



A meta-analysis of community-based studies on quality protein maize

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ABSTRACT

Biofortification, or the improvement of nutritional quality in food crops, is a promising strategy to combat undernutrition, particularly among the rural poor in developing countries. However, traditional methods of impact assessment are inadequate for biofortified crops, as they do not consider their nutritional benefits. Evidence for the nutritional impact of maize varieties with improved protein quality, collectively known as quality protein maize (QPM), was evaluated using meta-analysis of randomized, controlled studies in target communities. A new and generalizable effect size was proposed to quantify the impact of QPM on a key outcome, child growth. The results indicated that consumption of QPM instead of conventional maize leads to a 12% (95% CI: 7–18%) increase in the rate of growth in weight and a 9% (95% CI: 6–15%) increase in the rate of growth in height in infants and young children with mild to moderate undernutrition from populations in which maize is the major staple food. The proposed effect size and use of bootstrapping to determine statistical significance addressed some methodological limitations in the existing studies.

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Introduction

Undernutrition is estimated to directly cause 53% of child deaths (Caulfield et al., 2004; Habicht, 2008; Pelletier et al., 1995). Throughout the developing world, 32% of children under 5 years are stunted and 20% are underweight (Black et al., 2008). One approach to solving nutritional problems is to improve the nutritional quality of food crops (Johns and Eyzaguirre, 2007; Nestel et al., 2006; White and Broadley, 2005). This process, called biofortification, is a promising strategy to address an underlying cause of undernutrition, households' poor access to nutritious food. Biofortification is believed to be cost-effective (Meenakshi et al., 2007), more sustainable than nutrient supplementation (Bouis, 1999), and a viable complementary strategy to food fortification, which relies on central processing of food and therefore may be less accessible, particularly to the rural poor (Bouis, 1999; Horton, 2006; Johns and Eyzaguirre, 2007). Recent studies have demonstrated that biofortification can improve nutritional status, in particular using undermilled rice in the Philippines (Haas et al., 2005), low phytate maize in Guatemala (Hambidge et al., 2004), and orange-fleshed sweet potato in South Africa (van Jaarsveld et al.,

2005). "Golden rice" has been developed to have a relatively high concentration of pro-vitamin A carotenoids but its potential impact is yet to be tested (Enserink, 2008).

Several recent *ex ante* studies have analyzed the impact of biofortified crops on human health, using the disability-adjusted life year (DALY) method. The first analyzed the potential health benefits of golden rice in the Philippines (Zimmermann and Qaim, 2004). The method was expanded to crops biofortified with zinc and iron (Stein et al., 2005), and applied to a large number of countries in Africa, Asia, and Latin America (Meenakshi et al., Forthcoming). Given broad public support, golden rice could more than halve the disease burden of vitamin A deficiency in India (Stein et al., 2008a). Similarly, biofortification of rice and wheat could reduce the disease burden associated with iron deficiency by 19–58%, depending on the underlying assumptions (Stein et al., 2008b). A synthesis of *ex ante* studies suggests that biofortified crops can reduce the problem of micronutrient malnutrition in a cost-effective way (Qaim et al., 2007).

The first biofortified crop was *opaque-2* (*o2*) maize, bred for its improved protein quality (Mertz et al., 1964). At the time, this discovery was considered a potentially major breakthrough to help reduce protein deficiency, which was then considered the world's most pressing nutrition problem. Subsequent conventional breeding led to *o2* maize varieties with improved agronomic characteristics, now known as quality protein maize (QPM) (Vasal, 2000).

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QPM varieties are currently being disseminated in developing countries, particularly in Sub-Saharan Africa (Krivanek et al., 2007); however, there has been little published work on assessing the nutritional impact of these varieties.

Assessing the impact of conventional crop varieties such as those from the Green Revolution is a relatively straightforward exercise, based on yield increases and rates of adoption (Evenson and Gollin, 2003). Impact assessment of biofortified crop varieties such as QPM begins with the evaluation of their nutritional impact, to which traditional methods of impact assessment do not apply. Evaluating the nutritional impact of QPM would allow decisions to be made on the priority of improved protein quality as an objective in the breeding and dissemination of new maize varieties and would provide a model for the development, evaluation, targeting, and dissemination of other biofortified crops that are still in development. However, while studies have been done to determine the nutritional benefits of QPM, the results have not been widely accepted by the nutrition community, primarily because of issues concerning study design and methods as well as a dearth of peer-reviewed publications. Nutritional impact assessment is, of course, only a first step, and should be followed by a wider assessment of economic and social impact; this is, however, beyond the scope of this paper.

In this paper, background is provided on the history of QPM and the debate over the importance of improving dietary protein quality, a conceptual framework is developed for assessing the nutritional impact of biofortified crops, and this framework is applied to evaluate the existing evidence for nutritional impact of QPM. Evidence in community settings, as demonstrated by randomized, controlled studies, is evaluated using meta-analysis to quantify the impact of QPM on a key outcome, child growth. Further research needs are identified and methodological recommendations are made for comprehensive nutritional impact assessment of QPM and other biofortified crops.

Background

While the term “biofortification” (as coined by the HarvestPlus Challenge Program) most commonly refers to the process of increasing micronutrient levels, more recently it has been used in a larger sense to include other nutritional enhancements such as increased bioavailability of existing nutrients, improved oil profiles, or improved amino acid profiles resulting in improved protein quality (Unnevehr et al., 2007). The first biofortified crop, under this broader definition, was *opaque-2* (*o2*) maize, developed in the 1960s for its improved protein quality (Mertz et al., 1964). Maize is a staple food for millions of people in Sub-Saharan Africa, Latin America, and parts of Asia, and efforts to improve its protein quality date back to the 1950s (Bressani et al., 1953, 1960). Maize grain has poor protein quality due to deficiencies in lysine and tryptophan, two amino acids that are essential to the diets of humans and monogastric animals (FAO, 1992). In the early 1960s, Mertz et al. (1964) discovered that the natural *o2* mutation changed the protein composition of the maize endosperm, nearly doubling its lysine and tryptophan content. As a result, *o2* maize grain had improved protein quality, while its protein quantity remained the same.

The *o2* mutation, however, had several undesirable secondary effects including decreased yield, increased susceptibility to field and storage pests, and changes in kernel characteristics that made *o2* maize less appealing to both producers and consumers (Vasal, 2000). These drawbacks spurred years of breeding efforts at the International Maize and Wheat Improvement Centre (CIMMYT), resulting in maize varieties that retained the *o2* mutation and the quality protein trait but lacked the accompanying unfavorable agronomic characteristics (National Research Council, 1988). To

differentiate them from the earlier *o2* maize varieties and from “conventional maize” (CM) varieties, these new conventionally bred varieties were collectively referred to as “quality protein maize” (QPM). Aside from its modified amino acid profile, QPM varieties are comparable to CM varieties in their nutrient levels (Bressani, 1991; FAO, 1992).

Modern QPM varieties are currently being actively disseminated, particularly in Sub-Saharan Africa (Krivanek et al., 2007). Little is known, however, about where and under what circumstances QPM is likely to have nutritional impact. The *o2* mutation was identified at a time when protein deficiency was considered the world’s main nutrition problem, and the focus of nutritional interventions subsequently changed, first to the alleviation of energy deficiency in the 1970s and later to the alleviation of micronutrient deficiencies from the 1980s to the present day (Allen, 2003). Many nutritionists believe that other foods in maize-based diets compensate for the amino acid deficiencies in maize (Rahmanifar and Hamaker, 1999). The Nutrition Collaborative Research Support Program (CRSP), which studied nutrient adequacy in selected communities in Egypt, Mexico, and Kenya in the 1980s, in particular concluded that protein intake was unlikely to be a primary limiting factor for the growth and development of young children and little benefit could be expected from increasing the intake of limiting amino acids such as lysine (Beaton et al., 1992). However, others have argued that while this conclusion may hold for populations that are relatively “better-off”, it may not generalize to all populations, particularly those with limited access to high protein quality foods (Rahmanifar and Hamaker, 1999).

The decreased emphasis on protein deficiency as a nutritional problem was also accompanied by revision of human protein and amino acid requirements over the last 40 years (WHO, 1985, 2007). Using WHO (1985) protein and amino acid requirements to analyze regional and country-specific food availability data, Young and Pellett (1990) concluded that diets in a number of developing countries may be marginal for both lysine and utilizable protein. They recommended that improving dietary protein quality should remain a consideration in the design and implementation of food, nutrition, and agricultural programs and policies, particularly in countries where diets mainly depend on cereals. Further work by Pellett (1996) using regional and country-specific food availability data and dietary surveys from India and Pakistan also suggested that lysine may be lacking in many parts of the world where diets are heavily based on cereals.

The United States Institute of Medicine (IOM) (2005) has since said that individuals who consume little or no animal protein would be unlikely to obtain recommended amounts of lysine even if they obtain recommended amounts of total protein unless their diets were usually high in legumes, and even then, lysine intakes could be marginal. Recent protein and amino acid requirement guidelines, published in 2007 by the World Health Organization (WHO, 2007), recommend significantly higher intakes of lysine for adults compared with the earlier 1985 guidelines (WHO, 1985), and a study in India further concluded that the lysine requirements of chronically undernourished men are approximately 50% greater than the proposed values for well-nourished adults (Kurpad et al., 2003). The new WHO guidelines however have reduced the lysine requirements for well-nourished young children, primarily through a reduced protein:energy ratio that allows for higher energy expenditure.

Lysine fortification of wheat flour has been shown to improve indicators of nutritional and immunological status, chronic anxiety, stress, and morbidity in communities with adequate energy intakes and high dependence on cereal protein in Pakistan (Hussain et al., 2004), China (Zhao et al., 2004), and Syria (Ghosh et al., 2008; Smriga et al., 2004). When diets are deficient in energy, some protein is used to meet energy needs, but only in extreme cases where

chronic energy deficiency leads to progressive wasting will improving protein quality not have a benefit (WHO, 2007).

Methodology

Conceptual framework for nutritional impact assessment

The impact of any biofortified crop should be demonstrated at multiple levels to make sound, evidence-based decisions on its development, targeting, and dissemination. First, the change in the nutrient composition of the crop must result in increased *bioavailability* of the nutrient in question when consumed by target individuals. Bioavailability refers to the fraction of an ingested nutrient that is utilized by the body for normal physiological functions or storage (King, 2002). This increased utilization must then result in improved outcomes among target individuals who consume the biofortified crop instead of the conventional crop.

The impact of a biofortified crop on nutrition and health outcomes should be evaluated using both efficacy and effectiveness studies. *Efficacy* is “the extent to which a specific intervention, procedure, regimen, or service produces a beneficial effect under ideal conditions”, while *effectiveness* is “the extent to which a specific intervention, procedure, regimen, or service, when deployed in the field, does what it is intended to do for a defined population” (Last, 1988). As efficacy studies are conducted under well-controlled conditions, they primarily address biological factors, such as diet, nutritional status, and morbidity, relating to the effect of a technology or intervention. Effectiveness studies have less control over delivery of the technology or intervention to subjects and subjects’ compliance. Therefore, non-biological factors, such as the influence of community institutions, household decision-making, and cultural patterns of food preparation, allocation, and consumption, are more likely to modify the effect of the technology or intervention in this type of study. In particular, effectiveness also depends on market coverage, adoption rates, consumer acceptance, and allocation of food within households. Adoption rates are influenced by local adaptation of the biofortified varieties, but also seed distribution channels and farmers’ seed replacement rates; consumer acceptance is influenced by awareness, but also by other traits appreciated by consumers such as taste, texture, and visual appearance (Qaim et al., 2007). Finally, the impact of a biofortified crop should be evaluated in a broader context that, in addition to its nutritional and health effects, also investigates the agricultural, environmental, social, and economic effects of the technology and the effect of the institutional environment, in particular agricultural policy, regulations, and markets, on its adoption.

Several studies have been conducted to evaluate the bioavailability of protein in QPM and *o2* maize. Early studies on *o2* maize (reviewed in Bressani, 1991), involving children recovering from severe malnutrition in clinical settings in Colombia, Guatemala, and Peru, found that a greater proportion of protein from *o2* maize compared with CM is available for utilization by the body for normal physiological processes and growth. Similar results were observed in adults (Clark et al., 1977; Kies and Fox, 1972; Young et al., 1971), and Young et al. (1971) further concluded that the biological value of protein in *o2* maize was comparable to that of most animal proteins. Overall, these studies concluded that the improved quality protein in *o2* maize and QPM is more bioavailable than the protein in CM for humans. Further clinical work in Peru involving children recovering from severe malnutrition in a controlled environment found that children consuming an entirely QPM-based diet had similar growth rates as children consuming a modified cow’s milk formula (Graham et al., 1990).

Efficacy and effectiveness studies in community settings (to be reviewed below) have been conducted in several countries; how-

ever, only two (Ortega Alemán et al., 2008; Singh et al., 1980) have been published in the peer-reviewed literature, leading to a continued debate over the nutritional and health impact of QPM. These studies had a common design in which children were allocated into treatment groups, at least one of which received QPM and another of which received CM, and various outcomes, largely anthropometric, were monitored over time. The common hypothesis of these studies was that children on a QPM-based diet will have better growth than children on a similar diet based on CM. In this paper, the evidence provided by these studies on the nutritional impact of QPM is evaluated using meta-analysis.

Empirical model

Meta-analysis is a statistical method to integrate the results of independent studies that address the same research question (cf. Hedges and Olkin, 1985; Hunter and Schmidt, 2004). For each study a statistic, called an effect size, is calculated to quantify the effect of a treatment or intervention in a way that is interpretable and comparable across studies. A summary effect size is then calculated to quantify the overall effect of a treatment or intervention across studies.

For this analysis, a new effect size $\hat{\theta}_i$ was developed to quantify the effect of QPM relative to the effect of CM on growth in weight or height in a given study i :

$$\hat{\theta}_i = \frac{b_{QPM_i}}{b_{CM_i}} \quad (1)$$

where b_{QPM_i} is the average growth rate in the QPM group and b_{CM_i} is the average growth rate in the CM group in study i . The standard error of $\hat{\theta}_i$ was approximated using the delta method (Casella and Berger, 2002).

The summary effect size, $\hat{\theta}$, which quantifies the overall effect of QPM relative to CM, was calculated as:

$$\hat{\theta} = \sum_i w_i \hat{\theta}_i \quad (2)$$

where w_i is a weight corresponding to study i and $\sum_i w_i = 1$. As studies with larger sample sizes were expected to provide more precise estimates of the relative effect of QPM to CM, the w_i were proportional to the sample sizes of the respective studies. Two other sets of weights were also considered, one in which all studies were equally weighted and one in which the weights were inversely proportional to the variances of the respective $\hat{\theta}_i$. The latter weights, if known without error, are optimal in that they minimize the variance of $\hat{\theta}$.

An asymptotic 95% confidence interval (CI) for $\hat{\theta}$ was calculated as:

$$\hat{\theta} \pm 1.96 \text{SE}(\hat{\theta}) \quad (3)$$

where $\text{SE}(\hat{\theta}) = \sqrt{\text{var}(\hat{\theta})} = \sqrt{\sum_i w_i^2 \text{var}(\hat{\theta}_i)}$, which follows from Eq. (2) as the $\hat{\theta}_i$ are independent. For each set of weights, a 95% bootstrap percentile confidence interval (Efron and Tibshirani, 1993) was also calculated for $\hat{\theta}$. Bootstrapping is a statistical method in which resampling is used to approximate the sampling distribution of a statistic. Bootstrap percentile confidence intervals for $\hat{\theta}$ were calculated as follows. If n studies were available for a particular outcome, then a sample of size n of included studies was drawn with replacement from the set of included studies and a new summary effect size calculated. This process was repeated 5000 times, and the summary effect sizes calculated for each independent resample formed a bootstrap distribution that approximated the sampling distribution of $\hat{\theta}$. The 2.5% and 97.5% percentiles of the bootstrap distribution were used to construct a 95% bootstrap percentile confidence interval for $\hat{\theta}$. Sensitivity of the summary effect size to the influence

of individual studies was investigated by calculating summary effect sizes and bootstrap percentile confidence intervals while excluding one study at a time from the analysis.

Data

Internet searches for community-based studies on the nutritional impact of QPM were conducted on PubMed (<http://www.pubmed.gov>) and Google (<http://www.google.com>) using the keywords “QPM”, “quality protein maize”, “maize” and “protein quality”, and “opaque-2” in English, Spanish, and Portuguese. Proceedings of international conferences and books of abstracts on QPM and o2 maize were also searched. Information on unpublished work or work appearing in the grey literature was also solicited through electronic announcements and direct email or phone contact with researchers who had conducted nutritional research on QPM or o2 maize. As QPM was developed and disseminated by CIMMYT to other international and national research programs, researchers with possible knowledge of nutritional studies were identified by contacting past and current researchers working on QPM at CIMMYT; researchers at international or national research programs that had at one time collaborated with CIMMYT, received QPM germplasm from CIMMYT, or operated in a country where QPM varieties have been released; their colleagues; or others who had authored papers on nutritional aspects of QPM. Input from scientists who may not be part of this network was solicited through announcements on websites, scientific meetings, and the Plant Breeding News (PBN-L) electronic newsletter. Relevant research was considered from any country in any language. The meta-analysis of these studies was approved by the Purdue University Committee on the Use of Human Research Subjects.

To be included in the meta-analysis, a study needed to be a randomized, controlled trial with at least two treatment groups, one which received o2 maize or QPM and the other which received CM. Information and data had to be available, either from the principal investigator or from a relevant publication or presentation, on the study design, number of subjects, and mean changes in weight or height in each treatment group. Studies were excluded if they lacked a QPM/o2 maize or CM treatment group, information on the study design could not be obtained from an original report or principal investigator, or insufficient data were available to calculate the study's effect size. Separate meta-analyses were conducted to evaluate the impact of QPM on children's growth in weight and height.

Results

Included studies

Sixteen human nutritional studies on QPM or o2 maize were identified. Of these, one preliminary study in the 1970s from India (described by Singh et al. (1980)), a second study around 2000

from India (U. Singh, Rajendra Agricultural University, personal communication), a study in 1976–1977 from Guatemala (described by Bressani (1991)), and four studies in the 1990s or earlier from Brazil (Paes and Bicudo, 1994; M.C.D. Paes, Embrapa Milho e Sorgo, personal communication) were excluded because sufficient information and results from the studies could not be obtained from an original report or principal investigator. The remaining nine studies were identified as meeting the criteria for inclusion in the following analysis (Table 1). Of the nine studies, only two (Ortega Alemán et al., 2008; Singh et al., 1980) were peer-reviewed, while the others appeared as technical reports or dissertations. These studies were all conducted in Sub-Saharan Africa, Asia, or Latin America in communities where maize is the major staple food. Participating subjects were children under five years of age, with most under 24 months at baseline. All studies were conducted in locations where children exhibited mild to moderate undernutrition.

At baseline, children in study 1 had a mean weight-for-height z-score of -0.2 , while children in study 2 had a mean height-for-age z-score of -0.8 and a mean weight-for-age z-score of -1.1 (Akalu et al., 2008), based on the 2006 World Health Organization (WHO) growth standards (WHO, 2006). According to these standards, the reported mean heights, weights, and ages at baseline in studies 3–6 also indicate that study participants exhibited mild to moderate undernutrition in terms of height-for-age and weight-for-age, with a 20% prevalence of stunting reported in study 5 (Akuamo-Boateng, 2002). Children in study 7 were at 60% of expected weight-for-age or higher at baseline (Singh, 1977; Singh et al., 1980). In study 8, all children at baseline had at least first degree malnutrition, i.e., were between 76% and 90% of expected weight-for-age, according to the Gomez classification, and 9% of children were classified as having second or third degree malnutrition (75% or less of expected weight-for-age) (Morales Guerra, 2002). Children included in study 9 were classified as undernourished (one standard deviation or more below the expected value) according to two or more of the following indicators, based on the National Center for Health Statistics (CDC, 2006) growth standards: height-for-age, weight-for-age, and weight-for-height (Ortega Alemán et al., 2006, 2008). The levels of undernutrition in these studies suggest that children may also have limited intake of energy or micronutrients. However, randomization of children into treatment groups is expected to result in similar dietary patterns among children assigned to QPM or CM. The results of this meta-analysis can therefore be generalized to populations of infants and young children who show mild to moderate undernutrition and for whom maize is an important part of the diet.

The nine included studies varied significantly in size, from 48 to 486 children. All studies reported results on both weights and heights, except study 8 from Mexico, which reported results only on weight. Most studies provided the maize treatment (QPM or CM) to households in the form of seed, grain, or prepared but uncooked dough. These studies had higher sample sizes and longer

Table 1
Overview of nine available studies analyzing the impact of QPM on child growth.

| Study | Country | Study period | Duration of study (months) | Total participants | Age at baseline (months) | Form of treatment | Data on weight | Data on height | References |
|-------|-----------|--------------|----------------------------|--------------------|--------------------------|-------------------|----------------|----------------|---|
| 1 | Ethiopia | 2002–2003 | 13 | 135 | 5–29 | Seed | Yes | Yes | Akalu et al. (2008) |
| 2 | Ethiopia | 2005–2006 | 11 | 164 | 7–56 | Seed | Yes | Yes | Akalu et al. (2008) |
| 3 | Ghana | 1993–1994 | 12 | 83 | 4–23 | Seed | Yes | Yes | Akuamo-Boateng (2002) |
| 4 | Ghana | 1994–1995 | 12 | 78 | 4–15 | Dough | Yes | Yes | Akuamo-Boateng (2002) |
| 5 | Ghana | 1998–2000 | 12 | 317 | 4–9 | Dough | Yes | Yes | Akuamo-Boateng (2002) |
| 6 | Ghana | 2001 | 7 | 486 | 4–6 | Dough | Yes | Yes | Akuamo-Boateng (2002) |
| 7 | India | 1975–1976 | 6 | 67 | 18–30 | Meal | Yes | Yes | Singh (1977) and Singh et al. (1980) |
| 8 | Mexico | 2001–2002 | 14 | 67 | Most under 60 | Grain | Yes | No | Morales Guerra (2002) |
| 9 | Nicaragua | 2005 | 3.5 | 48 | 12–60 | Meal | Yes | Yes | Ortega Alemán et al. (2006) and Ortega Alemán et al. (2008) |

durations in which to observe an effect of QPM than the two studies that provided the treatment in the form of meals fed directly to the child. However, studies that provided maize to households had the drawback that they allowed behaviors and practices in the household as well as biological factors to have an effect on the impact of the treatment and, therefore, could not quantify the biological efficacy of QPM. All interventions were designed either to provide a daily meal to supplement children's typical diets or to substitute the maize in children's typical diets with either QPM (treatment) or CM (control). The included studies did not provide micronutrient supplementation and were not designed to ensure that children received adequate energy from their daily diets.

Studies 4 and 5 from Ghana and study 9 from Nicaragua were explicitly described as double-blind (Akuamo-Boateng, 2002; Ortega Alemán et al., 2006). Study 2 from Ethiopia was not blinded (Akalu et al., 2008). For the other studies, the degree of blinding was not described in the respective reports. However, as study 1 from Ethiopia and study 3 from Ghana both provided QPM or CM in the form of seed, it is likely that the researchers and enumerators could identify the treatment, even if the participating family could not. Although all studies were randomized, most studies did not have detailed descriptions of their study design, particularly regarding the unit (individual, village, or cluster of villages) that was randomized to either the QPM or CM group. The study design, including the unit of randomization, should be taken into account in the data analysis to have accurate standard errors and determination of statistical significance. Only study 4 from Ghana and study 9 from Nicaragua appeared to have a completely randomized design (Montgomery, 2005), in which individual children were randomized to the QPM or CM group. Of the other studies, only the Ethiopian studies specifically addressed the study design in their data analysis. Although the other studies also used randomized study designs, the methodologies were not sufficiently described to identify the design or unit of randomization. Informed consent was obtained from participants or their guardians prior to the start of the studies in Ethiopia, Mexico, and Nicaragua (Akalu et al., 2008; Morales Guerra, 2002; Ortega Alemán et al., 2006). The studies in Ethiopia and Mexico were also approved by an institutional review board (IRB) in their countries prior to commencement (Akalu et al., 2008; Morales Guerra, 2002). The ethical procedures followed in the other studies were not described in their respective reports.

Comparison of growth rates for weight in individual studies

While the nine included studies varied significantly in size, from 24 to 246 children per treatment group, the number of subjects was comparable across treatment groups in each study

(Table 2). Growth rates for weight in each treatment group were calculated from results presented in the respective publications for all studies except study 8 from Mexico and study 9 from Nicaragua. Growth rates for weight for study 9 were calculated from results provided by the principal investigator (Ortega Alemán, unpublished data). Study 8 did not use weight directly, but rather physical development (“desarrollo físico”), defined as the percentage of the median weight of a reference population of the same age. Observations of this variable for each child at the baseline and conclusion of the study were published (Morales Guerra, 2002) and used here to calculate growth rates for weight for study 8.

Growth rate, expressed as average weight gain per month, varied substantially between studies but was higher in the QPM groups (between 0.14 and 0.38 kg/month) than in the CM groups (between 0.05 and 0.35 kg/month) for all studies except studies 3 and 4 (Table 2). Generally, growth rates were higher in studies with younger children, which was expected as growth rate decreases with age. Standard errors of the growth rates were calculated from standard errors presented in the respective publications, from raw data in study 8, or from results provided by the principal investigator of study 9. These standard errors should be interpreted carefully, as they may be inaccurate for studies that did not use a completely randomized design and did not otherwise account for the study design in their data analysis. Generally, standard errors of growth rates for weight were higher in studies of smaller sample size and shorter duration.

For each study, the effect size was calculated as the relative growth rate of the QPM group compared with the CM group. Children in study 1 who received CM gained weight at an average rate of 0.15 kg/month over the duration of the study, while children who received QPM grew an average of 0.17 kg/month during the same period (Table 2). The effect size for this study is therefore 1.15, indicating a 15% increase in growth rate for weight in the QPM group compared with the CM group. Meanwhile, in study 3, children receiving CM grew an average of 0.20 kg/month, while children receiving QPM grew an average of 0.19 kg/month. The effect size of 0.96 for this study indicated a 4% decrease in the growth rate for weight in the QPM group compared with the CM group. The standard error of each effect size was approximated using the delta method and was based on the standard errors of the growth rates in the respective QPM and CM groups. Therefore, the effect size standard errors may be inaccurate for studies that did not appropriately account for the study design in their data analysis. Based on these standard errors, the effect sizes for studies 1, 2, 5, and 6 were greater than 1 with 95% confidence, indicating a significantly higher rate of growth in weight among children receiving QPM compared with children receiving CM in those four studies.

Table 2
Rate of growth in weight among children receiving conventional maize (CM) or quality protein maize (QPM) and the effect size for each study, calculated as the relative growth rate of the QPM group compared with the CM group.

| Study | Country | CM Growth rate (kg/month) | | | QPM Growth rate (kg/month) | | | Effect size Estimate | |
|-------|-----------|------------------------------|------|-----|-------------------------------|------|-----|-------------------------|-------|
| | | b_{CM} | SE | N | b_{QPM} | SE | N | (b_{QPM}/b_{CM}) | SE |
| 1 | Ethiopia | 0.15 | 0.01 | 70 | 0.17 | 0.01 | 65 | 1.15 | 0.07 |
| 2 | Ethiopia | 0.11 | 0.01 | 78 | 0.14 | 0.01 | 86 | 1.26 | 0.09 |
| 3 | Ghana | 0.20 | 0.02 | 40 | 0.19 | 0.02 | 43 | 0.96 | 0.11 |
| 4 | Ghana | 0.24 | 0.02 | 39 | 0.24 | 0.02 | 39 | 1.00 | 0.09 |
| 5 | Ghana | 0.19 | 0.00 | 157 | 0.21 | 0.00 | 160 | 1.10 | 0.04 |
| 6 | Ghana | 0.35 | 0.01 | 246 | 0.38 | 0.01 | 240 | 1.10 | 0.04 |
| 7 | India | 0.21 | 0.02 | 35 | 0.26 | 0.03 | 32 | 1.23 | 0.17 |
| 8 | Mexico | 0.25 ^a | 0.07 | 32 | 0.50 ^a | 0.06 | 35 | 1.97 | 0.60 |
| 9 | Nicaragua | 0.05 | 0.13 | 24 | 0.23 | 0.13 | 24 | 4.27 | 10.53 |

^a Weight was measured as physical development (percentage of the median weight of a reference population of the same age) instead of kg.

Comparison of growth rates for height in individual studies

For the eight studies that had data on height, growth rates for height in each treatment group were calculated from results presented in the respective publications or from results provided by the principal investigator of study 9 (Table 3). Similar to the analysis for weight, the analysis for growth in height indicated large variability between the studies and generally faster growth rates in studies with younger children. Except for study 1, all studies had faster growth rates, expressed as average height gain per month, in the QPM group (between 0.57 and 1.23 cm/month) than in the CM group (between 0.35 and 1.04 cm/month) and therefore had effect sizes greater than 1.

Standard errors of growth rates for height were again calculated from standard errors presented in the respective publications or from results provided by the principal investigator of study 9 and should be interpreted carefully, as they may be inaccurate for studies that did not appropriately account for the study design in their data analysis. Standard errors of effect sizes were based on standard errors of growth rates in the respective QPM and CM groups and therefore may also be inaccurate for the same reason. Based on these standard errors, the effect sizes of studies 2, 4, and 5 were greater than 1 with 95% confidence, indicating a significantly higher rate of growth in height among children receiving QPM compared with children receiving CM in those three studies.

Meta-analysis results

Summary effect sizes for growth in weight and height were determined using weights proportional to sample size in the respective CM groups and bootstrapping to calculate confidence

intervals (Table 4). The main data set for this analysis included the seven studies from Ethiopia, Ghana, and India. The two studies from Latin America were distinguished from the other included studies by their small sample size (35 or fewer children per treatment group), wide range of ages (up to 60 months or more at baseline), and, in the case of the Nicaragua study, short duration (3.5 months). In the meta-analysis, study 8 from Mexico and study 9 from Nicaragua were conservatively treated as outliers for weight and height, respectively, given their distinguishing characteristics and large effect sizes and standard errors compared with the other included studies.

The results indicate that consumption of QPM instead of CM leads to a 12% (95% CI: 7–18%, $p = 0.0002$) increase in the rate of growth in weight, and a 9% (95% CI: 6–15%, $p < 0.0001$) increase in the rate of growth in height in infants and young children with mild to moderate undernutrition from populations in which maize is a significant part of the diet. The comparable results for weight and height suggest that for these populations, the effect of QPM is largely an effect on growth in height, with an associated increase observed for growth in weight.

Summary effect sizes for growth in weight and height were also determined using equal and optimal weights with bootstrap percentile or asymptotic confidence intervals based on the main data set with and without outliers (Table 4). The summary effect sizes and confidence intervals were robust to alternative methods of weighting and calculating confidence intervals, and the positive effect of QPM on growth in weight and height remained statistically significant regardless of the methods used. The outlying studies from Mexico and Nicaragua generally had little effect on the summary effect size, as their study characteristics resulted in these studies having relatively low weights in the meta-analysis.

Table 3

Rate of growth in height among children receiving conventional maize (CM) or quality protein maize (QPM) and the effect size for each study, calculated as the relative growth rate of the QPM group compared with the CM group.

| Study ^a | Country | CM Growth rate (cm/month) | | | QPM Growth rate (cm/month) | | | Effect size Estimate | |
|--------------------|-----------|------------------------------|----------|------|-------------------------------|------|------|-------------------------|------|
| | | b_{CM} | SE | N | b_{QPM} | SE | N | (b_{QPM}/b_{CM}) | SE |
| | | 1 | Ethiopia | 0.77 | 0.02 | 70 | 0.77 | 0.02 | 65 |
| 2 | Ethiopia | 0.47 | 0.04 | 77 | 0.57 | 0.03 | 86 | 1.21 | 0.07 |
| 3 | Ghana | 0.83 | 0.06 | 40 | 0.88 | 0.05 | 43 | 1.07 | 0.10 |
| 4 | Ghana | 1.03 | 0.06 | 39 | 1.23 | 0.06 | 39 | 1.19 | 0.09 |
| 5 | Ghana | 1.01 | 0.01 | 156 | 1.09 | 0.01 | 161 | 1.08 | 0.02 |
| 6 | Ghana | 1.04 | 0.03 | 246 | 1.11 | 0.03 | 240 | 1.07 | 0.05 |
| 7 | India | 0.81 | 0.12 | 35 | 0.93 | 0.07 | 32 | 1.16 | 0.19 |
| 9 | Nicaragua | 0.35 | 0.19 | 24 | 0.58 | 0.36 | 24 | 1.66 | 1.37 |

^a The Mexico study did not have height data.

Table 4

Summary effect sizes and confidence intervals for growth in weight and height. Three weighting methods and two confidence interval methods were evaluated on the main data set with and without outliers.

| Outcome | Weighting method | CI method | Main data set ^a | | Main data set + outlier ^b | |
|---------|----------------------|------------|----------------------------|---------|--------------------------------------|---------|
| | | | Estimate (95% CI) | P-value | Estimate (95% CI) | P-value |
| Weight | Sample size | Bootstrap | 1.12 (1.07, 1.18) | 0.0002 | 1.16 (1.09, 1.31) | <0.0001 |
| | Equal | Bootstrap | 1.11 (1.04, 1.19) | 0.0007 | 1.22 (1.06, 1.46) | <0.0001 |
| | Optimal ^c | Bootstrap | 1.11 (1.07, 1.16) | 0.0006 | 1.11 (1.07, 1.16) | 0.0003 |
| | Optimal ^c | Asymptotic | 1.11 (1.06, 1.15) | <0.0001 | 1.11 (1.06, 1.15) | <0.0001 |
| Height | Sample size | Bootstrap | 1.09 (1.06, 1.15) | <0.0001 | 1.11 (1.07, 1.21) | <0.0001 |
| | Equal | Bootstrap | 1.11 (1.06, 1.16) | <0.0001 | 1.18 (1.07, 1.33) | <0.0001 |
| | Optimal ^c | Bootstrap | 1.07 (1.02, 1.14) | 0.0003 | 1.07 (1.02, 1.14) | 0.001 |
| | Optimal ^c | Asymptotic | 1.07 (1.04, 1.10) | <0.0001 | 1.07 (1.04, 1.10) | <0.0001 |

^a Main data set includes the seven studies from Ghana, Ethiopia, and India.

^b For weight, the outlier is the Mexico study; for height, the outlier is the Nicaragua study.

^c Inversely proportional to the variance of the effect size.

Inclusion of the outliers did increase the width of confidence intervals obtained from bootstrapping; however, in all cases, statistically significant positive effects of QPM on growth in weight and height were observed. Sensitivity to individual studies included in the main data set was also evaluated by omitting one study at a time and repeating the meta-analysis. The results were again robust to the inclusion or exclusion of individual studies.

Discussion

Meta-analysis can contribute significantly to policy and practice by changing the focus from individual studies to the overall body of existing research and by providing robust, quantitative results using a systematic and well-documented methodology. This study is the first systematic review of efficacy and effectiveness studies on a crop with improved nutritional quality. Taking into account the methodological limitations of existing studies, meta-analysis of these studies indicates a positive effect of QPM on growth of young children. Specifically, consumption of QPM instead of CM leads to a 12% increase in the rate of growth in weight and a 9% increase in the rate of growth in height in infants and young children with mild to moderate undernutrition from populations in which maize is a significant part of the diet. These results were robust to the influence of individual studies and to alternative methods of determining the summary effect size and its statistical significance, and, together with the clinical studies demonstrating the greater bioavailability of protein in QPM, indicate that substitution of CM with QPM in the diets of at-risk young children in maize-consuming areas could reduce the growth faltering that is widespread in these populations. These results are also the first step in evaluating the potential economic impact of QPM by establishing and quantifying a link between use of the improved crop and nutritional outcomes.

In meta-analyses of two-group studies to assess a treatment effect, the difference between the mean responses in the two groups, rather than their ratio, is typically used as the effect size, and this difference is often standardized using the pooled within-group standard deviation. However, in the studies considered here, the difference of means could increase with study duration or decrease with the age of subjects, as growth rates decrease with age. The effect size developed for this analysis removes the effects of study duration and child age in the comparison of QPM with CM. The effect size also did not depend on within-group standard deviations, which could have been calculated from results reported for individual studies but would have been inaccurate for those studies that did not appropriately account for the study design. The new effect size can be meaningfully interpreted as a relative growth rate and can be used in a wider range of applications in which the relative rate or percent change of an outcome in a treatment group compared with a control group has a meaningful interpretation.

The use of bootstrapping to construct confidence intervals also offered several advantages. It was not necessary to use potentially inaccurate within-group standard errors for individual studies or to approximate variances of individual effect sizes based on these standard errors. Also, unlike the asymptotic confidence intervals considered in this study, the bootstrap percentile confidence intervals took into account between-study variation. In this meta-analysis, variation among studies is expected as the included studies represent different populations, ages, diets, and other factors.

Future community-based evaluations of QPM or other biofortified crops should distinguish between efficacy and effectiveness and use methodologies that can separate the biological and non-biological effects of the biofortified crop. An efficacy study should

be conducted prior to an effectiveness study to quantify the biological impact of the improved crop while potentially confounding factors at the household or community level are controlled. Given the positive results of this meta-analysis, studies specifically designed to demonstrate the biological efficacy of QPM in a community-based setting would fill an existing knowledge gap.

Community-based studies on QPM or other biofortified crops should be conducted in areas where existing data indicate a risk of inadequate nutrient intakes that is likely to be reduced with the introduction of the biofortified crop. Study participants should represent population subgroups that are most vulnerable to risk of inadequate intakes and whose diets typically include high consumption of the crop in question. For example, QPM studies should target children aged 6–24 months, as this is a critical period of growth in which growth faltering due to an inadequate diet is most likely to occur (Shrimpton et al., 2001), and the diets of children in the study area should indicate a reliance on maize as a major source of protein.

In efficacy studies, the food-based intervention should be delivered to participants in a ready-to-eat or easily prepared form. Providing families with seed would yield information on the technology's effectiveness as it would reflect more typical conditions, but it would also introduce a host of confounding factors that limit the ability to measure biological efficacy. Monitoring, particularly of intake of intervention foods, total diet, and morbidity, is also an important component of community-based evaluations. Data should be gathered on other sources of the target nutrient in the diet as well as intakes of energy, other relevant nutrients, and compounds that affect the bioavailability of the target nutrient. Multiple, sensitive outcomes should be used to evaluate the biological impact of a biofortified crop. For QPM, biochemical indicators of protein and immune status should be considered in addition to morbidity and child growth. Studies should be randomized, controlled, and double-blind, and data analysis should take into account the unit of randomization (e.g., individual, village) used in the study. Finally, studies evaluating the nutrition and health of human subjects should first be reviewed by an IRB in the country in which the study is conducted, and documented informed consent should be obtained from study participants or their guardians prior to the start of the study.

In addition to its nutritional and health impact, several other questions must be addressed in a comprehensive impact assessment of QPM. Data are needed on typical diets, risk of inadequate intakes of quality protein, lysine, tryptophan, or energy, amount and sources of maize in the diet, and other factors that will determine the nutritional need for and potential impact of QPM in target populations and population subgroups. Sensory evaluation studies should be conducted to evaluate consumer acceptance of QPM varieties when used in the preparation of common local foods. QPM varieties must be agronomically competitive with other locally-available improved maize varieties, particularly with respect to yield and resistance to field and storage pests. QPM or other biofortified crops, particularly those for which the nutritional improvement is not visible, are unlikely to be well adopted and consequently to have impact if they do not perform at least as well as conventional varieties in the field. Nutrition messages to potential end users should not suggest that decreased yields are justified by increased nutritional quality. Quality control and extension activities are also needed to assure that the superior features of improved varieties, including the quality protein trait, are not lost due to poor seed stewardship by farmers who keep their harvested grain for use as seed in subsequent plantings. Finally, costs associated with QPM breeding, including extra time for breeding and costs of laboratory screening for the quality protein trait, need to be quantified. Similar questions need to be addressed for any biofortified crop in development.

Many maize breeding programs operating in Africa, Latin America, and parts of Asia are currently developing QPM varieties with the intention of having a positive impact on the nutrition and health of undernourished infants and children. However, while significant progress has been made on the breeding, genetics, and biochemical basis of QPM, progress in evaluating the impact of QPM in target populations has been limited. The highly significant results of the meta-analysis presented here justify greater attention and investment in evaluating the nutritional impact of QPM in sound scientific studies. QPM can meanwhile serve as a model for other biofortification efforts, as the conceptual framework and methodologies to evaluate evidence for nutritional impact, open questions in impact assessment, and methodological recommendations for future studies are directly applicable to other biofortified crops.

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