Impact of lysine-fortified wheat flour on morbidity and immunologic variables among members of rural families in northwest Syria

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Abstract

Background. *Previous studies have shown an effect of lysine fortification on nutrition and immunity of poor men, women, and children consuming a predominantly wheat-based diet.*

Objective. *To examine the lysine value of diets and the effect of lysine fortification on functional protein status, anthropometry, and morbidity of men, women, and children in rural Syria.*

Methods. *At baseline of a two-phase study using 7-day household food intake inventories* (n = *98), nutrient availabilities per adult male equivalent were estimated. In the intervention phase, a 16-week double-blind trial, households (*n = *106) were randomly assigned to control and lysine groups. Hematologic and anthropometric data were collected from men (*n = *69; 31 control, 38 lysine), women (*n = *99; 51 control, 48 lysine), and children (*n = *69; 37 control, 32 lysine) at baseline, 12 weeks, and 16 weeks. Total CD3 T lymphocytes as well as T lymphocytes bearing the receptors CD4, CD8, and CD56, IgM, IgG, IgA, complement C3, C-reactive protein, serum albumin, prealbumin, transferrin, retinol-binding protein, hemoglobin, and hepatitis B surface antigen were determined. Health status and flour usage were monitored. Paired- and independent-sample* t*-tests and chi-square tests were performed.*

Results. *Mean nutrient availability per adult equivalent was 2,650 ± 806 kcal, 70.1 ± 26.4 g protein, 65 ± 14% cereal protein, and 41.9 ± 0.8 mg lysine per gram of protein. Complement C3 was significantly higher in men receiving lysine than in controls (*$p < .05$*). Among women, there were significant differences between the control and lysine groups in diarrhea period prevalence (total number of diarrheal episodes during the period of intervention divided by the total number of observations), (20 in the control group, 6 in the lysine group;* $p = .014$*), the mean number of days ill (0.4 ± 0.7, control, 0.14 ± 0.4, lysine,* $p = 0.03$*), and the number of diarrheal episodes per person per year (1.39 in the control group, 0.47 in the lysine group). No other significant differences between the lysine and the control groups were observed.*

Conclusions. *Lysine fortification of wheat flour demonstrated a positive effect on diarrheal morbidity in women. The effect could be attributed to an improvement in protein utilization but possibly also to a direct effect of lysine in gastrointestinal tract. Studies in populations with higher diarrheal prevalence and significant dietary lysine deficiency are needed to determine whether the reported effects on diarrheal prevalence are replicable and whether they are pharmacological or nutritional. It would be particularly desirable to study the effect of lysine on diarrhea in preschool children, who have much higher morbidity and mortality rates from this disease than school-age children or adults.*

Key words: Complement C3, diarrhea, flour fortification, immunity in women, lysine, morbidity, protein, Syria

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Introduction

In diets with more than 50% of protein from cereal sources, lysine is a key limiting amino acid [1–4].

Studies in China and Pakistan [5, 6] have shown a positive impact of lysine-fortified wheat flour on nutritional and immunologic variables of men, women, and children of poor socioeconomic backgrounds consuming a diet with wheat as the principal source of protein. The present study was conducted to examine the impact of lysine-fortified wheat flour on the nutritional, immunologic, and morbidity status of rural households in northwest Syria.

Nitrogen balance studies in adults and children from the 1940s through the 1960s and 1970s examined the impact of lysine on nitrogen retention when lysine was added to adult or child diets largely composed of wheat or maize [7–14]. Following the demonstration of favorable effects of lysine in these studies and the findings and recommendations of the 1969 Conference on Amino Acid Fortification of Protein Foods [15], a population-based intervention was conducted in Tunisia in 1970–75, in which wheat was fortified with lysine, iron, and vitamins and provided to a malnourished population in the southern part of the country. Detailed data were collected on the results of physical examinations, anthropometry, hemoglobin and hematocrit, morbidity, and mortality. No beneficial health effects were observed, but there were serious flaws in the study design and execution: there was no control for differences in morbidity from infections in the two areas, and contraband unfortified flour was introduced and consumed by the population assigned to consume only lysine-fortified flour.

Two recent studies conducted in Pakistan and China [5, 6] found that lysine fortification of wheat flour significantly improved sensitive indicators of nutritional status in populations consuming diets in which 57% to 67% of the protein originated from wheat. The addition of lysine to the daily diet resulted in significant improvements in weight and height in Chinese and Pakistani children, hemoglobin levels in Chinese women, prealbumin levels in Pakistani and Chinese men and women, and transferrin levels in Chinese men, women, and children. Significant improvements were observed in immunologic indicators (T lymphocytes with CD4 and CD8 receptors and complement C3) in all three groups in Pakistan, whereas in China significant improvements were seen in total CD3 lymphocyte levels in women and children, complement C3 and IgG in men, IgA in women, and IgG, IgA, IgM, and complement C3 in children.

Growth status and dietary patterns of Syrian children

Syria, with a population of around 18 million, is classified by the United Nations as a low-income country. About 5.1 million of the Syrian population live below the poverty line. National stunting and underweight rates are 20.8% and 12.9%, respectively [16]. Mokbel [17] examined differences in nutritional status and dietary patterns in two rainfall zones of Aleppo Province and found that 50% of rural children were underweight and 17% of preschool children and schoolchildren were below the 90th percentile of height-for-age. Baba et al. [18] found stunting and underweight rates as high as 41% and 14%, respectively, in children of settled Bedouins who lived as herders of government livestock. The trends in the overall Syrian dietary pattern according to Food and Agriculture Organization (FAO) food balance sheet data were examined and the availability of total protein, cereal protein, and animal protein for selected years from 1961 to 2001 were analyzed. Although there was an increase in total protein availability, a significant proportion of total protein was obtained from cereals. Fluctuations occurred in the availability of animal protein and pulse protein; however, in 2001 lysine availability was found to be 44.5 mg per gram of protein and total protein availability approximately 75 g, with 57% of protein from cereals, 26% from animal sources, and 4.7% from pulses and soybeans. Thus, the dietary availability of lysine and good-quality protein is limited in the Syrian diet, with availability values lower than those observed in Pakistan [6] and China [5]. A lysine fortification trial, replicating the design of the Pakistan and China studies, was conducted in the Syrian Arab Republic during 2002 and 2003.

Methods

The study was conducted in two phases, a baseline phase and an intervention phase. The major objectives of the baseline phase (February to May 2002) were to determine lysine values in rural Syrian diets and to test the stability of lysine in thin Arabic bread. The lysine intervention (March to July 2003) examined the effects of lysine fortification of wheat flour on men, women, and children. Its objective was to determine any effects of improved protein quality on protein status, immunologic status, growth, body mass index (BMI), and morbidity from diarrhea and acute respiratory infections.

The protocol was reviewed and approved by the committee on the use of humans as experimental subjects of the International Center for Agricultural Research in the Dry Areas, Aleppo, Syria. Village meetings were held, and consent forms translated into Arabic were distributed. In each village, two or three key informants (headmen or village elders) were asked to hold meetings after the consent forms were handed out. Further clarification was provided as needed. Each household head was asked to accept or decline participation in the trial. Consent forms were returned to the field team with signatures or initials of the household heads.

Study area and characteristics

The Khannaser Valley is located in northwestern Syria, 80 km southeast of the city of Aleppo. The area is classified as semiarid and is located at the fringe of the Syrian steppe [19]. The valley has approximately 40 villages and a population of 11,000. The main forms of agriculture are rain-fed farming, irrigated farming, and livestock rearing; however, the primary source of income for most people is agricultural wage labor. An anthropometric assessment of children under 10 years of age in this marginal area found higher levels of stunting (23%) and underweight (14.3%) than in villages in other areas (13% and 3.5%, respectively). Girls had the highest prevalence of stunting (28.3%) with boys in the same villages averaging at 17% [20].

Characteristics of the sample

The selection of the geographic area and the villages was purposive. Thirty-five villages were surveyed, of which seven reported using home-made bread. Three of these villages were large enough to meet sample size requirements. Household lists were generated for these three villages with the help of the village elders and official heads of the villages. Ninety-eight households completed the interview, which had two parts. In the second phase, all 98 households were approached, but only 66 households consented to participation. In order to maintain a sample size similar to that in China and Pakistan (n = 80) [5, 6], two more villages were surveyed, and 50 more households with a similar dietary pattern as the sampled households and using home-made bread were identified. Forty of these households consented to participate in the trial, bringing the sample size in the intervention trial to 106 households. The villages that were surveyed were taken from the list of 35 villages in the Khannaser Valley, which has predominantly marginal rainfed mixed-crop and livestock farming [21, 22] and belongs to agroecological zone 4, in which the households have a lower consumption of animal products (dairy products, meat, and eggs) [17, 18, 23]. Thus, the villages selected to enroll additional households were part of the universe from which the initial sample had been drawn.

Intervention study design

The 16-week study was a randomized, double-blind, controlled intervention conducted from March to July 2003. The study design was based on the China and Pakistan studies [5, 6]. One hundred six households were randomly assigned to a control or lysine group (4.2 g of lysine HCl per kilogram of flour). Ninety-three households completed the study (48 control and 45 lysine). In each household, one adult male, one adult female, and one child aged 4 to 15 years participated in the study. In 10 households, two women volunteered for the study, which changed the demographic features very slightly (**table 1**). Blood was drawn at baseline and at 12 weeks after the beginning of fortification. Height and weight data were obtained at baseline and at 16 weeks. Morbidity data were obtained at baseline (start of the study), postfortification (end of the study), and every 2 weeks in between the baseline and post fortification through the entire trial period. The additional time allowed for observation of possible effects on growth and BMI.

An important feature of the design was that the fortification took place at the village mill, where premixes, with and without lysine, were added to the villagers' own grain after it had been ground to flour. This avoided the possibility of dietary change resulting from the economic benefit to the subjects of free flour.

TABLE 1. Characteristics of study population during the intervention phase

	Men		Women		Children	
Characteristic	Control	Lysine	Control	Lysine	Control	Lysine
No. at baseline	31	38	51	48	37	32
No. excluded because of severe anemia	0	0	1	3	1	0
No. pregnant	0	0	13	11	0	0
No. postfortification (morbidity data)	31	38	50	44	36	32
No. postfortification (hematologic data)	25	29	48	44	36	31
Total no. (hematologic and anthropometric data)	25	29	35	33	36	31
No. with normal C-reactive protein	20	27	26	32	36	29
Mean ± SD age (yr)	38.97 ± 11.16	36.82 ± 9.64	37.49 ± 10.71	34.74 ± 11.11	9.65 ± 2.91	9.42 ± 3.11
Mean ± SD no. days of fortification	88.0 ± 4.88	89.0 ± 6.16	86.2 ± 4.00	86.4 ± 4.80	86.14 ± 4.26	86.82 ± 5.26

Baseline phase

The baseline phase included a dietary assessment, a socioeconomic assessment, and a test of lysine stability in Arabic bread. Dietary intake patterns were assessed with the use of a 7-day household food intake inventory ($n = 98$), and data were expressed as nutrient availability per adult equivalent. The method is a combination of the food account and household food record method [24]; although not validated independently, it is based on previously validated work [25]. The procedure involved collecting data in two interviews a week apart on all food bought, cooked, eaten, and wasted by the household and the total number of persons present at each meal, their ages, and their sexes. Quantities expressed in household measures were converted to total grams of each food item used by the household through the week.

Nutrient analyses for total household consumption were conducted at the Massachusetts Nutrient Data Bank of the University of Massachusetts, Amherst, Massachusetts, USA, using the US Department of Agriculture nutrient database and the Food Tables for the Middle East [26]. Availabilities per adult equivalent were calculated by adding the total number of individuals consuming the total grams of each food item. Then the energy requirement (FAO/WHO/UNU requirement) of an adult male involved in moderate to heavy work [27] was considered, a factor of 1.0 was assigned to adult males in the household, and equivalency factors for individual age and sex groups (the proportion of the adult male equivalency factor of 1.0) were calculated.

Using this approach, we expressed the total number of persons consuming a food item in a household in terms of the number of adult male portion equivalents ("consumption units") of the food item**.** (Consumption units and adult equivalents are used to define per capita usage of food rather than food consumed by a certain number of men, women and children.) The calculation was modeled after a similar study conducted in the West Bank and Gaza [28]. The calculated factors are shown in **table 2**. Since the completion of this study, new energy requirements have been released [29]. To give due consideration to any changes that might have occurred to individual age and sex dependent requirements, a comparison of the energy values per age group was conducted between the 1985 and the 2001 requirements. While the comparison did show a difference on converting to adult equivalent factors, the differences between the 1985 and the 2001 factors was minimal such that it was considered appropriate to use the original analysis with the 1985 values.

TABLE 2. Factors used for estimation of adult male portion equivalents (consumption units)

Age/sex group	Equivalent factor
Adult males (≥ 18 yr)	1
Adult females (≥ 18 yr)	0.81
Children (10 to 17.99 yr)	0.80
Children (5–9.99 yr)	0.63
Children (0–4.99 yr)	0.40

To estimate the loss of lysine due to the Maillard reaction, which would be especially significant in flat bread, a lysine stability study was conducted prior to the actual fortification trial. Arabic bread with unfortified flour and with fortified flour (3 g of lysine per kilogram) was tested for lysine levels, and on the basis of the results it was estimated that there was an overall 12% loss in lysine HCl in baking. The planned original level of 3.75 g of lysine HCl per kilogram of flour or 3 g of lysine per kilogram of flour used in the China and Pakistan studies was increased to 4.2 g of lysine HCl per kilogram of flour or 3.4 g of lysine per kilogram of flour (14% protein) to allow for baking loss.

A socioeconomic questionnaire was administered initially to all households based on the Living Standards Measurement Survey of the World Bank [30]. Almost 40% of the total gross income in the interviewed households was from agricultural wage labor.

Intervention phase

The intervention phase began with a blood sample from each participant followed by collection of anthropometric and health data. Households were then asked to provide wheat to be ground at the village mill set up with a chrome V-shaped blender that is used to mix flour with small aliquots of premix of lysine HCl. The blender is filled with flour and the premix (lysine or placebo), and run for 10 minutes. This ensures adequate mixing of the wheat flour with premix**.** Trained staff were designated to perform the task of fortifying the flour with either lysine or placebo premix. The flour was returned to the households on the same day. Any extra flour present in the household before baseline was also collected and mixed with the appropriate quantities of premix at the beginning of fortification. The teams monitored morbidity and compliance throughout the 16 weeks. The flour team dispensed fortified flour as needed by the household.

Biochemical analyses

Ten milliliters of venous blood was collected from adult men, adult women, and children and analyzed for total CD3 T lymphocytes as well as T lymphocytes bearing the receptors CD4, CD8, and CD56, IgM, IgG, IgA, complement C3, C-reactive protein, serum albumin, prealbumin, transferrin, retinol-binding protein, hemoglobin, and hepatitis B surface antigen (HBsAg). Serum albumin was measured by a colorimetric technique using bromcresol green (Technicon RA-XT with

the use of Albumin-KIT). Cortisol and HBsAg were measured by automated chemiluminescent immunoassays (IMMULITE 1000 Automated Immunoassay Analyzer, Diagnostic Products Corporation) using an IMMULITE Cortisol KIT and an IMMULITE HBsAg KIT, respectively. IgG, IgA, IgM, complement C3, prealbumin, transferrin, retinol-binding protein, and C-reactive protein were measured by nephelometry using the Behring Nephelometer (100 Analyzer) with N antisera to human immunoglobulins IgG, IgA, and IgM and N antisera for human complement C3, prealbumin, transferrin, retinol-binding protein, and C-reactive protein, respectively. Hemoglobin, hematocrit, and lymphocyte counts were measured with the use of a blood counter (SF300 SYSMEX). CD3, CD4, CD8, and CD56 cell counts were conducted by Flow Cytometry (Becton Dickinson, FACS Sort) using monoclonal antibodies B-F5, human CD4 FITC/MCD8, human CD8 R-PE/B-B11, human CD3 Cy-Q and c5.9, and human CD56 PE (IQ Products).

Anthropometric data

Height and weight were measured on men, women, and children at baseline and after 16 weeks of fortification with the use of a Shorr Height Board and a SECA digital scale (accuracy, 0.05 g). Data were entered in EpiInfo 2000, and height-for-age, weight-for-age, and weight-for-height z-scores were calculated. The z-scores were then compared with National Center for Health Statistics/World Health Organization (NCHS/WHO) 1978 reference standards. The BMI was calculated for all subjects.

Baseline and monitoring health data

Detailed health data were collected and physical examinations were performed at baseline, and morbidity was monitored throughout the 16-week period at intervals of 2 weeks. Data were entered in Excel and SPSS and merged to calculate the total number of episodes, period prevalence (total number of diarrheal or respiratory episodes during the period of intervention divided by the total number of observations), the mean number of episodes, the number of episodes per person per year, and the mean number of days of illness for both diarrhea and respiratory infections.

Distribution of premix and monitoring of flour consumption

Samples of flour with premixes A and B and samples of the premixes themselves were sent to Ajinomoto, Japan, for determination of lysine content and checking the adequacy of the mixing procedure. Consumption of the flour and acceptability of its flavor and taste were monitored every 2 weeks.

Statistical analysis

Statistical analyses, including paired- and independent-sample *t*-tests, univariate analysis of variance (ANOVA), cross tabulations, chi-square tests, and Pearson chi-square tests, were performed with SPSS statistical software.

Results

Baseline phase

Dietary assessment

The mean nutrient availability per adult equivalent (consumption unit) was 2,650 ± 806 kcal for energy and 70.1± 26.4g for total protein, with 65 ±14% cereal protein and 41.9 ± 0.8 mg lysine per gram of protein (**table 3** and **fig. 1**). It was estimated that the addition of 4.2 g of lysine HCl per kilogram of wheat flour provided about 23.7 mg of lysine per gram of wheat protein (20.8 mg per gram of wheat protein after baking losses). When added to the available lysine in the flour, this increased the total average lysine intake to 51.1 mg per gram of protein (49.7 mg per gram of protein after losses). This is above the estimated 45 mg per gram of protein allowance proposed by FAO/UNU/WHO [29].

Intervention phase (comparison of baseline with postintervention)

In evaluating changes in immunologic and other hematologic variables, consideration must be given to the possible effect of inflammation. In our study, C-reactive protein values were used to identify persons

TABLE 3. Mean availability of energy, protein, and essential amino acids in 98 households of Khannaser Valley

Nutrient (n = 98)	Mean ± SD
Energy (kcal)	2,651 ± 806
Protein (g)	70.1 ± 26.4
Lysine (mg/g protein)	41.9 ± 8.2
Tryptophan (mg)	840.6 ± 360.3
Threonine (mg)	2,245.8 ± 856.3
Isoleucine (mg)	2,810.9 ± 1,070.1
Leucine (mg)	5,147.3 ± 1,972.8
Lysine (mg)	2,917.0 ± 1,189.7
Methionine (mg)	1,221.4 ± 477.6
Cysteine (mg)	1,077.2 ± 516.4
Phenylalanine (mg)	3,456.8 ± 1,353.6
Tyrosine (mg)	2,317.4 ± 893.3
Valine (mg)	3,517.7 ± 1,334.2
Arginine (mg)	3,329.8 ± 1,276.3
Histidine (mg)	1,673.2 ± 647.8

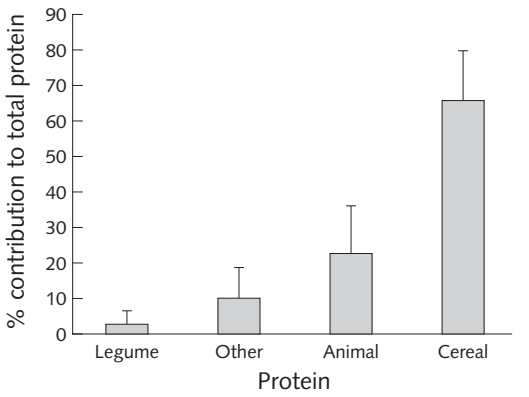

FIG. 1. Sources of dietary protein for 98 households interviewed in phase 1

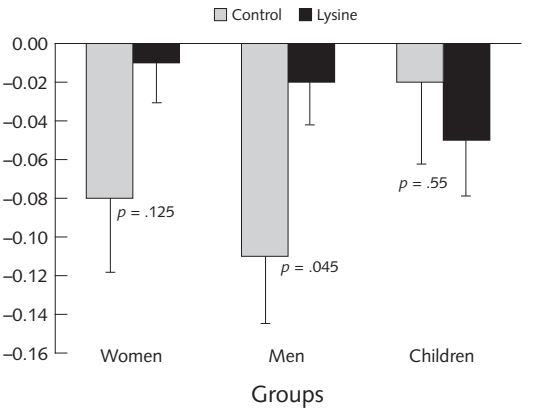

FIG. 2. Delta complement C3 values in men, women, and children with normal C-reactive protein levels after 12 weeks of fortification

with active inflammation. All those with C-reactive protein levels greater than 10 mg/dL at the time blood samples were drawn were considered to be in a state of active inflammation [31]. Of the entire sample (including pregnant women), 19 persons had an active inflammation at baseline, as defined by their C-reactive protein levels. However, by chance, only five were in the lysine group. After fortification, only seven persons in the control group and three in the lysine group had elevated C-reactive protein levels. Thus, we could not find any evidence for an effect of lysine fortification on inflammation, as judged by this criterion. Nevertheless, our final analyses excluded persons with elevated C-reactive protein levels at either the beginning or the end of the study. Including them would not have altered our conclusions.

Complement C3 and selection of C-reactive protein

Complement C3 values after 12 weeks of fortification were significantly higher in lysine-fortified men (p = 0.04) with normal C reactive protein (n = 20 control, 27 lysine) than in men of the control group. While both women and children in the lysine-fortified group had higher values, differences between fortified and control groups were not statistically significant (**fig. 2**).

Morbidity

In general, morbidity rates were low. Unfortunately, we were not able to include preschool children, who have much higher rates of diarrheal disease, in the study. Total morbidity from diarrheal and respiratory disease in men and school-age children was about half that in women. There were no significant differences between the control and lysine groups in the total number of days ill for men, women, or children. Among women, the total number of diarrheal episodes was significantly higher in the control than in the lysine group (20 vs. 6, p = .014). The mean number of days ill with diarrhea over 16 weeks was 1.22 for women in the control group and only 0.43 for women in the lysine group. The mean number of diarrheal episodes per person was 0.4 in the control group and 0.14 in the lysine group (p = .03, independent-sample t-test).

There were no differences in the number of days ill with acute respiratory infections between the lysine and control groups in men, women, or children (**tables 4** and **5**).

Other results

The results were also analyzed to examine differences in hematologic and immunologic variables for persons with no active inflammation either at baseline or postfortification. Women who were pregnant at baseline, during monitoring, or postfortification were excluded (**table 1**). No significant differences were observed between groups in delta values of most of the variables. In the case of transferrin, despite a high delta change value (–0.18) between the control and lysine groups, the differences were not significant (p = .105) (data not shown). No differences between fortified and control groups were observed in anthropometric variables in children (data not shown). **Table 6** depicts the differences in BMI between adults in the lysine fortified versus control groups. Changes in BMI from baseline to post fortification were not significant between the fortified and control group.

Discussion

Lysine is the first limiting essential amino acid in diets with wheat as the major source of protein. Current recommendations indicate a lysine requirement of 45 mg per gram of protein [29]. Animal foods contain on average 85 mg of lysine per gram of protein. Legume

TABLE 4. Diarrheal morbidity in women, men, and children during 15 weeks of lysine fortification

	Women		Men		Children	
Variable	Control	Lysine	Control	Lysine	Control	Lysine
No. of subjects	50	44	31	38	36	31
No. of episodes in 15 weeks	20[a]	6[a]	7	6	1	5
Period prevalence	5.1	1.7	3.0	2.1	0.4	2.0
Episodes per person per year[b]	1.39	0.47	0.8	0.5	0.1	0.6
Total days ill from diarrhea	61	19	18	19	2	20
Mean ± SD no. of episodes per person	0.4 ± 0.7[c]	0.14 ± 0.4[c]	0.23 ± 0.6	0.16 ± 0.4	0.03 ± 0.17	0.15 ± 0.5
Mean ± SD no. of days ill per person	1.2 ± 2.7	0.4 ± 1.3	0.58 ± 1.5	0.5 ± 1.7	0.06 ± 0.3	0.6 ± 2.1

a. $p = .014$, chi-square test.
b. Extrapolated from a 15-week period prevalence.
c. $p = .03$, independent-sample t-test.

TABLE 5. Morbidity from acute respiratory infection in women, men, and children during 15 weeks of lysine fortification

	Women		Men		Children	
Variable	Control	Lysine	Control	Lysine	Control	Lysine
No. of subjects	50	44	31	38	36	31
No. of episodes in 15 weeks	73	81	28	26	29	24
Period prevalence	18.6	23.5	11.8	9.1	10.2	9.6
Episodes per person per year[a]	5.1	6.4	3.1	2.4	2.8	2.7
Total days ill from acute respiratory infection	320	326	108	112	101	89
Mean ± SD no. of episodes per person	1.5 ± 1.3	1.8 ± 1.7	0.9 ± 1.27	0.7 ± 0.9	0.06 ± 0.9	0.7 ± 1.3
Mean ± SD no. of days ill per person	6.4 ± 6.4	7.4 ± 7.8	3.5 ± 4.6	2.9 ± 4.0	0.8 ± 0.9	0.7 ± 1.3

a. Extrapolated from a 15-week period prevalence.

TABLE 6. Mean ± SD body mass index before and after intervention for men and women in the control and lysine groups

	Control (n = 35 women, 31 men)[a]			Lysine (n = 34 women, 27 men)[b]			Difference between change in control and lysine groups	
Sex	Before	After	Change	Before	After	Change		p
Female	28.7 ± 6.0	28.1 ± 6.2	–0.6 ± 1.4	29.1 ± 7.0	27.9 ± 7.0	–0.8 ± 1.3	0.2	0.5
Male	25.4 ± 4.4	25.5 ± 5.3	–0.4 ± 1.0	25.2 ± 4.2	25.4 ± 5.4	–0.7 ± 1.0	0.3	0.3

a. Control men: n = 24 postfortification.
b. Lysine men: n = 28 postfortification.

proteins are also good sources of lysine, with an average of about 65 mg per gram of protein, but cereals are a poor source of lysine, containing an average of 30 mg per gram of protein [4, 32].

Significant improvements in protein and immunologic status were observed with lysine fortification of wheat flour in Pakistan and China [5, 6]. Our study was similar in design and time period to the Pakistan and China studies, and the results should be comparable. In our study, the total number of diarrheal episodes and the mean number of days ill were significantly decreased among women in the lysine-fortified group. Among men and children, baseline diarrheal disease rates at the onset of fortification were lower than in women; this difference may help account for the fact that an effect of lysine fortification was not apparent for men and children.

The efficacy of lysine-fortified flour in women can be attributed to an improvement in protein utilization but possibly also to a direct effect of lysine in the gastrointestinal tract. It is known that dietary components such as fiber [33] can influence gut motor functions and that amino acid solutions given orally inhibit gastrocolonic and small intestinal transit [34, 35]. Recent animal studies have shown that lysine deficiency per se can strongly increase the incidence of diarrhea [36] and that lysine administration alone blocks gastric emptying and stress-induced diarrhea through a direct intestinal action on serotonin receptor 4 [37]. These results indicate the possibility of functional changes (e.g., serotonergic changes) in the gut of persons ingesting lysine-deficient diets, which may be independent of gut pathologies induced by poor hygiene and malnutrition. Our sample of women included 24 who

were pregnant (at baseline and throughout the study) and at least 37 who were lactating (also at baseline and throughout the study), who had different protein and energy needs. Nevertheless, their lysine requirement expressed as milligrams per gram of protein remains the same. However, if they are not meeting their energy and protein needs, they could have an additional benefit from the added lysine.

As a response to infection, complement C3 levels increase in well-nourished persons but remain the same or fall in undernourished persons. For example, in a study in rural India, well-nourished children had increases in complement C3 proportional to the number of days of fever, whereas poorly nourished children had a decrease [38]. In the present study, men in the control group had a greater decrease in complement C3 levels after fortification than did the men in the lysine-fortified group. Protein availability apparently was adequate to maintain complement C3 levels only in the lysine-fortified group. Although the men had little diarrhea, the total prevalence of disease was apparently sufficient to cause a fall in the complement C3 levels in the control group. Among children, either the burden of infection was not sufficient to produce a significant effect on complement C3 levels or the degree of protein deficiency was too marginal to limit complement C3 synthesis. There were no other significant differences in laboratory findings between the lysine and control groups. An apparent difference in the number of CD8 cells between women in the control and lysine groups disappeared after correction for elevated C-reactive protein.

An issue to be addressed is the dietary assessment of energy and protein intakes. Because of the common platter system of food consumption, it was impossible to estimate individual food intake. The dietary data were collected on the basis of total household intake and estimated per capita in proportion to estimated individual caloric requirements. Despite this limitation, the diets appeared to be at least as deficient as those in the China and Pakistan studies [5, 6].

Respiratory diseases are highly seasonal. The study began in a period of high prevalence of respiratory disease and ended in a period of relatively low prevalence. This may be the reason that no effect of lysine fortification on acute respiratory disease could be detected. The Aleppo area is dry, and diarrheal rates are low compared with those in South Asia. Diarrheal prevalence was highest in women in this study. The observation of a significant decrease in diarrhea among women receiving the lysine-supplemented flour is of potential health significance. This is also true for the improved ability of the men receiving lysine-fortified flour to maintain adequate complement C3 levels.

Studies in populations with higher diarrheal prevalence and significant dietary lysine deficiency are needed to determine whether the reported effects on diarrheal prevalence are replicable and whether they are pharmacological or nutritional. It would be particularly desirable to study the effect of lysine on diarrhea in preschool children, who have much higher morbidity and mortality rates from this disease than school-age children or adults. A study during a season with a high rate of respiratory disease would also be desirable.

Acknowledgments

Ajinomoto (Tokyo, Japan) provided financial support for this study.

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