroom Scale for children who could stand on their own and Infant Weighing Scale for children

who could not stand. Vertical height (cm) and supine length were measured using a Height Board for children who could stand and a Length Board for children who could not stand respectively. Triceps skin fold reading was obtained using Holtain<sup>®</sup> Skin fold Calipers and Mid Upper Arm Circumference (cm) (MUAC) using a MUAC Tape. All anthropometric procedures were conducted using standard methods [15].

### Data Quality Management

The study tools and procedures were pre-tested on 10 randomly selected child-mother/caregiver pairs in the adjacent Ngumo/Kenyatta Market Estate. On completion, the study tools and procedures were adjusted where applicable to generate the required data.

Data collection procedures were standardized and all equipment were calibrated and recalibrated after every 10 measurements taken. All measurements were taken in triplicate at an accuracy of 0.01 before the mean between the three measurements was computed. Finally, before the mean value was entered on the tool provided all values were compared with established reference standards to enhance accuracy. Variation of the mean collected from the maximum allowable difference in the reference standards resulted in discarding of the values recorded and fresh ones being taken [16]. Data collection forms were reviewed every day to determine completeness of data collected and to detect any errors of omission as well as data outliers. Statistical outliers were identified using a Grubbs' test on the Graph Pad software and expunged from the dataset.

### Study Procedure

Study children in the treatment group received leucine supplement capsules (150 mg/kg/day) while those in the control group received a placebo that was indistinguishable from the leucine capsule. Capsules given were sufficient for one week and therefore were to be administered orally at home by the child's caregiver. Both capsules (active treatment and placebo) were packaged in identical, opaque and securely sealed containers that were marked with only one of the unique study codes representing each study child.

The capsules could either be administered as a whole but in the case this was not possible the capsule(s) was opened and the content inside dissolved in a clean cup of 150 - 250 ml potable water at room temperature (20 - 25°C). The child was required to ingest the entire content of the cup.

A supplementation adherence form was given together with the capsules to enable weekly monitoring of adherence to the prescribed dosage. This supplementation adherence form was a written record by the caregiver of the times in a day and the number of capsules that were administered to the child.

Capsules prescribed were administered every day for a period of four weeks. The children continued eating their regular diet and taking any medication and therapeutic feeds prescribed. Information on medication and therapeutic feeds prescribed to the study children was documented under 'Medical History' section of the Study Case Report Form.

Clinic visits were scheduled after every seven days when the capsule refills were also done. During this clinic visits: the weight, height, triceps skin fold and MUAC were measured as well as

monitoring for adverse reactions. Adherence to the previous week's prescribed dosage was also observed before finally supplement refills were administered. Data collection forms were reviewed daily by the enumerators to ensure completeness of data collected

### Description of capsules used

Two types of capsules were administered to the study children; the active treatment and placebo.

The treatment capsules administered contained all three Branched Chain Amino Acids (BCAAs); L-leucine (3000mg), L-Valine (2000 mg) and L-Isoleucine (1000 mg). This is because all the BCAAs compete for cell transport and metabolism and therefore ingestion of leucine alone may lead to depletion of plasma valine and isoleucine. All three amino acids therefore need to be consumed concurrently to prevent plasma depletion of any one of the BCAAs. Leucine should however be the predominant nutrient because valine and isoleucine are ineffective in modulating nutrition status in stress situations [17].

Other ingredients used in formulation of the capsule include; gelatin, magnesium stearate and microcrystalline cellulose. All the children in the treatment group were required to consume 150 mg/kg/day of leucine and thus capsule prescription was based on the individual weight of each study child in the treatment group. The active treatment capsules were shipped from Milton Keynes, United Kingdom as they could not be sourced locally.

The placebo in turn was a capsule similarly formulated with gelatin, magnesium stearate and microcrystalline cellulose but in place of the three BCAAs was glucose (7000 mg). This was locally assembled in a laboratory at the study site. The placebo was ensured to be indistinguishable in size, colour, shape as well as taste from the active treatment.

### Monitoring of adherence to prescribed supplement dosage:

Monitoring of the adherence by the study participants to the prescribed dosage was done using the Pill Counts method together with the scrutiny of the supplement adherence forms. The pill counts method involved counting the number of pills (if any) in the container compared to the number given out on the previous visit. The difference between the two values was assumed to reflect the number of pills consumed. Supplement adherence forms were then inspected to compare the home-based capsule administration with the prescription given. Discrepancies between the two monitoring methods necessitated further probing of the caregiver.

**Safety of study children:** Under acute dietary conditions intakes of leucine that exceed 500 mg/kg/day could potentially increase the risk of adverse events [17] and therefore was proposed as the upper limit for leucine. It is recommended that when chronic ingestion studies are designed, leucine intake levels should be in the range of 250-300 mg/kg/day [18].

This study used a very modest dosage of 150 mg/kg/day for a period of 28 days. Notwithstanding, the study was keen to observe and document adverse reactions related to leucine supplementation in children that included: diarrhoea, vomiting, scaly pigmented rash on skin, swollen mouth and/or bright red tongue, depression, disorientation and apathy [17]. Caregivers were strongly encouraged to report any of these adverse reactions and events or any other suspicious reaction(s) to the investigators if and when they occurred

**Table 1:** Demographic characteristics of care givers.

		Treatment group (%) n=72	Control group (%) n=72	p-value
Sex	Male	0	0	1.00
	Female	72 (100)	72 (100)	1.00
Mean age (years)		28.68	28.88	0.20
Education level	Post-secondary	9 (12.50)	12 (16.70)	0.48
	Secondary	33 (45.90)	26 (36.10)	0.23
	Primary	28 (38.90)	33 (45.80)	0.40
	No education	2 (2.80)	1 (1.40)	0.56
Marital status	Married	63 (87.50)	64 (88.90)	0.80
	Separated	6 (8.30)	5 (6.90)	0.75
	Single	3 (4.20)	3 (4.20)	1

through a designated 24-hour hotline. All adverse reactions were recorded and the participation of the affected study children discontinued permanently.

### Contraindications

Leucine supplementation has no known contraindications.

**Study dropouts and replacement strategy:** Study children who developed any form of adverse reactions and those who defaulted more than one clinic visit were dropped from the study. Data collected from the study children who developed adverse reactions was however retained in the database but that collected from the defaulters was discarded. All defaulters were immediately replaced with new study recruits who begun the study procedure afresh. In total, nine adverse reaction cases were reported while 12 study children defaulted. Each of the 12 defaulters were notified on being dropped from the trial, their study codes were reclaimed then allocated to their immediate replacement child.

**Statistical analysis:** On completion of the study, descriptive statistics (means, medians, modes, percentages and standard deviations) were computed for the socio-demographic factors and anthropometric variables. Differences between the treatment group and the control group were analysed using Analysis of Variance (ANOVA) and Chi-square. A p-value of <0.05 was considered significant in all the analyses. The cohen's d [19] was then computed to quantify the effect size in end-of-study differences between the two study groups. This was used to measure the magnitude of the effect of leucine (treatment effect) on the anthropometric measurements of the study children.

**Table 2:** Baseline child characteristics.

		Treatment group n=72	Control group n=72	p-value
Mean age (months)		12.05	14.05	0.59
Sex (%)	Male	34 (47.22)	35 (48.61)	0.87
	Female	38 (52.78)	37 (51.39)	0.87
Mean weight (Kg)		7.00	7.03	1.00
Mean height (cm)		70.93	72.15	0.86
Mean skinfold thickness (mm)		9.70	9.68	1.00
Mean MUAC (cm)		12.44	12.45	1.00

**Table 3:** Changes in mean anthropometric measurements in the treatment group.

	Baseline	Day 7	Day 14	Day 21	Day 28
Mean weight (Kg)	7.00	7.14	7.32	7.42	7.50
Mean height (cm)	70.82	70.86	70.93	70.98	70.98
Mean skinfold thickness (mm)	9.70	9.91	10.15	10.35	10.52
Mean MUAC (cm)	12.44	12.58	12.74	13.01	13.18

Finally, a multiple linear regression model was used to study the correlation between leucine supplementation and other independent variables. Unblinding of the study was done by the statistician in the presence of the principal investigator after completion of data analysis.

## Results

### Demographic characteristics of child caregivers

As shown in Table 1, all of the child caregivers in both the treatment group and control group were female. The mean age of caregivers in the treatment group was  $28.68 \pm 6.54$  years while in the control group was  $28.88 \pm 6.07$  years. There was no significant difference in the sex, age, education level and marital status of the respondents between the treatment group and control group ( $p > 0.05$ ) (Table 1).

### Child characteristics

The study comprised of a sample of 144 children; 72 (52.78 % girls and 47.22 % boys) in the treatment group and 72 (51.39 % girls and 48.61 % boys) in the control group. The mean age of the study children was  $12.05 \pm 4.54$  months in the treatment group and  $14.05 \pm 4.29$  months in the control group. As shown in Table 2, there was no significant difference ( $p > 0.05$ ) in any of the baseline child characteristics between the two study groups (Table 2).

### Changes in anthropometric measurements in the treatment group

As displayed in Table 3, the mean weight of study children in the treatment group increased from 7.00 Kg to 7.50 Kg over the study period. There were also changes over the study period in the mean skinfold thickness (9.70-10.52 mm), mean MUAC (12.44-13.18 cm) and mean height from 70.82 cm to 70.98 cm (Table 3).

### Changes in anthropometric measurements in the control group

As shown in Table 4, the mean weight of children in the control group increased from 7.0 kg at baseline to 7.3 kg at the end of the study. The mean height also changed over the study period from 72.1 cm to 72.7 cm. Changes also occurred in the mean skin fold measurement (9.7-10.2 mm) and the mean MUAC (12.5-12.7 cm) (Table 4).

**Table 4:** Changes in mean anthropometric measurements in the control group.

	Baseline	Day 7	Day 14	Day 21	Day 28
Mean weight (Kg)	7.03	7.08	7.15	7.24	7.32
Mean height (cm)	72.14	72.32	72.69	72.73	72.74
Mean skinfold measurement (mm)	9.68	9.87	10.02	10.17	10.24
Mean MUAC (cm)	12.45	12.49	12.56	12.64	12.73



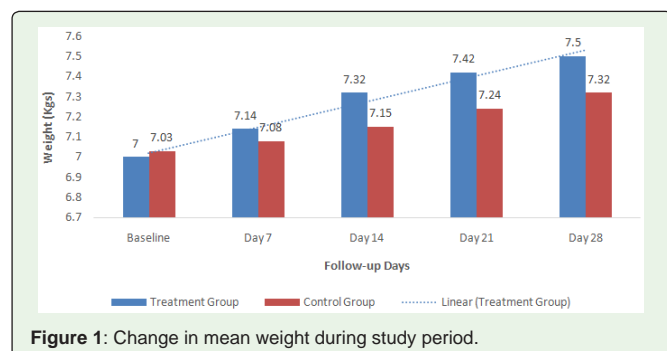


Figure 1: Change in mean weight during study period.

### Comparison of anthropometric measurement changes between the two study groups

**Mean weight:** The mean baseline weight of the treatment group ( $7.00 \pm 0.83$  kg) and control group ( $7.03 \pm 1.16$  kg) were not statistically different ( $p = 0.92$ ). However, as shown in Figure 1, the final mean weight of the treatment group ( $7.50 \pm 0.96$  kg) was significantly different from that of the control group ( $7.32 \pm 1.15$  kg) where ( $p = 0.02$ ). This represented a mean change in weight of  $+0.50 \pm 0.33$  kg in the treatment group and  $+0.29 \pm 0.25$  kg in the control group. This difference was also statistically significant ( $p = 0.02$ ) with a Cohen's  $d$  of 0.68 implying a medium effect size.

The mean rate of weight gain among children in the treatment group ( $2.55$  g/kg/day) was significantly different ( $p < 0.00$ ) from that of the control group ( $1.48$  g/kg/day). The largest mean weight increment in the treatment group occurred between day 7-14 ( $+0.18$  kg) while in the control group was between day 14-21 ( $+0.09$  kg) (Figure 1).

**Mean height:** Mean baseline heights of study children in the treatment group ( $70.82 \pm 4.85$  cm) and in the control group ( $72.14 \pm 6.75$  cm) were not significantly different ( $p = 0.38$ ). The final mean height of the treatment group was  $70.98 \pm 5.20$  cm while that of the control group was  $72.74 \pm 6.75$  cm and this difference was not statistically significant ( $p = 0.08$ ). This represented an overall change in mean height of study children of  $+0.16$  cm in the treatment group and  $+0.50$  cm in the control group however, this difference was not significant ( $p = 0.46$ ).

**Weight for height Z-scores:** The effectiveness of leucine supplementation in the treatment of moderate wasting was determined by comparing the differences in weight for height Z-scores between the two study groups over the trial period.

At the end of the study, 67 (93.05 %) of the children in the treatment group recovered from moderate wasting (z-score equal to or greater than -2) compared to 43 (40.28 %) in the control group and this difference was statistically significant, ( $p = 0.00$ ). None of the children in the control group had a weight-for-height greater than -1 at the end of the study with a majority still having a weight-for-height Z-score equal to or less than -2 (Table 5). In comparison, 16 (22.22 %) of the study participants in the treatment group had a weight-for-height Z-score greater than -1 (Table 5).

**Mean mid upper arm circumference:** As shown in figure 2, baseline MUAC readings for both treatment group and control group were almost similar (12.44 cm; 12.45 cm respectively). The final MUAC reading in the treatment group was  $13.18 \pm 0.80$  cm while in the control group it was  $12.73 \pm 0.84$  cm however this difference was not

Table 5: Weight-for-height Z-scores of study children.

Wasting category	Z-score	Treatment group (%) n=72	Control group (%) n=72
Severe	< -3	0 (0)	0 (0)
Moderate	<-2 but $\geq$ -3	5 (6.95)	43 (59.72)
At Risk	<-1 but $\geq$ -2	44 (61.11)	25 (34.72)
Normal	$\geq$ -1	23 (31.94)	4 (5.56)
Total		72 (100)	72 (100)

statistically significant ( $p = 0.11$ ). At the end of the trial, 58 (80%) of respondents in the treatment group had a MUAC equal to or greater than 12.50 cm (at risk of acute malnutrition) compared to 41 (57.10 %) in the control group. This difference had a borderline statistical significance ( $p = 0.08$ ).

The overall mean rate of MUAC gain was 0.03 cm/day for the treatment group and 0.01 cm/day for the control group and this difference was statistically significant ( $p = 0.03$ ) (Figure 2).

**Mean skin fold thickness:** The mean baseline skin fold thickness for the treatment group and the control group was almost similar ( $9.70 \pm 1.48$  mm;  $9.68 \pm 0.97$  mm). At the end of the study the mean skin fold thickness for the treatment group was  $10.52 \pm 1.45$  mm while that of the control group was  $10.24 \pm 0.92$  mm but this difference was not statistically significant ( $p = 0.17$ ). As a consequence of this change, there was a significant difference between the mean baseline and end-of-study skin fold thickness in both the treatment group and control group ( $p = 0.00$ ).

The mean skin fold reading increased by  $+0.82$  mm in the treatment group and  $+0.56$  mm in the control group over the study period however this difference was not significant ( $p = 0.17$ ).

The mean rate of gain in skin fold for the treatment group (0.03 mm/day) was almost similar ( $p = 0.92$ ) to that of the control group (0.02 mm/day).

**Correlation between changes in anthropometric measurements and child characteristics in the treatment group:** Multivariate regression models were run to investigate the correlation between change in anthropometric measurements and child characteristics in the treatment group. As shown in Table 6, there was a positive

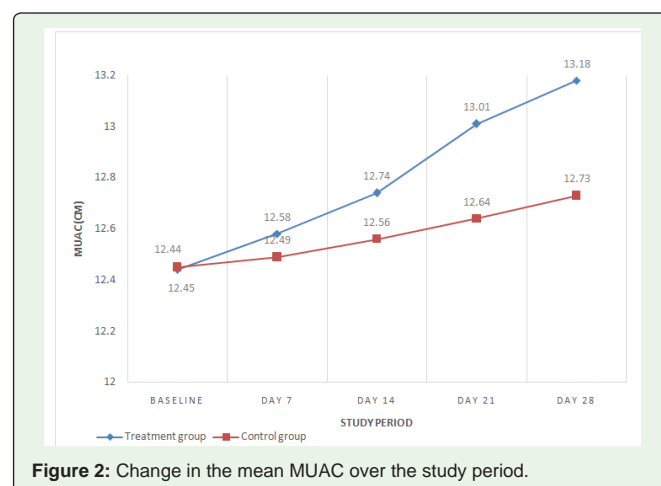


Figure 2: Change in the mean MUAC over the study period.

**Table 6:** Correlation between changes in anthropometric measurements and child characteristics in the treatment group.

Anthropometric measurement	Child characteristics			
	Age		Sex	
	r	p	r	p
Mean weight (Kg)	0.41	0.02	-0.11	0.28
Mean height (cm)	0.11	0.55	0.27	0.63
Mean skin fold measurement (mm)	0.19	0.15	0.09	0.31
Mean MUAC (cm)	0.41	0.01	-0.26	0.08

correlation observed between the age of the study children and their weight gain ( $r = 0.41$ ,  $p = 0.02$ ). There was however no correlation between the sex of the child and their weight gain during the study period in the treatment group ( $r = -0.11$ ,  $p = 0.55$ ). Change in MUAC of the study children in the treatment group was positively correlated with their age ( $r = 0.41$ ,  $p = 0.01$ ) (Table 6).

**Adverse reactions observed in the treatment group:** In total, nine (12.50 %) children from both study groups developed clinician-documented adverse reactions during the study period with six (66.67 %) and three (33.33 %) in the treatment group and control group respectively however this difference was not significant ( $p = 0.25$ ). Among the children in the treatment group, two (22.22 %) experienced diarrhoea while four (44.44 %) experienced emesis while in the control group one (11.11 %) had diarrhoea and two (22.22 %) had both diarrhoea and vomiting.

There was no correlation between development of adverse reactions and the age of the study participant ( $r = -0.08$ ,  $p = 0.63$ ) nor their sex ( $r = 0.15$ ,  $p = 0.36$ ). There was however a negative correlation between baseline weight and the development of adverse reactions ( $r = -0.03$ ,  $p = 0.84$ ). Respondents who weighed 6.50 kg and above at baseline were 0.5 times more likely to develop adverse reactions than those who weighed less than 6.50 kg at baseline.

## Discussion

At the baseline level, there was no significant difference between the treatment and control groups, indicating that the randomization procedure was effective. At the end of the study most of the children in the treatment group had recovered from moderate wasting while majority of those in the control group were still moderately wasted. The rate of weight gain in the treatment group was significantly higher than that in the control group as well as that attributed to lipid based nutrient supplements and corn soy blend, both of which are currently used in the treatment of moderate wasting [8]. These differences can therefore be directly related to supplementation with leucine.

Wamiti et al [11] showed leucine supplementation to be an effective strategy in accelerating muscle protein synthesis in humans and rodents, especially during states of muscle wasting. The mechanism through which this occurs is that leucine supplementation activates messenger ribonucleic acid translational machinery through mammalian target of rapamycin which results in up-regulation of protein translation leading to cell growth and proliferation [20-22]. This accelerates muscle protein synthesis which then directly translates to an increase in body weight.

Changes in skin fold thickness for study children were not significantly different between the treatment group and control group. This implies that leucine supplementation has no direct impact (positive or negative) on body fat accumulation. Wasting represents a depletion of the body's fat and lean tissue mass but since lean tissues account for the largest body compartment, their rate of loss is the most significant determinant of total body weight in most cases of wasting [23]. Wasting in children occurs due to accelerated protein breakdown during states of insufficient caloric intake. The breakdown of cell proteins, particularly in skeletal muscle, increases to provide the body with amino acids essential for gluconeogenesis, energy production and new protein synthesis. This acceleration of protein breakdown resulting in muscle wasting mainly occurs due to activation of the ubiquitin (Ub) proteasome pathway [24].

To therefore effectively treat moderate wasting muscle protein synthesis must be accelerated to make up for the muscle protein broken down. Current treatment methods fail to correct moderate wasting because of their emphasis on heavy calorie loading to enable gain in body fat which is wrongfully assumed to contribute to overall gain in body weight.

There was a trend of increase in the MUAC from baseline to end-of-study in both study groups however this was higher in the treatment group. The end-of-study MUAC reading was higher in the treatment group than in the control group however this difference was not statistically significant. During leucine supplementation, gain in body weight is recognized to be as a result of a gain in lean body mass (muscle protein). It can therefore be deduced that a change in MUAC is strongly related to a change in lean body mass rather than body fat.

There was no significant difference in changes in height over the study period between the two study groups. This implied that leucine supplementation had no immediate impact on linear growth however, it could possibly have an impact over a longer duration of time.

This is because episodes of wasting may contribute to stunting (low height-for-age) depending on the severity, duration and recurrence especially if there is inadequate nourishment to support recovery [25]. By reducing the episodes and duration of wasting it would be possible to prevent the occurrence of chronic malnutrition and hence prevent stunting.

The occurrence of adverse reactions was not significantly different between the two study groups. These adverse reactions are thus adjudged to be as a result of poor hygiene in handling and administration of the capsules.

## Conclusion and Recommendations

Leucine supplementation in moderate wasting results in a significantly greater weight gain than any of the treatment methods presently in use. Treatment of moderate wasting should be built on a mechanism that accelerates muscle protein synthesis to make up for the significant losses incurred during the accelerated breakdown triggered by malnutrition. This study has proven that this can be achieved with a large safe level dose of leucine in which increased muscle protein synthesis directly contributes to a weight gain in the affected children. Leucine supplementation is an effective way of

treating moderate wasting in children. As a follow-up to this study, leucine will be incorporated into a therapeutic food that will be designated and validated for use in the treatment of moderate wasting in children.

Leucine supplementation does not improve linear growth over 28 days. There is need to conduct a study to determine the impact of leucine supplementation on linear growth over a longer duration of time.

Change in MUAC in moderately wasted children is related to a gain in lean mass rather than fat mass.

### Study limitations

This study was conducted during a time when nurses in public hospitals in Kenya had gone on strike. This limited the number of patients attending public hospitals including Mbagathi Hospital as patients sort alternative facilities for healthcare services. As such, attaining the pre-determined sample size took longer to attain and consequentially, the study ran for a longer period of time than first scheduled.

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