Effect of lysine supplementation on health and morbidity in subjects belonging to poor peri-urban households in Accra, Ghana¹−³

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ABSTRACT

Background: Lysine affects diarrhea and anxiety via effects on serotonin receptors, enhanced intestinal repair, and sodium chloride–dependent opioid peptide transport.

Objective: The objective was to investigate the effects of lysine supplementation on morbidity, growth, and anxiety in children and adults of peri-urban areas of Accra, Ghana.

Design: In a double-blind randomized trial, the effect of lysine supplementation (1 g lysine/d) compared with that of placebo was examined in 2 groups of men, women, and children (n = 271). Primary outcomes included diarrheal and respiratory morbidity, growth, and anxiety and complement C3, C-reactive protein, serum cortisol, transferrin, and ferritin values. Independent-sample t tests, odds ratios, generalized estimating equations, 4-parameter sinusoid regression, and generalized linear models were used.

Results: Thirty percent of men, 50% of women, and 15% of children were at risk of lysine inadequacy. Supplementation in children reduced diarrheal episodes [19 lysine, 35 placebo; odds ratio (OR): 0.52; 95% CI: 0.29, 0.92; P = 0.046] and the total number of days ill [21 lysine, 47 placebo; OR: 0.44; 95% CI: 0.26, 0.74; P = 0.034]. Mean days ill per child per week (0.058 ± 0.039 lysine, 0.132 ± 0.063 placebo; P = 0.017) were negatively associated with weight gain with control for baseline weight and study group (P = 0.04). Men had fewer coryza episodes (23 lysine, 39 placebo; OR: 0.60; 95% CI: 0.36, 1.01; P = 0.05), total number of days ill (lysine: 130; placebo: 266; OR: 0.51; 95% CI: 0.28, 0.93; P = 0.03), and mean days ill per person per week (lysine: 0.21 ± 0.23; placebo: 0.41 ± 0.35; P = 0.04). Serum ferritin (P = 0.045) and C-reactive protein (P = 0.018) decreased in lysine-supplemented women but increased in placebo-supplemented women.


INTRODUCTION

Lysine is the first limiting amino acid in cereal diets and affects protein quality in many developing country diets (1–4). Its requirements are increased during acute infectious disease states—a common occurrence in many developing countries (5–9). Protein intake at 9 mo of age is positively associated with later height and weight status (10). Protein quality affects linear growth, possibly through a specific amino acid effect (eg, arginine and lysine) on growth hormone release (10).

Two studies found inverse associations between arginine and high lysine intakes (3.8–4.6 g/d) and fat mass index in prepubertal lean girls (3 and 6 y of follow-up) (10, 11). Lysine fortification enhanced child growth in ethnically and culturally diverse populations of Pakistan and China consuming lysine-deficient diets containing 57–67% wheat protein (12–14). Improvements in serum proteins, immune variables, and immunoglobulin concentrations were also observed (13, 14).

Lysine benefits go beyond protein quality and growth (6, 15, 16). Fortification and supplementation lowered diarrheal morbidity and decreased diarrhea prevalence in women in a Syrian rural population (17) and urban slums in Bangladesh (18), respectively. In Syria, fortification lowered chronic anxiety (Trait and State Anxiety Inventory) (6) and improved stress outcomes in Eastern European subjects with high baseline anxiety (19).

The effects of dietary lysine on the gut and/or brain-gut function in marginally deficient diets are not fully understood. Rodent models show that lysine deficiency contributes to serotonin-mediated anxiety and diarrhea through a direct effect on serotonin receptors (20, 21). Diarrheal incidence could also be affected through enhanced intestinal repair or through effects on the sodium- and chloride-dependent opioid peptide transport systems (22). Whereas the mechanism may not be understood, the need to investigate the concomitant effects of dietary lysine on stress, diarrhea, growth, and morbidity in developing countries is based on lysine being reported effective in I) protecting the gut irrespective of the diarrhea trigger (stress, bacterial, or viral infection) (17, 18, 20), 2) alleviating chronic anxiety across different populations (6, 19), and 3) potentially enhancing growth in children with poor-quality diets (13, 14, 23, 24) while potentially playing a role in body composition (10, 11).

Protein quality enhancement and its effects can be achieved through long-term nutritional strategies (improving access to...
animal food sources and complementary vegetable protein sources), but the effect on stress and diarrhea, as observed by Smriga et al (6) and Ghosh et al (17, 18), is likely a direct effect. On the basis of previously published experiences (6, 13, 14, 17, 18), we hypothesized that lysine supplementation of men, women, and children belonging to poor peri-urban households in Accra, Ghana, would reduce chronic stress in men and women; improve functional indicators of protein in men, women, and children; reduce diarrheal morbidity in women and children; reduce respiratory morbidity in all 3 groups; and affect growth in children.

SUBJECTS AND METHODS

A 16-wk double-blind, randomized, placebo-controlled trial was conducted from January to May 2008 in 90 households of 2 peri-urban areas of Accra, Ghana. The study protocol was reviewed and approved by the Committee on the use of Humans as Experimental subjects of the University of Ghana, Legon, Ghana. Permissions were obtained from the village chief and elders in both settlements and from the local councilman. Households were recruited by actively visiting and discussing the trial, and consent forms were provided at the end of the visit. All recruited households provided a signed informed consent form. Socioeconomic and demographic analyses indicate that, in the recruited households, >50% of the men were drivers, masons, electricians, and unskilled laborers, whereas 60% of the women were street food vendors with a mean monthly household income of US$180. Ninety-two percent of households had access to electricity; 70% used charcoal as their main household fuel. Educational levels were similar in men and women (~60% had primary or middle school qualifications), although 12% of the women had no education.

Sample size and randomization

A total of 271 men, women, and children participated in the study. Sample size determinations for each group (men, women, and children) were based on findings from Smriga et al (6). The power and significance was set at 95%. The minimum sample needed was in the range of 15–20 subjects (men and women) per group, but considering that the trial was being implemented in a different country and environment and included measures of morbidity, we enrolled 45 men, 45 women, and 45 children each for the lysine and placebo groups. All community households were invited to participate in the study. Recruitment criteria included the following: presence of at least one adult male (15–45 y of age), one adult female (15–45 y of age), and one child (2–12 y of age); commitment to participate in the study; intent to reside in the area for ≥1 y; absence of serious illness (eg, diabetes mellitus, heart diseases, renal diseases, and malignancy); nonpregnant and nonlactating; and absence of severe anemia. Each household was assigned a numerical code and was randomly placed in the lysine or placebo group by a research technician at the University of Ghana who was not involved in the study. Randomization was conducted at the household level to ensure consistent supplement (lysine or placebo) use and to prevent mixing of supplements between household members. The trial was double-blind, with all investigators, field staff, and participants being unaware of the coding; the supplements were identical in appearance and taste.

Supplement dosage

Lysine concentrations in Ghana have been found to be marginal (41.1 mg/g protein) (4). On the basis of these estimates, the addition of 15.2 mg/g protein or 900 mg lysine/d would improve the diets of vulnerable groups such as women, children, and individuals with infections. After the baseline data were collected, the subjects were provided 2 lysine-HCl or placebo (dibasic calcium phosphate) tablets each day for 16 wk. Thus, a total of 1200 mg placebo or lysine HCl (1000 mg lysine) was provided per day. Supplements were formulated in India (Disto Pharmaceuticals, Hyderabad, India) and packaged in similar plastic bottles labeled only with unique identification codes.

Data collection

Baseline data collection included an in-depth dietary assessment with 3 nonconsecutive 24-h recalls (25), data on health status, height and weight, a venous blood sample (10 mL collected from 0500 to 0900 from all subjects), skin conductance measurements (6), and a complete clinical assessment including measurements of blood pressure and blood glucose concentrations in adults (25). The baseline health status was measured for 1 wk (the week before the start of the trial). Heights were measured by using a Shorr Height Board (0.1 cm), and weights were measured by using a Seca Scale (±0.1 kg) with appropriate anthropometric techniques (26). In addition, midupper arm circumference was measured (in triplicate). Skin conductance responses were measured noninvasively before, during, and after blood sampling (total measurement time: 5 min). Two Ag/AgCl electrodes were placed in a contact area of 6 mm in diameter on the middle phalanxes of the fore and index fingers of the left hand by using an adhesive collar. Hypoallergenic gel provided good skin contact, and a computerized module (UFI, San Francisco, CA) amplified the electrical signal by a circuit of constant voltage (0.6 V). An artifact-free change in skin conductance ≥0.1 μSiemens was considered a response. Chronic anxiety was evaluated by a local language (Ga) translation of trait-STAI (t-STAI)—an inventory composed of 20 items that has been successfully used in multietnic neighborhoods (27). Because of high levels of illiteracy, the adult subjects replied orally to the t-STAI items read to them. The t-STAI was administered separately from the blood drawing 1 wk before the start of the supplementation and 4 wk into supplementation. All measurements were repeated after testing, and morbidity was monitored weekly (diarrheal, respiratory, and other infectious diseases), which occurred from January to May 2008 by a field team at the 2 settlements. A standard operating manual that described all procedures was provided to all field staff at training and was used throughout the study.

Supervisors assessed all measurements for consistency and accuracy. Supplement compliance was monitored, and refills were provided to participants weekly by field enumerators and monitors. The participants were asked to take the tablets with food. They were also required to mark a monitoring form provided to them; if they were traveling, they were asked to inform...
the monitors so that appropriate amounts of tablets could be given to them.

Data calculations and analysis

The data entry and management system was designed by using MS Access 2000 (Centers for Disease Control, Atlanta, GA) and MS Excel 2003 (Microsoft Corp, Redmond, WA) were used for data calculations, and SPSS 14 (SPSS Inc, Chicago, IL), SigmaPlot 9.0 (Systat Software Inc, San Jose, CA) and SAS 9.0 (SAS Institute, Cary, NC) were used for data analysis. To estimate nutrient intakes, a list of foods commonly consumed in the study areas was generated, and weights of average portion sizes were determined. For composite preparations, field staff collected data on ingredients and amounts of ingredients used in the composite foods that were identified in the list of foods from food vendors in the local community. Wooden and plastic food models and standard ladles and spoons were used to determine portion sizes. Because of the lack of amino acid data in the Ghanaian food tables (28), nutrient composition data were obtained from the US Department of Agriculture (29). Nutrient analysis included total energy, protein, fat, carbohydrate, type of protein (animal, legume, vegetable, and roots and tubers), utilisable protein levels based on the PDCAAS method and expressed as total nitrogen in grams per day total utilisable protein per day and utilisable protein g/kg body weight (30), lysine, total sulfur amino acids, threonine, and tryptophan (mg/d and mg/g protein).

Comparisons of all calculated data—including total utilisable protein per day, lysine, total sulfur amino acids, threonine, and tryptophan (mg/kg per day and mg/g protein)—were made to newly published WHO requirements (30). Risk of protein inadequacy was estimated by log transforming available nitrogen in mg/kg body weight and estimating the mean deficit based on mean intake by sex and age group versus mean requirement for determining protein intake and risk of inadequacy in populations (30). For children, we examined the risk of inadequacy in children older than 2 yrs. We also examined the expected changes in total nitrogen, utilisable protein, and lysine (mg · kg⁻¹ · d⁻¹) and the effect on amino acid balance (using amino acid reference patterns) based on the addition of 1000 mg lysine HCl to the baseline diets of adult men, women, and children in the lysine group. The addition of 1000 mg lysine resulted in a total of 0.19 g of additional nitrogen based on the molecular weight of lysine (146.19 g/mol) and the mass percentage of nitrogen in lysine (19.163%) (31).

Blood samples at baseline and after testing were analyzed for complement C3, C-reactive protein, cortisol, serum albumin, transferrin, ferritin, retinol-binding protein, and hemoglobin. Blood was collected into EDTA-containing and plain tubes and transported to the Noguchi Medical Institute (University of Ghana, Accra) and centrifuged (3000 rpm, 10 min). The samples were stored at −70°C. Complement C3, C-reactive protein, transferrin, ferritin, and retinol-binding protein were measured with quantitative competitive sandwich enzyme immunoassay techniques by using enzyme-linked immunosorbent assay (ELISA) kits (AssayPro, St Charles, MO) and an ELISA multispec plate reader (Thermo Electron Corporation, Shanghai, China). Cortisol was measured by using a competitive assay (EIA kits from Cayman Chemical Company, Detroit, MI). Hemoglobin was assayed by using the Sysmex Auto Analyzer KX-21 (Sysmex, Kobe, Japan). Serum albumin was measured by Bromo Cresol Green reagent on the Flexor E Clinical Chemistry Analyzer (Vital Scientific, Dieren, Netherlands).

Anthropometric data were used to calculate body mass index (for adults) and z scores for 3 indexes: height-for-age, weight-for-age, and weight-for-height for children by using EpiInfo 2000. A −2 SD z score was used as a cutoff for prevalence estimation (32). Skin conductance was measured in tandem with blood sampling with venipuncture from 0500 to 0900 as a cause of stress.

Morbidity data were coded based on type of condition. Diarrheal data were coded as acute or persistent/chronic. Acute diarrhea was defined as ≥3 liquid or semiliquid stools per day. Acute diarrhea with blood and mucus in the stool was defined as dysentery. Diarrhea incidents were considered separate if separated by ≥3 diarrhea-free days. An episode of diarrhea was coded as persistent/chronic if it lasted for ≥14 d with <3 diarrhea-free days within the period of diarrhea occurrence. Data on respiratory morbidity included symptoms of coryza, cough, fever, runny nose, bronchitis, and pneumonia. Incidents of respiratory morbidity were considered distinct if separated by ≥7 incident-free days. The prevalence of morbidity symptom per week, average duration of symptom per week per subject, and mean days ill per subject across 16 wk were calculated.

To examine the effect of lysine longitudinally by using general estimating equations (GEE), follow-up data on each subject were divided into 15 person-periods of 7 d each (starting from week 2 of supplementation through the end of week 16). Person-periods are used to account for the longitudinal study design and the reporting of multiple incidents of a disease by a single subject. In this study, a person-period is a 7-d period in which incidence of diarrhea and/or respiratory morbidity are estimated. Data are expressed as incidence over 15 person-periods for each subject. For an effect to occur, we assumed that ≥1 wk of supplement consumption would be necessary (17).

Statistical analysis (SAS 9.0 and SPSS 14.0) included baseline data comparisons between groups for differences in diets, anthropometric measures, serum variables, and stress variables by using independent-sample t tests. Baseline and posttest comparisons within groups were conducted by using paired t tests. Change in variables were calculated for both lysine and placebo groups (men, women, and children), and comparisons of the difference between the change in the lysine group with that in the placebo group were made by using independent-sample t tests. Significance level was set at 0.05. Morbidity analysis, including differences in number of episodes and duration of illness between the lysine and placebo groups longitudinally, was examined by using chi-square tests and GEE logistic regression analysis to account for repeated measures of the same individuals over 15 wk (33). GEE does not assume independence of events and therefore prevents underestimation of variance that results because of correlation of morbidity within the same subject. This also accounts for disproportional missing data in either group (34, 35).

Specifically for children, we examined the time profile between diarrheal incidents in both groups over the period of the trial by using a 4-parameter sinusoid function (36).
\[ f(D) = y(0) + a \times [\sin(2\pi D/b) + c] \tag{1} \]

where \( D \) corresponds to days of the trial, \( b \) represents the period of the waveform, and \( a \) is the amplitude of the waveform (SigmaPlot 9.0). The Kolmogorov-Smirnov statistic and the constant variance statistic were used to test for assumption of normality and to test for the assumption that the variance of the dependent variable in the source population is constant regardless of the value of the independent variable, and the \( P \) value was calculated by the test. The effect of days ill with diarrhea on height and weight gain was tested by using the generalized linear model with control for baseline values of height and weight (respectively) and study group type (lysine compared with placebo) (37).

RESULTS

A total of 428 households (1284 individuals) were screened for eligibility, 90 of which met the eligibility criteria and were randomly assigned in the study [\( n = 271 \) individuals: 44 men and women and 45 children in the placebo group (there was an additional child because of a pair of twins in one household) and 46 men, women, and children each in the lysine group]. Twenty-two individuals did not show up for the second blood drawing, 5 women discontinued the intervention because of pregnancy, and 7 participants were dropped because their intake of tablets in the first 2 wk of the supplementation period was <50% of the total tablets per week (Figure 1).

Baseline characteristics

In children, no significant differences were observed in age, diarrhea prevalence, or nutrition and growth indicators, including weight-for-age, height-for-age, and weight-for-height. Whereas more children had below-normal serum ferritin concentrations in the placebo group, the numbers (4 children in the placebo group compared with 1 in the lysine group) were relatively small. Among adults at baseline, no significant differences in age, diarrhea prevalence, or BMI were observed (Table 1). Habitual dietary energy levels in men, women, and children were 1740 ± 391, 1420 ± 416, and 1094 ± 256 kcal (data not shown). About 37% of men, 57% of women, and 16% of children were at risk of protein inadequacy (Table 2) (30). Lysine was found to be the first limiting amino acid in all diets, with about one-third of men, 50% of women, and 15.6% of children not meeting their lysine requirement (total mg · kg\(^{-1}\) · d\(^{-1}\)).

**FIGURE 1.** Trial profile. Poor compliance is defined as consumption of \( \leq 50\% \) of the allocated weekly supplement tablets. Seven subjects dropped out because of poor compliance in the first 2 wk of supplementation. *Reflects households; no. of individuals in brackets.
An examination of the differences in habitual dietary intakes between the lysine and placebo groups (before the addition of the 1000 mg lysine to the diets of the lysine group) showed no differences in mean energy, protein (total and utilizable), and lysine (in mg/kg body weight and mg/g protein) in men, women, and children (Table 2). The addition of 1000 mg lysine led to an additional 0.19 g N, which accounted for additional nitrogen at 2.3% in men, 2.7% in women, and 4.2% in children based on habitual intake (percentage values not shown). The addition of lysine and the percentage increase in nitrogen did not add significantly to the total nitrogen intake in the lysine group compared with that in the placebo group for men, women, and children; however, it resulted in a decreased risk of protein inadequacy in all 3 groups, and the difference in protein inadequacy was significantly different only between the lysine-supplemented and placebo children (Table 2). The addition of 1000 mg lysine to the diets of the lysine group (83%) worked together to maintain the compliance of the children. Compliance with tablet consumption was similar among men in the lysine-supplemented men and women: placebo: 45 episodes (OR: 0.88; 95% CI: 0.48, 1.6; P = 0.046).

No significant differences were found in the total number of diarrheal episodes, total number of days ill, and mean days ill per person per week between the lysine and placebo groups, both in men and women. Whereas the number of episodes was fewer in the lysine-supplemented women than in the placebo women, the difference found by GEE was not significant (lysine: 39 episodes; placebo: 45 episodes (OR: 0.88; 95% CI: 0.48, 1.6; P = 0.667)). Lysine-supplemented men had a total of 24 episodes of diarrheal, and the placebo group had 26 episodes. The difference by GEE was not significant in the men (OR: 0.95; 95% CI: 0.49, 1.88) (Table 4).

**Effect on diarrhea incidence, diarrhea duration, and time between episodes**

The total number of episodes of acute diarrheal in children in 15 (7-d) person-periods per child was 19 and 35 in the lysine and placebo groups, respectively (Table 4). Using GEE logistic regression models to account for repeated measures, we found that children supplemented with lysine were 48% less likely to have an episode of diarrhea during the study than were children receiving a placebo [odds ratio (OR): 0.52; 95% CI: 0.29, 0.92; P = 0.046].
Children were a total of 21 and 47 d ill from diarrhea reported in children of
women (0 in the lysine group, 3 in the placebo group), and 1 episode
in children (1 in the lysine group, 0 in the placebo group). There
were a total of 21 and 47 d ill from diarrhea reported in children of
the lysine and placebo groups, respectively. The GEE model indicated
that the total days ill was lower in children supplemented
with lysine than with placebo ($P = 0.034$). The mean days ill per
child per week were also significantly fewer in the lysine group
than in the placebo group ($P = 0.017$). Among men and women,
no significant differences were found in mean days ill per person
per week between the lysine and placebo groups.

No persistent or chronic diarrhea was observed in any of the
subjects. We found a total of 7 episodes of dysentery with 3 epi-
sodes in men (2 in the lysine group, 1 in the placebo group), 3 in
women (0 in the lysine group, 3 in the placebo group), and 1 episode
in children (1 in the lysine group, 0 in the placebo group). There
were a total of 21 and 47 d ill from diarrhea reported in children of
the lysine and placebo groups, respectively. The GEE model indicated
that the total days ill was lower in children supplemented
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### Table 2

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**Men**

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**Women**

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**Children >2 y of age**

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$^a$ No significant differences were observed between the lysine and placebo groups before the addition of lysine (independent-sample t test).

$^b$ This group reflects the expected improvement in baseline nutrient values of the lysine-group diets on the addition of 1000 mg lysine, which is equivalent to the amount of supplement provided.

$^c$ Percentage at risk of protein inadequacy calculated based on a protein requirement of 0.66 g/kg for adults and 0.73 g/kg for children >2 y of age (30).

$^d$ Adult amino acid requirements: lysine, 30 mg · kg$^{-1}$ · d$^{-1}$; sulfur amino acids, 15 mg · kg$^{-1}$ · d$^{-1}$; threonine, 15 mg · kg$^{-1}$ · d$^{-1}$; tryptophan, 4 mg · kg$^{-1}$ · d$^{-1}$ (30).

$^e$ Significantly different from placebo group, $P < 0.001$.

$^f$ Child amino acid requirements: lysine, 35 mg · kg$^{-1}$ · d$^{-1}$; sulfur amino acids, 18 mg · kg$^{-1}$ · d$^{-1}$; threonine, 18 mg · kg$^{-1}$ · d$^{-1}$; tryptophan, 4.8 mg · kg$^{-1}$ · d$^{-1}$ (30).

Four-parameter sinusoid regression of the disease prevalence passed normality (K-S statistic $P = 0.7868$) and constant variance (constant variance test, $P = 0.7545$) tests for children in both the lysine and placebo groups. The period of the wave-form was significantly prolonged in the lysine group (8.54 ± 1.38 d) when compared with the placebo group (3.41 ± 0.16 d) at $P < 0.05$ ($f$ test), whereas the amplitude of the regression sinusoid remained comparable between the groups (0.067 ± 0.009 occurrences per day in the lysine group compared with $P = 0.072 ± 0.001$ occurrences per day in the placebo group), which indicated that lysine treatment affected predominantly the interval between single diarrheal episodes (data are not shown).
There were no significant differences in dietary intakes were observed between the lysine and placebo groups before the addition of lysine (independent-sample t tests).

This group reflects the expected improvement in baseline nutrient values of the lysine-group diets after the addition of 1000 mg lysine, which is equivalent to the amount of supplement provided.

Adult amino acid reference pattern: lysine, 45 mg/g protein; sulfur amino acids, 22 mg/g protein; threonine, 23 mg/g protein; tryptophan, 6 mg/g protein. Child amino acid reference pattern: lysine, 48 mg/g protein; sulfur amino acids, 24 mg/g protein; threonine, 25 mg/g protein; tryptophan, 6.6 mg/g protein.

Mean ± SD (all such values).

**Effect on anthropometric measures**

No significant differences were observed in any anthropometric indicators of midupper arm circumference and weight-for-age, height-for-age, and weight-for-height z scores (Table 5) when the changes in the lysine and placebo groups were compared.

**Effect of diarrhea duration on growth of children**

Days ill per child with diarrhea were significantly fewer in the lysine than in the placebo group (P = 0.034). In addition, we observed a significantly lower weight gain in the placebo group (0.70 ± 2.18 kg) than in the lysine group (0.87 ± 0.92 kg). Change in height was similar between the lysine and placebo groups (lysine: 2.02 ± 1.18 cm; placebo: 2.26 ± 0.83 cm). Using a generalized linear model, we found that the mean number of days ill per child per week with diarrhea for each child was significantly negatively associated with their change in weight over the study period, after control for baseline weight and study group (P = 0.04) despite no significant difference in weight change between the lysine and placebo groups (data not shown).

**Effect on respiratory symptoms**

Of all the respiratory symptoms, we found a change only in the incidence of coryza (“colds”) in men (Table 4). There were significantly fewer incidents of coryza in lysine-supplemented men (lysine: 23; placebo: 39; OR: 0.60; 95% CI: 0.36, 1.01; P = 0.05) as well as a significantly fewer number of days ill (lysine: 130; placebo: 266; OR: 0.51; 95% CI: 0.28, 0.93; P = 0.03) and mean days ill per person per week (lysine: 0.21 ± 0.23; placebo: 0.41 ± 0.35; P = 0.04). No significant differences in respiratory disease were observed in women or children between the 2 groups (Table 4).

**Effect on blood variables**

No differences were observed in hemoglobin or serum albumin between groups (Table 6). Posttest data for serum cortisol, serum retinol-binding protein, serum transferrin, and complement C3 were analyzed, and substantial differences in concentrations (5–10-fold increases or decreases) were found from the baseline values. However, a closer examination of the data showed measurement error in specific ELISA plates for the indicators. Unfortunately none of the samples had been retained for reanalysis because of local laboratory conditions. Given this, we were unable to verify whether there was an effect of lysine on any of the abovementioned indicators. In women, C-reactive protein decreased in the lysine group but increased in the placebo group (P = 0.018). Thus, women in the lysine group suffered less acute inflammation. No differences were observed in
**TABLE 4**

Morbidity incidence (acute diarrhea and coryza) and duration in men, women, and children in the lysine and placebo groups

<table>
<thead>
<tr>
<th></th>
<th>Acute diarrhea</th>
<th>Acute coryza</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lysine group</td>
<td>Placebo group</td>
</tr>
<tr>
<td><strong>Men</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>n</strong></td>
<td>46</td>
<td>44</td>
</tr>
<tr>
<td>Number of 7-d person-periods</td>
<td>627</td>
<td>653</td>
</tr>
<tr>
<td>Incident episodes of morbidity (person-periods)</td>
<td>24</td>
<td>26</td>
</tr>
<tr>
<td>Total days ill</td>
<td>33</td>
<td>38</td>
</tr>
<tr>
<td>Mean days ill per person per week</td>
<td>0.052 ± 0.058</td>
<td>0.077 ± 0.071</td>
</tr>
<tr>
<td><strong>Women</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>n</strong></td>
<td>44</td>
<td>43</td>
</tr>
<tr>
<td>Number of 7-d person-periods</td>
<td>604</td>
<td>612</td>
</tr>
<tr>
<td>Incident episodes of morbidity (person-periods)</td>
<td>39</td>
<td>45</td>
</tr>
<tr>
<td>Total days ill</td>
<td>67</td>
<td>60</td>
</tr>
<tr>
<td>Mean days ill per person per week</td>
<td>0.208 ± 0.087</td>
<td>0.184 ± 0.075</td>
</tr>
<tr>
<td><strong>Children</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>n</strong></td>
<td>46</td>
<td>45</td>
</tr>
<tr>
<td>Number of 7-d person-periods</td>
<td>678</td>
<td>667</td>
</tr>
<tr>
<td>Incident episodes of acute morbidity (person-periods)</td>
<td>197</td>
<td>35</td>
</tr>
<tr>
<td>Total days ill</td>
<td>21</td>
<td>47</td>
</tr>
<tr>
<td>Mean days ill per person per week</td>
<td>0.058 ± 0.039</td>
<td>0.132 ± 0.063</td>
</tr>
</tbody>
</table>

1 Person-periods were used to account for longitudinal study designs and the reporting of multiple incidents of a disease by a single subject. In this study, a person-period included 7 d of data on incidence of diarrhea and/or respiratory morbidity.

2,3,7,8 Significantly different from placebo group, within disease group (general estimating equations logistic regression model accounting for repeated measures): 2P = 0.05, 3P = 0.03, 7P = 0.046, 8P = 0.034.

4 Significance of difference between means tested by using a nonparametric Mann-Whitney U test.

5 Mean ± SD (all such values).

6,9 Significantly different from placebo group (nonparametric Mann-Whitney U test): 6P = 0.04, 9P = 0.017.

**Effect on stress variables**

Changes in sympathetic arousal as reflected by skin conductance due to venipuncture stress were examined in men, women, and children. A lowered response in skin conductance (in μSiemens) indicates a reduction in acute anxiety. All 3 groups (men, women, and children) had similar baseline values of skin conductance ranging from 10.5 to 11.8 μSiemens and posttest values ranging from 8.8 to 10.9 μSiemens. No significant differences were observed across groups (data not shown).

Changes in chronic anxiety as reflected by Trait scores obtained in men and women before supplementation and 2 wk after supplementation were examined. A decrease in T-STA1 score indicates a reduction in long-term anxiety. Men and women in both the lysine and placebo groups started at similar baseline scores (range: 42.7–44.5) and posttest scores (range: 37.7–39.2) (data are not shown). No significant differences were observed between groups, but, within groups, all subjects reported a reduction in chronic anxiety.

**DISCUSSION**

The effects of lysine supplementation (after 16 wk, 1.0 g/person per day) on morbidity and stress measured in Ghana extend previous evidence from Syria (6, 17) and Bangladesh (18). Baseline data indicated that 37% of men, 57% of women, and 16% of children were at risk of protein inadequacy. Lysine is the first limiting amino acid in nearly all developing countries (1–4), and indeed, one-third of the men and one-half of the women did not meet their total lysine requirement (mg/C1 kg/d) (30). Because of these baseline observations, only lysine was fortified in this study. The added nitrogen from the 1 g lysine did not significantly add to the total nitrogen available to the lysine group (Table 2), and the amino acid balance calculations (Table 3) indicated that no other essential amino acids would have an effect on protein quality. Moreover, including another experimental group would have added enormous practical burden on the field staff. Thus, the potential benefits of having an added intervention control were judged as insufficient from both nutritional and practical viewpoints.

We found significant lysine-triggered decreases in diarrheal morbidity and an effect of diarrhea incidence on weight gain in children, without biochemical measures being significantly affected. The potential effect of the diarrhea findings for developing...
**TABLE 5**  
Comparison of baseline and posttest anthropometric measurements of participants enrolled in the study

<table>
<thead>
<tr>
<th></th>
<th>Lysine</th>
<th>Placebo</th>
<th>Difference between change in lysine and placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Posttest</td>
<td>Change</td>
</tr>
<tr>
<td><strong>Men</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>46</td>
<td>46</td>
<td>46</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.60 ± 3.41[^3]</td>
<td>23.19 ± 3.39</td>
<td>0.13 ± 0.57</td>
</tr>
<tr>
<td><strong>Women</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>45</td>
<td>45</td>
<td>45</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.67 ± 5.28</td>
<td>24.95 ± 4.60</td>
<td>−0.05 ± 0.63</td>
</tr>
<tr>
<td><strong>Children</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>41</td>
<td>41</td>
<td>41</td>
</tr>
<tr>
<td>Weight-for-age z score</td>
<td>−1.24 ± 1.26</td>
<td>−1.25 ± 1.33</td>
<td>−0.01 ± 0.28</td>
</tr>
<tr>
<td>Height-for-age z score</td>
<td>−0.84 ± 1.39</td>
<td>−0.83 ± 1.39</td>
<td>0.016 ± 0.26</td>
</tr>
<tr>
<td>Weight-for-height z score[^4]</td>
<td>−1.25 ± 1.05 [19]</td>
<td>−1.43 ± 0.90 [19]</td>
<td>−0.18 ± 0.51 [19]</td>
</tr>
<tr>
<td>Midupper arm circumference (cm)</td>
<td>17.92 ± 2.48</td>
<td>18.28 ± 2.74</td>
<td>0.36 ± 0.69</td>
</tr>
</tbody>
</table>

[^1]: No significant differences were observed between the lysine and placebo groups at baseline in men, women, or children.
[^2]: No significant differences were observed in difference in changes between the lysine and placebo groups for men, women, or children (independent-sample t tests).
[^3]: Mean ± SD (all such values).
[^4]: n values in brackets.
countries is clear: there is an estimated 5 billion cases of diarrhea in children per year, which equates to ≥2.5 billion preventable deaths under 5 y of age (39, 40). Every additional diarrheal episode before age 2 y contributes to stunted growth (41), and diarrhea is a risk factor for poorer school performance and diminished long-term cognitive function later in life (42). Diarrhea and protein-energy malnutrition are synergistic, especially in developing countries where cereal-based diets are marginal or deficient in protein (43–46).

Whereas previous lysine studies (in Syria and Bangladesh) found a reduction in diarrhea in women (17, 18), this study found the effect only in children. This discrepancy could be due to lower compliance in the lysine-supplemented women (P = 0.003), but most likely was due to insufficient doses, given that a higher percentage of the women than of the children did not meet their protein and lysine requirements. The addition of lysine reduced protein and lysine inadequacy but not to the extent observed in children. In Syria and Bangladesh, total lysine (mg/d) was adequate (17, 18) in women, but the amount of lysine (mg/g lysine) required for a balanced dietary essential amino acid pattern was not met.

We found favorable effects of lysine on diarrhea in a geographic region where bacterial and viral causes predominate (39, 40), similarly to Bangladesh (47). The efficacy of lysine in reducing diarrhea in diverse areas such as Ghana, Syria, and Bangladesh suggests a significant effect of lysine in population groups with a high prevalence of diarrhea (6, 17, 18), without regard to the infectious agent or demography. The effect, however, could be contingent on the protein and lysine contents already in the diet as well as the adequacy of the amount of added lysine.

At least 3 mechanisms could have contributed to the observed effect on diarrhea in children. First, the effect could have been due to an improvement in protein status. The provision of high-quality animal protein diminishes intestinal damage and reduces the effect of the rotavirus infection (48, 49). Lysine supplementation significantly improves the protein quality of high-cereal–based diets (6, 17, 18). Thirty-five percent of children in the lysine group did not meet their requirement for lysine (mg/g protein) in their baseline diet; thus, it is likely that the addition of lysine improved the protein quality of their diet.

A second more rapid and potent mechanism is possible because the effect was observed consistently from the second week of supplementation. In humans, lysine partially but significantly blocks serotonin-induced diarrhea (20, 21) and inhibits the opioid transport system in the intestines (22). Both systems are involved in bacterial diarrhea, although their role is not fully understood (46, 50). Whereas intestinal fluid secretion through activation of the intestinal enteric nervous system is one of the main theories of bacterial diarrhea (50), viral diarrhea activates ≥2 intracellular regulators of protein synthesis (51–53). In in vitro models of rotavirus diarrhea, arginine is suggested as a possible protein synthesis stimulator during diarrheal episodes (52), and a new class (human intestinal G-protein coupled receptor) with high affinity for arginine and lysine has been described (54, 55).

A third possibility is an indirect effect through reduced long-term stress, based on findings in Syria where fortification reduced

### Table 6

Comparison of baseline and posttest serum variables between the lysine and placebo groups

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Posttest</th>
<th>Change</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Men</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>36</td>
<td>36</td>
<td>36</td>
</tr>
<tr>
<td>Hemoglobin (g/100 mL)</td>
<td>14.39 ± 1.25</td>
<td>14.11 ± 1.18</td>
<td>−0.29 ± 0.98</td>
</tr>
<tr>
<td>Serum ferritin (g/mL)</td>
<td>110.42 ± 106.37</td>
<td>183.13 ± 157.59</td>
<td>72.70 ± 155.62</td>
</tr>
<tr>
<td>Serum albumin (g/L)</td>
<td>42.34 ± 2.58</td>
<td>42.12 ± 2.57</td>
<td>−0.18 ± 1.88</td>
</tr>
<tr>
<td>C-reactive protein (mg/L)</td>
<td>3.33 ± 4.42</td>
<td>5.02 ± 4.22</td>
<td>1.70 ± 5.06</td>
</tr>
<tr>
<td><strong>Women</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>35</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>Hemoglobin (g/100 mL)</td>
<td>12.06 ± 1.44</td>
<td>11.86 ± 1.24</td>
<td>−0.19 ± 0.81</td>
</tr>
<tr>
<td>Serum ferritin (g/mL)</td>
<td>94.34 ± 111.79</td>
<td>62.54 ± 58.16</td>
<td>−31.81 ± 104.02</td>
</tr>
<tr>
<td>Serum albumin (g/L)</td>
<td>41.14 ± 2.98</td>
<td>41.11 ± 2.00</td>
<td>−0.03 ± 3.31</td>
</tr>
<tr>
<td>C-reactive protein (mg/L)</td>
<td>4.19 ± 4.68</td>
<td>3.53 ± 2.69</td>
<td>−0.67 ± 4.71</td>
</tr>
<tr>
<td><strong>Children</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>38</td>
<td>38</td>
<td>38</td>
</tr>
<tr>
<td>Hemoglobin (g/100 mL)</td>
<td>11.37 ± 1.15</td>
<td>11.23 ± 1.08</td>
<td>−0.139 ± 0.71</td>
</tr>
<tr>
<td>Serum ferritin (g/mL)</td>
<td>61.16 ± 54.16</td>
<td>77.49 ± 58.70</td>
<td>16.34 ± 91.69</td>
</tr>
<tr>
<td>Serum albumin (g/L)</td>
<td>41.34 ± 3.30</td>
<td>41.29 ± 2.09</td>
<td>−0.05 ± 2.92</td>
</tr>
<tr>
<td>C-reactive protein (mg/L)</td>
<td>3.41 ± 4.94</td>
<td>4.32 ± 3.74</td>
<td>0.90 ± 5.67</td>
</tr>
</tbody>
</table>

**Notes:**
- No significant differences were observed between the lysine and placebo groups at baseline in men, women, or children.
- Normal range: 11–16 g/100 mL.
- Mean ± SD (all such values).
- Normal range: 15–300 mg/mL.
- Normal range: 35–50 g/L.
- Normal range (no infection): <10 mg/L.
- Significant difference in change between lysine and placebo groups, P = 0.045 (log transformation and correction for C-reactive protein, independent-sample t test).

Data on arithmetic mean, SD, and change in mean are shown in the table.

Significant difference in change between lysine and placebo groups, P = 0.018.
anxiety and stress response (6). Parallel findings were reported in experimental animals (20, 21). In this study, stress-triggered sympathetic arousal and chronic anxiety were not significantly influenced by added lysine because of a high placebo-like effect of the treatment per se. Unfortunately, serum cortisol values were not available because of unexpected technical problems in the local laboratory. A likely factor in the placebo response and a lack of specific effects was the provision of treatment in the form of capsules, whereas lysine supplementation in Syria was in the form of fortification to the local diet (6).

Diarrhea has a detrimental effect on weight gain in young children (39–42, 56, 57). Parallel to the earlier observations, we found a significant negative association between days ill from diarrhea and change in weight gain and a positive effect of lysine supplementation on weight gain in children when baseline weight and group type (lysine compared with placebo) were controlled for. No significant difference in height gain was observed between children in the lysine and placebo groups; however, a 16-wk-long study may have been too short to observe such an effect given that the period between a diarrhea episode and detection of a subsequent adverse effect on height gain is 2 mo (58).

In Pakistan and China, lysine fortification effects in adults were most pronounced on the immune system, as observed by changes in CD cells (eg, CD4, CD3, and CD8), immunoglobulins (eg, IgG, IgA, and IgM), and complement C3; however, Ghana did not have the facilities to replicate the same battery of immune tests. In this study the limited indicators of immune function were not sex-consistent. Reduced coryza incidents were observed in men but not in women. In women, C-reactive protein was significantly lower in lysine-supplemented women, which suggested less inflammation. Serum ferritin was not affected in men, but it was slightly lower in lysine-supplemented women after accounting for nonnormality of the data and any effect of acute inflammation, although it was not below the normal, physiologic level.

Finally, the group with the highest inadequacy of total utilizable protein (women) did not show a significant improvement in variables. This led to the hypothesis that the level of lysine currently provided was not sufficient to bring levels of utilizable protein and lysine above the requirement for maintenance in women; even with supplementation, 11% of women in the lysine group were still not meeting their daily lysine requirement. The effects on diarrhea in children and immune endpoints in men point to an improved protein quality and possibly a direct effect of free lysine on gastrointestinal health, as has been suggested in humans (17, 18) and measured in experimental animals (20, 21). Considering the human and societal effect of diarrhea in the developing world and the availability of lysine for fortification, the current results warrant further investigation of lysine’s interaction within the gut and its effect on protein quality.

In summary, this double-blind randomized study in poor peri-urban communities of Accra, Ghana, showed significant benefits of 16 wk of lysine supplementation on diarrhea morbidity and weight gain in children. A positive effect of lysine on respiratory disease outcomes was found in men. Varying dietary levels of lysine and utilizable protein, as well as specific age- and sex-related doses, need to be given more consideration in the future. The results suggest that lysine could be a useful nutritional intervention for decreasing diarrhea morbidity and improving the nutritional status of populations in some developing countries.

We thank all field staff and the participants of the trial from the peri-urban areas of Teiman and Gyari, the Noguchi Medical Institute for conducting the analyses of blood variables, and Nicholas Strutt (Research Assistant and graduate student at the Friedman School of Nutrition Science and Policy) for assisting with data cleaning and management.

The authors’ responsibilities were as follows—SG: responsible for the conceptualization of the research questions, development and oversight of trial implementation, data management oversight, analysis, interpretation, and preparation of the manuscript draft for publication; MS: involved in the conceptualization of the research questions relating to stress, joint oversight on the trial implementation, data analysis, interpretation, and preparation of manuscript related to stress and intestinal function; FY (Field Director): responsible for organization and management of the trial, data entry process management, and work on the manuscript; DS: responsible for the statistical data analysis, interpretation, and preparation of the manuscript; HM: responsible for the stress (sympathetic and chronic) data collection and supervised the trial; SMA: responsible for the dietary data collection and assisted in the nutrient calculations; and NSS (Senior Advisor): responsible for oversight from conceptualization through interpretation of the results and final editing of the manuscript. MS is an employee of Ajinomoto Co, Inc. No other conflicts of interest were reported.

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