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FOOD AND NUTRITION BULLETIN

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Pan American Health Organization Regional Consultation of the Americas on Diet, Physical Activity and Health —*Enrique R. Jacoby, guest editor*

- A call to action
- Improving food and nutrition in Latin America
- Promoting physical activity in the Americas
- Public health framework for chronic disease prevention and control
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Submissions. Unsolicited manuscripts of articles of the type published in this and previous issues may be sent to the Managing Editor at the address given above electronically or by mail. For mail submissions, manuscripts must be typed, double-spaced, with complete references, original copy for any figures used, and should include the file on a floppy disk. **We strongly encourage electronic submissions.** Figures and tables may also be submitted electronically. (Please refer to the Note for Contributors in the back of this issue.)

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Also of interest are "Short Communications," defined as papers that explore a topic with the customary scientific rigor but that do not need extensive analysis and discussion of the data or that have useful information that is not sufficient for a full article. These would be identified as such at the beginning of each article.

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If two reviewers agree that the paper should be published in the *Bulletin*, it is accepted and either sent immediately for copy-editing or returned to the authors for consideration of suggestions from the reviewers and the editor. If both reviewers agree that the paper should not be accepted, the authors will receive a letter from the editor explaining the reason and enclosing the comments of the reviewers anonymously. If the reviewers do not agree with each other, either the paper is sent to a third reviewer or a decision is made by the editor. In these cases, the authors are usually given a chance to respond to the reviewers' comments.

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Lysine fortification: Past, present, and future

Peter L. Pellett and Shibani Ghosh

Introduction

The papers by Hussain et al. [1] and Zhao et al. [2] are further episodes in the long-running saga on whether lysine fortification in free-living, cereal-dependent populations can produce significant nutritional and health benefits. These studies were performed in Pakistan and China, in 1996–97 and 1999, respectively. Over the years, many investigations involving lysine have taken place with human subjects, but despite the care taken in experimental design, the study outcomes in free-living populations have remained equivocal, and the practice of lysine fortification of human dietaries has not been implemented other than under experimental conditions. In contrast, in developed economies, lysine fortification of cereal-based animal feeds is widespread for the purpose of producing more rapid and greater weight gain and hence improved profitability.

In this paper, we will discuss the assessment of lysine status from food balance sheet data, previous human fortification studies, lysine in animal feeds, and some thoughts for the future of lysine fortification.

Nutritional background

Staple food availability at the national, regional, and household levels is a cornerstone of nutritional well-being. Aggregate estimates of food availability at global, regional, or country levels, while often indicative, cannot truly reflect household or individual food consumption. Factors affecting the latter include the abilities of households to produce or procure food, which themselves are functions of income levels and distribution, food availability and wastage, and prices and consumer choices.

The majority of the deprived and undernourished population in the world subsists on diets heavily based on cereals. Such diets are likely to be low in a number of micronutrients, including the amino acid lysine. When comparisons have been made between food availability data for various countries, it has been demonstrated that as wealth (gross national product or GNP) decreases, not only is food energy availability reduced, but there are also major changes in the pattern of foods selected. In particular, there are significant decreases in the availability of animal protein foods and increases in the dependency on cereals [3–8]. Further analysis also indicates that, of the essential amino acids, lysine is the amino acid for which the largest differences occur between the diets of the rich and the poor. It was also observed that the amino acid compositions of animal, pulse, and cereal proteins are sufficiently distinctive from each other to enable food-group data to be used for simple predictions of the lysine value of diets [8]. From examination of standard tables of amino acid composition [9], it was demonstrated that the lysine content of most cereals ranges from 26 to 38 mg per gram of protein, whereas the lysine content of animal foods is much higher, ranging from 70 to 100 mg per gram of protein [6, 8]. These relationships thus permit the estimation of lysine value from considerations of the amounts of animal protein and cereal protein present.

Thus, the socioeconomic status of both households and populations strongly influences the quality of available dietary protein. The effect of wealth status on the diet is illustrated in table 1 [8]. Although these data are for 1992–93, the relationships remain relevant. An increase in socioeconomic/wealth status (signified by per capita GNP) corresponds to an increase in the availability of total food energy, total protein, and animal protein. Between those with GNPs of less than

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TABLE 1. Total population, nutrient availability, mortality rate, and life expectancy for world economic classes (data from 122 countries)

GNP (US$/ person/yr)	No. of countries	Population (millions)	Food energy (kcal/day)	Total protein (g/day)	Animal protein (%)	Cereal protein (%)	Pulse-soy protein (%)	Mortality, children < 5 yr (/1000)	Life expectancy at birth (yr)
< 500	37	2,990	2,070	51	20	53	11.4	171	52
500–2,000	41	862	2,570	65	31	50	7.0	76	64
2,000–10,000	21	548	2,913	78	45	39	5.6	39	69
> 10,000	23	806	3,335	101	61	24	2.7	9	77

GNP, Gross national product. Source: ref. 8. Data for 1992–93.

$500 per capita and those with GNPs greater than $10,000 per capita, food energy availability increased by some 50% and total protein availability doubled, while the percentage of animal protein increased threefold. Although not shown in table 1, lysine would be expected to increase along with the increase in animal protein. For the same comparisons (under $500 to above $10,000 per capita) the mortality rate among children under five years of age declined from 171 to 9 per thousand live births.

Essential amino acid requirements and supply

Human lysine requirement remains the subject of current collaborative international research, and future agreement on the actual value is essential for the assessment, on a global basis, of those at risk for deficiency, as well as for the assessment of whether diets meet essential amino acid needs. Thus, reference standards are needed for comparison. This remains true whether values are expressed in absolute terms of milligrams per day or in quality terms of milligrams per gram of protein. The earlier 1991 requirement [10] of lysine for the adult was 58 mg per gram of protein, which translates to 2,840 mg per day for a young 65-kg adult male whose protein requirement is 49 g per day. Alternative recommendations, based on stable isotope studies, have been proposed [11], in which the lysine requirement for the adult has been reduced to 50 mg per gram of protein or 2,450 mg per day for the same young adult male.

From recent international deliberations, the value for the adult is likely to be reduced even further to 45 mg per gram of protein by the Food and Agriculture Organization/World Health Organization (FAO/WHO) expert group concerned with protein and amino acid requirements whose report is awaiting publication [12]. The new lower adult values will be used in the present assessment. In practice, however, population requirements are higher, because the needs of babies, children, and pregnant and lactating women are all greater than those of the adult male, with the requirement of the young baby being as high as 69 mg per gram of protein [12]. The requirements for the other essential amino acids have not been changed to any significant degree, and thus lysine remains the first limiting amino acid in many world dietaries. An approximate rule of thumb is that a population average at or below the adult requirement value of 45 mg per gram of protein may be indicative that a significant proportion of the population is at risk for lysine deficiency.

Although risk assessment can only be made with accuracy by using estimates based on individual variability of intake, the best estimates of those at risk must continue to be based on population average values, such as those derived from food balance sheets. These are the only readily available data that can be used to make population-wide estimates of nutritional availability. Provided that the limitations of such procedures are recognized, useful conclusions can be drawn.

Typically, food balance sheet data for each year and for each country or region consist of 100 to 150 food items. Thus, even with computer assistance, data entry and subsequent calculations are time-consuming. It has, however, been observed [6, 8] that, in practice for countries and regions, a much smaller number of food items (some 20 to 30) provide the majority of the protein, allowing for a simplified calculation procedure. These dietary components include the major cereals, such as wheat, rice, corn, millet, and sorghum; the starchy roots cassava, sweet potato, and potato; the pulses soybean, groundnut, and common beans; and the animal food products, primarily meat, milk, fish, and eggs. The estimation of lysine value from food balance sheet data has been further simplified by Pellett [8], who developed prediction equations from multiple regression analysis. The best prediction (R^2 = .98) involved the three groups of major protein sources:

$$\text{Lysine (mg/day)} = 86.3\text{AP} + 19.8\text{CP} + 63.6\text{PSP} + 599$$

where AP, CP, and PSP were animal, cereal and pulse (including soy) protein, respectively, in grams per day. Cereal protein (grams per day) by itself is a nonsignificant predictor of daily lysine availability. This is because in developing-country diets, although cereal protein may constitute a high proportion of the total protein, the amount of cereal protein in absolute terms (grams per day) is often similar to that in developed countries. Because of the high degree of predictability

(R^2 = .98) of dietary lysine from animal, cereal, and legume protein contents, the prediction equation above was used to calculate lysine availability (milligrams per day) from food balance sheet data. Lysine (milligrams per gram of protein) was subsequently calculated from daily lysine by dividing by total protein per day.

Some calculations showing estimated lysine values, using the procedures described above, are given in table 2 for selected FAO regional and economic groupings of countries using FAO on-line food balance sheets for 2001 [13]. A very significant difference between nutrient availabilities in developed and developing regions can be seen. Developed regions, as a group, had average availabilities of 3,285 kcal and 99.4 g of protein per day, with 56.1% of the protein being of animal origin. In contrast, the developing countries had a lower average availability of food energy of 2,675 kcal, together with 69.6 g of protein per day. Only 29.5% of this protein was of animal origin. When lysine values were estimated, only 3,454 mg per day were available, on average, in the developing countries, compared with 6,167 mg per day for the developed regions. A comparison between the North America Developed and the Sub-Saharan Africa Regions shows even more extreme differences in availabilities of 7,419 and 2,466 mg/day, respectively. In terms of milligrams per gram of protein, the values are 65.5 for North America and 44.9 for Africa. With the probable requirement value for the adult at 45 mg per gram of protein, it could thus be anticipated that a significant proportion of those in Africa might be at risk of lysine deficiency.

Further examples from selected individual countries are shown in table 3. According to the same criteria, Syria, Bangladesh, Nigeria, Egypt, Morocco, and Côte d'Ivoire would all be at risk, with the latter two countries having lysine values below 40 mg per gram of protein. The contrast with Japan and the United States, which have lysine values above 65 mg per gram of protein, is obvious and results primarily from the far greater availability of animal protein in the latter countries. Animal protein supplies some 63% of the total protein in the United States, but the value is less than 20% in a number of the poorer countries, with Bangladesh, for example, having only 12.8% of the total protein from animal foods.

Strategies for intervention

Strategies and activities to alleviate micronutrient deficiencies must include several approaches. The first involves improving dietary diversity by stimulating the production and consumption of micronutrient-rich foods. A complementary approach is the direct fortification of cereals with micronutrients, including synthetic amino acids. The former option of increasing the availability of animal foods and legumes is highly constrained in most societies by social and economic factors. Fortification of basic foods is, nevertheless, a short-term intervention that has proven to be very effective (e.g., iodization of salt). Fortification is especially applicable to cereals used in a milled form, but difficulties arise where there are multiple small-scale producers. Where large centralized milling facilities exist, it is more straightforward. Additional requirements for successful fortification are the adoption and enforcement of appropriate legislation as well as the convincing of both consumers and professionals that such plans are to their benefit.

TABLE 2. Food energy, protein (total, animal, cereal, and pulse-soy), and estimated lysine values for selected groups of countries

Region	Population (millions)	Food energy (kcal/day)	Total protein (g/day)	Animal protein (%)	Cereal protein (%)	Pulse-soy protein (%)	Lysine (mg/day)	Lysine (mg/g protein)
North America developed	316.9	3,708	113.2	62.9	22.2	2.5	7,419	65.5
European Union	377.2	3,539	108.2	60.3	24.9	2.3	6,917	63.9
South America	350.6	2,854	75.8	49.9	29.8	9.8	4,779	63.1
Developed countries	1,317.6	3,285	99.4	56.1	29.2	2.8	6,167	62.0
World	6,110.4	2,807	76.0	37.0	42.5	7.8	4,039	53.1
Asia	3,706.6	2,701	71.3	29.9	48.0	9.5	3,547	49.7
Developing countries	4,792.6	2,675	69.6	29.5	47.8	9.6	3,454	49.6
Sub-Saharan Africa	620.4	2,229	53.9	19.5	49.5	12.6	2,466	45.8
Africa	809.5	2,444	61.5	21.0	53.3	10.2	2,762	44.9

Source: Food and Agriculture Organization/Faostat (2004) [13]. Data for 2001.

Lysine values in milligrams per day were calculated from the equation: lysine = (86.3 × animal protein g/day) + (19.8 × cereal protein g/day) + (63.6 × pulse-soy protein g/day) + 599 [8]. Lysine values in milligrams per gram of protein were derived by further dividing by the amount of total protein.

TABLE 3. Food energy, protein (total, animal, cereal, and pulse-soy), and estimated lysine values for selected countries

Country	Population (millions)	Food energy (kcal/day)	Total protein (g/day)	Animal protein (%)	Cereal protein (%)	Pulse-soy protein (%)	Lysine (mg/day)	Lysine (mg/g protein)
Japan	127.3	2,746	90.3	55.5	23.8	10.7	5,965	66.1
USA	285.9	3,766	114.5	63.3	22.1	2.1	7,509	65.8
Thailand	63.6	2,486	55.5	41.1	41.8	7.0	3,274	59.0
Pakistan	144.9	2,457	62.5	37.4	50.9	5.9	3,483	55.7
China	1,262.6	2,961	85.8	35.0	38.9	9.6	4,371	50.9
South Africa	43.7	2,961	85.8	35.0	38.9	9.6	4,371	50.9
Syria	16.6	3,038	74.6	25.7	57.1	4.7	3,322	44.5
Bangladesh	140.4	2,187	46.8	12.8	75.9	6.2	2,004	42.8
Nigeria	116.9	2,747	61.8	13.8	51.3	13.8	2,501	40.5
Egypt	69.1	3,385	96.5	20.4	61.2	7.0	3,902	40.4
Morocco	30.4	3,046	83.4	18.7	68.1	4.9	3,331	39.9
Côte d'Ivoire	16.3	2,594	51.2	17.8	49.2	1.0	1,915	37.4

Source: Food and Agriculture Organization/Faostat (2004) [13]. Data for 2001.

Lysine values in milligrams per day were calculated from the equation: lysine = (86.3 × animal protein g/day) + (19.8 × cereal protein g/day) + (63.6 × pulse-soy protein g/day) + 599 [8]. Lysine values in milligrams per gram of protein were derived by further dividing by the amount of total protein.

Human studies of lysine fortification

In some studies using the 1973 Food and Agriculture Organization/World Health Organization [14] scoring pattern, it was observed that the content of sulfur-containing amino acids and tryptophan in cereal proteins was substantially higher than requirement values, but that lysine was limiting. Despite this lack, however, it was possible to achieve useful amounts of protein intake from cereals if they were consumed in sufficient quantities. However, it was later shown [15] that the consumption of sufficient amounts of cereal protein to meet protein and lysine requirements was difficult for infants and preschool children because of the large volume of food required. Graham and his associates [16] demonstrated in 1969 that a one-year-old infant with a lysine requirement of 90 mg per kilogram of body weight per day and a food energy requirement of 90 kcal per kilogram of body weight per day, consuming only wheat flour (11% protein, 2.5 g lysine/100 g protein), would have to eat 327 g of wheat flour daily to meet his or her lysine requirement. Not only would this volume of food be impossible to ingest, but such an intake would lead to excessive food energy intake, with low levels of micronutrients.

Studies conducted in the 1950s and 1960s on children recovering from protein–energy malnutrition demonstrated that lysine was important in improving nitrogen retention when wheat or corn was the staple food. Scrimshaw and his associates [17] examined the effects of amino acid imbalances and the addition of simple amino acids on the nitrogen retention of children consuming a corn masa diet. Despite the limited number of trials and their short durations, it was found that the children were sensitive to small changes in the amino acid content of their diets. When lysine and tryptophan (the two limiting amino acids) were added to corn masa in the diet (3 g protein per kilogram of body weight per day and 100 kcal per kilogram of body weight per day), so as to approximate the then recommended FAO reference pattern, nitrogen retention was markedly improved in both cases. Working at the Institute of Nutrition of Central America and Panama (INCAP), Bressani et al. [18] observed similar results using 2.0 and 1.5 g protein per kilogram of body weight per day and a corn masa basal diet. They then conducted a study using wheat as the source of plant protein [19]. It was noted again that in the utilization of wheat protein, lysine was the most limiting amino acid. Addition of lysine to the basal diet resulted in sustained nitrogen retention similar to that obtained with milk feeding, and when the basal diet was supplemented with all of the limiting amino acids, the retention matched that of the FAO reference pattern. Further confirmation was later reported by the same group [20]. Similar studies were conducted using lime-treated corn and rolled oats as basal diets [21, 22]. The corn basal diet was improved by the addition of lysine and tryptophan, whereas the rolled oats basal diet had less marked amino acid deficiencies than the wheat or corn diet.

In a study of adults under hospital conditions in 1949, Hoffman and McNeil [23] found that patients given a gluten preparation with 4% lysine had a significantly higher nitrogen balance index than those given a gluten preparation without lysine. Bricker and associates [24] had even earlier shown increased nitrogen retention when a diet based on white bread was supplemented with lysine.

Population studies

Based on the findings and recommendations of the 1969 Conference on the Amino Acid Fortification of Protein Foods held at MIT in Cambridge, Massachusetts, USA [25], three population-based studies were designed in the early 1970s. These large-scale village trials were subsequently conducted in Thailand (1971–75), Tunisia (1970–75), and Guatemala (1972–76). In Thailand, rice in the diets of preschool children was fortified with lysine, threonine, thiamine, riboflavin, vitamin A, and iron. In Tunisia, wheat was fortified with lysine, iron, and vitamins in a malnourished population in the southern part of the country. In Guatemala, corn (maize) was fortified with lysine, vitamins, and minerals. All three studies involved physical examination, anthropometry, collection of blood samples for hemoglobin and hematocrit, and collection of morbidity and mortality information. None of the studies reported any significant beneficial health effects. Some years after the completion of the studies, a task force was formed [26], with one of its objectives being to examine each of the studies (design, approach, analysis and interpretation of data, project management, and budget costs). For all three studies, examination revealed serious flaws in design, methods, or analysis.

As a result of the recognition of these failures, together with a reduction in international interest in protein deficiency and protein requirements, no further lysine-fortification trials took place until the currently reported studies from Pakistan and China [1, 2]. No attempt will be made to outline these studies, since they can be reviewed in the abstracts. Nonetheless, the results of both indicate that lysine fortification of wheat flour may significantly improve sensitive indicators of nutritional status in populations consuming diets in which 57% to 67% of the protein originates from wheat. It should be noted that at the time the studies were conducted, the average dietary lysine values from food balance sheet data were lower than the relatively acceptable values from 2001 shown in table 3. Furthermore, in the region of China where the study took place, near Huixian City in Henan Province, wheat was the major staple rather than rice.

As has been indicated earlier, the addition of a synthetic form of lysine to improve protein quality is not a new concept, and fortification with synthetic amino acids is a procedure that is widely used for animal feeds in the developed regions of the world. Production and use of l-lysine in animal feed started in Japan in the 1960s. l-Lysine HCl is especially important in pig feed, as it is the first limiting amino acid for growing pigs. The rationale for the addition of crystalline amino acids is that it is a more efficient form of converting feed proteins to meat, as it reduces the costs of additional protein sources [27]. It may also lead to an improved efficiency of utilization of limited arable land. Furthermore, nitrogen excretion due to animal farming can be a considerable threat to human health because of pollution of soil and water by ammonia or nitrates and nitrites. By supplementing the diet with amino acids, one can actually decrease the overall protein level in feeds, thereby reducing the risk of nitrogen pollution. Worldwide production of lysine for feed is considerable, and it has been estimated that 500,000 to 600,000 metric tons of l-lysine HCl was produced in the year 2000 [27].

Summary and conclusions

Fortification with lysine to improve the protein value of human diets that are heavily based on cereals has received support from the results of these recent studies [1, 2]. Support also comes from examination of average food and nutrient availability data derived from food balance sheets. Whereas nutritional status is influenced by the nutrient content of foods consumed in relation to need, the requirements for protein and amino acids are influenced by many additional factors [10, 12, 14, 28, 29]. These include age, sex, body size, physical activity, growth, pregnancy and lactation, infection, and the efficiency of nutrient utilization. Even if the immune response was influenced by the added lysine, adequate water and basic sanitation would remain essential.

Acute and chronic undernutrition and most micronutrient deficiencies primarily affect poor and deprived people who do not have access to food of adequate nutritional value, live in unsanitary environments without access to clean water and basic services, and lack access to appropriate education and information [30]. A further variable is the possible interaction between protein and food energy availability [31]. This could affect the protein value of diets when food energy is limiting to a significant degree. Thus, the additional effects of food energy deficiency on protein utilization could well be superimposed on the very poorest.

The improvement of dietary diversity must be the long-term aim, with dietary fortification considered only a short-term solution. The former should take place as wealth improves and the gaps between rich and poor diminish. Although such changes are taking place, they are highly uneven. Over the last several decades, increases have occurred in the availability of food energy, total protein, and animal protein for both developed and developing countries. However, for the very poorest developing countries over the same period, changes have been almost nonexistent, and the values for some nutritional indicators have even declined. For estimated lysine value, the developed countries showed increases in per capita availability from 5,400 to 6,167 mg per day and the developing countries from 2,400 to 3,454 mg per day, while in contrast, the very poorest

countries remained static at about 2,400 to 2,500 mg per day.

Thus, although lysine fortification may be theoretically only a short-term solution, in the very poorest countries changes in wealth such that dietary diversity and lysine availability may increase by natural progression remain remote. If we can justify using lysine to fortify animal feed in the rich regions of the world for economic gain, perhaps we should now consider adding lysine to the flour consumed by the deprived people in the poorest regions of the world to improve both their nutrition and their resistance to disease.

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Lysine fortification of wheat flour improves selected indices of the nutritional status of predominantly cereal-eating families in Pakistan

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Abstract

Wheat provides more than 50% of the protein and calorie intake of the population of Pakistan. Legumes and animal protein that could complement the amino acid pattern of wheat, in which lysine is the first limiting amino acid for utilization of protein, are not affordable by members of lower socioeconomic groups in developing countries. The purpose of the study was to determine whether lysine fortification of wheat flour would have a positive impact on populations consuming a predominantly wheat-based diet. A double-blind study was carried out for three months on the outskirts of Peshawar, Pakistan. Forty families received wheat flour fortified with lysine, and 40 families received wheat flour without lysine. Wheat provided 59% of the protein for men, 65% for women, and 58% for children. The weight and height of the children in both groups increased during the study, but the increase was significantly greater in the lysine group. Hemoglobin increased significantly in the women receiving lysine-fortified flour. Transferrin levels increased significantly in men, women, and children in the lysine group as compared with those in the control group. Prealbumin increased significantly in adults receiving additional lysine but decreased in children. Men, women, and children in the lysine-supplemented families had significant increases in CD4, CD8, and complement C3 as compared with controls. These results indicate that lysine fortification of wheat flour can significantly improve sensitive indicators of nutritional status in a population consuming a diet in which 58% to 65% of the protein, depending on age and sex, is supplied by wheat.

Key words: Fortification, immunological status, lysine, nutritional status, Pakistan, wheat flour

Introduction

Mild to moderate protein-energy malnutrition affects 52% of all children two to five years of age in Pakistan, as indicated by low weight-for-age [1]. At the national level, 25% of newborns have birthweights less than 2,500 g, indicating a high prevalence of maternal undernutrition. Approximately 65% of children under five and 55% of women are anemic [2]. The infant mortality rate is 90 per 1,000 live births, and maternal mortality is 500 per 100,000 [2].

The poor of developing countries have traditionally complemented the protein of their predominantly cereal diet with a source of better-quality protein that supplies enough of the limiting amino acid in cereal protein to increase the overall utilization of dietary protein to an acceptable level. In Latin America the source is a combination of maize and beans; in China and East Asia it is rice and soybean; and in South Asia it is most commonly wheat and a variety of legumes, including mung bean, Bengal gram, green gram, and groundnut (peanut). Cereal diets are frequently supplemented by small amounts of fish or other animal protein when these can be afforded.

The main dietary staple of Pakistan is wheat flour, consumed three times daily in the form of a circular flat bread, along with small amounts of legumes and pulses cooked in the form of a curry. Wheat constitutes approximately 80% of the total cereal intake, and it contributes 50% of total energy and 60% of total

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protein intake [2]. Since lysine is the first limiting amino acid for utilization of wheat protein, high-wheat diets must be complemented in some way to improve their amino acid pattern [3]. Either animal or legume protein will do this. Unfortunately, sufficient animal protein is usually not affordable, and legume production has lagged [4] and its price has soared beyond the reach of the most vulnerable groups [5].

The nutritional quality of food protein is determined by how well the content of the specific essential amino acids in the protein meets the requirement for each of these. The amino acid score of a protein is calculated by comparing its amounts of individual essential amino acids with the pattern of the requirement for each per unit of its protein. On the basis of the Food and Agriculture Organization/World Health Organization (FAO/WHO)[6] reference amino acid pattern, wheat has a protein score of 50, with lysine the first limiting amino acid in cereal. Nitrogen balance studies in rats [7] and humans [8] indicate a net protein utilization of about 50% for wheat protein, compared with nearly 100% for meat, milk, and eggs [9].

Improvement in nitrogen retention of wheat protein with lysine supplementation has been repeatedly confirmed in experimental animals [10]. It has also been confirmed by child growth studies [11–14] and nitrogen balance studies in infants [15–17], children [18–20], and adults [21–25]. All of these studies have been performed with "captive" populations (in hospitals, orphanages, schools, and universities). The only major population field trial of lysine supplementation of wheat flour was reported in 1976 from Tunisia [26]. No effect was found, but the study was seriously flawed because the investigators were unable to control either the entry of contraband flour or differences in infectious disease between experimental and control areas. Nevertheless, the failure of this trial and the decreased interest in protein deficiency have discouraged any further studies of lysine fortification of wheat flour for humans since 1976.

As a result of the increasing cost of legumes as well as animal protein to complement the predominantly wheat diets of Pakistan, the protein quality of these diets often fails to meet optimal protein needs. There is a need to re-explore the possible value of lysine fortification of wheat flour distributed to individuals who receive a majority of their dietary protein from wheat. If fortification could be shown to be effective, there are many other developing countries to which the findings would be immediately applicable. Moreover, fortification of wheat flour with iron and folic acid is already being implemented in some of these countries, including Pakistan. Therefore, the cost of adding lysine to the fortification premix would be reduced.

In order to determine whether lysine fortification of wheat flour could benefit populations consuming a diet in which wheat was the major source of protein, a controlled double-blind study was carried out in a poor community near Peshawar, the capital city of the North West Frontier Province (NWFP). The Research Project Appraisal Committee of the Faculty of Nutrition Sciences, NWFP Agricultural University, approved the study, and the field director personally obtained the consent of all of the families after a detailed explanation of the study.

Materials and methods

Sample

The study village of Palosi is situated 2 km from the main campus of the NWFP Agricultural University and about 10 km from Peshawar. It has about 500 households and a population of 3,500 to 4,000 people. Eighty families of low socioeconomic status, consuming wheat-based diets, were identified. Two groups of 40 families were selected randomly from the list of 80 families. The families selected agreed to consume only the flour that was provided without charge during the study period, to provide socioeconomic and dietary data, to furnish weekly morbidity data, and to allow blood samples to be drawn at the beginning and end of the study. The father, mother, and one child between 5 and 10 years old in each family were selected for collection of dietary data, stool samples for detection of parasites, and initial and final blood samples for a number of biochemical and immunological determinations.

Socioeconomic data

Socioeconomic information was gathered during visits to households by direct observation, interviews with mothers and fathers, and a questionnaire.

Food consumption

Before the study started, food-consumption data were obtained from each of the three target individuals in each family by 24-hour dietary recall on two occasions a week apart, and the results were averaged. The mothers provided help in dietary recall for their children. Cooking utensils were measured to improve the accuracy of the food-consumption data. Nutrient intakes were calculated using the food-composition table for Pakistan [27].

Anthropometry

Weight, height, and triceps skinfold thickness of the 240 subjects were measured at the beginning and the end of the three-month experimental period according to World Health Organization guidelines [28]. Of the targeted children, 56% were stunted or

wasted. Contributing causes included poverty, intestinal helminths, poor sanitation, and lack of personal hygiene. No cases of kwashiorkor or marasmus were seen, but fatigue and listlessness among children were common.

Morbidity

All families in the study were visited by a woman physician in their homes every two weeks to record morbidity. The data were compiled during 18 visits over six months from October 8, 1996, to March 30, 1997. The focus was on respiratory and diarrheal infections among children and their mothers.

Provision of fortified and unfortified wheat flour

The wheat flour was purchased from government utility stores. A ribbon-type blender was used to add 120 g of l-lysine monohydrochloride, supplied by Ajinomoto (Tokyo, Japan), to each 20 kg of wheat flour for fortification at a rate of 0.6 g lysine/100 g wheat flour. This was designed to bring the proportion of lysine in the protein of the fortified wheat to the levels of the 1991 FAO/WHO reference protein [6]. Subsequent FAO/WHO/United Nations University (UNU) expert consultations have reached tentative agreement on a new amino acid reference protein pattern based closely on that proposed by Young et al. [29]. The proportion of lysine, 0.45 mg per gram of protein, was the same in both patterns, but the values for several of the other amino acids differed slightly. The bags of fortified and unfortified flour were distinguished only by a slight difference in the shade of the gray thread with which the bags were stitched. The color that corresponded to the fortified flour was known only to the project coordinator in Islamabad.

The wheat flour was distributed weekly to all 80 families in the study. In agreement with the families, the quantity was based on the estimated household need. If they demonstrated that they needed more, it was provided. No attempt was made to monitor the intake of individuals.

Monitoring wheat consumption

Before the start of the experiment, each family's flour need was estimated, and this amount was provided. No family required more than 20 kg of wheat flour per week. A questionnaire was used to monitor wheat flour consumption every two weeks. None of the members of the 80 families complained about the taste, color, or acceptability of the wheat flour.

Examination for intestinal parasites

Stool samples were collected in their homes from each target child and preserved in 10% formate saline (10% formate +0.9% NaCl). The specimens were emulsified in formate saline, and an aliquot was washed with tap water and filtered. One drop of the resultant filtrate was stained with Lugol's solution and examined microscopically under a cover glass.

Laboratory determinations

From each subject, 8 ml of venous blood was collected, of which a few drops were used for hemoglobin determination with a HemoCue [30]. Then 3.5 ml was transferred to tubes with heparin EDTA to prevent clotting. The remainder was centrifuged at 3,000 RPM for 10 minutes within two hours of collection. The serum was separated, and 50 µl of sodium azide per milliliter was added as a preservative. The serum was packed in ice and transported by air from Peshawar to the laboratory in Islamabad on the same day it was collected, and it was stored at –70°C until analyzed. The plasma fraction for lymphocyte cell subsets was processed within 48 hours.

Prealbumin, transferrin, and complement fraction C3 were measured by radial immunodiffusion (RID) kits (Bindarid, Birmingham, England). Ferritin was measured by a specific enzyme immunoassay (EIA) kit (Ramco Laboratories, Houston, Tex., USA). The lymphocyte cell subsets were counted using immunofluorescence (Immunology Manual, Department of Immunology, St. Thomas Hospital, London). Monoclonal antibodies that bind specifically to individual T-cell types were used to identify CD4 (helper) and CD8 (suppressor) cells.

Statistical procedures

The paired *t*-test, assuming a two-tailed distribution, was used to determine the significance of the differences between the lysine and control groups for all variables.

Results

Socioeconomic characteristics

Data on the socioeconomic characteristics of the study village are presented in table 1. Having a larger number of sons in the family is considered security against death from family feuds and other causes. Such an attitude reflects a lack of education. Eighty-eight percent of the men were illiterate, and none of the women had any education. Of the 12% of the men who were educated, 20% had an education up to the 10th grade, 8% up to the 4th grade, and the remainder had only a limited religious education. All inhabitants of the village were Muslim.

TABLE 1. Characteristics of families ($N = 80$)

Characteristic	Value
No. of persons in the household	7 ± 2 (SD)
Any education (%)	
Mother	0
Father	12
Housing (%)	
Owned	96
Rented	4
Mud	94
Cement	6
Ownership of farm animals (%)	
Cow	0
Goat or sheep	30
Chicken	60
Water source (%)	
Tube well	37
Unprotected hand-dug well	63
Nonpit latrine (%)	90
Land availability >4 kanal (2 acres)(%)	96
Major source of income for men (%)	
Farming	20
Unskilled labor	45
Low-grade government employee	30
Other	5
Average monthly household income (rupees)[a]	2,200
Children 6-14 yr old attending school (%)	60

a. 46 rupees = US$1.

All households were headed by and economically dependent on a male. Nearly all families (96%) owned their houses, and the remainder lived in houses that were rented or offered free by landlords in return for service. A typical house had one or two rooms with mud walls and dirt floors and no proper ventilation. Most families lived in these mud houses, and only 6% lived in cement houses. However, 83% of the houses had electricity.

The majority of the families lived in walled compounds. A small, partially enclosed area with no sewer or drainage was reserved for defecation of women and children. Men used the fields for defecation. The majority of the families (63%) used water from an open, unprotected, hand-dug, uncemented well, either in the house or outside in the compound. The economic costs of constructing pipelines and the annual or biannual water charges levied by the municipality are beyond the reach of these families. The remaining families had tap water in their houses flowing from tube wells constructed by the municipality. Food was usually prepared in a separate area located inside the house. Most had an earthen oven (tandoor) for baking bread, and 91% used wood as fuel for cooking and baking. The remaining families prepared foods using an electric or gas burner.

Household possessions were limited largely to cooking utensils, serving plates, and earthen pots for storing wheat grain and water. Half of the families surveyed had a radio, but none had television. However, 25% of the families had a bicycle, and two had motorcycles. None of the families possessed a cow or buffalo, but 30% owned one or two goats or sheep and 60% raised poultry, a traditional profession for the poorer segment of rural society areas.

Forty-five percent of the men worked as daily wage laborers; 30% were low-grade employees in governmental or semigovernmental departments (autonomous bodies mostly funded by the government); 20% were agricultural laborers; and 5% worked in various other activities. Due to the rapid growth of the population (2.9%), much of the cultivatable land is being converted to housing, and the number of persons in the village involved in farming is slowly declining. The average monthly household income was Rs 2,200/– (US$48 by the exchange rate at the time of the study).

An important change in the village seems to be the realization of the importance of education. Although the village has a very low literacy rate for adult males and a zero rate for adult females, it is worth noting that 60% of the children aged 6 to 14 were enrolled in schools either within or outside the village. The village has one basic health unit, which also provides maternal and child health care.

Dietary intake

Data on average protein and energy intakes are given in table 2. The average daily energy intakes of the children, women, and men of the sample population, calculated from the two dietary recall surveys, were 1,185, 2,057, and 2,269 kcal, respectively. These average energy intakes were all lower than the Pakistani recom-

TABLE 2. Protein and energy intake and sources

	Protein				Energy			
Subjects	Total (g/day)	Wheat (%)	Animal (%)	Other (%)[a]	Total (kcal/day)	Wheat (%)	Animal (%)	Other (%)[a]
Men	64	59	20	21	2,269	63	20	17
Women	57	65	18	17	2,057	58	22	20
Children	33	58	24	18	1,185	53	27	20

a. Mainly legumes.

mended dietary allowance (RDA), which is 1,265 kcal for children aged 6 to 14 years, 2,160 kcal for women, and 2,550 kcal for men.

The average daily protein intake of the children (33.0 g) was lower than the RDA for Pakistani children aged 6 to 14 years (42–56 g) [31]. However, the values of 57 g for women and 64 g for men were similar to the Pakistani RDAs. It should be noted that the Pakistani RDAs for protein allow for the poor quality of the protein in the predominantly cereal diet and for this reason are higher than those estimated from multicountry studies sponsored by UNU [32] and FAO/WHO/UNU [33].

Wheat flour contributed 58%, 65%, and 59% of the protein intake and 53%, 58%, and 63% of the energy intake of children, women, and men, respectively (table 2). Among children, wheat provided 58% of dietary protein, with 18% from other vegetable sources and 24% from animal sources. Among women, wheat provided 65% of dietary protein, with 17% from other vegetable sources and 18% from animal sources. For men, the corresponding percentages were 59%, 21%, and 20%. The total daily protein intake ranged from 44 to 68 g for women and from 18 to 65 g for children. Cereal protein intake ranged from 10 to 52 g for men, from 30 to 48 g for women, and from 10 to 35 g for children.

Anthropometry

Baseline data for weight, height, body mass index, and triceps skinfold thickness for adults are given in table 3. They did not change significantly during the study. Both groups of children showed the expected increases in weight and height during the three-month period, but the gains in weight and height were significantly greater in the lysine group (table 4). However, the initial weights and heights were higher in the control group due to large random differences in the age distribution of the children in the two groups. The control group had only 2 children in the 5- to 7-year age group, compared with 12 in the lysine group. By contrast, the control group had 14 10-year-olds and the lysine group only 4.

Laboratory findings

Parasites

Parasitic infections were found in 68% of the children. *Ascaris lumbricoides* (33%), *Entamoeba histolytica* (25%), *Hymenolepis nana* ova (18%), and *Giardia lamblia* cysts (16%) were the most common parasites in this population.

Transferrin and prealbumin

The values for transferrin and prealbumin are given in table 5. In both the control and the lysine groups, the increase in transferrin at the end of the study was significant for women. However, the difference for men and women in the increase in transferrin was far greater in the lysine than in the control group. For men and women, prealbumin increased significantly in both treatment groups, but the increase was greater in individuals receiving lysine-supplemented flour. Prealbumin decreased significantly in the control group of children and increased significantly in the lysine-supplemented group.

Immunology

The results for complement fraction C3 and for CD4 and CD8 T cells are given in table 5. All three measurements increased significantly in all family members in the group receiving lysine as compared with those in the control group. CD4 and CD8 cells generally decreased in the control group and increased significantly in the group receiving lysine. The significance

TABLE 3. Selected baseline anthropometric measurements for adults ($N = 39$ for each group)

	Men		Women	
Measurement	Control	Lysine	Control	Lysine
Weight (kg)	57.8	56.8	57.8	56.2
Height (cm)	165.7	162.6	156.5	154.8
Body mass index (kg/m^2)	21.0	20.6	23.6	23.4
Triceps skinfold thickness (mm)	12.1	13.3	7.7	7.8

TABLE 4. Anthropometric measurements of children at the beginning and end of the study ($N = 39$ for both groups)

	Control			Lysine			
Measurement	Start	End	C[a]	Start	End	L[b]	L – C[c]
Weight (kg)	26.1	26.6	*	22.7	23.6	*	*
Height (cm)	130	132	*	118	121	**	*
Body mass index (kg/m^2)	15.5	15.3	NS	16.3	15.9	NS	NS
Triceps skinfold thickness (mm)	6.8	6.1	NS	7.1	6.6	NS	NS

* $p < 0.05$; ** $p < 0.01$; NS, not significant.

a. C is the difference between the initial and final values for the control group.

b. L is the difference between the initial and final values for the lysine group.

c. L – C is the difference between the preceding two values, i.e., the change in values for the lysine group minus the change in values for the control group.

TABLE 5A. Transferrin, prealbumin, and T-cell populations at the beginning and end of the study in men

	Control			Lysine			
Measurement	*N*	Start	C[a]	*N*	Start	L[b]	L – C[c]
Transferrin (mg/dl)	35	431	4	32	384	135*	132*
Prealbumin (mg/L)	36	280	21*	34	260	27*	6*
Complement C3 (mg/L)	36	995	206*	34	979	349*	143*
CD4 cells (%)	24	11.5	–1.5	21	8.0	3.7*	5.4*
CD8 cells (%)	23	5.9	–0.8	20	3.9	7.0*	3.4*

* $p = < 0.05$.

TABLE 5B. Transferrin, prealbumin, and T-cell populations at the beginning and end of the study in women

	Control			Lysine			
Measurement	*N*	Start	C[a]	*N*	Start	L[b]	L – C[c]
Transferrin (mg/dl)	34	438	39*	30	412	126***	87**
Prealbumin (mg/L)	35	251	13*	30	264	40*	27*
Complement C3 (mg/L)	27	1,262	86*	29	1,044	378*	292*
CD4 cells (%)	22	14.6	–1.5	21	13.5	2.4*	3.9*
CD8 cells (%)	22	6.6	0.3	20	8.3	1.7*	1.4*

*$p = < 0.05$; **$p = < 0.02$; ***$p = < 0.01$.

TABLE 5C. Transferrin, prealbumin, and T-cell populations at the beginning and end of the study in children

	Control			Lysine			
Measurement	*N*	Start	C[a]	*N*	Start	L[b]	L – C[c]
Transferrin (mg/dl)	35	431	4	32	380	146**	30**
Prealbumin (mg/L)	36	218	22	33	249	–13	35*
Complement C3 (mg/L)	36	1,080	42	34	979	349*	43*
CD4 cells (%)	24	18.5	–1.7	22	7.8	3.6*	5.3*
CD8 cells (%)	24	8.6	–2.2	22	3.9	4.2*	1.9*

*$p = < 0.05$; ** $p = < 0.01$.

a. C is the difference between the initial and final values for the control group.

b. L is the difference between the initial and final values for the lysine group.

c. L – C is the difference between the preceding two values, i.e., the change in values for the lysine group minus the change in values for the control group.

of the randomly lower initial values for CD4 and CD8 among children in the lysine group is unknown. C3 also increased significantly in men and women in the control group, but not as much as in the group receiving lysine. The values were on the low side of the normal range of 910 to 1,567 mg/L.

Hemoglobin

Among women receiving fortified flour, there was a significant increase in hemoglobin that may have been influenced by the lower initial values. There were no other differences in hemoglobin levels between the control and lysine fortification groups (table 6).

TABLE 6. Hemoglobin values (g/dl) at the start and end of the study ($N = 39$ for both groups)

	Control		Lysine	
Subjects	Start	End	Start	End
Men	11.2	11.7	11.6	11.8
Women	9.6	9.6	8.8	9.6[a]
Children	9.4	9.4	9.5	9.8

a. The difference in hemoglobin between the start and end of the trial was significant for women ($p < 0.01$). Other differences between the start and end of the trial and between control and lysine groups were not significant ($p > 0.05$).

Disease frequency

There were no significant differences between the control and lysine groups in morbidity from diarrheal and respiratory diseases. In the two visits during the first month, an average of 43% of the children had respiratory infections and 46% had diarrhea. In the two visits

during the final month, 45% had respiratory disease and 33% had diarrhea. A small number of adult males had throat and respiratory infections. Seasonal variation was the most likely reason for the small decrease in child morbidity from the first to the third month.

Discussion

Visceral serum proteins, such as albumin and transferrin, are directly related to protein nutritional status. However, because of its relatively long half-life of 20 days, albumin is slower to reflect short-term nutritional changes. With its shorter half-life of 7 to 10 days, transferrin is more responsive to improvement in protein status [34], as is serum prealbumin [35]. Both transferrin and prealbumin significantly increased in the men and women receiving lysine as compared with the control group, with the effect more marked for transferrin. For children, only the improvement in transferrin with lysine supplementation was significant.

Most immune responses involve the production of proteins with specific functions. Specific immune responses such as T-cell subtypes, complement C3, and delayed cutaneous hypersensitivity are useful indicators of malnutrition [36–38].

Unfortunately, the antigens to test delayed cutaneous hypersensitivity lost their potency by the time they reached the study site. However, the measures of transferrin, prealbumin, complement C3, and C4 and C8 T cells all showed significant increases in the group receiving lysine-fortified wheat flour. This can reasonably be attributed to the effect of lysine in improving the overall protein quality of the diet.

Serum C3 also increased in the control group, but less than in the fortification group. In well-nourished individuals, C3 increases in response to infection, but in those who are poorly nourished, it remains the same or falls [39]. The increased C3 in all groups could be attributed to a seasonal increase in infection, and the greater rise in C3 in the families in the lysine-fortification group to better nutritional status.

An attempt was made to determine whether the positive responses of transferrin, the C3 complement fraction, and CD4 and CD8 T cells were limited to those in the lower socioeconomic groups, but this was not the case. However, this suggests that a relatively poor protein digestibility and quality made most of the protein intakes suboptimal.

The significant increase in hemoglobin among women in the lysine-supplemented families was not anticipated, since there was no difference in the iron content of the two diet groups. It can be speculated that the increase in transferrin, the transport protein for iron, was sufficient to improve iron absorption and/or transport. Serum ferritin, an indicator of iron stores, was also measured at the end of the study, but the overall differences between the two groups were not statistically significant.

Where significant improvements were observed in the control group, it is presumed that an increase in wheat available to the family or the cash saved by receiving free wheat may have been responsible. It is noteworthy that these effects of improving the protein quality of the diet with lysine fortification were observed in a population with apparently adequate mean total protein intake. As described above, the predominantly wheat-based diet of Pakistan has a lower protein quality than diets with more protein from legumes and animal sources. Moreover, although the mean protein consumption is adequate, there is wide variation in intake, so that a large number of individuals actually have marginal or low protein intake.

For the 40% of Pakistan's 140 million people who are below the poverty line, improving the protein quality of their diet through increased consumption of animal food such as meat, eggs, and milk is not economically feasible. Moreover, the traditional sources of complementary amino acids, such as legumes and pulses, are no longer affordable by a large segment of the Pakistani population.

A national survey found that 76% of the protein intake in Pakistan is derived from vegetable sources, and cereal accounts for 65% of the total protein intake [2]. In the present study, the percentages of total protein provided by cereal were similar: 59% for men, 65% for women, and 58% for children. This supports the suggestion that the benefits of lysine fortification of wheat flour found in this study are likely to apply to most other populations in Pakistan. The main cereal, wheat, is consumed in the form of bread by rich and poor alike.

The results indicate that lysine fortification of wheat flour can significantly improve some indicators of nutritional status in a population of individuals consuming a diet in which nearly two-thirds of the protein, depending on age and sex, is supplied by wheat. Iron deficiency is even more prevalent, and iron and folate fortification of cereal flour in Pakistan is already planned. Wherever this is the case, the cost of adding lysine to the premix would be reduced. Since policies cannot be based on one study in one location, it is important that the study design be replicated as soon as possible in another country and population, if possible with some additional indicators of nutritional and health status. There is an obvious need to try to link the positive findings of this study to tangible health benefits.

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Lysine-fortified wheat flour improves the nutritional and immunological status of wheat-eating families in northern China

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Abstract

The purpose of this study was to determine the impact of the fortification of wheat flour with lysine on selected health indicators among farm families obtaining 58% to 67% of their dietary protein from wheat. A man, a woman, and a child aged 5 to 12 years were studied from each of 88 families in a village near Huixian City, Henan Province, China. Half of the families received wheat flour fortified with 3 g of lysine per kilogram for three months, and the other half received wheat flour without fortification. The results showed a significantly greater gain in the height and weight of children receiving lysine-fortified wheat flour. Hemoglobin values were not affected. The mean prealbumin values of adult men and women were higher in those receiving lysine. The numbers of CD3 T cells increased significantly in women and children, as did the complement fraction C3 and IgG in men, IgA in women, and IgG, IgA, IgM, and C3 in children. These results indicate that lysine fortification of wheat flour can significantly improve some indicators of the nutritional status and immune function of family members consuming a wheat-based diet.

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Key words: China, fortification, immunological status, lysine, nutritional status, wheat flour

Introduction

A diet dependent on cereal protein must be complemented by a lysine source to provide a balanced amino acid pattern in the total diet. Protein from either animal sources or legumes will meet this need. However, the former is often prohibitively expensive for those at risk, and legume prices have soared in recent years. Fortification of a staple food offers a possible way of correcting specific nutritional deficiencies in an underprivileged population.

Although the quality of the diet of the Chinese people has improved with the economic development of the last two decades, dietary protein continues to be derived primarily from cereals and cereal products. According to the national nutrition survey conducted in 1992 [1], an average of 66.8% of dietary protein in rural China came from cereals. The amount of legumes and animal products in the diet is still low for most rural populations. Despite the predominance of rice in the South, the staple food of 44% of the Chinese population is wheat flour [1]. On the basis of the Food and Agriculture Organization/World Health Organization (FAO/WHO) [2] amino acid reference pattern, the protein score of wheat is 50, because of its deficiency in lysine. This is confirmed by nitrogen balance studies in humans indicating a net protein utilization for wheat of about 50%, as compared with 100% for meat, milk, and eggs [3, 4].

When wheat protein is fortified with lysine, improvement in nitrogen retention has been repeatedly observed in experimental animals [5], young children [6, 7], and adults [8, 9]. In 1996–97, a field study was conducted in a predominantly wheat-eating community outside Peshawar, Pakistan [10]. For three months, wheat flour, which supplied up to 65% of the protein in the diet, was given either with or without

added lysine to 80 families with 240 subjects. In this double-blind study, men, women, and children from the lysine-supplemented families had significant increases in serum transferrin, hemoglobin, and CD4 and CD8 cells. Given the potential practical importance of these findings and the need to replicate them, a double-blind study similar to that in Pakistan was carried out in Zhaoliuhe Village, Jitun Town, Huixian County, Henan Province, China, from March to June 1999. The proportion of dietary protein from wheat was comparable to that of the Pakistani population studied previously.

Methods

A total of 88 families were assigned randomly to either the experimental or the control group. The project provided all of the wheat flour consumed by these families for the three months of the study, but there was no alteration of their dietary pattern and food behavior. In each family, a man, a woman, and a child aged 5 to 12 years were given a physical examination, and each provided a 5-ml blood sample at the beginning and the end of the study.

There was no significant difference in the annual income of 920 yuan (US$115) for the control group and 904 yuan (US$113) for the lysine-fortification group. Food-consumption data were obtained for each of the three family members by two methods: 24-hour recall for three consecutive days at the beginning and end of the trial, and food-frequency questionnaires (FFQs) at the beginning of the study, based on their usual diet for the previous year.

Before and after the study, weight, height, triceps skinfold thickness, and mid-upper-arm circumference were measured, and the body mass index for adults was calculated. Albumin, prealbumin, and transferrin in blood serum were determined in the Henan Provincial Health and Anti-Epidemic Station. A Clinical Chemistry Analyzer System (SPACE, Fairfield, NY, USA) was used for these assays with reagents imported from the United States. Hemoglobin was determined at the survey site using a 721 spectrophotometer (HICN 721, Shanghai Third Analytic Plant, Shanghai, China).

The immunological assays were done at the Department of Immunology in the Capital University of the Medical Sciences of China. The reagents were purchased from Coulter-Immunotech (Beijing, China). T-cell numbers and T-cell subsets (CD3, CD4, CD8), and the numbers of natural killer cells (NK) in blood, were determined by using a Flow Cytometer (FACS, Coulter, Miami, Fla., USA). Interleukin-2 in serum was measured by ELISA. Complement fraction C3, IgM, IgG, and IgA in serum were measured by simple agar diffusion methods. A CO_2 incubator was used for the lymphocyte transformation test [11].

Institutional approvals

The protocol was reviewed and approved by the Committee on the Use of Humans as Experimental Subjects of the National Institute of Nutrition, the Henan Provincial Health and Anti-Epidemic Station, and the health and antiepidemic authorities of Huixian County, in which the study village was located. The study was explained in detail to each family, and written informed consent was obtained. All families were visited weekly to record any complaints, with the use of a follow-up questionnaire, and were interviewed for information on morbidity and food consumption.

Lysine-fortification procedure

In order to ensure adequate mixing of the lysine with the wheat flour, a pretest was carried out and a three-step mixing method was adopted. The local wheat flour contains an average of 250 mg of lysine per 100 g. Therefore, 3.75 g of l-lysine monohydrochloride, equal to 3 g of lysine, was added to make 1 kg of fortified wheat flour. This resulted in a lysine concentration of 550 mg per 100 g of the final product. This complies with the latest published FAO/WHO reference amino acid pattern [2]. Subsequent FAO/WHO/UNU expert consultations have reached tentative agreement on a new amino acid reference protein pattern based closely on that proposed by Young et al. [12] The proportion of lysine, 0.45 mg per gram of protein, is the same in both patterns, but the values for several of the other amino acids differ slightly. The lysine was supplied by Ajinomoto, Tokyo, Japan. Analyses of successive batches of the wheat flour before and after fortification confirmed that the mixing method was satisfactory.

The sacks of fortified and nonfortified flour were marked with threads of slightly different color, but their significance was not known to the field team. The flour was distributed weekly to each household in amounts based on the household intake data collected during the preparatory stage. All data were entered into the Institute of Nutrition and Food Hygiene (INFH) computers in Beijing, and the SAS 6.12 (SAS Institute, Cary, NC, USA) program was used for their analysis. Analysis of all indicators was performed separately for men, women, and children. ANOVA and chi-square tests were used to compare the trends of differences between the control and lysine group. The level of significance was set at $p < .05$.

Results

Dietary data

The three-day food-consumption data collected before and after the experiment indicated a wheat intake of

433 to 675 g per day for adults and 253 to 319 g for children. The average intake of animal products was only 30 to 50 g per day for adults and 18 to 28 g for children. There were no fish or milk products in the diet. Food consumption was not significantly different in the lysine and control groups ($p > .05$).

The intake of selected nutrients was calculated by using the food-consumption data and the Chinese food-composition table [13]. The average nutrient intake data from the baseline survey are shown in table 1. There were no significant differences between the control and lysine groups at the baseline survey ($p > .05$). The average daily energy intakes of adult men, adult women, and children were about 3,300, 2,700, and 1,700 kcal, respectively.

Approximately 60% of dietary energy for adults was obtained from wheat flour, 23% from other cereals, 1% from legumes and legume products, and 3% from animal foods (mainly pork). Among children, 55% of the energy was derived from wheat flour, 26% from other cereals, 0.7% from legumes and legume products, and 3% from animal foods. Protein intakes were around 105 g, 80 g, and 50 g, respectively, for men, women, and children; 58% to 67% of their dietary protein was derived from wheat flour, 16% to 19% from other cereals, 2% to 3% from legumes and their products, and 3% to 6% from animal food.

At the end of the study, there were no significant differences ($p > .05$) in nutrient intakes between the lysine and control groups, except for retinol equivalents, niacin, and selenium for men and retinol equivalents for children. The average lysine intake of the subjects was 2,370 ± 622, 1,931 ± 389, and 1,142 ± 262 mg/day for control men, women, and children, respectively, and 4,238 ± 1,038, 3,235 ± 832, and 2,166 ± 644 mg/day, respectively, for the lysine-fortification groups.

Anthropometry

For adult subjects, there were no significant differences between the two groups before and after the study in weight, height, body mass index, mid-upper-arm circumference, and triceps skinfold. For children, this was also true for body mass index, mid-upper-arm circumference, and triceps skinfold, but as shown in table 2, the lysine group grew taller and gained significantly more weight by the end of the study.

Laboratory determinations

Biochemical results

There were no significant differences between the control and lysine groups in hemoglobin, albumin, prealbumin, and transferrin for children either at baseline or at the end of the study ($p > .05$) (table 3). For adults this was also true for hemoglobin, albumin, and transferrin levels, but at the end of the study the serum prealbumin concentration was significantly higher in the lysine group than in the control group.

Immunological results

The mean immunological values, including T-cell numbers (CD3), T-cell subsets (CD4 and CD8), NK cells, interleukin-2, lymphocyte transformation test (stimulation index), immunoglobulins IgG, IgM, and IgA, and complement fraction C3, are shown in table 4 for men, women, and children. There were no significant differences in these measurements between

TABLE 1. Nutrient intakes of subjects from baseline survey (mean ± SD) compared with the Chinese recommended dietary allowances (RDAs)

Nutrient	Men	Women	Children
Energy (kcal)	3,348.9 ± 775.2[a]	2,659.8 ± 558.5[a]	1,693.6 ± 451.8[a]
Protein (g)	105.2 ± 28.4[a]	82.3 ± 18.7[a]	50.3 ± 14.6[a]
Carbohydrate (g)	605.1 ± 157.5	477.2 ± 105.3	293.9 ± 78.4
Fat (g)	54.2 ± 21.3	46.5 ± 21.7	40.2 ± 18.5
Retinol Eq (μg)	125.6 ± 92.3[b]	116.4 ± 95.2[b]	64.7 ± 58.1[b]
Thiamine (mg)	2.5 ± 0.65[a]	1.9 ± 0.4[a]	1.2 ± 0.4[a]
Riboflavin (mg)	1.1 ± 0.3[b]	0.8 ± 0.2[b]	0.5 ± 0.1[b]
Niacin (mg)	23.6 ± 6.7[a]	18.5 ± 4.7[a]	10.9 ± 4.0[a]
Ascorbic acid (mg)	143.6 ± 84.1[a]	128.3 ± 68.1[a]	57.4 ± 34.1[a]
Vitamin E (mg)	38.3 ± 9.3[a]	33.6 ± 10.6[a]	25.5 ± 8.4[a]
Calcium (mg)	682.9 ± 237.1[a]	578.6 ± 188.1[b]	287.6 ± 92.8[b]
Iron (mg)	38.8 ± 10.3[a]	31.6 ± 6.9[a]	18.5 ± 4.8[a]
Zinc (mg)	17.0 ± 4.5[a]	13.5 ± 3.0[a]	8.0 ± 2.3[a]
Selenium (μg)	49.5 ± 13.1[a]	38.6 ± 11.0[b]	24.0 ± 7.3[b]

a. ≥ 80% of RDA.
b. < 80% of RDA.

TABLE 2. Anthropometric measurements of subjects at baseline and end of the study (mean ± SD)

	Baseline		End	
Measurement	Control (n = 44)	Lysine (n = 44)	Control (n = 41)	Lysine (n = 43)
Men				
Body mass index (kg/m^2)	21.0 ± 2.1	21.3 ± 2.3	21.7 ± 2.2	21.9 ± 2.7
Height (cm)	169.4 ± 5.9	169.7 ± 5.4	169.7 ± 6.0	169.4 ± 5.2
Weight (kg)	60.5 ± 8.8	61.5 ± 8.5	62.8 ± 9.2	63.1 ± 9.2
Mid-upper-arm circumference (cm)	27.9 ± 2.2	28.4 ± 2.5	28.6 ± 2.4	28.9 ± 2.7
Triceps skinfold thickness (mm)	7.6 ± 2.2	8.3 ± 3.9	9.1 ± 3.2	10.6 ± 5.9
Women				
Body mass index (kg/m^2)	22.2 ± 3.3	22.7 ± 2.7	22.8 ± 3.1	23.4 ± 3.0
Height (cm)	156.3 ± 5.0	156.5 ± 4.8	155.9 ± 6.7	156.6 ± 4.7
Weight (kg)	55.9 ± 7.5	55.6 ± 7.2	55.8 ± 8.7	57.3 ± 7.7
Mid-upper-arm circumference (cm)	28.1 ± 2.8	28.4 ± 2.7	27.9 ± 3.6	28.8 ± 2.9
Triceps skinfold thickness (mm)	15.4 ± 5.1	16.5 ± 6.1	18.9 ± 6.9	20.1 ± 6.8
Children				
BMI(kg/m^2)	14.8 ± 1.3	14.9 ± 1.9	15.1 ± 1.9	15.2 ± 2.1
Height (cm)	120.4 ± 12.8	125.4 ± 11.6	122.6 ± 13.6	128.4 ± 11.9*
Weight (kg)	21.7 ± 5.4	23.7 ± 5.9	22.3 ± 7.9	25.4 ± 6.7*
Mid-upper-arm circumference (cm)	17.3 ± 1.9	17.7 ± 2.1	18.1 ± 3.3	18.1 ± 2.4
Triceps skinfold thickness (mm)	7.1 ± 2.3	7.1 ± 2.7	8.1 ± 3.8	7.56 ±2 .6

*$p < 0.05$.

TABLE 3. Biochemical measurements of subjects at baseline and end of the study (mean ± SD)

	Baseline		End	
Measurement	Control (n = 41)	Lysine (n = 43)	Control (n = 41)	Lysine (n = 43)
Men				
Hemoglobin (g/dl)	16.2 ± 1.6	15.9 ± 1.8	15.4 ± 1.0	15.2 ± 0.9
Albumin (g/L)	44.1 ± 7.2	41.0 ± 7.5	39.9 ± 4.0	40.2 ± 3.62
Prealbumin (g/L)	0.29 ± 0.06	0.26 ± 0.09	0.25 ± 0.05	0.27 ± 0.06**
Transferrin (g/L)	2.74 ± 0.59	2.66 ± 0.67	2.37 ± 0.44	2.45 ± 0.05
Women				
Hemoglobin (g/dl)	13.7 ± 1.6	13.9 ± 1.6	13.1 ± 1.2	13.2 ± 1.0
Albumin (g/L)	45.0 ± 9.2	44.3 ± 7.9	38.7 ± 2.6	38.6 ± 3.4
Prealbumin (g/L)	0.24 ± 0.06	0.24 ± 0.06	0.20 ± 0.04	0.23 ± 0.04*
Transferrin (g/L)	3.36 ± 0.76	3.34 ± 0.90	2.56 ± 0.46	2.70 ± 0.46
Children				
Hemoglobin (g/dl)	13.1 ± 1.5	13.2 ± 1.6	12.7 ± 1.0	13.2 ± 0.8
Albumin (g/L)	41.0 ± 9.6	39.7 ± 10.4	39.7 ± 3.3	38.8 ± 3.5
Prealbumin (g/L)	0.18 ± 0.07	0.19 ± 0.06	0.18 ± 0.03	0.18 ± 0.04
Transferrin (g/L)	2.72 ± 0.67	2.72 ± 0.65	2.51 ± 0.37	2.53 ± 0.38

*$p < 0.05$, **$p < 0.01$.

the lysine and control groups for any of the age and sex groups before the study. After the study, however, adult male subjects in the lysine group showed a significant increase in CD8 ($p < .05$) as well as in IgG ($p < .01$) and C3 ($p < .01$), as compared with those in the control group. Adult female subjects in the lysine group had higher values of CD3 ($p < .01$) and IgA ($p < .05$) at the end of the study. Among children, CD3 ($p < .01$), IgG ($p < .001$), IgA ($p < .01$), and IgM ($p < .01$) increased significantly, as did C3 ($p < .05$).

Discussion

The results of this study, together with those of the nearly identical prior study in Pakistan [10], indicate that lysine fortification can improve the nutritional status of families for whom wheat is the major source of dietary protein. Although the positive findings were not identical in this study and the similar one in Pakistan [10], more than half of the parameters measured were significantly increased in both studies. In

TABLE 4. Immunological measurements of subjects at baseline and end of the study (mean ± SD)

	Baseline		End	
Measurement	Control	Lysine	Control	Lysine
Men	(n = 43)	(n = 43)	(n = 43)	(n = 41)
CD3 (%)	66.90 ± 5.46	67.29 ± 5.53	67.70 ± 5.52	69.13 ± 6.57
CD4 (%)	37.20 ± 5.39	35.61 ± 5.94	37.67 ± 6.51	36.10 ± 7.31
CD8 (%)	25.86 ± 4.99	27.74 ± 5.64	26.27 ± 6.83	29.43 ± 6.83*
NK (%)	16.27 ± 5.73	15.56 ± 6.81	15.11 ± 5.93	17.14 ± 6.84
IL-2 (OD405)	0.31 ± 0.15	0.32 ± 0.18	0.41 ± 0.06	0.44 ± 0.11
LTR (%)	0.32 ± 0.14	0.31 ± 0.14	0.58 ± 0.05	0.60 ± 0.07
IgG (g/L)	15.65 ± 5.95	15.44 ± 7.84	13.82 ± 4.40	18.83 ± 6.99**
IgA (g/L)	3.04 ± 1.33	2.85 ± 1.57	2.78 ± 1.41	3.37 ± 1.88
IgM (g/L)	1.25 ± 0.44	1.16 ± 0.29	1.36 ± 0.49	1.55 ± 0.56
C3 (g/L)	0.91 ± 0.19	0.93 ± 0.25	0.99 ± 0.10	1.10 ± 0.26**
Women	(n = 40)	(n = 43)	(n = 40)	(n = 43)
CD3 (%)	66.90 ± 6.14	67.51 ± 5.40	67.94 ± 5.55	71.15 ± 5.51**
CD4 (%)	38.60 ± 6.69	37.82 ± 5.35	37.58 ± 6.07	40.12 ± 6.07
CD8 (%)	25.07 ± 3.73	26.46 ± 4.83	27.30 ± 4.85	27.87 ± 5.97
NK (%)	16.72 ± 6.24	15.63 ± 5.74	14.61 ± 4.57	15.35 ± 6.73
IL-2 (OD405)	0.33 ± 0.22	0.29 ± 0.11	0.44 ± 0.08	0.42 ± 0.10
LTR (%)	0.33 ± 0.15	0.30 ± 0.12	0.61 ± 0.06	0.58 ± 0.06
IgG (g/L)	18.30 ± 8.70	17.3 ± 7.35	16.90 ± 7.73	20.15 ± 8.26
IgA (g/L)	1.50 ± 0.56	1.43 ± 0.46	1.67 ± 0.59	1.95 ± 0.69*
IgM (g/L)	2.99 ± 1.62	2.71 ± 1.64	2.88 ± 1.60	2.98 ± 1.64
C3 (g/L)	0.99 ± 0.23	0.92 ± 0.20	1.04 ± 0.14	1.05 ± 0.16
Children	(n = 40)	(n = 43)	(n = 40)	(n = 43)
CD3 (%)	66.83 ± 6.15	68.20 ± 5.47	66.26 ± 6.34	70.96 ± 6.34**
CD4 (%)	34.77 ± 5.78	35.32 ± 5.08	35.50 ± 6.94	37.62 ± 5.77
CD8 (%)	26.87 ± 3.78	28.18 ± 5.03	26.94 ± 4.16	29.06 ± 5.82
NK (%)	14.84 ± 5.83	13.61 ± 4.89	14.79 ± 5.50	13.81 ± 5.29
IL-2 (OD405)	0.26 ± 0.08	0.29 ± 0.14	0.42 ± 0.08	0.43 ± 0.09
LTR (%)	0.33 ± 0.14	0.31 ± 0.13	0.59 ± 0.06	0.59 ± 0.06
IgG (g/L)	12.67 ± 4.40	12.34 ± 6.19	11.51 ± 4.53	17.42 ± 8.55***
IgA (g/L)	1.27 ± 0.37	1.28 ± 0.34	1.34 ± 0.32	1.66 ± 0.52**
IgM (g/L)	1.64 ± 0.86	1.57 ± 0.76	1.52 ± 0.63	1.97 ± 1.03**
C3 (g/L)	0.98 ± 0.18	0.95 ± 0.24	1.01 ± 0.16	1.10 ± 0.19*

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.
CD3, CD4, CD8, and NK are T-cell subsets; IL-2 is interleukin-2; LTR is lymphocyte transformation response to a mitogen (stimulation index); IgG, IgA, and IgM are immunoglobulins; and C3 is a complement fraction.

addition, CD3 cells, not studied in Pakistan, increased in all three groups in this study, as did a number of the immunoglobulins. Wheat flour is the staple food in the north and northwest provinces of China, and lysine is known to be limiting in wheat protein. The preferred approach to correcting the lysine deficiency is improving the overall quantity and quality of the diet. However, this would require an increase in animal or legume proteins or both. For the poor of developing countries, these are costly or in short supply, and an alternative approach is required.

As summarized in the Introduction, in experimental trials in rats and metabolic studies in humans, the protein quality of wheat was improved by lysine fortification. However, evidence for this in human populations at the household level is limited to the recent study in Pakistan, after which this study is patterned [10]. A study in the 1960s in Tunisia that failed to find an effect of lysine fortification of wheat flour was seriously confounded by contraband unfortified flour entering the experimental area, possible differential effects of infection in the villages, and low dietary energy intakes that may have been limiting [14].

In this latest study in China, the average protein intake of children of around 46 to 54 g per day was only 79% to 89% of the recommended dietary allowance (RDA), but energy intakes were adequate. Given this relatively low intake of available protein,

an improvement in protein quality with lysine fortification of wheat flour would be expected. It is not surprising, therefore, that the height and weight of children were observed to increase significantly in the lysine-fortification group as compared with those in the control group.

Although the total protein and energy intake of the adults in this study was adequate, the protein quality was poor, because the predominantly wheat diet was deficient in lysine. Serum proteins, such as albumin, prealbumin, and transferrin, are directly related to protein nutritional status. The level of prealbumin was significantly higher at the end of the study in the women receiving the lysine-fortified wheat flour, suggesting improved protein status, but no other significant changes in these serum proteins were observed.

Immune responses are adversely affected by poor nutritional status, including that of protein [15]. In subjects whose protein status is suboptimal, increasing the amount of available protein in the diet can result in improvement in sensitive immunological parameters [16].

In the current study, the women and children who received lysine-fortified flour showed significant increases in CD3 T-cell numbers. Complement C3 also increased significantly in men and children in the lysine-fortification group. The increase in all three immunoglobulins measured was highly significant in children, and the increases in IgG in men and IgA in women were significant. It is concluded that lysine fortification improved the nutritional quality of the dietary protein, as judged by an increase in some measures of immune function.

The three-month test period was too short and sanitation was too good to allow any effect on morbidity from infectious disease to be detected. During the entire study, there were only seven cases of diarrheal disease: two among children in the control group, two among women in the control group, and three among men in the lysine group. Respiratory disease was seasonally rare in the population studied, and the distribution of the small number of cases was almost identical in the lysine and control groups.

The evidence that individuals whose dietary protein comes mainly from wheat, with only limited legume and animal protein, show improvement in some indicators of nutritional status in similar studies conducted in two different countries justifies further efficacy trials. The cost-effectiveness of multiple fortification of wheat flour should also be explored.

According to the Chinese national nutrition survey conducted in 1992 [1], the protein intake of children aged 2 to 5 years averaged only 81% to 86% of their respective RDAs, and that of children aged 6 to 17 years was 89% to 93% of their RDAs. It is not surprising, therefore, that lysine fortification of wheat flour significantly improved their growth and a number of immunological indicators.

There are 600 million Chinese people in officially defined poor areas. They are dependent on a very simple diet consisting predominantly of cereals. To improve the protein quality of their diet by increasing sufficiently the intake of animal foods such as meat, fish, eggs, and milk is currently not economically feasible, and as pointed out in the Introduction, this has become true for legumes as well. This study and the previous one in Pakistan suggest two potential approaches. There is currently a major international effort to promote the multiple fortification of cereal flours with vitamins and minerals. For some populations, the addition of lysine to this premix might be justified. Another approach could be the application of biotechnology to improve the lysine content of wheat, as has been done successfully for maize by conventional breeding [17], but conventional breeding efforts for wheat have proved more difficult.

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Fast and reliable salt iodine measurement: Evaluation of the WYD Iodine Checker in comparison with iodometric titration

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Abstract

Iodine deficiency persists as the leading cause of preventable brain damage and reduced intellectual capacity in the world. The most effective method for the elimination of iodine deficiency is the consumption of adequately iodized salt. Ensuring that a population receives adequately iodized salt demands careful monitoring of the salt iodine content. We evaluated the WYD Iodine Checker, a hand-held instrument that quantitatively measures the salt iodine content on the basis of a colorimetric method, and compared its performance with iodometric titration. Performance testing results indicated that the WYD Iodine Checker is a highly precise, accurate, and sensitive tool for measuring salt iodine content. It is a user-friendly instrument that is based on a simple methodology and a straightforward salt sample preparation and testing procedure. We recommend further testing to examine the field performance of the WYD Iodine Checker when measuring iodate salt samples.

Key words: Colorimetry, low-technology, portable instrument

Introduction

Iodine-deficiency disorders are the leading cause of preventable brain damage and mental retardation, with over a billion people at risk worldwide. The consequences of iodine-deficiency disorders not only severely threaten the growth and development of a child, but its subsequent implications also thwart the socioeconomic development of an entire population. The most cost-effective and sustainable intervention to eliminate iodine-deficiency disorders is universal salt iodization (USI), which involves the adequate iodization of all edible salt. It is critical to monitor the iodine content of salt at the production, distribution, retail, and consumption levels to ensure the quality of iodized salt and to verify advancement toward achieving USI.

Various methods are available for testing the iodine content in salt. One such method is the rapid salt testing kit, which is a field-friendly, inexpensive, highly sensitive test. The test qualitatively or semiquantitatively detects iodine in salt. To obtain a more accurate, quantitative measurement of the salt iodine concentration, iodometric titration is recommended [1]. Titration is conducted for validation, but it requires skilled personnel and is time-consuming and costly, which limits its applicability for routine monitoring of the salt iodine concentration.

One method that can be used for routine monitoring and requires simple laboratory equipment is the colorimetric procedure. Evaluation of one spectrophotometric method, based on a modified Dustin and Ecoffey iodine quantitative method, indicated that the method was highly precise, accurate, sensitive, and specific. The method also had added advantages, such as its ease of use and simple methodology [2]. A similar colorimetric method developed by the Salt Research Institute of the China National Salt Industry Corporation is the WYD Iodine Checker. The WYD Iodine Checker was evaluated by the Centers for Disease Control and Prevention (CDC) and compared with the iodometric titration method. This evaluation does not constitute an endorsement by CDC. CDC's evaluation of the method was conducted independently, and thus it was in no way commissioned or supported by the instrument manufacturer.

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Experimental methods

Apparatus

The WYD Iodine Checker is a single-wavelength spectrophotometer that measures the iodine level (mg/kg) in salt based on the absorption of the iodine-starch blue compound at 585 nm. The manufacturer specifies that the instrument's range of measurement is 10 to 90 mg/kg and that it has an analytical error of less than two parts per million. Its weight (500 g) and dimensions (175 × 135 × 60 mm) make it easily transportable; the manufacturer states that it can withstand a damp and corrosive environment. It functions on 220V AC voltage or DC 9V, which requires six AA batteries [3].

Solutions and reagents

The CDC chemists followed the procedures provided in the WYD Iodine Checker Manual for the preparation of solutions for testing salt iodized with potassium iodate (KIO_3) and iodide (KI), except that 18 MΩ-cm ultra-pure water was used for the preparation of solutions and testing of salt samples. The WYD Iodine Checker manual states that distilled water, purified drinking water, or cooled boiled water can be used [3]. Measurement of salt iodized with iodate requires the preparation of a KIO_3 standard solution and an iodine working standard solution, which can be used for up to six months and is equivalent to the concentration of 50 mg/kg in an iodized salt sample. It further requires the preparation of two reagents: solution A, a starch-based solution; and solution B, a sulfuric acid solution. Measurement of salt iodized with iodide requires oxidation of iodide to iodate using 3% (w/v) bromine solution, and the excess bromine solution is neutralized by 10% (w/v) sodium formate solution. After this oxidation, the same steps are performed as for salt iodized with KIO_3 [3]. The cost of reagents per salt sample tested is approximately US$0.07.

Salt samples

Morton Salt samples with known concentrations of iodide or iodate (25, 50, 75, and 100 mg/kg) were analyzed. The Morton Salt samples did not contain a free-flow agent. Potassium iodate was used as the fortificant for the iodate salt samples, and KI was used as the fortificant for the iodide salt samples, which were stabilized with dextrose and sodium bicarbonate. Market salt samples obtained from Argentina, Brazil, Chile, and Mexico that had unknown concentrations of iodine were tested by the iodate procedure.

Analytical methods

WYD Iodine Checker. All procedures were performed according to the instructions provided in the WYD Iodine Checker manual [3]. After a zero point calibration, the instrument is calibrated using the gray glass or KIO_3 standard solution to an LCD readout of 50 ± 0.1. One gram of iodized salt sample is used for the measurement. An iodate salt sample can be analyzed in about three or four minutes, which does not include the time for weighing of the salt sample. The measurement of salt fortified with KI requires oxidation of iodide to iodate before the same procedure can be followed as for salt with iodate. Therefore, the measurement of one iodide salt sample takes approximately five or six minutes, minus the sample weighing time. Testing a batch of salt samples slightly decreases the analysis time per sample, so that 20 to 30 samples can be analyzed in one hour.

Reference method

Iodometric titration. The reference method for measuring the iodine content of salt is iodometric titration with the thiosulfate-starch reaction as the external indicator [1, 4]. Ten grams of iodized salt is used for the measurement; the cost of reagents per salt sample tested is approximately US$0.06. One iodate salt sample is analyzed in roughly 15 to 20 minutes, and one iodide salt sample in about 17 to 22 minutes (minus the sample weighing time). As with the WYD Iodine Checker, testing a batch of salt samples decreases the analysis time. An estimated 12 to 15 salt samples can be measured in one hour.

Procedures

We assessed the within-assay, among-assay, and total assay imprecision of the WYD Iodine Checker and iodometric titration. To determine the within-assay imprecision of the WYD Iodine Checker, five replicates from each of the 25 and 100 mg/kg iodate and iodide Morton Salt samples were analyzed within one day. The experiment was repeated over five individual days. First computing the standard deviation of the results measured on the same day and then dividing by the mean of the results for that day obtained the within-assay imprecision for one day. Thus, five within-assay coefficients of variation were calculated. To give a reflection of the variability of the within-assay coefficient of variation from day to day, the range of within-assay coefficients of variation was reported.

To compute the among-assay imprecision, the standard deviation from the mean result of each day was divided by the average of the mean results of each day. The total assay imprecision was determined by the standard deviation of all 25 measurements (five replicates per day over five days) divided by the grand mean of the 25 measurements. For iodometric titration, the same computations were used to determine the within-

assay, among-assay, and total assay imprecision, except that the number of replicates within one day and the number of days were three instead of five.

Because the WYD Iodine Checker can take multiple measurements from one preparation or vial (i.e., the sample solution vial contains 50 ml and the measurement cell for the WYD Iodine Checker holds approximately 4 ml), the within-vial imprecision was calculated. Five consecutive measurements from each vial of the 25 and 100 mg/kg iodate and iodide Morton Salt samples were taken. This assessed the instrument's reproducibility.

To assess the mixing accuracy (recovery) of the WYD Iodine Checker, we prepared a panel of eight salt samples containing different proportions of the noniodized, 25 and 100 mg/kg iodide or iodate Morton Salt samples (table 1). Three replicates from each of the prepared salt samples were measured. The averages of the three measurements were compared with the expected concentrations to determine the mixing accuracy of the WYD Iodine Checker for iodide and iodate samples.

The limit of detection for the WYD Iodine Checker was calculated by measuring 10 samples of noniodized Morton Salt. The samples were measured using the procedure for measuring iodide in salt. The mean plus three standard deviations of the 10 measurements indicated the limit of detection for the instrument.

A method comparison was performed between the WYD Iodine Checker and iodometric titration. Multiple measurements from the four Morton Salt samples containing iodide were conducted by both methods. A total of 47 salt samples fortified with KIO_3 were measured by the WYD Iodine Checker and iodometric titration. These iodate salt samples included 4 Morton Salt samples and 43 market salt samples obtained from Argentina, Brazil, Chile, and Mexico. Since the two methods were run independently by two scientists, separate salt aliquots were used for each method. For future investigations, it is recommended instead to use subaliquots from the same salt aliquot once it has been dissolved for method comparison.

Results and discussion

Table 2 presents imprecision data for the WYD Iodine Checker and iodometric titration using Morton Salt samples with concentrations of 25 and 100 mg/kg iodate and iodide. The total assay variability of the WYD Iodine Checker was approximately 9% when salt iodized with iodide was measured and 6% when salt iodized with iodate was measured (table 1). The total assay variability of the titration method was higher: about 12% to 14% for salt iodized with iodide and about 7% to 12% for salt iodized with iodate. The

TABLE 1. Mixing accuracy (recovery) for the WYD Iodine Checker

No.	Ratio of proportions: sample 1 + sample 2	Amount of sample 1 (25 mg/kg KI or KIO_3)	Amount of sample 2	Expected concentration (mg/kg)	Average measured concentration (mg/kg)	Mixing accuracy (%)
KI						
1	1 + 4	5 g	20 g noniodized salt	5.0	6.2	124.0
2	1 + 2	5 g	10 g noniodized salt	8.3	10.8	130.5
3	1 + 1	20 g	20 g noniodized salt	12.5	14.8	118.4
4	2 + 1	30 g	15 g noniodized salt	16.7	17.8	106.4
5	4 + 1	40 g	10 g noniodized salt	20.0	21.6	108.2
6	2 + 1	30 g	15 g 100 mg/kg KI	50.0	50.0	100.1
7	1 + 1	20 g	20 g 100 mg/kg KI	62.5	58.3	93.3
8	1 + 2	15 g	30 g 100 mg/kg KI	75.0	70.1	93.5
Average						109.3 ± 13.9 (SD)
KIO_3						
1	1 + 4	5 g	20 g noniodized salt	5.0	5.0	100.7
2	1 + 2	5 g	10 g noniodized salt	8.3	8.6	104.0
3	1 + 1	20 g	20 g noniodized salt	12.5	12.6	100.5
4	2 + 1	30 g	15 g noniodized salt	16.7	16.5	98.8
5	4 + 1	40 g	10 g noniodized salt	20.0	19.9	99.7
6	2 + 1	30 g	15 g 100 mg/kg KIO_3	50.0	45.6	91.1
7	1 + 1	20 g	20 g 100 mg/kg KIO_3	62.5	51.9	83.1
8	1 + 2	15 g	30 g 100 mg/kg KIO_3	75.0	66.7	89.0
Average						95.9 ± 7.2 (SD)

TABLE 2. Assay imprecision for the WYD Iodine Checker and iodometric titration

Method	Mean and imprecision			
	Mean (mg/kg)	Total assay[a] CV (%)	Among-assay[b] CV (%)	Within-assay[c] CV (%) range
WYD (5 replicates/day, over 5 days)				
25 mg/kg KI	26.0	8.9	7.1	2.4–11.7
100 mg/kg KI	110.0	8.7	6.9	3.8–11.4
25 mg/kg KIO_3	25.0	5.8	4.7	3.2–5.4
100 mg/kg KIO_3	105.0	5.8	3.4	2.1–10.3
Titration (3 replicates/day, over 3 days)				
25 mg/kg KI	26.8	11.5	11.7	2.5–8.8
100 mg/kg KI	118.8	13.9	5.0	3.0–20.9
25 mg/kg KIO_3	28.6	12.4	1.8	4.0–13.9
100 mg/kg KIO_3	114.1	7.2	7.6	2.0–3.8

CV, Coefficient of variation

a. Standard deviation (SD) of all measurements divided by the grand mean of all measurements.

b. SD from the mean result of each day divided by the average of the mean results of each day.

c. SD of the results measured on the same day divided by the mean of results for that day; range reflects variability of the within-assay CV from day to day.

among-assay imprecision for salt fortified with iodide was 6.9% to 7.1% with the WYD Iodine Checker versus 5.0% to 11.7% with iodometric titration. For salt with iodate, the among-assay imprecision was 3.4% to 4.7% with the WYD Iodine Checker and 1.8% to 7.6% with iodometric titration.

The average within-assay imprecision for the WYD Iodine Checker was 5.8% (range, 2.4%–11.7%) when measuring salt iodized with iodide and 4.4% (range, 2.1%–10.3%) when measuring salt iodized with iodate. In comparison, the average within-assay imprecision for titration was 9.4% (range, 2.5%–20.9%) for salt iodized with iodide and 4.9% (range, 2.0%–13.9%) for salt iodized with iodate (table 2). The results of precision testing for the iodate and iodide Morton Salt samples indicated improved or comparable total assay and within- and among-assay precision for the WYD Iodine Checker versus iodometric titration. Less subjectivity associated with the WYD Iodine Checker compared with titration (i.e., reading the result directly from the instrument's display versus noting the color change of the sample) may contribute to the improved precision of the WYD Iodine Checker.

The average within-vial imprecision for the WYD Iodine Checker was 0.7% (range, 0.2%–1.1%) for the iodide samples and 1.2% (range, 0.0%–1.8%) for the iodate samples. This very low within-vial imprecision indicated the excellent reproducibility of measurements by the WYD Iodine Checker and suggested that one measurement from each prepared salt sample is fully adequate.

The mean recoveries for a panel of eight iodide and iodate salt samples obtained through proportional mixing were 109.3% ± 13.9% and 95.9% ± 7.2%, respectively, and thus fell in the suggested range of 85% to 115% (table 1) [1]. To visualize the results from this mixing accuracy test, we plotted the expected concentration versus obtained concentration (fig. 1): obtained iodate concentration = 0.848 * expected iodate concentration + 1.854, $r^2 = .996$; obtained iodide concentration = 0.901 * expected iodide concentration + 3.060, $r^2 = .998$. The excellent correlations between the expected and obtained concentrations verified the high accuracy of the WYD Iodine Checker in detecting all iodine present in both iodate and iodide salt samples.

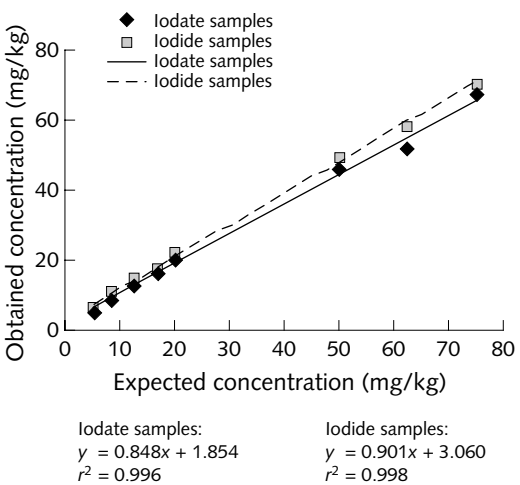

FIG. 1. Comparison of expected and obtained concentrations in a panel of eight iodide and iodate salt samples as measured by the WYD Iodine Checker in a mixing accuracy (recovery) test

The limit of detection of the WYD Iodine Checker was determined by 10 replicate measurements of the noniodized Morton Salt sample. One measurement of 5.5 mg/kg exceeded two standard deviations from the mean of the samples, and therefore it was confirmed as an outlier and rejected from the calculations. The mean of the nine remaining measurements was 0.43 mg/kg, with a standard deviation of 0.47 mg/kg. Therefore, the limit of detection of the WYD Iodine Checker was 1.84 mg/kg. This low limit of detection indicated that the instrument can detect very low levels of iodine. The outlier illustrated the necessity of taking proper precautions against contamination. Preventing contamination of the sample was most critical during the sample preparation process, because contamination was most likely to occur during this process. Precautionary steps such as the maintenance of a clean laboratory environment (e.g., the use of distilled water, clean spatulas, and 50-ml sample vials) and proper equipment maintenance (e.g., clean cell holders) can help prevent contamination problems.

Using the eight samples of Morton Salt with known concentrations of iodide or iodate (25, 50, 75, and 100 mg/kg), we compared the results obtained by the WYD Iodine Checker and iodometric titration (figs. 2 and 3). Multiple measurements of all samples were performed, and the error bars in figures 2 and 3 represent the mean ± 1 SD for each concentration. For salt samples fortified with iodide, we obtained a regression equation of WYD Iodine Checker = 0.935 * titration – 2.458, r^2 = .989 (fig. 2). For salt samples fortified with iodate, we obtained a regression equation of WYD Iodine Checker = 0.963 * titration – 3.659, r^2 = .993 (fig. 3). These results show that when fine, homogeneous salt is used, a very good correlation is obtained between the two methods. As shown earlier in the precision calculations, the WYD Iodine Checker reported less variability from multiple measurements than titration. The lower variability was particularly notable for the 100 mg/kg iodide salt sample, where the standard deviation for the WYD Iodine Checker (9.5) was more than half the standard deviation for iodometric titration (16.5).

Comparison of all iodate salt samples (n = 47; 43 market samples and 4 Morton Salt samples) measured by iodometric titration and the WYD Iodine Checker yielded a regression equation of WYD Iodine Checker = 1.112 * titration – 1.113, with a slightly lower correlation coefficient (r^2 = .850) than for the Morton Salt samples (fig. 4). This somewhat lower correlation might be a consequence not of the methods, but of varying iodine concentrations within the market salt samples. Some of the market samples measured in replicates displayed large standard deviations and broad ranges (table 3). This level of variation did not occur among the Morton Salt samples. Therefore, the iodine concentrations of these market samples may have varied because of the uneven distribution of iodine within the bags. A lack of homogeneity within bags of salt is reported to be a product of poor mixing at the production level [5].

The variation in the measurements of the iodate salt

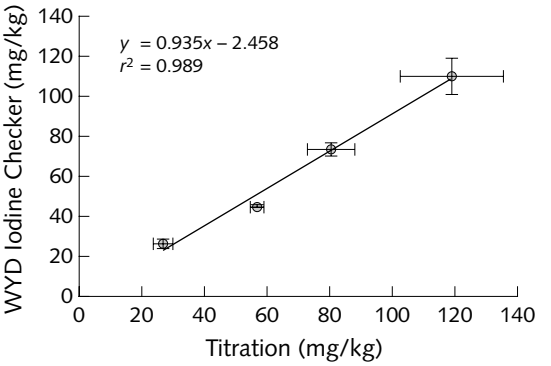

FIG. 2. Comparison of results obtained for four Morton Salt samples fortified with iodide as measured by titration and the WYD Iodine Checker

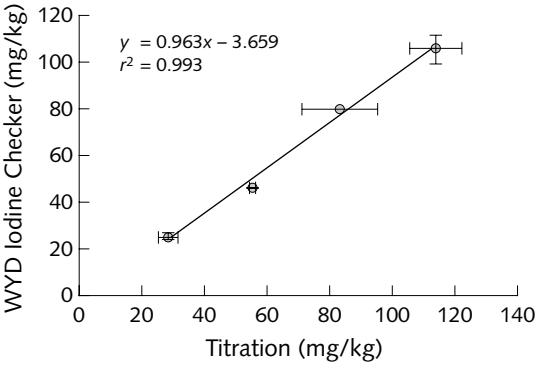

FIG. 3. Comparison of results obtained for four Morton Salt samples fortified with iodate as measured by titration and the WYD Iodine Checker

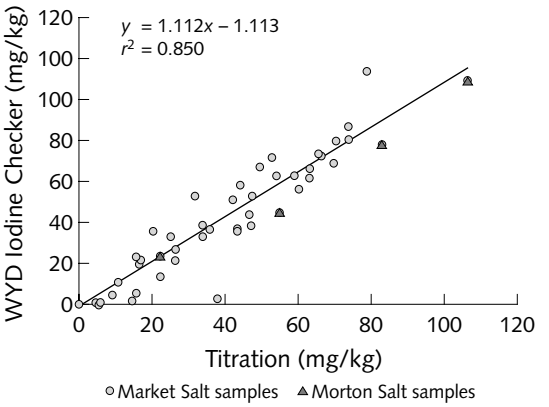

FIG. 4. Linear regression analysis to compare results obtained for iodate salt sample (43 market samples and four Morton Salt samples) as measured by iodometric titration and the WYD Iodine Checker

TABLE 3. Comparison of results obtained for iodate salt samples as measured by iodometric titration and the WYD Iodine Checker

	Titration			WYD Iodine Checker		
No.	*n*	Mean ± SD (mg/kg)	Range (mg/kg)	*n*	Mean ± SD (mg/kg)	Range (mg/kg)
1	12	28.6 ± 3.2	22.2–31.7	25	25.15 ± 1.3	23.1–27.9
2	3	55.2 ± 1.1	54.0–56.1	2	45.9 ± 0.1	45.8–46.0
3	3	83.2 ± 12.2	76.2–97.3	2	79.7 ± 0.4	79.4–79.9
4	9	114.1 ± 8.2	102.6–129.96	25	105.3 ± 6.3	91.9–114.7
17	2	78.9 ± 6.7	74.1–83.6	4	116.4 ± 34.1	92.2–166.7
24	2	16.4 ± 14.3	6.3–26.5	5	19.9 ± 16.7	0.8–45.5
26	2	43.4 ± 1.5	42.3–44.4	2	36.5 ± 2.1	35.0–38.0
29	2	20.3 ± 23.6	3.6–37.0	4	36.4 ± 9.9	28.4–49.2
30	2	13.3 ± 3.7	10.6–15.9	3	44.8 ± 20.7	27.0–67.5
31	2	22.3 ± 1.5	21.2–23.3	4	14.1 ± 7.3	5.0–20.5
32	2	47.1 ± 12.7	38.1–56.1	4	39.2 ± 37.8	14.7–95.5
33	2	33.9 ± 10.5	26.5–42.3	4	39.5 ± 17.3	17.4–56.1
34	2	38.1 ± 20.9	23.3–52.9	4	2.8 ± 1.6	0.9–4.5
35	2	4.8 ± 2.2	3.2–6.3	4	0.6 ± 0.1	0.5–0.7
36	2	31.8 ± 14.9	21.2–42.3	4	54.2 ± 14.6	39.1–72.9
37	2	5.9 ± 3.7	3.2–8.5	4	0.6 ± 0.2	0.5–0.9
38	2	43.4 ± 3.0	41.3–45.5	4	37.6 ± 9.7	30.6–52
39	2	14.3 ± 9.8	7.4–21.2	2	2.0 ± 0.6	1.5–2.4
40	2	9.0 ± 3.7	6.3–11.6	2	4.7 ± 3.4	2.3–7.1

samples was also hypothesized to be greater with the WYD Iodine Checker than with titration because the WYD Iodine Checker requires 1 g of salt for analysis, versus 10 g for titration. If the iodate was not uniformly distributed within the bag, the smaller amount of sample required for analysis by the WYD Iodine Checker increased the probability of variation among multiple analyses of the same iodate salt sample. To minimize the potential for variation when using the WYD Iodine Checker, 10 g of the salt sample should be diluted in a 50-ml vial. From the 50-ml vial, 1 ml is then used for analysis following the WYD Iodine Checker instructions.

We illustrated the bias between the two methods by plotting for each iodate salt sample ($n = 47$) the mean of the results (x axis) and the difference between the results of the two methods (y axis) (fig. 5). The mean difference was 3.4 mg/kg; however, this was a statistically nonsignificant bias (95% confidence interval, –0.06 to 6.94). This means that bias-free agreement existed between the two methods. The central 0.95 interval (mean difference ± 2SD) indicated that 95% of the determinations by the WYD Iodine Checker were 20.55 mg/kg lower to 27.43 mg/kg higher than the concentrations determined by iodometric titration. We detected slight concentration dependency of the difference between the two methods ($y = 0.195x - 4.803$, $r^2 = .192$), with the difference increasing with increasing iodate concentration of the salt.

Important final discussion points regard the ease of use, cost, safety considerations, and field applicability

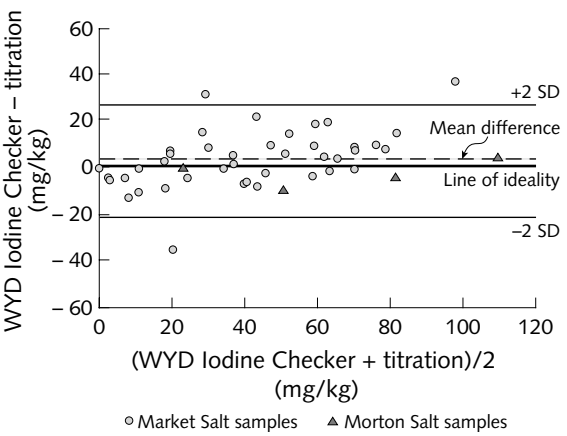

FIG. 5. Bland-Altman bias analysis to compare the difference between the WYD Iodine Checker and iodometric titration for iodate salt samples (43 market samples and four Morton Salt samples)

of the WYD Iodine Checker. The laboratory worker operating the WYD Iodine Checker in this study was a nonchemist with minimal experience preparing salt samples for analysis. The nonchemist worked with a chemist who prepared the solutions (e.g., standards, bromine solution, and sodium formate solution) required for the salt sample testing. The nonchemist reported the WYD Iodine Checker to be easy to operate, given the relatively simple sample preparation process and straightforward use of the instrument.

The WYD Iodine Checker kit costs approximately US$360 and is equipped with 100 ml each of solutions A and B. The cost of the burette and burette stand needed for titration is roughly US$260. When the supply costs required for both methods are calculated, the titration costs per sample are estimated to be US$1.50, compared with just over US$1 per sample with the use of the WYD Iodine Checker. The cost of the reagents used per sample is similar for both methods: US$0.06 for titration and US$0.07 for the WYD Iodine Checker.

Preparation of the solutions necessary for salt testing by the WYD Iodine Checker introduced important safety concerns and limitations in the field applicability of the instrument. Bromine solution, which is used for testing iodide salt, should be handled with caution and stored in a chemical fume hood. The safety concerns associated with handling bromine solution limit the field applicability of the instrument for measuring salt fortified with iodide. Second, in preparing the sulfuric acid solution or solution B, the acid must be added to the water slowly. Neither precaution is specified in the WYD Iodine Checker Manual, but both are significant safety concerns.

The 100 ml each of solutions A and B included in the WYD Iodine Checker kit can test about 45 to 48 samples. A chemist should prepare these solutions, plus the iodine working standard solution, bromine solution, and sodium formate solution when necessary. If only iodate salt is measured, the WYD Iodine Checker could be a useful tool for the field monitoring of salt iodine levels. To prevent contamination problems and to ensure proper functioning of the instrument and other necessary equipment (e.g., analytical balance), the WYD Iodine Checker should remain in a central field location for salt sample testing. Assessment of the performance of the WYD Iodine Checker in field conditions warrants further investigation.

Conclusions

The WYD Iodine Checker achieved better total assay and among- and within-assay precision than iodometric titration. This improved precision, along with the very good mixing accuracy of the WYD Iodine Checker (i.e., the accuracy of the measurement when salt samples with different iodine concentrations are mixed in different proportions) and its low limit of detection, verified that the instrument is highly precise, accurate, and sensitive. The measurements by the WYD Iodine Checker correlated well with titration measurements when iodide and iodate Morton Salt samples were tested.

We found a somewhat lower, but still acceptable, correlation between the WYD Iodine Checker and titration when testing iodate market salt samples. This lower correlation may not result from the analytical methods but rather from a lack of homogeneity of the salt being measured and/or the amount of sample used for analysis. One recommendation to minimize error caused by poor homogeneity of the salt would be to sample 10 g instead of 1 g of salt when using the WYD Iodine Checker.

The safety issues and chemical training requirements for the WYD Iodine Checker limit the field applicability of the instrument, particularly for measuring salt with iodide. However, further testing should be done to evaluate the field applicability of the WYD Iodine Checker for measuring iodate salt. A nonchemist reported the WYD Iodine Checker to be a user-friendly instrument based on a simple methodology with clear instructions for the sample preparation and testing of salt samples. The WYD Iodine Checker is easier to use by personnel who are not highly technically trained and is slightly less time-consuming than the titration method. Evaluation of the WYD Iodine Checker by the CDC indicated that the instrument is an accurate and reliable tool for the quantitative monitoring of the iodine concentrations of iodide and iodate salt.

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Adding an oxidant increases the stability of iodine in iodized salt

Huimin Shi

Abstract

It has been shown that moisture plays a critical role in the stability of iodine and that reducing agents in iodized salt reduce the stability of iodine. We question whether this is valid in all cases, and have found that the reducing agent may play a more important role than moisture in decreasing the stability of iodine.

We reviewed current methods to enhance iodine retention in iodized salt, and propose methods to produce stable iodized salt and to analyze its stability. Our experiments showed that when reducing impurities are removed, iodine remains stable in iodized salt, even when the salt is "wet."

We suggest that the stability of iodine in iodized salt can be improved by oxidizing iodized salt with sodium hypochloride, and that the iodine content of iodized salt, after heating at 120°C for one hour, can be used to reflect the quality of iodized salt. We have demonstrated that reducing agents play a critical role in the stability of iodine in iodized salt.

We have shown a method of purifying salt by removing reducing materials, which can be used to produce iodized salt with sufficient stability at lower cost. We also propose an analytical method to determine the stability of iodine in iodized salt. These methods could be further developed to achieve better accuracy, precision, and reliability and be applied to a greater variety of iodized salts.

Key words: Iodine, iodized salt, sodium hypochloride, stability

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Introduction

Diosady et al. have suggested that moisture plays a critical role in the stability of iodine, writing that "potassium iodate (KIO_3) can be reduced to elemental iodine by a variety of reducing agents in the salt" [and] "elemental iodine readily sublimes and is then rapidly lost to the atmosphere through diffusion" [1, 2]. We question whether this is valid in all cases, and in fact we have found that the reducing agent may play a more important role than moisture in decreasing the stability of iodine. Potassium iodate is known to be extremely stable at temperatures below 200°C without a reducing agent and will not decompose until the temperature reaches 500°C. Potassium iodate and its solutions are widely used as standard substances for many analyses. However, if there are reducing impurities in iodized salt, potassium iodate in iodinated salt will be reduced to iodine, and the iodine in iodized salt will be lost. Our experiments showed that when reducing impurities are removed, iodine remains stable in iodized salt, even when the salt is "wet." All samples used by Diosady [1] (including the Canadian reference sample) may contain different amounts of reducing agents, so moisture may play a greater role in decreasing the stability of iodine in their study cases.

Diosady also states that "salt purification is the best technical means of preventing iodine loss; however, this would be prohibitively expensive in the short term for many developing countries" [2]. The list of impurities includes carbonates, bicarbonates, total carbonates, calcium, magnesium, barium, potassium, iron, strontium, and sulfur [2]. The amounts of these impurities in the brine used to produce salt, especially calcium, magnesium, potassium, and sulfur, are relatively high. Purifying salt and removing these "impurities" would truly be prohibitively expensive for many developing countries. However, if our aim is only to retain iodine in iodized salt in the form of potassium iodate, the term impurities should be restricted to reducing agents, and salt purification means just the removal of those reduc-

ing agents. The cost of salt purification would not then be prohibitively expensive.

We analyzed whether salt produced in developed countries contains reducing agents and whether it needs advanced purification. Although many developed countries chlorinate the brine used to produce salt to remove reducing materials (such as hydrogen sulfide) before vaporization, the reducing materials in brine may not be completely removed. During vaporization, the facilities must be kept in a nonoxidizing condition to prevent metal corrosion. To achieve this condition, some vaporizing processes (including those in Canada) introduce reducing materials (such as sodium sulfite) to feed brine to remove oxygen. Although the concentration of such reducing materials in feed brine is relatively low, the concentration in salt-brine slurry could be high as a result of recycling of the mother liquid. In this case, salt dewatered from the salt-brine slurry could contain a certain amount of reducing materials. This may be the reason that Canadian salt packed in low-density polyethylene film bags loses 22.7% of its iodine after 12 months at 40°C and 60% humidity.

Diosady also writes, "by packaging salt in an effective moisture barrier such as solid low-density polyethylene bags, iodine losses can be significantly reduced" [1]. This is true for some samples then tested, but there was still a great loss of iodine after 12 months of storage. In some samples, such as the Chinese salt tested by Diosady et al., the losses were even more significant. Because the losses of iodine in various iodized salts are obviously different, retaining the iodine involves more than improving the packaging and storage of iodized salt.

The article also mentions that "in order to make allowances for the probable losses of iodine, countries must determine iodine losses from local salt iodized under local conditions, as these will be greatly affected by salt source, quality and processing technology" [1]. However, iodine losses from local iodized salt under local conditions are difficult to determine. In developing countries, there are many plants producing iodized salt. Even at the same plant, the iodine losses of iodized salt differ according to when it was processed, although the storage conditions are the same. In some plants, for example, the concentration of sodium thiosulfate in the initial feed brine can be as low as 10 mg/L. As the brine is vaporized, the concentration of sodium thiosulfate in the resulting salt-brine slurry gradually rises to more than 1,500 mg/L. As time goes by, the salt dewatered from this salt-brine slurry contains more and more reducing material. As a result, iodine losses in iodized salt are usually inconsistent.

Current methods of iodine retention

The following methods of enhancing iodine retention in iodized salt are in current use.

Enhanced packaging. Enhanced packaging can significantly lessen iodine losses by reducing the moisture content of iodized salt and the permeation of gases in the period of storage. However, this method cannot prevent oxidation-reduction due to residual reducing materials in salt, which can generate significant iodine losses.

Increasing alkalinity of salt. Adding soda to iodized salt increases the pH and decreases $[H^+]$, thereby decreasing iodine losses. However, there are some problems associated with this method. First, wet salt with a large amount of soda solution is difficult to dry in a fluidized-bed drier due to the high content of water in wet salt. Second, the public may dislike such alkali salt. Third, the cost of this treatment is high.

Heat-iodized salt. Iodized salt that has been heated at high temperatures for several hours is stable. However, there are disadvantages. First, the iodate added to the salt is partially reduced and lost during the heating procedure. Therefore, the cost of iodizing the salt will rise. Second, heating iodized salt at high temperatures for several hours is difficult to achieve on an industrial production scale. Third, as the content of reducing materials in iodized salt varies, the amount of lost iodate, the necessary excess amount of iodate, and the content of iodate in iodized salt vary. Finally, the iodine lost in the production process might give rise to an increase in the content of reducing materials in salt slurry, which could in turn give rise to a vicious cycle of addition and loss.

Production of salt with purified brine. Using such purified salt would improve the stability of iodine. However, brine purification removes a relatively large amount of impurities, such as Ca^{+2}, Mg^{+2}, and SO_4^{-2}, and is very costly. Therefore, according to Diosady [1], "it would not be technically or economically feasible in the short term in many developing countries." In addition, the iodized salt is not completely stable, even when purified salt is used.

Proposed processing and analytical methods

It is crucial to explore the processing methods used to produce iodized salt and the analytical methods used to determine the stability of iodine in iodized salt. The following are some suggestions.

Purifying salt

To purify salt, an oxidant is added to wet salt to remove the reducing materials. Since the quantities of reducing materials in wet salt are less than those in brine or in salt-brine slurry, purifying salt is easier and less costly than purifying brine. Removal of reducing materials by

this method is more efficient than removal by the use of purified brine.

Analytical method

Ten grams of iodized salt is placed in a 200-ml flask and heated in a oven at 120°C for one hour, and then is analyzed by titration. The iodine content of the salt is referred to as the MCIS (minimum content of iodate in iodized salt). The quantity of iodine lost after heating (L value) is regarded as the content of reducing materials in the iodized salt. We propose that the L value and the MCIS value be used to reflect the quality of this iodized salt. The MCIS value, especially, can be used as the stability index of iodized salt. Although there are many suppositions in this method, the quality and stability of iodized salt can be at least partly evaluated.

To show that the reducing agent plays a critical role in the stability of iodine, and to examine the suggestions given above, we performed the experiments described below.

Background of the experiments

The reaction that gives rise to the iodine losses from iodized salt is an oxidation-reduction reaction, expressed as:

$$IO_3^- + 6H^+ + \text{“reducing material”} = \tfrac{1}{2}I_2 + 6H_2O + \text{“oxidized material”}$$

Since the velocity of an oxidation-reduction reaction is relatively lower than the diffusive velocity of iodine vapor, this reaction is a control reaction for the velocity of iodine losses from iodized salt.

The oxidation potential of potassium iodate would be $E(V.)$.

$$E = 1.2 + 3.22 \times 10^{-5} \times T \times \log([IO_3^-][H^+]^6[I_2]^{-1/2})$$

E is a function of $[IO_3^-]$, $[H^+]$, and $[I_2]$. The higher the $[H^+]$, the larger the E will be.

Reducing materials include all materials (such as Fe^{+2}, I^-, Br^-, S^{-2}, $S_2O_3^{-2}$, SO_3^{-2}, $S_2O_4^{-2}$, and organic compounds) with oxidation potentials lower than the above-mentioned E. Therefore, the term “reducing material” refers to all materials that can reduce iodate in iodized salt to iodine rather than some specific substances. Since E is a function of $[IO_3^-]$ and $[H^+]$ in iodized salt, the quantity of reducing materials in iodized salt changes with $[IO_3^-]$ and $[H^+]$ in iodized salt. In this case, we would like to work out some analytical methods and special indices, like COD (chemical oxygen demand) in water analysis, to show the quantities of reducing material, and to show the minimum content of iodate in iodized salt stored in certain conditions for several months. Let us suppose that we can, and use the MCIS index to reflect and manage the quality of iodized salt.

When the reducing material is added to iodized salt, the reaction velocity is a function of $[IO_3^-]$ and $[H^+]$, as well as the temperature at which the iodized salt is stored. According to chemical kinetics, a reaction between ions in solution is quicker than one in the solid phase. As a result, the higher the moisture content of iodized salt, the quicker the reaction is. The higher the $[H^+]$ of iodized salt (i.e., the lower the pH), the more iodine will be lost. Diosady noted, however, that “the effect of pH was also not clear-cut” [1]. The pH of salt was determined by these researchers at a time when the samples were received but not after the samples had been stored. The pH of the salt had continuously changed during the period of storage due to absorption of gases such as CO_2 and SO_2. Second, they compared the effects of pH among different samples rather than within the same sample. Third, the pH they found was in the range between 6.25 and 9.77, but not higher.

The higher the temperature, the quicker the reaction. This kind of oxidation-reduction reaction would supposedly have a reaction velocity thousands of times faster at higher temperatures, for instance 120°C, compared with a reaction at ambient temperature.

Materials and methods

The materials (salt, potassium iodate, sodium hypochlorite solution, and potassium ferrocyanide) used in our study were all of industrial grade.

Iodized salt, from which reducing materials had been removed by adding oxidants, was stored for 1, 2, 3, 6, 10, 12, and 18 months. The iodine content in these samples was then analyzed to determine whether the reducing agent played a critical role in the stability of iodine. The experiments on the removal of reducing materials in iodized salt were performed at industrial production scale (160,000 tons of salt per year), and the chemical analyses for iodized salt were performed according to GB/13025.3-91, GB/13025.4-91, GB/13025.5-91, GB/13025.6-91, GB/13025.7-91, and GB/13025.8-91 [3]. The sodium hypochlorite solution was analyzed by the titrimetric method (iodimetric analysis and alkalimetric analysis).

Results

Experiments using the analytical method

Samples taken from several plants were heated in a controlled-temperature oven at 120°C for one hour. The iodine in these samples, before and after heating, was determined by titration. The potassium iodate content is shown in table 1.

It is clear from these data that there were some losses of iodine during heating, and heating can be used to

TABLE 1. Potassium iodate content of iodized salt from several plants, before and after heating

Measurement	Plant no.					
	1	2	3	4	5	6
KIO_3 (mg/kg) before heating	65.3	20.2	57.6	31.2	38.2	40.0
KIO_3 (mg/kg) after heating (MCIS[a])	59.8	13.2	36.7	29.7	34.5	26.1
Loss (%)	8.4	35	36	4.7	9.6	35

a. MCIS, Minimum content of iodate in iodized salt.

show the stability of iodized salt.

Experiments in removing reducing materials in iodized salt

We used a sodium hypochlorite solution as our oxidant and used the following procedure:

wet salt (from centrifuge) → addition of sodium hypochlorite solution by spraying on transfer belt → mixing → drying in fluidized-bed drier → addition of potassium iodate solution and potassium ferrocyanide by spraying on transfer belt → mixing and transfer to salt storehouse with transfer belt → packing

In our first experiment, we added 84 L of sodium hypochlorite solution (NaOCl 110 g/L, NaOH 20 g/L) to 151.7 ton of salt. This is equal to 0.557 L (0.061 kg) of sodium hypochlorite per ton of salt. The salt produced was packed in polyethylene bags. Two bags of salt (about 15 kg), with different amounts of KIO_3 added and poorly sealed, were stored in a humid place near a water pool in our laboratory. Analyses were performed on those samples; the results are shown in table 2.

When exposed to intense humidity, the salt in the bags gradually changed, but it can be seen from these data that the iodized salt sprayed with sodium hypochlorite solution was relatively stable. Although the exact moisture content of the salt was not determined, the surface of the salt was wet to the touch, suggesting that moisture does not play a critical role in the stability of iodine in this case. The MCIS values were never higher than the potassium iodate content of iodized salt, even after 18 months of storage. Therefore, that MCIS value might be used to reflect the quality of iodized salt.

In our second experiment, we added sodium hypochlorite solution (NaOCl 121.5 g/L, NaOH 8 g/L) to wet salt. This was equal to 0.557 L (0.068 kg) of sodium hypochlorite per ton of salt. The cost of this oxidant is extremely low (less than US$0.04 per ton of salt). The salt produced was packed in polyethylene bags (50 kg per bag). The bags were adequately sealed and stored six bags high and six bags across in a common salt storehouse (table 3).

The content of these iodized salts was NaCl 99.49%, H_2O 0.25%, insoluble in water 0.2%, $CaSO_4$ 0.12%, $CaCl_2$ 0.05%, and $MgCl_2$ 0.03%.

As mentioned earlier, iodized salt sprayed with sodium hypochlorite solution is relatively stable. Thus, we confirmed that a reducing agent plays a critical role in the stability of iodine. The amount of sodium hypochlorite added in our experiments was about three times the amount that was theoretically necessary to remove the reducing materials. The excess sodium hypochlorite fully decomposed, and the resulting oxygen was blown out in a fluidized-bed dryer at an elevated temperature (120°C) for about 6 minutes. The temperature of dried salt at the outlet of the dryer was about 50°–60°C. The odor of the processed salt, which is similar to the odor of bleach powder, fully disappeared while the salt was packed.

Conclusions

We have demonstrated that reducing agents play a critical role in the stability of iodine in iodized salt. We have shown a method of purifying salt by removing reducing materials, which can be used to produce iodized salt with sufficient stability at lower cost. We have also

TABLE 3. Stability of iodine in iodized salt, oxidated with sodium hypochloride in the second experiment

Original KIO_3(mg/kg)	KIO_3 (mg/kg) after storage		
	1 mo	6 mo	12 mo
101.5	103.6	96	116.3

TABLE 2. Stability of iodine in iodized salt, oxidated with sodium hypochloride in the first experiment

Bag no.	Original KIO_3 (mg/kg)		KIO_3 (mg/kg) after storage					
	Unheated	Heated 1 h at 120°C (MCIS[a])	1 mo	2 mo	3 mo	6 mo	10 mo	18 mo
1	53.8	52.1		54.5		54		
2	26.4	25.3	26.7		28.1		27.4	27.3

a. MCIS, Minimum content of iodate in iodized salt.

proposed an analytical method to determine the stability of iodine in iodized salt. These methods could be further developed to achieve better accuracy, precision, and reliability and could be applied to a greater variety of iodized salts.

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Commentary on "Adding an oxidant increases the stability of iodine in iodized salt"

The author makes the important point that humidity by itself is not an independent causal factor in the reduction of iodine content in iodized salt. This offers an explanation of why better packaging does not necessarily prevent the effect of humidity when reducing agents are present.

We know from work in the 1950s at the Institute of Nutrition of Central America and Panama (INCAP) that iodate is more stable than iodide [1]. The oxidation-reduction reaction of iodide is much quicker than that of iodate. This has been the basis for recommending the use of iodate in hot and humid climates. However, even when iodate is used for salt iodization, some decomposition to elemental iodine happens in the presence of reducing agents. At a given concentration of reducing agents, the reaction is quicker at lower pH (higher H^+ concentration) and in the presence of humidity.

Regarding the practical implications of using NaOCl/NaOH as proposed by the author, there are two things to note. Heating and drying the salt at 120°C after treatment needs evaluation as to its cost and the organoleptics of the end product; moreover, the use of the oxidant NaOCl will influence the titration value of iodine. To assure the stability of iodized salt, therefore, it is most important that the amount of reducing agents be minimized. When reducing agents are present in salt, the second consideration for stability is the pH (H^+ concentration) and humidity. A low pH and a relative humidity above 75% promote the reaction.

The practical relevance of these factors to the stability of iodate in iodized salt therefore depends on the source of the salt, the production method, or both.

Sea salt. Sea or solar salt normally has a pH close to neutral but is high in organic and chemical impurities. Additives are not always used in its production; when they are used, anticaking or free-flow agents are more usual. Sea salt is comparatively humid when dispatched (even when bagged in polyethylene). In terms of iodine stability, iodized sea salt is often the least stable among the various types of salt produced in the world.

Evaporated salt. Without prior brine purification, evaporated salt normally also has a pH close to neutral, but it has many impurities, mostly chemical. This salt is more expensive to manufacture than sea salt. Additives, such as anticaking agents, are often used. This is the most common type of salt produced in China. In China, many brine sources are high in sulfide ("black brine"), which accelerates the decomposition of iodate, as indicated by the author. The author proposes research to establish the value and feasibility of adding an oxidant plus raising the pH. He proposes a hypochlorite/sodium hydroxide solution. These chemicals are cheap, but the economics of managing this extra step must be considered too.

Evaporated (rock) salt after purification of brine. The evaporated salt produced from purified brine (PVD, or pure vacuum-dried salt) is still more expensive. PVD salt has a high pH (alkaline: thus a low H^+ concentration) and hardly any impurities, and anticaking agents are usually added. This would seem the type of salt in which iodate is stable (this is the type that was used when salt iodization started with iodide in the West).

Most of the world consumes salt other than PVD, and we cannot wait to eliminate iodine deficiency until PVD salt is provided to and accessed by all, which makes the proposed research important. We need to know more about the removal of reducing agents and its potential in fine-tuning recommended levels of iodate in consumption salt as well as potential savings in its manufacture.

The author also proposes the MCIS (minimum content of iodate in iodized salt) criterion as a normative index for defining the "true" iodine content of iodized salt. Again, this would be most relevant for non-PVD salt (see above). For quality assurance by producers and for food inspection at production, the MCIS might be less subjective than the present titration results in the absence of further chemical information, such as reducing agents, pH, and humidity. Again, more data on different salt sources and types are needed.

In summary, the author's observations on stability are relevant for the usual situation in most of the world, where large populations depend on the salt supply from the sea or from underground sources with simple evaporation.

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Biocultural diversity in the sustainability of developing-country food systems

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Abstract

The policy implications of a model of contemporary food systems for developing countries that integrates nutrition, reduction of disease risk, culture, income generation, and biodiversity are reviewed within a theoretical and empirical examination of the relevance of nutrition to the priorities put forward at the World Summit on Sustainable Development in Johannesburg, South Africa, 2002. Agricultural, health, economic, and social policies with local reach are necessary responses to the increase in noncommunicable disease associated with the globalization of food systems. Nutrition offers a nexus for the changes in individual behavior and motivation essential for fundamental shifts in production and consumption patterns. Mutual consideration of biocultural diversity and nutrition can guide policy, research, promotion, and applied action in developing countries. Benefits from enhanced use of biodiversity must legitimately flow to the undernourished poor, while potential negative consequences must be minimized and mitigated. Quality and quantity of food need not be mutually exclusive. Functions related to energy density, glycemic control, oxidative stress, and immunostimulation define important research priorities. Tests of the hypothesis that biodiversity equates with dietary diversity and health might combine quantitative indicators of dietary and biological diversity with nutrition and health outcomes. Biodiversity, where it is part of traditional agricultural and food systems, can be best conserved and enhanced through rational use within a broad-based developmental focus on small-scale and low-input production. The fact that traditional systems, once lost, are hard to recreate underlines the imperative for timely documentation, compilation, and dissemination of eroding knowledge of biodiversity and the use of food culture for promoting positive behaviors.

Key words: Agro-biodiversity, dietary diversity, functional food, nutrition transition, wild food, WSSD

Introduction

Antecedents to profound dietary changes that are rapidly redefining nutrition and health priorities in developing countries parallel those that constrain environmental sustainability. Healthy diets for populations depend on availability and accessibility, within a context that promotes and supports healthy behaviors, of a variety of plant and animal foods. Although both these resources and positive behaviors are characteristic of traditional food systems, contemporary trends simultaneously erode biodiversity and the sociocultural context in which it is conserved.*

Nutrition policies, research, and applications should be guided by concerns for sustainable development. The World Summit on Sustainable Development (WSSD) in Johannesburg, South Africa, 2002, re-established and extended a steep challenge to the international community [3]. Specifically, its Plan of Implementation calls for the promotion of three interdependent and mutually reinforcing pillars of sustainable development: economic development, social development,

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* *Biocultural* emerges conceptually from an anthropological consideration of the manner in which human societies adapt to the varied biological circumstances in which they live. *Biocultural diversity* is concerned with the relationships among traditional knowledge, biological diversity, and cultural diversity [1, 2].

and environmental protection. In seeking to define tangible and relevant approaches to global problems it put forward five key focal areas—water, energy, health, agriculture, and biodiversity (WEHAB)—with an accompanying call to pursue the interrelatedness among these themes [4] along the lines put forward in the Indaba Declaration on Food, Nutrition, Health and Sustainable Development from the WSSD Implementation Conference, 2002 [5]. Nutrition, being intrinsically multidisciplinary, offers timely heuristic lessons for making the concerns of WEHAB mutually reinforcing, as well as a strategic role in providing key linkages with the emerging Global Strategy on Diet, Physical Activity and Health [6]. For nutrition, linkages arise most naturally between health and agriculture, as well as in relation to water and sanitation.

The present review extends the interconnections, first with concern for the nutritional consequences of economic and environmental changes on food systems, and more amply in considering the importance of biodiversity for dietary diversity and health. Its focus is consistent with the insights and important contributions of food systems research and interventions linking nutrition and agriculture [7, 8], while extending an emphasis on biodiversity. It draws on evidence-based research and consolidates the few initiatives and contributions in the literature that are addressing these issues in theory and practice. In addition to presenting a theoretical and empirical basis for the increasing interconnection of nutrition and environmental considerations, this review identifies the policy implications of a desired model for improving contemporary food systems by integrating nutrition, reduction of disease risk, culture, income generation, and biodiversity (fig. 1). International and national policies that build on the biodiversity and cultural strengths inherent in traditional food systems optimize the chances for vulnerable populations to adapt to changing conditions in a sustainable manner.

Forestalling the imminent extinction of up to one-quarter of the world's wild species and the loss of important agro-biodiversity, while at the same time assisting the 800 million undernourished humans and some 1.2 billion living in extreme poverty, sets a formidable task [9]. Simply feeding the world's growing population by 2030 brings a threat of large-scale natural destruction. Meanwhile, dietary patterns are changing, and obesity accelerates at unprecedented rates [10]. Solutions are neither obvious nor realistic when taken in isolation. A biodiversity-focused strategy therefore has relevance within a multipronged approach that includes improved and sustainable production technologies, changes in trade agreements and food-pricing policies [11], poverty reduction, education, and improved health care.

Fundamental changes in human behavior can be founded on economic incentives, health benefits, values, and knowledge. Although extensive diversity may not be necessary for humans to satisfy basic nutritional needs, within a sociocultural context traditional biodiversity use is a potentially powerful vehicle for maintaining and enhancing health-positive behaviors [12, 13]. Conversely, health and economic gain can be mutually reinforcing of biodiversity conservation, as

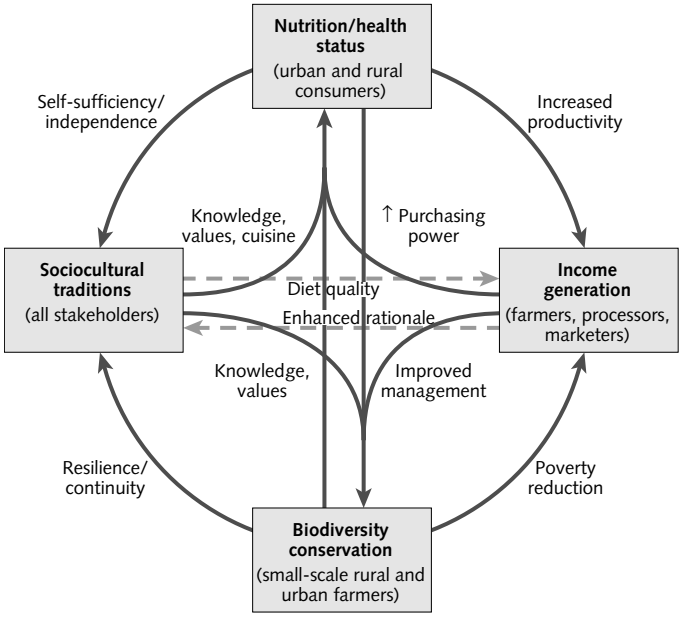

FIG. 1. Population-level synergies linking biodiversity conservation and human nutrition in developing countries

they provide the impetus for positive practices and sustainable development.

While formal declarations related to environment or nutrition acknowledge the other in condensed terms or development jargon codewords such as food security or health and sustainability, respectively [e.g., 14–16], the WSSD presents a new imperative to move beyond mutual deference. In retrospect, the World Declaration and Plan of Action for Nutrition (WDPAN) [17] is attentive to sustainability, with clear calls that improved nutrition and health should be founded on environmentally sound development, including the conservation and protection of biodiversity and traditional resources. Food-system approaches to nutrition also characteristically emphasize the sustainable use of resources [7]. More recent summits, symposia, and some intervention initiatives have taken up these themes [18].

Contemporary changes in food systems

Forces of globalization, commercialization, population increase, and urbanization change patterns of production and consumption and profoundly affect human diets. High-input, high-yield agriculture and long-distance transport increase the availability and affordability of refined carbohydrates (wheat, rice, and sugar) and edible oils [6, 11, 19, 20]. While making greater numbers of people secure in terms of energy, they also underpin the so-called nutrition transition [10, 21] and can undermine the self-sufficiency and economic viability of local producers [11, 19]. India, for example, has in the last five years gone from self-sufficiency to being the world's largest importer of edible oils. At the same time as changing trade policies undermine the livelihoods of local farmers, health changes associated with changes in the Indian diet [22] are further exacerbated. Edible oils, imported rice, and wheat also replace traditional cereals as the main energy sources in African countries [23]. In Sub-Saharan Africa, importation of food has increased the yearly per capita consumption over the past 30 years by about 7 kg for rice and wheat and 2 kg for edible oil [20]. Among periurban populations of Dakar, Senegal, we observed in a recent survey that imported rice, wheat bread, and sugar accounted for the majority of calories in diets, with high rates of micronutrient deficiencies. Nonetheless, traditional millet foods, which account for only 12% of energy, remain the largest contributor of iron (providing 46% of the intake although bioavailability reduces the contribution).* In addition, globalization of culture and commercial activities promulgate a westernization of developing-country food systems and diets [19]. For example, Bourne et al. report that in South Africa, increased westernization of diets is occurring even in rural areas [24].

Such changes also profoundly affect local systems of production. Fewer farmers engage in subsistence agriculture in the classic sense. Most are increasingly oriented to markets for both income and food purchase [18, 24–26]. Market factors alter traditional cropping patterns and, in general, result in erosion of the agricultural biodiversity represented by traditional crops and varieties [19]. Commercialization can, however, provide opportunities through which biodiversity can be retained and enhanced, as has been documented in Vietnam [25]. In other contexts, home gardens that serve as important complementary resources for diet and medicine [27] as well as important repositories of biodiversity may assume less importance [25, 28, 29].

Commercial monocropping can offer economic benefits to rural populations and reduced food costs to consumers, but it has mixed impacts on nutritional status [30, 31], in part because of reduction in traditional dietary diversity [31]. Changes in land use, including disturbance, deforestation, and appropriation of natural areas, diminish opportunities for gathering and hunting the essential wild components of many traditional food systems [1, 32]. Contamination from industrial and agricultural activities further undermines traditional and indigenous food systems and health [32], while climatic change will probably adversely affect crop production and nutritional status, at least in Africa [18]. Growing pressure on water resources also directly affects productivity and food security [18].

Since most of the world's impoverished populations live in countries harboring the largest amounts of biodiversity, conservation and poverty cannot be addressed independently [4, 14]. Sustainable development thus requires coupling investment in rural enterprise and infrastructure with sound resource management. The model put forward here assumes in its simplest form that small-scale farmers can manage and use traditional agro- and wild biodiversity to comparative economic advantage on the premise that the products marketed are desired by, and offer nutritional and sociocultural benefits to (increasingly urban) consumers. Linking biodiversity and health is both a response to the consequences of economic growth and a way to direct growth in a positive manner.

Diet-related trends in developing-country demographics and health

Food insecurity and undernutrition

In the face of persistent food emergencies and the scale of global hunger, addressing nutrient deficiencies remains an immediate priority. Food deficit and low

*Spigelski, Johns, and Gray-Donald, unpublished results.

dietary diversity in both animal and plant foods simultaneously characterize food inadequacy for the chronically undernourished [33], although the current low cost of staples relative to nonstaples means that diets can be simultaneously adequate in energy and deficient in micronutrients [34]. The fact that access to quality food is often a problem of affordability rather than availability underlines the fundamental importance of poverty alleviation. The fact that large-scale agriculture in India produces surpluses while 250 million rural farmers remain malnourished [18] suggests that advanced technology is not a complete solution. Unless policies and programs for food security approach food systems in holistic terms, they will exacerbate malnutrition and disease in the long term.

Nutrition transition

Increasing numbers of the malnourished poor live in urban areas. Consumption of a diet derived from high-energy foods of plant and animal origin coincides with low energy expenditure. The greater diversity, including fruits and vegetables, generally available to urban populations does not necessarily translate into consumption [21], particularly for the poor. An increasing number of urban residents depend on "junk food" or street foods, which are too often fried foods of low nutrient density [33, 35, 36]. Processed foods available for purchase through contemporary market systems, while potentially variable in brand and formulation, may have limited actual biological diversity, often related to the use of imported replacements for local foods.

The nutrition transition is leading to emerging epidemics of type 2 diabetes mellitus, cardiovascular disease, obesity, cancer, and other chronic noncommunicable diseases, even within poor countries [6, 10, 21]. The consequences of a high-carbohydrate, high-fat diet are further complicated and compounded among the disadvantaged in developing countries, where dietary changes in combination with poverty and high rates of infectious disease and undernutrition create a double burden [10, 21].

In Latin America and elsewhere, cheap food energy combined with low diversity and nutritional quality produces a pattern of obesity, particularly of women, in combination with household undernutrition [34]. Early childhood malnutrition (fetal programming) probably increases susceptibility to diabetes and other conditions in later life [21]. Epidemics of chronic noncommunicable diseases can be expected to further accelerate in countries with aging populations.

Promising models of food systems in transition

In the transition to lifestyles more characteristic of Western industrial societies, countries that retain a strong traditional food system in which diet has recognized health, cultural, and ecological roles best avoid the often concomitant increases in chronic noncommunicable diseases [21]. Asian and Mediterranean diets [37] provide the clearest examples. Kim et al. [10, 38] offer sociocultural explanations for the lower than expected rates of chronic noncommunicable diseases in South Korea. The congruence of physiological, cultural and ecological function is well represented by the concept of *Sin-To-Bul-Yi* ("A body and a land are not two different things"). Strong social marketing emphasizes the higher quality of traditional dishes based on the interpretation that "a person should eat foods produced in the land where he or she was born and is living." Okinawan food, which has been strongly influenced by Chinese ideas of longevity through traditional diet, offers a further example [39].

Traditional systems often see food, medicine, and health as interrelated [40, 41]. Food may have strong symbolic and religious value and is highly associated with cultural identity and social well-being [41]. The foods of indigenous peoples form part of rich knowledge systems [2]. They typically draw on indigenous resources, are based on local production, and are associated with the land and environments from which they are obtained. The merits of such concepts for guiding contemporary adaptation are testable in general terms, in the first instance in relation to scientific evidence for the health benefits of traditional food biodiversity, and second for their validity as a sociocultural basis for positive systems.

Empirical base for risk-reduction potential of developing-country foods

The fact that traditional food systems provide the inspiration for seminal insights into the relationship of diet and health (for example, the importance of fiber, omega-3 fatty acids, and antioxidants in African, Inuit and Mediterranean, and Asian diets, respectively [42]) underlines the theoretical value of their investigation [43]. For societies in transition, diets characterized by indigenous cereals, legumes, and fruits and vegetables provide lower energy content and higher fiber than the staple commodities [39] and presumably reduce the risk of disease. In general, however, the evidence for the health benefits of traditional foods is circumstantial and is rarely based on randomized, double-blind clinical trials or epidemiological studies. In sum, the evidence is similar but marginally less than the body of data supporting the disease-risk-reduction values of foods generally [39]. Nonetheless, a number of dietary factors of potential importance for specific health conditions in a developing-country context can be considered.

Dietary diversity

A handful of epidemiological studies underline the benefits of a varied diet, particularly one including fruits and vegetables, in increasing longevity and reducing the rates of chronic degenerative diseases [36, 44] and in improving nutritional quality and child growth in developing countries [36, 45–47]. The diversity of indigenous crops and wild plant and animal species available in most tropical countries, in addition to providing essential nutrients, presumably offers broad benefits to health [45, 46]. Considering the difficulty in precisely identifying optimal diets, a diverse and balanced diet, including legumes, fruits, vegetables, and animal-source foods, provides an intrinsic buffer against the uncertainties of change and remains the preferred choice for human health [48].

Nutritional value of traditional edible species and varieties

Although wild and cultivated biodiversity in most developing regions is ignored in dietary surveys, compositional analyses, Food and Agriculture Organization food balance sheets, and policy and decision-making [49], such resources unquestionably make essential contributions to dietary adequacy [36, 50, 51]. Studies of home gardens have made the links between diversity and nutritional status [27, 36, 52]. In exceptional cases, the contribution of specific nutrients from gathered species has been clearly demonstrated [46, 53], while many indigenous species have exceptional nutritional properties [54].

Documentation of the contribution of intraspecific diversity to nutrition and health has received little attention and analytical resources. Farmer-based research demonstrates the wealth of traditional knowledge and beliefs concerning the health, sensory, and culinary properties of local crop varieties [55]. Screening of major crops [36, 52, 55, 56], while incomplete, clearly documents wide variation in nutritional and functional properties that undoubtedly has implications for the nutritional status of populations and individual consumers (in addition to its usefulness to plant breeders). The potential genetic variation in nutrient composition within neglected and underutilized species [50, 52, 57] has been even less documented.

Reduction of the risk of cardiovascular disease and diabetes

The role of foods and food constituents such as soy protein, flaxseed, cereal fiber, plant sterols, omega-3 fatty acids, fish, and lycopene in reducing risk factors for cardiovascular disease [58] has implications for developing country diets. Less-studied and widely distributed foods, such as whole-grain cereals including buckwheat [59], grain amaranth [60], and millet [61], various leafy vegetables [62], grain legumes [63], fermented foods, and foods high in antioxidants, offer similar potential as part of various traditional food systems.

Variation in the glycemic response to foods that comprise major portions of developing-country diets, including varieties of rice [64], finger millet [65], and buckwheat [59], has profound health implications. Soluble and insoluble fiber, digestibility-inhibiting phytochemicals in food, and the nature of particular carbohydrates improve glycemic control [66]. A number of foods contain compounds that directly affect insulin resistance, e.g., bitter gourd and fenugreek [66]. High intake of fruits and leafy vegetables is associated with low glycosylation of hemoglobin and may contribute to the prevention of type 2 diabetes [67]. Common polyphenolics, such as the isoflavonoid genistein and curcumin, inhibit the formation of advanced glycation end-products [68], low-density lipoprotein peroxidation [68], and lens aldose reductase [69].

Blindness and vision impairment

Ingestion of the xanthophyll carotenoids lutein and zeaxanthin, which comprise the major macular pigments, may reduce the risk of age-related macular degeneration and cataracts [71], the leading cause of blindness worldwide. The benefits of non-nutrient carotenoids in leafy vegetables, which represent rich biodiversity in African and many Asian food systems [50], may exceed those attributable to beta-carotene or other nutrients. For example, vegetable diets that make modest contributions to improving vitamin A status result in significant increases in serum levels of lutein [72]. Nigerian patients with cataracts had consistently lower intakes of fruits and vegetables than control subjects [73]. Even for xerophthalmia, food-based strategies that increase dietary variety offer benefits in directly increasing vitamin A intake and improving its utilization [74].

Lens aldose reductase inhibitors, which include the common flavonoid quercitin and other antioxidants, may mediate diabetes-related retinopathy [68]. Hyperinsulinemia may contribute to increasing rates of myopia and other diseases in populations with high consumption of rapidly digestible refined carbohydrates [75], an observation that suggests a mechanism through which dietary modification can mediate such conditions.

Communicable disease

Probiotic [76], immune-stimulant [77], and antibiotic [36, 41] properties of traditional foods offer largely unexplored benefits in reducing diarrheal and other infectious diseases.

Other potential functional properties

Similarly, insights into cancer risk reduction attributable to antioxidants, flavonoids, carotenoids, lutein, phytoestrogens, and cruciferous vegetables extrapolate to indigenous foods [78]. Within developing-country contexts, spices [79] and other foods may also offer specific anticancer benefits. A number of other functional properties, such as further antioxidant activities, cognitive improvement, antidepression, and modulation of xenobiotic stress, demonstrate the largely unexplored potential benefits of traditional diets.

Merging empirical evidence with sociocultural values in practice

Authentic global sustainability depends on a paradigm shift in human values and behavior leading to profound changes in production and consumption patterns [3]. Accordingly, the following disparate examples suggest the beginnings of a common coalescence in the way traditional culture can combine with empirical evidence to both meet human needs and increase the value of biodiversity. Extensive efforts and published works related to food-systems approaches over a number of years provide a conceptual and practical context for these and other initiatives [7, 8, 13, 23].

Food-based dietary guidelines

Food-based dietary guidelines (FBDGs), as developed from the WDPAN and subsequent initiatives, emphasize the use of locally available foods, food variety, traditional cuisines, and culturally sensitive methodologies to address both undernutrition and the nutritional transition [80]. An evidence-based approach to nutrition and health function helps direct the production, preparation, processing, and development of foods. FBDGs serve as the basis of both public education and sound public policy.

In their most elaborated presentation, that for the Western Pacific Region [80], FBDGs identify the environmental contributions from promoting local and traditional foods as reduction in fossil energy use and pollution from long-distance transportation and intensive agriculture. Surprisingly, while extolling food variety, the discussion is silent on the benefits for enhancing biodiversity.

Lessons in synthesizing sociocultural values, economic development, and scientific research from the Asian regions [38, 39] where poverty has declined most significantly in the past 30 years offer potential guidance for countries, particularly in South Asia and Sub-Saharan Africa, where poverty and malnutrition have not declined.

Nutrition of indigenous peoples and the environment

Both the WDPAN and the WSSD acknowledged the special case of indigenous peoples. The unique lifestyles, knowledge systems, and other means by which indigenous communities meet their nutritional needs offer extant and badly needed models of how humans can adapt using the local resources available in varied environments [32]. Indigenous communities are both sentinels of environmental distress and stewards of important biodiversity [81]. At the same time, they are among the most marginalized and impoverished in the contemporary world. As victims of sociopolitical factors outside their control, economic development, and environmental change, they are profoundly implicated in environmental issues and play important symbolic, political, ethical, and practical roles of leadership in the struggle for universal sustainability.

In consort with initiatives such as the Centre for Indigenous Peoples' Nutrition and Environment (CINE) (http://cine.mcgill.ca) [81], the IUNS Task Force on Indigenous Food Systems and Nutrition (http://www.iuns.org/taskforces.htm) [23], and others [82], indigenous communities engage in community-based scientific research to guide the course along which they can continue to meet their subsistence, economic, and social aspirations.

On-farm conservation of agricultural biodiversity

In Nepal, community-based approaches to agro-biodiversity conservation promote the value of landraces through cultural linkages, market incentives, and health associations [83], while a project of the International Plant Genetic Resources Institute (IPGRI) on traditional leafy vegetables [50] is developing along similar lines in Sub-Saharan Africa.

Functionality as a physiological, environmental, and philosophical construct

For developing countries, something approaching functionality is emerging de facto as communities around the world adapt traditional systems and values to modern socioeconomic situations, while synthesizing traditional knowledge with ideas drawn from the global information arena.

Food functionality as a contemporary concept embraces aspects of scientific research (and conjecture), changing consumer values, and entrepreneurial initiative [39, 84]. It likewise presents one step toward the change in consumption and production patterns in which the WSSD states developed countries, given their past records, must take a lead [3].

A desired outcome of reducing the risk of chronic noncommunicable diseases on the one hand, and issues

of production, marketing, and regulation on the other, define policy discussions of functional foods in developed and, to a growing degree, developing countries [85]. Nonetheless, philosophical and ethical considerations that embrace aspects of self-sufficiency, spirituality, nostalgia for the past, and environmentalism, as well as physical health (real or imagined), motivate consumers of functional foods and dietary supplements [84]. Organic foods, vegetarianism, and the resistance in some jurisdictions to genetically modified foods further illustrate the passions, perceptions, and misperceptions—with interesting links to sustainability—that food evokes. The actual products purchased are further influenced by promotions combining both health and environmental messages. The fact that countries with very distinct food cultures, such as Japan and the United States, embrace functionality suggests that the concept has broad cross-cultural transference.

Modern popular ideas parallel traditional concepts of health. Contemporary food can assume physiological, social, cultural, and ecological function without the need to express its role in such terms. Perhaps not surprisingly, consumers and marketers look to developing countries as sources of new functional foods and beverages [86].

In this context, personal health, being of more immediate self-interest to consumers than the health of remote systems of production, offers a useful entry point to sustainability. Set within a cultural context, a coupling of human health, ecosystem health, and shared values provides the beginnings of the paradigm shift that re-establishes the local and global links between production and consumption, and the interests of people of rich and poor countries alike.

Reconstructing sustainable food systems: policy and practice

Developing countries are challenged to reconstruct their food systems in positive ways. They differ in their robustness to global commodities, economic forces, and westernization, depending on their needs and culture. For example, South Asia, Ethiopia, and the West African region have strong identifiable food traditions, while ethnic, economic, and historical factors may make those of other countries in Sub-Saharan Africa less cohesive. In any case, progressive nutrition and food policies can proactively integrate and direct the evolution of the food system in optimal ways.

Although professionals comprehend at least the nature of the changing demographic, dietary, and health realities of the developing world, redefining the priorities of institutions responsible for nutrition and food, health, agricultural, economic, and educational policies lags behind. Food security, as usually operationalized, prioritizes energy and micronutrient requirements, issues that primarily draw on targeted, reductionist, and technological approaches to nutrition and health. Vested interests in the marketplace and economic structures further contribute to a focus on a limited diversity of staple crops.

Thus, most nutrition interventions address the symptoms of a problem, rather than the causal factors and the whole health or systematic contexts from which real and lasting solutions must come.

Novel and more sophisticated approaches to developing-country nutrition seem necessary and timely [87]. Efforts fostered by private or public interests can form part of a broad-based development focus on small-scale and low-input production [3, 18]. Specifically, they should be relevant to health needs, scientifically valid, ethical, economically viable, culturally appropriate, and based on sustainable use of resources.

Nutrition and health

Optimal diets must respond both to undernutrition and to overconsumption of energy [83, 88]. Nutrition policy must continue to prioritize food security, particularly for the poor in Sub-Saharan Africa and South Asia. But emphasis is best placed on nutritional balance as well as functional diversity. Quality and quantity of food need not be mutually exclusive. Functions that address the health outcomes of the nutritional transition, particularly through attention to energy density, glycemic control, and oxidative stress, define obvious priorities. Immunomodulating and related activities offer a response to conventional infectious diseases and HIV/AIDS. Stress suffered by urban populations, particularly the poor, from pollution of air, water, and food may be mitigated in part through diet [89].

Diversification of diet with indigenous fruits, vegetables, and whole grains and by moderate use of animal-source foods in both intact and processed forms is a first priority for policy and funding. Second, exotic nonstaples for which nutrient-specific (e.g., calcium in dairy products) and recognized health benefits exist can make important contributions to diet quality. Novel foods enhanced through processing or biotechnology with nutrient or functional components may be considered on a case basis relative to the well-defined needs of the target population.

Biofortification of cereal staples, while a potentially powerful tool to address specific micronutrient deficiencies, would further simplify food systems, decrease dietary diversity, and add to the economic advantage of large-scale producers [36]. Dietary diversification and attention to function in food processing, fortification, and FBDGs can complement narrowly targeted strategies and offset their potential adverse ecological, sociocultural, and biological consequences for human and ecosystem health.

Prior to intervention in any location, however,

it is essential to understand the existing nutrition-related strengths and weaknesses of the community's food system.

Research and scientific priorities

Research defined by an implicit hypothesis of this review, that dietary diversity contributes to health and that biodiversity equates with dietary diversity, can catalyze the integration of these converging perspectives. Specific tests of the hypothesis might combine quantitative indicators of dietary and biological diversity with nutrition and health outcomes [36]. Program research relevant to the nutrition transition also requires establishing the validity of novel outcome measures. In addition to nutritional indicators such as child development, anemia, infection, and obesity, this undertaking might also draw on the disease-risk-reduction activities presented above within the framework of Dietary Quality Indices [88], or as represented by appropriate biomarkers [90]. Intake assessments could consider a measure of traditional foods in sustainability of the diet.

Analysis of food function, composition, digestibility, and safety takes high priority in its own right, as do biodiversity conservation and sociocultural and agronomic aspects of small-scale agriculture. Food processing can be simultaneously attentive to traditional food culture and to modern, more globalized, tastes. Evaluation of the impact of biotechnology on biodiversity and the well-being of vulnerable populations is also essential. Biotechnology as part of a balanced approach to development and improved food security can contribute to improvement of local crops that benefit and enhance local self-sufficiency [48].

Policy-oriented research can define and direct the manner in which socioeconomic, technological, and political factors affect human and ecosystem health. Research at the local, national, and international levels might explore farmer transaction costs and the pricing, institutional, technological, informational, sociocultural, and organizational factors that affect homestead production of nonstaple crops. Successful interventions are likely to be multisectoral, multidisciplinary, and problem-focused.

Wild foods, which are typically understudied, deserve particular attention both for basic characterization [46, 50–52] and for ecological, agronomic, and marketing research [91]. Developing-country scientists with knowledge of local resources, customs, and cultural values will play a fundamental role in identifying sustainable approaches to diet; external interventions must respect local qualification, insight, and commitment. A growing body of peer-reviewed data generated in developing countries addresses the health properties of indigenous foods. However, although large nations such as India, China, or Brazil can support extensive research and development programs, in general progress depends on improvements in the scientific resources, opportunities, and infrastructure needed for adapting to, as the WSSD promotes for Africa, "world-class technologies" [3]. Full access to information technology is essential.

Economic viability

Food-based approaches to health in developing countries must provide bona fide health benefits and value to consumers. Nonetheless, recognition of foods on the basis of enhanced quality presumably equates with economic value. In this context, function provides valid income-generating opportunities for producers, processors, and marketers, which in turn improves market stability. Appropriate products may be simply intact foods with recognized quality, in some cases identified varieties or genotypes, including potentially those enhanced through genetic modification. Processed cereal and legume-based complimentary food mixes provide models of nutritionally rational products [92].

Although investment in small-scale and sustainable agriculture offers the largest potential gains to productivity [18], governments continue to ignore this sector. Investments in rural infrastructure and small enterprises should accompany improved extension to small farmers in relation to sustainable practices, biodiversity conservation, and crop selection [3, 18]. Furthermore, credit access, organizational supports, and policies that reduce input costs and improve returns to farmers, as well as support development of marketing and processing sectors for traditional crops, are needed.

Agricultural subsidies and tariffs in developed countries constrain poverty reduction and agricultural development in developing countries [18], although the beneficiaries and benefits of economic globalization remain uncertain [11, 19, 93, 94]. With or without this "leveling of the playing field, " small-scale producers, when provided with appropriate supports, have comparative advantage in producing or wild-harvesting semiperishable and locally demanded crops, principally fruits, vegetables, traditional cereals, and animal-source foods, for urban and rural markets [18]. In South Asia, the fact that demand for such high-value commodities is rising three times faster than demand for staples underlines their key role in changing economies [18]. Given that in many cases the majority of rural farmers are women, this focus has important potential for alleviating gender inequities.

Culturally appropriate foods and food systems

The key public policy needed to elevate food culture as a vehicle for ensuring that healthful foods form part of a socially, ecologically, and economically sustainable

system is simply to acknowledge, respect, and promote the fact. Decision makers with personal links to their own traditions may look to international, donor, and scientific support for the license to express this in public policy. The fact that traditional systems, once lost, are hard to recreate underlines the imperative for timely documentation, compilation, and dissemination of eroding knowledge of biodiversity and its uses. Food culture is an underutilized vehicle for promoting positive behaviors that should be part of the process of education and awareness-raising. Supporting cultural traditions within extension and public health activities, including recipe books and cooking classes, represents a tangible step [95].

Local biodiversity resources

Cultivated and wild biodiversity, where it is part of traditional agricultural and food systems, can be best conserved and enhanced through rational use. On-farm conservation of intraspecific diversity and neglected and underutilized species is a priority for increased agricultural investment in biodiversity management. Adding value to biodiversity by coupling it to the market and to health increases farmers' likelihood of conserving and enhancing diversity [18], although raising awareness of farmers and others is essential [83]. Home gardens and urban agriculture offer contexts where functional diversity can be usefully promoted. Broader promotion of the combined role of biodiversity in human and ecosystem health provides a philosophical platform for positive individual and community action.

Ethical and safety issues

Benefits from the enhanced use of biodiversity must legitimately flow to the undernourished poor, while potential negative consequences must be minimized and mitigated. Indigenous and traditional knowledge must be protected through legal and other mechanisms where appropriate [3]. Compensation for knowledge and genetic resources [3], as well as fair return for products, is a right of indigenous and local communities.

The promotion of foods for health benefits, whether for public health or commercial purposes, constitutes a health claim, stated or not. Even normal dietary constituents have potential consequences for safety, nutritional status, and health when their consumption is increased [96]. Although traditional diets offer many benefits, they are not inherently safe or all positive. Safeguards against adverse biological and social risks of promoting the use of traditional resources must be based on conscientious analysis and procedures for implementation. Because local communities often lack the basic information and empowerment to make decisions, efforts to promote novel foods and technologies are challenged to satisfy the essential principles of the right to informed choice and the right to democratic participation. Safety, environmental impact, perceived risks and benefits, transparency, accountability, and equity also must be addressed [97].

Novel food products, whether they have enhanced nutritional or functional properties, can be tested by using in vitro, animal, and human studies and potentially followed through postmarket surveillance. Although international standards of evidence and evaluation may not be affordable in developing countries, claims remain subject to scrutiny and regulation based on credible evidence. Many products may be approved generically in other jurisdictions. Thus, improved access to information by developing countries has a high priority.

The promotion of indigenous foods requires special consideration. As well as being little studied for safety, these foods vary in genetic and environmentally determined composition. As the basis for building culturally appropriate dietary behavior and sustainable livelihoods, and as foods of longstanding use, they can be considered safe when consumed as part of a total diet, but should be a priority for research.

Principles that would minimize inappropriate decisions include ensuring that traditional and novel foods have good nutritional value; that they are evaluated within the context of total nutritionally balanced diet and are appropriate for ad libitum consumption; that they are relevant to the target population and national health and nutrition policies; and that the level recommended in the diet to obtain benefit is achievable and sustainable by the target population [97].

Awareness and promotion

Perhaps the most immediate priority involves simply increasing awareness of the issues and raising the level of education among health-care personnel, policy specialists, and decision makers along the objectives for development of the WHO Global Strategy on Diet, Physical Activity, and Health [6]. Nutritionists can ensure that insights emerging from scientific research are available and are applied to best serve populations in need. Policies can incorporate these data into public health recommendations.

Subsequent efforts at public health education are likely to be most fruitful when they are two-way. Social-marketing methodologies that build on existing food culture and positive beliefs, participatory action, and context-appropriate forms of communication and promotion offer useful guidance [95].

Food and diet are of fundamental personal interest to all humans and thus provide a highly visible vehicle with local and global impact for linking health and sustainability. Nutritionists can play an important leader-

ship role in linking dietary and biocultural diversity. Diversity enriches the quality of life in health, sensory, social, intellectual, and moral terms and increases options and resilience for building livelihoods in the short term and for the future.

Conclusions

Biocultural diversity provides a positive vantage point on priorities put forward at the WSSD, particularly for sustainable development in Africa and impoverished countries in Asia [3], as well as on global changes in health [6]. Nutrition offers a practical integration of the WEHAB themes, as well as, when placed within a sociocultural context, a nexus for changes in individual behavior and motivation essential for the fundamental shifts in production and consumption patterns upon which sustainability ultimately rests. Mutual consideration of biocultural diversity and nutrition can instigate reflective development research and applied action. A desirable dietary culture links human and ecosystem health.

Optimization of diet includes both physiological factors, as it mediates the risk of disease, and cultural factors, as it embraces values and seeks to define health-positive forms of human behavior. Strategies for reduction of the risk of disease can draw on empirical evidence relating to the nutrient content and functional properties of foods as well as the benefits of dietary diversity. Empirical data on the effectiveness, economic viability, acceptability, and sustainability of programs, policies, technologies, and interventions can also rationally direct the best ways to use biodiversity to meet nutrition and health needs. Nonetheless, a policy consensus related to the environment and human well-being involves choices that must be based on philosophical and ethical considerations and common human sociocultural values. Without deliberate and concerted action, neither biodiversity nor health objectives can be realized.

In our opinion, approaches to complex interconnected phenomena are typically desegregated and inadequate for solving environmental health problems. Conversely, rational efforts that draw collectively on dietary diversity and biodiversity, rather than being dismissed as romantic, should be recognized as immediately pragmatic and ultimately essential. Faced with the scope of global poverty, social and dietary change, and environmental distress, anything less is a policy of despair and inevitable failure.

Education of nutritional, health, and agricultural professionals themselves with a holistic and responsive message can take a higher priority. Current policies for dealing with both health and resource use in developing countries limit expectations equally for the deliverers and the recipients of dietary interventions. A fundamental and authentically optimistic outcome of an integration of social, economic, and environmental considerations with health can be that of receptivity to greater possibilities based on the strengths inherent in traditional biological, cultural, and dietary diversity, and in evidence-based science.

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