

## Review

## Interventions to improve calcium intake through foods in populations with low intake

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**Calcium intake remains inadequate in many low- and middle-income countries, especially in Africa and South Asia, where average intakes can be below 400 mg/day. Given the vital role of calcium in bone health, metabolism, and cell signaling, countries with low calcium intake may want to consider food-based approaches to improve calcium consumption and bioavailability within their population. This is especially true for those with low calcium intake who would benefit the most, including pregnant women (by reducing the risk of preeclampsia) and children (by reducing calcium-deficiency rickets). Specifically, some animal-source foods that are naturally high in bioavailable calcium and plant foods that can contribute to calcium intake could be promoted either through policies or educational materials. Some food processing techniques can improve the calcium content in food or increase calcium bioavailability. Staple-food fortification with calcium can also be a cost-effective method to increase intake with minimal behavior change required. Lastly, biofortification is currently being investigated to improve calcium content, either through genetic screening and breeding of high-calcium varieties or through the application of calcium-rich fertilizers. These mechanisms can be used alone or in combination based on the local context to improve calcium intake within a population.**

**Keywords:** calcium; food fortification; biofortification

### Purpose

In March and April 2021, the Nutrition Science Program of the New York Academy of Sciences, in partnership with the Children's Investment Fund Foundation, convened a Calcium Task Force and

hosted two virtual meetings. This task force is composed of experts in micronutrients, malnutrition, pediatrics, gynecology and obstetrics, biochemistry, public health, and strategies for supplementation and fortification. During these two virtual meetings,

doi: 10.1111/nyas.14743

the task force assessed the evidence on global calcium deficiency and its health consequences, useful indicators of calcium status or intake. It also considered potential interventions, such as calcium supplementation for pregnant women to improve pregnancy outcomes and associated implementation challenges, as well as food-based interventions to improve calcium intake, especially in populations with low dietary intake. The group was also commissioned to identify research gaps and provide guidance for interventions and policies based on the most current available evidence. The following paper, as a consensus viewpoint, describes the task force's discussions and conclusions with regard to population-wide, food-based interventions to improve low calcium intake.

## Background

Calcium plays integral roles in skeletal structure, smooth muscle contraction, and neuronal signaling. It makes up approximately 1.9% of the total body weight and as such is the fifth most abundant element in the body. However, a recent review found that many countries, especially those in Southeast Asia and South America, have average intakes less than 400 mg/day, well below the recommended intakes, which range between 700 and 1300 mg/day for individuals over 19 years of age.<sup>1–3</sup> Based on these data of low calcium intake and availability, it is difficult for much of the world to obtain adequate calcium from food, and the lowest calcium availability is generally found in low-income countries in Asia, Africa, and Latin America.<sup>4</sup>

This paper includes the conclusions of the task force and considers different food-based, population-level approaches that can be used to increase calcium intake and bioavailability. Food-based approaches to improve calcium intake can reach all members of the population who consume the promoted calcium-containing food. These include: promoting and facilitating access to foods naturally containing calcium through policies and education; food processing techniques that improve the bioavailability of dietary calcium; fortification of foods with additional calcium; and biofortification of crops to achieve higher calcium density. Considerations for these interventions should include the potential challenges and opportunities that arise from the food systems within which they are embedded as well as consumer preferences,

organoleptic properties of calcium-rich or -fortified foods, and issues of bioavailability. Food-based approaches are also less likely than supplementation to exceed tolerable upper intake levels (ULs) for calcium. The task force concluded that food-based approaches may be particularly important for reaching especially vulnerable populations, including women who enter pregnancy with low calcium intake and are at risk of preeclampsia, children who are at risk of rickets during rapid growth, and older adults who are at risk of bone fracture. While any population-level intervention would improve calcium intake of the entire population, women and children are the most vulnerable to low calcium intake and stand to benefit most from such an intervention.

One of the main challenges with studying calcium intake and status in populations is the limited availability of intake data and a lack of acceptable biomarkers of calcium status, which further complicates identifying populations that would most benefit from increased calcium intakes.<sup>3</sup> While nationally representative surveys of calcium intake are not available for all countries, Food and Agriculture Organization of the United Nations' food balance sheets and household consumption and expenditure surveys can provide indirect evidence on the availability of calcium and household-level consumption. As a result, information from multiple data sources, including various forms of dietary assessment, target requirements, and health outcomes, should be triangulated to determine when and for whom a calcium intervention is most appropriate.

## Promoting the consumption of naturally calcium-rich foods

In regions where naturally bioavailable calcium-containing foods are available, affordable, and acceptable, promoting the consumption of these foods may be effective in improving calcium intake. A review of the food balance sheets shows that availability of calcium-rich foods is insufficient to cover the needs of many low- and middle-income country (LMIC) populations.<sup>5</sup> There are also wide variations in the availability in different regions or intake frequency that can affect the specific recommendations for a particular region. Therefore, assessments of locally available, acceptable, and accessible foods that are rich in bioavailable calcium may

be helpful in identifying the most appropriate foods to promote within populations. While a number of foods are naturally high in calcium, there are many necessary considerations, which include biological access (e.g., bioavailability of the calcium), cultural acceptability (e.g., lactose intolerance rates may cause avoidance of dairy foods high in lactose), and economic sustainability (e.g., affordability of the foods recommended).<sup>6</sup> In addition, the promotion of consumption of calcium-rich foods requires behavioral change and improvement of access to culturally acceptable foods and should, therefore, be considered among long-term strategies. The cost-effectiveness of these strategies needs to be evaluated.<sup>7</sup>

Promoting the consumption of calcium-rich foods can be achieved through a number of strategies via policies and education of the public. Policies, such as commodity-specific (e.g., dairy) vouchers or cash transfers, can be used to help make food more affordable and have been especially effective in increasing the uptake of nutritious foods.<sup>8–10</sup> It may also be possible to increase food availability through agricultural subsidies geared toward increasing production or through improvements to the supply chain that reduce waste. Public education on the health benefits and the importance of calcium in the diet can also be valuable in increasing awareness. This can be done through public health messaging, marketing, or nutrition counseling. The NOURISHING Framework was developed to help countries implement nutrition policies related to food environments and food systems and that incorporate behavior change communication to tackle these issues and promote healthier diets.<sup>11</sup> Hawkes *et al.* also provide a list of policies that countries associated with development of the framework have implemented.<sup>11</sup>

### *Animal-source foods*

Dairy products, such as milk, cheese, kefir, amasi (sour milk), and yogurt, are some of the richest food sources of naturally occurring calcium and are sources of protein, potassium, and magnesium.<sup>12</sup> Compared with plant-based foods, as discussed below, bioavailability of calcium is also relatively high in dairy foods. However, the high costs and limited shelf-life of dairy products make them more unaffordable to many lower-income households. Despite the overall high costs of dairy foods, when

measured in terms of the cost per mg of calcium, dairy products are the least costly source of calcium owing to their relatively high levels of calcium compared with other foods.<sup>13</sup> Lactose intolerance or malabsorption can be another barrier to the consumption of dairy products. It is estimated that two-thirds of the world's population suffers from some form of lactose maldigestion/malabsorption, especially in South Asia, the Middle East, and in Southern Africa.<sup>14</sup> However, some forms of dairy, including some aged, hard cheeses, yogurt, and other sour milk products, can contain lower levels of lactose and may be well tolerated.

When consumed with bones, small fish and fish meal are also naturally high in calcium, as well as sources of iron and n-3 fatty acids. An analysis of the nutritional composition of 367 fish species in 43 countries<sup>15</sup> revealed that species from tropical areas contain higher concentrations of calcium (as well as iron and zinc) when compared to temperate and cold areas. Results from animal studies also support the use of small fish in population groups with low intakes of milk and milk products.<sup>16</sup> In one species caught in the Caribbean region, where there is a high prevalence of calcium deficiency, 100 g of the raw, edible portion of the fish provided more than 200 mg of calcium (elemental calcium is implied unless otherwise stated).<sup>15</sup> In an experiment in Bangladesh, the Darkina fish (a small indigenous fish) was selected for its high iron, calcium, and zinc content as a means to increase mineral intake in the diet. These fish were ground into a powder and prepared as a chutney. A 30 g portion was prepared with the usual rice meal and provided 360 mg of calcium, contributing 35% of the recommended dietary intake for pregnant and lactating women.<sup>17</sup> In another study, dried and ground fish, providing 952 mg/day of calcium, were used to treat Nigerian children (6 months to 12.5 years of age) with rickets.<sup>18</sup> Over 24 weeks, the children responded positively to the treatment. There was a decrease in serum alkaline phosphatase and improvements in calcium, 25-hydroxyvitamin D, and bone mineral density (BMD), and there was no difference between the ground-fish treatment arms and the limestone treatment arm, which received a similar dose of calcium.

Insects are consumed by as many as 2 billion people around the world and can be naturally high in calcium.<sup>19–21</sup> Insects have been proposed as a

sustainable solution to the rising costs of food animal production, food insecurity, population growth, and demand for protein.<sup>21,22</sup> In many parts of the world, insects are considered a delicacy. For example, in Zimbabwe, where worms are popular among locals in rural and urban areas, farming of mopane worms has become a multimillion dollar industry.<sup>23</sup> An analysis of six insects considered publicly acceptable in Central Europe found that the *Bombyx mori* (silk moth caterpillars) had a similar level of calcium to a glass of milk, with approximately 100 mg per 100 g of dry weight.<sup>20</sup> A study in Manipur, India, where aquatic insects are commonly consumed, found all species to be high in calcium (24–96 mg of calcium per 100 g of dry weight), as well as magnesium and protein.<sup>19</sup> The authors noted that the calcium content of these aquatic insects was higher than reported in a separate study of terrestrial insects, which contained just 0.0012–0.126 mg per 100 g of dry weight.<sup>24</sup> Insects also contain phenols and tannins, which have some antinutritive properties, and the bioavailability of calcium and other minerals has not been well studied.<sup>25</sup> Further work is also needed to consider the safety implications specifically of wild-caught bugs, which may have been exposed to pesticides and other toxins, as well as the allergenicity of insect proteins that are similar to those of mollusks and shellfish, which can lead to severe allergic reactions.<sup>20,26</sup>

### *Plant-source foods*

A number of edible plants are also naturally high in calcium, although the quantity and bioavailability are lower than animal-source foods, especially dairy. The role of calcium and its distribution in plants has been extensively discussed elsewhere.<sup>27–30</sup> Briefly, plant roots are involved in tightly regulating the amount of calcium that the plant takes up via  $\text{Ca}^{2+}$ -permeable cation channels. Once within the cytosol, calcium concentrations are maintained at submicromolar concentrations and generally incorporated into various calcium-dependent proteins or stored in the vacuoles. From the roots, calcium is transported into shoots and ultimately to leaves, where the majority of calcium is found in plants. The amount of calcium transported into the shoots is dependent on the phytoavailability of calcium in the rhizosphere, the area of the soil surrounding the roots. Fruits, seeds, and tubers often contain less calcium than roots and leaves.<sup>31</sup>

As much of the calcium is distributed into the leaves, leafy green vegetables, including kale and spinach, contain calcium. However, the presence of oxalates and phytates in plants can significantly reduce calcium's bioavailability. For example, spinach by weight contains more calcium than kale; but kale has lower oxalate levels, making it a better source of bioavailable calcium per serving than spinach. Other leafy green vegetables with relatively high levels of bioavailable calcium include Chinese spinach, Chinese mustard greens, and bok choy.<sup>32</sup> Table 1 provides several examples of plant-based sources of calcium, the fractional and total absorption of calcium in one serving, and how they compare with a serving of milk.<sup>12</sup> Cereal grains, especially finger millet and teff,<sup>33,34</sup> are relatively high in calcium as well but not as high as dairy sources. Finger millet and teff contain significant concentrations of phytate (679–1419 mg/100 g), which may affect calcium bioavailability.<sup>33,34</sup> Some root vegetables, including sweet potatoes, are also natural sources of calcium. In root vegetables, some of the calcium is bound by oxalate, thus reducing its bioavailability.

### *Water*

In some places, the naturally occurring calcium in water is high enough that it can contribute to the dietary intake of calcium. Although most tap and bottle waters contain little calcium, waters naturally containing calcium amounts of 300 mg/L have been reported from specific locations in Italy, Spain, the UK, and France.<sup>35</sup> Another study from Poland showed that tap water with around 68–114 mg/L of calcium can contribute to 6–14% of the total calcium intake of young women, and a study in the UK showed that calcium intake in areas of water with 300 mg/L can contribute to 8% of total calcium intake of adolescents.<sup>36,37</sup> However, in much of the world, including LMICs, there is very little calcium in drinking water. A study in Argentina shows that the calcium concentration of tap water ranged from 6 to 105 mg/L, while most bottled waters had calcium levels well below 50 mg/L.<sup>38</sup> Groundwater in Brazil showed a mean calcium concentration of 47.6 mg/L,<sup>39</sup> whereas one in Algeria showed concentration above 150 mg/L.<sup>40</sup> Some bottled mineral waters contain even larger amounts, with more than 500 mg/L.<sup>41</sup> One study showed that consumption of calcium-rich water can provide a quarter of the total calcium daily intake for adult

**Table 1. Food sources of bioavailable calcium<sup>12</sup>**

Food source	Serving size <sup>a</sup> (g)	Calcium content (mg/serving)	Estimated absorption efficiency <sup>b</sup> (%)	Absorbable calcium/serving <sup>c</sup> (mg)	Servings needed to equal 1 cup milk
Milk	240	290	32.1	93.1	1.0
Beans, pinto	86	44.7	26.7	11.9	8.1
Beans, red	172	40.5	24.4	9.9	9.7
Beans, white	110	113	21.8	24.7	3.9
Bok choy	85	79	53.8	42.5	2.3
Broccoli	71	35	61.3	21.5	4.5
Cheddar cheese	42	303	32.1	97.2	1.0
Chinese cabbage flower leaves	85	239	39.6	94.7	1.0
Chinese mustard green	85	212	40.2	85.3	1.1
Chinese spinach	85	347	8.36	29	3.3
Kale	85	61	49.3	30.1	3.2
Spinach	85	115	5.1	5.9	16.3
Sugar cookies	15	3	91.9	2.76	34.9
Sweet potatoes	164	44	22.2	9.8	9.8
Rhubarb	120	174	8.54	10.1	9.5
Whole wheat bread	28	20	82.0	16.6	5.8
Wheat bran cereal	28	20	38.0	7.5	12.8
Yogurt	240	300	32.1	96.3	1.0

<sup>a</sup> Based on a one-half cup serving size (~85 g for green leafy vegetables), except for milk (1 cup or 240 mL) and cheese (42.5 g).

<sup>b</sup> Adjusted for load using the equation for milk (fractional absorption = 0.889–0.0964 ln load [23]), then adjusting for the ratio of calcium absorption of the test food relative to milk tested at the same load, the absorptive index.

<sup>c</sup> Calculated as calcium content × fractional absorption.

men and women.<sup>42</sup> The ionic nature of calcium in water makes it bioavailable and facilitates absorption as long as the pH is sufficiently low, and absorption is further increased if water intake is spread throughout the day.<sup>35</sup>

### Utilizing food processing techniques that can increase calcium levels, bioavailability, or shelf-life

In addition to increasing the consumption of foods naturally rich in calcium, some food-processing methods incorporate calcium as a functional ingredient, leading to foods with greater calcium content or can improve the bioavailability of calcium in a food. For example in baking, calcium carbonate and calcium phosphate are used to provide structure to breads by retaining the CO<sub>2</sub> in the gluten and reduce the stickiness of dough. Calcium chloride, lactate, or carbonate are used in dairy and snack products to add firmness and structure. Dried foods, such as milk powders, can also have a much longer shelf-life than fresh milk, which can increase availability and accessibility of dairy products.

### Reducing phytate levels

Phytate or phytic acid is found in the highest amounts in cereals, legumes, nuts, and oil seeds, where it can account for 60–80% of total phosphorus content, and is found in lower amounts in roots, tubers, fruits, and berries.<sup>43,44</sup> It can make up between 1% and nearly 5% of the mass of the seed, fruit, or grain.<sup>45</sup> Phytic acid tightly binds calcium and other divalent cations, such as magnesium, iron, and zinc, and prevents them from being readily absorbed or hydrolyzed *in vivo*.<sup>46</sup> It has been estimated that a three-fold increase in phytic acid results in a 25% reduction in calcium absorption.<sup>47</sup> Thus, processing methods that hydrolyze phytate can have a significant impact on calcium bioavailability. While phytate is generally considered an antinutrient because it reduces the bioavailability of minerals and proteins, it is also an antioxidant that is especially important for seed viability.

Phytate can be broken down during food processing through either an enzymatic process, such as the use of phytase (a phytate-specific phosphatase), or through nonenzymatic processes, such as high

temperatures or pH. Enzymatic hydrolysis can occur during fermentation, malting, and sprouting and can help lower the phytate levels in vegetal foods, resulting in more bioavailable calcium and can lead to a longer shelf-life of the food.<sup>30,44</sup> This is especially important in the breadmaking process, as wheat and other cereal grains can contain a significant amount of phytate depending on the level of extraction. However, fermentation with lactic acid, such as during sourdough fermentation, has been shown to help the grain's natural phytase (phytogenic phytase) breakdown of the phytate by as much as 100% with rye, 95–100% with wheat, and 39–47% with oat.<sup>48</sup> The level of phytate reduction was directly correlated with the levels of phytase contained in the cereal and was not directly due to the lactic acid itself, although it creates the favorable conditions to facilitate the enzymatic degradation by lowering the pH.

Fermented foods can play a role in traditional diets and substantially increase calcium content. For example, in Southern Ethiopia, *enset* or false banana is fermented into *kocho*, which can increase calcium content<sup>49</sup> and is a major source of calcium for children in the area.<sup>50</sup> *Injera*, another common Ethiopian food that is made from fermented *teff*, is another example of improved calcium bioavailability that leads to higher intakes of bioavailable calcium and improved health outcomes.<sup>51</sup> In the mid-20th century, calcium intake in Ethiopia was estimated to be 1075 mg/day, with relatively low preeclampsia rates.<sup>52,53</sup> However, in more recent years, the estimated calcium intakes have decreased in Ethiopia to a mean intake of 501 mg/day for women, possibly due to the increase in maize and wheat flour in the diets and the relatively high cost of *teff*.<sup>54</sup>

Other strategies have also been proposed to reduce phytate in breads, for example, through bifidobacteria, which have higher levels of phytate-degrading enzymes than typical lactic acid bacteria.<sup>55</sup> Alternatively, a commercially available fungal phytase from *Aspergillus niger* has been shown to reduce phytate levels in the breadmaking process and improve the overall quality of the bread, with better shape, softer crumbs, and reduced proofing times.<sup>56</sup> These strategies all can result in a bread with lower phytate levels and improved bioavailability of divalent cations, such as calcium.

Germination or soaking seeds in water overnight can also hydrolyze phytate. Germination over 10 days of fava beans resulted in a decrease in phytate levels by 71.2–77.3%, depending on the cultivar, and was concomitant with an increase in phytase activity, which peaked around 6 days.<sup>57</sup> A more recent study found that germination significantly increased the bioavailability of calcium in all legume seeds studied, including lentils, chickpeas, cowpeas, and green gram.<sup>58</sup> Soaking appears to decrease phytate levels but does so to a lesser degree than malting or fermentation in sorghum.<sup>59</sup> However, another study looked at the subsequent bioavailability of iron and zinc after soaking and found it likely did not improve despite the reduction in phytate (calcium was not measured).<sup>60</sup>

There are gut bacteria capable of phytase activity, such as *Bifidobacterium dentium*, *Lactobacillus reuteri*, and *Lactobacillus salivarius*, that could break down these compounds. Promoting growth of these bacteria through probiotics can help improve calcium absorption and, as mentioned above, have been proposed for use in the breadmaking process.<sup>61</sup> Finally, phytase could be added to supplements and micronutrient powders to help make calcium and the other divalent cations more available.<sup>62–65</sup>

### *Nixtamalization*

*Nixtamalization* is a process that refers to soaking boiled corn overnight in a lime solution of calcium hydroxide.<sup>66</sup> After soaking, some of the outer layer of the corn, which can contain aflatoxins, is removed. This is the traditional home process used in Mesoamerica that makes corn dough malleable enough to prepare tortillas, and it results in a 20-fold increase in calcium content compared with raw maize and increased calcium absorption compared with commercially available tortillas.<sup>67–69</sup> In fact, the *nixtamalized* tortillas in Guatemala provides diets that are relatively high in calcium and lower rates of preeclampsia. This led to the hypothesis that diets with adequate calcium could reduce the risk of preeclampsia.<sup>52</sup> Work is ongoing to adapt this process to make maize dough from maize for traditional maize dishes in sub-Saharan Africa (personal communication). Inserting the additional *nixtamalization* step to increase calcium intake in such setting may face initial acceptability hurdles.

### *Parboiling rice*

In many rice-consuming communities around the world, slender, long, white grains are the most desired by consumers, but the milling and polishing processes required to achieve the end-product remove the nutrient-rich bran layer. It is estimated that 10% milling of raw rice can lead to a loss of  $57.2 \pm 2.5\%$  of the calcium in unmilled grain.<sup>70</sup> Parboiling is a technique that transfers the nutrients of the bran to the rice grain prior to milling, resulting in greater grain nutrient content. Parboiled rice loses significantly less calcium ( $48.3 \pm 3.2\%$ ) than raw rice.<sup>70</sup> However, the product of parboiling rice is a more yellow grain than traditional polished white rice, which is less desirable for consumers in many countries. It was recently shown that parboiling the whole grain could increase calcium content by over 200% in the finished, milled rice.<sup>71</sup> In another study, the soaking water used in parboiling rice was fortified with calcium lactate and iron (ferrous sulfate heptahydrate), resulting in a rice containing nearly 1000 mg of calcium per kg of milled rice.<sup>72</sup> Thus, the consumption of 400 g of this cooked, calcium-soaked, parboiled rice can provide about 15% of the recommended dietary allowance of calcium.

### *Eggshells and fish bones*

It is also possible to mix calcium-rich foods with staple foods, such as flour, to produce a higher calcium food. This has been proposed for calcium-rich foods like fish bones and eggshell-fortified foods. Ground tuna bones were used to create a fortified cracker that increased calcium, phosphorus, and protein levels and was tested in Thailand.<sup>73</sup> The study found that up to 30% of the bone mix could be added to the cracker (by weight) without affecting sensory properties, although it did create a more dense and less porous cracker compared with controls. These methods of using otherwise waste products, such as bones, may create a cost-effective way to fortify foods while reducing waste.

Eggshells also contain a high amount of calcium and with a similar bioavailability to calcium carbonate (~39%).<sup>74</sup> One gram of eggshells provides approximately 380 mg of calcium and covers half of the calcium needs of a sub-Saharan African adult female, making eggshell a highly equitable solution to fill the calcium intake gap of rural sub-Saharan Africa (where village poultry ownership is common).<sup>74</sup> In a study in piglets, eggshell powder

added to either the soy- or casein-based diets was more absorbable than purified calcium carbonate, though this difference was only significant in the soy-based diets.<sup>75</sup> However, the eggshells had no impact on the absorption of magnesium or crude fat digestion, suggesting that the eggshells are not interfering with the absorption of other nutrients.

While Salmonella and other egg-associated pathogens are a risk to be considered, it has been proposed that eggshells boiled for 10 min when preparing hard-boiled eggs, with a further 20 min cooking of crushed eggshell in staple foods, would eliminate identified egg-associated pathogens.<sup>74</sup> In a study done in Tanzania, where the diet is low in energy and micronutrients, especially calcium, zinc, and iron, ground eggshells were used to improve calcium content. In this study, the eggshells were boiled for 20 min and then dried, crushed, and ground to the finest powder particles, reducing the impact on overall meal texture, and added to the mixture of any prepared meal.<sup>74</sup> In one study, eggshell powder was tested as a calcium fortificant for bread, resulting in a better appearance of the crust, the color of the crumb, flavor, and overall acceptability compared with the control bread.<sup>76</sup> The optimal level of calcium was around 200 mg calcium/100 g bread.<sup>77</sup>

The acceptability and safety of these approaches as well as the impact on the absorption of other nutrients requires further research. While these methods could reduce waste, behavior modification would be needed to incorporate eggshells and fish bones into the diet and how to prepare the powders if they were to be made in the home.

### **Implementing calcium food fortification programs**

Food fortification is an effective means of increasing micronutrient intakes, including calcium. Staple food fortification is a population-based approach, which aims to shift intakes of a nutrient among all consumers of a food vehicle. Ideally, fortification is implemented without a change in behavior, unlike supplementation regimes. To realize such population-level impact, a high proportion of the target population needs to consume the fortified food regularly. Calcium food fortification has been successfully implemented in many, mostly high-income countries, with demonstrated cost-effective impacts on health outcomes in some age

groups, especially in children and postmenopausal women.<sup>78</sup>

### *Impact of calcium fortification*

The UK (and Newfoundland, a former part of the UK) is the only country that currently has mandatory calcium fortification, which is for wheat flour (235–390 mg calcium carbonate (94–156 mg of elemental calcium)/100 g wheat flour) and was implemented in 1943. With this fortification policy, it is estimated that the fortified wheat flour contributes to 13–14% of the total calcium intake in the UK, and without this policy, an additional 10–12% of adolescents would not meet the recommended intake.<sup>37,79</sup>

For more than 30 years, Denmark had a mandatory calcium fortification policy for wheat and rye flours. In 1985, while this fortification policy was in effect, 7% of women and 6% of men were not meeting their calcium recommendations. However, in 1993–1994, after the fortification program ended, the number of women and men not meeting the daily calcium recommendations increased to 24% and 21%, respectively.<sup>80</sup> As a result, it was estimated that the calcium-fortified foods contributed to about 30% of the total calcium intake in the diet. While these two examples from the UK and Denmark are in high-income settings and where dairy is widely consumed and the average calcium intake is higher than in many parts of the world, the policies were effective in raising the calcium intake of the population and preventing inadequate intake, especially in low-income groups.

For young children, very low calcium intakes, as well as low vitamin D status, can put them at risk for rickets and osteomalacia. In trials of calcium-fortified foods, children and adolescents receiving the fortified foods had greater gains in height and BMD than children who did not receive these foods. In a randomized, placebo-controlled trial in Finland, calcium supplemented through cheese (1000 mg/day) over 2 years in adolescent girls (10–12 years old) resulted in a significantly greater percent change in cortical thickness of the tibia over the study period (37.1 mm  $\pm$  1.3), when compared with 1000 mg/day calcium with 200 IU vitamin D supplementation (31.7 mm  $\pm$  1.3), 1000 mg/day of calcium supplementation alone (29.8 mm  $\pm$  1.4), and placebo (31.1 mm  $\pm$  1.4).<sup>81</sup> Higher total BMD

was also observed in all calcium groups compared with the controls as long as compliance was greater than 50%. Similar results in girls (10 years old) were found in another 2-year randomized controlled trial in China using fortified milk powders with 300, 600, or 900 mg of calcium with 200 IU of vitamin D in all groups. The high-calcium group (consuming a total of 1100 mg/day of calcium) showed greater BMD accretion in the hip (2.3%), femoral neck (2.7%), and shaft (2.6%) than those in the low-calcium group (consuming a total of 655 mg/day of calcium).<sup>82</sup> However, these results were not significant for boys, and more work needs to be done to better understand the effect of sex differences and calcium intake on bone outcomes. It should also be noted that the trials to date have been conducted in just a few settings, including China, the United States, Australia, New Zealand, and Western Europe.<sup>78</sup> While some targeted fortification programs, including calcium, have been successfully implemented in LMICs for children,<sup>83–85</sup> additional work is needed to see if the results with calcium-fortified foods have similar health impacts on bone density and other health outcomes.

Adequate calcium intake in early pregnancy is important for improving pregnancy outcomes.<sup>86,87</sup> As it is very hard to identify women very early in pregnancy, especially in LMICs, a food fortification program that reaches women of reproductive age could help women consume more adequate levels of calcium before they become pregnant. However, the benefits of such a strategy have not yet been tested through fortification. Most of what is known about the impact of calcium in pregnancy is through supplementation trials, whereas food fortification may not reach the same intake levels as supplementation. Work is ongoing to better understand if lower doses of supplementation (500 mg/day) can be as effective as the higher (1–2 g/day) doses currently in use to prevent preeclampsia in pregnant women.<sup>88</sup> If the lower doses can have similar impact, it would be worth investigating whether this level could be achieved in a fortification program without exceeding the UL for any other group in the population and the impact of a calcium fortification program on pregnancy outcomes.

Women of reproductive age and postmenopausal women are another group likely to benefit most from fortification efforts. In a study of 141



postmenopausal Chinese women, daily consumption of cow milk containing 250 mg calcium increased BMD in the hip (2.8%) and femoral neck (2.5%) over 18 months.<sup>89</sup> This is a significant difference from the control group, which decreased BMD in the hip and femoral neck over the study period. However, there were no differences in the BMD of the lumbar spine, where all groups showed a decrease in BMD, and the endline outcomes for calcium-fortified soymilk (250 mg calcium) were not different from controls. In another study of postpartum women over a 12-month period in China, consuming 40 g of milk powder/day with either 300, 600, or 900 mg of calcium showed no differences over the study period in the BMD.<sup>90</sup> Interestingly, the previous study of postmenopausal women also saw no significant differences at the 12-month time point either, and it was only the 18-month time point that showed significant gains. Longer-term studies are, therefore, perhaps needed to understand the potential benefits and risks of calcium-fortified foods.<sup>89</sup>

To date, the only study reporting on cost-effectiveness of calcium with health outcomes assessed the impact of a hypothetical calcium fortification program on bone fractures in postmenopausal women in Germany. This modeling exercise showed that the voluntary fortification of an unspecified food that would provide an additional 800 IU vitamin D (as cholecalciferol) and 200 mg of calcium would prevent 36,705 bone fractures in German women over 65 years old by 2050, with an annual savings of €315 million.<sup>91</sup>

### *Calcium fortification policies*

As with all fortification policies, calcium fortification can either be mandatory or voluntary. Mandatory fortification is used as a population-based approach to address a public health concern and is generally recommended for improving micronutrient intakes.<sup>92</sup> In the case of calcium, the aim is to address inadequate calcium intake to prevent the health consequences of inadequate intake, such as rickets and preeclampsia. Mandatory food fortification requires minimal behavior change and for calcium can be a cost-effective intervention.<sup>91</sup> By contrast, voluntary fortification relies more on the food manufacturers and consumer demand and access to address the public health issue, and typically many options are available to the consumers.<sup>93–95</sup> In

these cases, the equity of availability and affordability must also be considered to ensure that the most at-risk population groups can benefit from these products, especially if the costs of food fortification are passed on to the consumer.<sup>96,97</sup> However, mandating fortification of staple foods (i.e., all commercial production of the specified food must be fortified by law) enhances the potential to realize this benefit and ensures equity.

The food vehicle selected for mandatory food fortification programs should be based on usual intakes in the population of a food that should be consumed regularly by a large proportion of the population and is industrially produced to facilitate effective fortification. In setting the fortificant level, population subgroups with the lowest and highest intakes of the food should be taken into consideration to maximize the potential for impact while minimizing the risks of excess intake. Food selection for voluntary fortification is commonly at the discretion of industry, or may be included in legal documents that allow for (but not obligate) the fortification of certain food types. The WHO provides additional information on how to establish a food fortification program, with specific guidance for calcium.<sup>98</sup> It is also important to monitor coverage of the food fortification program, and the Fortification Assessment Coverage Toolkit (FACT) has been developed by the Global Alliance for Improved Nutrition.<sup>99</sup>

While the UK is the only country with a mandatory calcium fortification policy (for wheat flour), several countries have voluntary fortification policies for calcium-fortified staple foods and are listed in Table 2. Standards generally suggest 1250–2400 mg/kg of product for a variety of calcium salts.<sup>100</sup> The extent and quality of implementation and the population-level impact of voluntary calcium fortification has not been thoroughly assessed. A recent review of the fortification policies of 15 countries suggests that coverage of fortified staple foods is highly variable, especially with voluntary fortification policies.<sup>7</sup> Limited evaluations of the impact of fortification programs on health outcomes, such as bone density, incidence rate of osteoporotic bone fractures, prevalence of hypertension, population blood pressure, and preeclampsia, have been conducted to date. However, these have assessed the impact of calcium-fortified dairy products and not staple foods.<sup>101</sup>

**Table 2.** Staple foods currently fortified with calcium<sup>100</sup>

Country	Nutrient level in standard (mg/kg)
<b>Maize flour</b>	
The United States of America	1375
Zambia	1278
<b>Rice</b>	
Belize	650
The United States of America	1650
<b>Wheat flour</b>	
Antigua and Barbuda	1250
Bahamas	1250
Bangladesh	53
Barbados	1250
Belize	1250
China	2400
Colombia	1280
Dominica	1250
Grenada	1250
Guyana	1250
Jamaica	1250
Jordan	14.15
Kuwait	2115
Qatar	2115
Saint Kitts and Nevis	1250
Saint Lucia	1250
Saint Vincent and the Grenadines	1250
Saudi Arabia	2115
Suriname	1250
Trinidad and Tobago	1265
United Arab Emirates	2115
The United Kingdom	3125
The United States of America	2112
Zambia	1278

NOTE: The low values appear to indicate the minimum allowable amount added in the standard.

### Calcium salts for food fortification

When considering the form of calcium to be used in food fortification, a number of factors must be considered, including the bioavailability, solubility, sedimentation, acceptability, organoleptic properties, shelf-life, and cost of the calcium salt. Thus, the choice of calcium salt will be dependent on these properties as well as the properties of the food vehicle being fortified.

There are many calcium salts suitable for human consumption and used by the food industry. These calcium salts have a wide range of elemental calcium content and water solubility, and the most

commonly used in fortified foods are listed in Table 3.<sup>76,102</sup> Despite the range in physical properties, the fractional absorption of calcium (i.e., the percent of calcium absorbed in the intestine) is fairly similar among the most common salts, including calcium carbonate, tricalcium phosphate, calcium citrate, and calcium citrate malate. In general, calcium salts are fairly water soluble (except for calcium oxalates), but the bioavailability or fractional absorption can be low. Absorption is more dependent on calcium load and other factors, such as foods consumed with the salt, than on the form of the salt itself.

Calcium is a relatively bulky nutrient and relatively large amounts are needed in comparison with other nutrients to help meet the calcium requirements of a population, especially where intakes are very low. As a result, a bioavailable salt with high elemental calcium that does not have adverse acceptability or organoleptic properties is needed.<sup>76</sup> Fortunately, most calcium salts are odorless and white, which can help with acceptability and avoid discoloration of foods. Yet, some salts have a tart or acidic flavor that can affect acceptability and the amount that can be used when fortifying foods. Different salts are also used in different types of foods. For example, solid foods, such as cereals and flour, are often fortified with calcium carbonate, while liquids, such as juices, are fortified with different salts depending on the properties of the final food product in terms of pH, flavor, stability, and so on.<sup>103</sup> Acceptability studies have shown that up to 600 mg of calcium citrate malate (or 66 mg of elemental calcium) in 240 mL of orange juice did not reduce acceptability by consumers.<sup>104</sup> Similarly, calcium content in calcium-fortified tortillas could be increased by nine-fold with the addition of 114 mg of elemental calcium to a 48 g wheat-flour tortilla without affecting consumer acceptability. In fact, the calcium carbonate-fortified tortillas were preferred over the unfortified tortillas and the tortillas fortified with either calcium lactate or calcium citrate.<sup>105</sup>

Interactions between calcium and the fortified foods or other nutrients should also be considered. Calcium can interact with anthocyanins, contained in some blue, purple, or black foods (e.g., berries and black soybeans), causing a color change.<sup>106</sup> Adding calcium to beverages high in protein, such as soy and nut-based beverages, can cause

**Table 3.** Properties and uses of the most commonly used calcium salts in food fortification<sup>76,102</sup>

Calcium source	Calcium content [%]	Solubility [mmol/L] <sup>a</sup>	Fractional absorption <sup>b</sup>	Sensory properties	Uses
Calcium carbonate	40	0.14	0.296 ± 0.054	Odorless; white or off-white; chalky, soapy, or lemony taste	Direct food additive; dough strengthener; firming agent; thickener; stabilizer; pH control
Tricalcium phosphate	38	0.97	0.252 ± 0.130	Odorless; sandy or bland taste	Food additive to control pH in dough and lard; yeast nutrient in flour; supplement in livestock and poultry feeds
Calcium citrate	21	7.3	0.242 ± 0.049	Odorless; tart, acidic taste	Food additive; sequestrant
Calcium citrate malate	30	80	0.363 ± 0.076	Odorless	Food additive; sequestrant

<sup>a</sup> Solubility in water at neutral pH.

<sup>b</sup> Determined in women using isotopic tracer techniques on test loads of 200–300 mg calcium.

sedimentation.<sup>107</sup> However, a soy lecithin coating of the calcium can help avoid this issue. There have also been reports of calcium interfering with iron absorption in supplementation studies, but this was found to be a short-term issue that is adapted to within a few months.<sup>108,109</sup> Some studies have also cited the interactions between calcium and zinc absorption, but longer-term studies are needed to determine if this effect is limited to the short-term, as it is for iron.<sup>110</sup> For more information on this, see the paper on supplementation in this special issue.<sup>86</sup>

When fortifying foods, it is important to consider the percent of the population at risk of reaching or exceeding the UL with the fortified foods. However, for calcium, the amount that would typically be added to a fortified food would be relatively low due to calcium's effect on organoleptic properties and acceptability when compared with the relatively high UL (2500 mg/day for most adults). Two recent analyses modeled the impact of calcium fortification of water and flour for several countries.<sup>35,111</sup> Flour fortification at the level of 156 mg per 100 g of flour would result in less than 2% of individuals (6 months and older) exceeding the UL in the six of the seven countries modeled from five continents.<sup>111</sup> However, in two of these countries, the Lao PDR and Bangladesh, flour consumption is too low to have an impact on calcium intake. In a similar mod-

eling exercise of calcium-fortified water (500 mg of calcium/L), which included five LMICs and two high-income countries, the populations from the LMICs had the greatest potential to benefit from fortification, with very few individuals exceeding the UL.<sup>35</sup>

### Specialized foods

Specialized foods are a form of fortified foods used to target a specific segment of the population, such as pregnant women or young children, for a limited period of time. Unlike staple-food fortification, these products generally rely on special delivery mechanisms to reach individuals. Several of these specialized foods are available, though these may not be used by the entire population. These include fortified blended foods of partially precooked and milled cereals, soya, beans, and pulses that contain 350–450 mg of calcium as tri- or di-calcium phosphate per 100 g of product, and they have been used in a few settings.<sup>112–114</sup> For example, Super Cereal, a corn-soy blend, targets pregnant and lactating women and contains 362 mg of calcium per 100 g.<sup>115</sup> Many varieties of micronutrient powders and ready-to-use therapeutic foods (RUTFs) also contain 300–800 mg per 100 g food calcium. RUTFs also contain 300–600 mg per 100 g and are used for the treatment of severe acute malnutrition in children.<sup>116</sup>

## Supporting the production of calcium-biofortified foods

Biofortification of calcium in various food crops has been suggested as a potentially economic and environmentally advantageous method to increase calcium intake. While calcium content in plants is driven by both environmental and genetic factors,<sup>117,118</sup> known strategies for improving the uptake and storage of calcium of food crops like rice require further research into the molecular mechanisms involved and the potential side effects to plant health and productivity.<sup>33,119</sup> While there are biological limits on how much calcium can be added to a food via biofortification, and, even with the help of biofortification, some plant foods will remain well below the calcium levels of dairy products. However, it is a potential solution, especially in areas with very low calcium intakes or where plant-based diets are consumed.<sup>30</sup> A combination of lower-phytate crops with higher mineral calcium has recently been proposed as a potentially viable path forward to provide bioavailable calcium that can meet the needs of the most vulnerable groups worldwide.<sup>30</sup>

### *Agronomic biofortification*

Agronomic biofortification seeks to alter the environment, while conventional breeding and genetic modification take advantage of the genetic properties of the plant that can improve calcium content and bioavailability. The calcium content in the plant is primarily limited by the supply and phytoavailability of calcium within the rhizosphere.<sup>31</sup> Soil properties, such as pH, water content, soil structure, and microbial activity, can also alter the calcium being taken up by the plant. In general, higher soil pH improves calcium availability.<sup>120,121</sup> In acidic soils, aluminum cations may inhibit calcium uptake, while sodium inhibits calcium uptake in saline soils.<sup>36</sup> As such, altering the properties of the soil (e.g., increasing the pH) or adding fertilizers containing phytoavailable calcium can improve the uptake of calcium into the plant and accumulation into edible tissues. Common calcium-containing fertilizers, including lime (CaO and CaCO<sub>3</sub>), gypsum (CaSO<sub>4</sub>), calcium phosphate, and calcium nitrate, have been shown to increase the calcium content in tubers and leaves but not always in the fruits and seeds.<sup>31</sup> However, adding lime to the soil can also reduce the bioaccessibility of magnesium to the roots due to carbonate formation and excess

calcium in the alkaline soil.<sup>36</sup> Some plants can also alter the rhizosphere to improve the bioavailability of the soil's mineral content, as can some arbuscular mycorrhizal fungi.<sup>122</sup>

In one example of agronomic biofortification, durum wheat grown on peat in mineral solutions resulted in a 76% increase in calcium content in the roots and shoots and doubled the calcium content in the grains without toxicity.<sup>123</sup> Similarly, calcium content was increased in mustard and lettuce grown in hydroponic solutions of calcium.<sup>124</sup> This treatment also increased the number of leaves without decreasing biomass.

Calcium fertilizers can help improve soil health, especially in sub-Saharan Africa. Unfortunately, adding calcium to the soil and leaves does not improve the crop yield, so there is little incentive for a farmer to use these relatively expensive methods to improve calcium content in their crops.<sup>125</sup> There is also concern for negative environmental impacts, so precise application strategies should be used.<sup>31,122</sup>

Foliar application of calcium can also improve calcium content, though this is most often used to prevent calcium deficiency and can stimulate more uptake of calcium, especially in the leaves. Unfortunately, this is a more expensive approach and is readily washed away by rain.<sup>122</sup> Foliar applications of calcium applied to Rocha pear trees in which the leaves were treated with either Ca(NO<sub>3</sub>)<sub>2</sub> or CaCl<sub>2</sub> showed an increase in calcium content in the fruit, both in the epidermis and the central region of the fruit.<sup>126</sup> Importantly, there was no toxicity to the plant and no significant change to the sensory acceptability of the fruit. In another study, sunflower sprouts were treated with CaCl<sub>2</sub>, which increased calcium as well as total flavonoids and phenolics in the sprouts.<sup>127</sup>

Due to the higher costs of manufacturing and distributing calcium fertilizers, agronomic biofortification is recommended in fairly niche areas and as a complement to breeding programs to improve calcium. To date, there are few studies that show a direct link between the application of such fertilizers and the impact on increased intake and health.<sup>128</sup>

### *Conventional plant breeding*

Biofortification through conventional plant breeding requires existing and useful genetic variation for targeting a nutrient of interest. For calcium, relatively little is known about the genetic control or

physiological mechanisms that can lead to higher calcium content in the grain of staple crops. As a result, this requires additional research to identify crops with high amounts of calcium.

While biofortification through breeding is still in the early stages of understanding the genes involved in calcium regulation, there are several potentially good starting points to explore plant breeding to increase calcium content. Currently, finger millet is known to have relatively large amounts of calcium at 344 mg/100 g of dry weight compared with pearl millet, which contains 42 mg/100 g of dry weight, and other cereal grains.<sup>33</sup> Finger millet also has high grain yield potential and environmental sustainability,<sup>33</sup> as well as many accessions in gene banks from around the world,<sup>129</sup> which makes this form of millet a good starting point for conventional breeding programs. Other cereals, such as wheat, have fairly high calcium values, and their genetic variability could be explored. Legumes also provide some potential based on their genetic variability, especially in the wild lines that contain up to nine times higher amounts of calcium than the cultivated versions.<sup>130</sup> Use of lower-phytate corn can help improve the fractional absorption of calcium in corn by about 30%.<sup>131</sup> However, any alteration of phytate content in the originating plant must be considered for its potential effects on yield, either direct<sup>132</sup> or indirect.<sup>119</sup>

### *Transgenic modification*

The major genetic targets for transgenic modifications to increase calcium in plants are overexpression of calcium transport proteins, calcium channels, and other calcium-binding proteins. Most of the research to date has focused on cation exchanger 1 (CAX1), which exchanges  $\text{Ca}^{2+}$  and  $\text{H}^+$  and can thereby increase calcium content.<sup>133</sup> Ensuring the health of the plant can also be a challenge because the overexpression of some of these genes has resulted in defects in the plants that could lead to mineral imbalances and growth defects.<sup>134</sup>

Other opportunities remain for genetic modification of plants, such as lowering phytic acid levels, which could increase the bioavailability of micronutrients, including calcium.<sup>135,136</sup> Calcium-dependent protein kinases could be a future target that can increase a plant's stress resistance. This may

prove especially important for climate change and improving the drought resistance of plants. This has been done for potatoes and rice to enhance their tolerance to drought, stress, and diseases.<sup>137,138</sup> It will also be important to monitor the heavy metal uptake in transgenic plants as it may be possible for ions similar to calcium, such as cadmium, to be transported into the plants along with the calcium, and increasing calcium uptake could lead to higher levels of these toxic ions.<sup>139</sup>

### **Interactions with other food components**

Several food components may affect the absorption of calcium present in many foods. Oxalates and phytates (described above) are plant components that create insoluble salts with calcium, resulting in reduced bioavailability.<sup>32</sup> Oxalate salts are found in strawberries, spinach, rhubarb, beets, nuts, wheat bran, chocolate, tea, and coffee.<sup>140</sup> In particular, calcium oxalate crystals are insoluble at the intestinal pH.<sup>141</sup> However, some gut bacteria can break down the oxalates, including *Oxalobacter formigenes*, *Lactobacillus*, and *Bifidobacterium*. These bacteria have been proposed for use in the form of probiotics for oxalate degradation.<sup>140</sup> However, this is under investigation, and the effect on increasing calcium absorption is rather small, that is, <5%.<sup>47,142</sup>

Sodium consumption can increase the elimination of calcium through urinary excretion (calcinuria).<sup>143</sup> As the amount of sodium consumed is on the rise, largely due to the increased consumption of processed foods, including bread, this is an especially important consideration. As a result, reducing sodium consumption can improve the retention of calcium.

Vitamin D can help promote the absorption of calcium depending on age and health/vitamin D status. Vitamin D status influences active calcium absorption through vitamin D- and parathyroid hormone-dependent upregulation of transport proteins, which takes longer than can be accomplished to influence coingested calcium.<sup>144</sup> Nevertheless, combining calcium with vitamin D can assist with adherence to taking both nutrients through supplementation. A recent study using isotopic techniques has shown that vitamin D<sub>3</sub> supplementation in the short term causes positive bone mineral balance, leading to better calcium deposition into the bone.<sup>145</sup>

Based on animal models, lactose may also help in the passive absorption of calcium. But calcium absorption from various dairy products that differ widely in lactose content is similar in humans.<sup>146</sup> Milk proteins, such as casein phosphopeptides,<sup>12</sup> can also promote calcium absorption, but the effect is modest.<sup>147</sup> Some amino acids (i.e., lysine and arginine) can help convert calcium into a more soluble form in the lumen.<sup>148</sup>

Prebiotic fibers can increase calcium absorption and retention in children and adults.<sup>149–153</sup> In one study, adolescents consumed short- and long-chain inulin-like fructans, which increased calcium absorption and BMD, and this effect was influenced by the genotype of a vitamin D receptor gene, *FokI*.<sup>152</sup> Soluble corn fiber (10 and 20 g/day) was also shown to increase calcium retention in postmenopausal women and improved calcium balance by 50 mg/day.<sup>153</sup> Similar results were found in adolescents as well.<sup>150,151</sup> It is thought that the soluble corn fiber is fermented by one group of bacteria and the metabolites are fermented further by a second group of bacteria, which in turn results in increased calcium absorption.<sup>151</sup>

There have been concerns raised over the interactions of calcium with other divalent cations, such as iron and zinc. Short-term supplementation studies showed that increasing calcium can interfere with the intestinal absorption of iron, but this does not appear to have any long-term effects on iron status. A recent systematic review suggests the short-term effect is small, but more work is needed to better understand the effect.<sup>154</sup> This is described in more detail in the supplementation article in this special issue.<sup>86</sup> With respect to foods, the quantities of micronutrients consumed at any one time are likely to be smaller and thus have a less of an impact on absorption.

### Remaining research questions

There are a number of unanswered research questions that would better inform policies and interventions for food-based approaches to improve calcium intake. One such issue that remains unknown, especially for calcium, is the potential impact of calcium interventions on the burden of disease. It would be helpful to have a validated indicator of impact for modeling interventions, whether this is in terms of disability adjusted life years or lives saved from maternal and perinatal mortality.

The effectiveness and cost-effectiveness of the various calcium interventions discussed here are also largely unknown and would be useful in advocating for interventions, such as fortification or the impact of water fortification on infrastructure. However, it is also unclear whether the costs of agronomic biofortification can be overcome to increase the calcium intake in a meaningful way among those most vulnerable, and if fertilizer formulations can be optimized to reduce costs.

Also, there is a need to better understand which foods and staples are amenable to conventional or transgenic calcium biofortification and if there is an ideal “basket” of foods that can be biofortified/fortified to reach all vulnerable populations. Investments are needed to characterize the genes involved in the acquisition and mobilization of calcium and other nutrients among millets, other cereals, and legumes in order to identify future candidates for biofortification. High-quality reference genomes must be created in order to apply the increased capabilities of next-generation sequencing platforms that will enable doing so at increasingly faster speeds.

There are also remaining needs in overcoming the impacts of antinutrients, such as phytates in whole grain flours, in conventional or transgenic biofortification methods and/or food processing methods, by using phytase to increase calcium absorption. The answer could lie in traditional practices and crops that had relatively high levels of calcium, such as millet and other ancient grains.<sup>155</sup> Lastly, the elevated CO<sub>2</sub> from climate change is likely to decrease nutritional quality, which may impact the calcium content of crops and is expected to affect the availability and affordability of foods, including those rich in calcium.<sup>156</sup> The effects of climate change are also expected to result in a decline of small local fish, which are a natural source of calcium for some populations.<sup>15</sup> Determining how climate-related challenges will impact calcium intake and developing solutions will be an important step in providing adequate dietary calcium in the future.

These research gaps would also help inform which policy levers would be available to increase local supplies of natural calcium-source foods to reach vulnerable populations if increasing scale is a viable option (e.g., production subsidies, trade policies, etc.).

## Conclusions

Calcium intake in many LMICs remains well below the recommended levels. To help improve calcium intake, there are a number of interventions that can be implemented. These include promoting the consumption of foods naturally high in calcium, using food processing techniques that can improve calcium content or bioavailability, staple food fortification, and biofortification to produce higher calcium-containing crops. These interventions are available to policy makers interested in improving calcium intake. Monitoring and evaluation of the coverage and health impacts of such interventions is strongly encouraged. Addressing some of the research gaps will also help optimize interventions and should lead to greater understanding of the health impacts of improving calcium intake.

## Acknowledgments

We gratefully acknowledge Christine Stewart for her critical review and comments on this paper. The meetings of the Calcium Task Force, the development of this paper, and its open access were supported by funding from the Children's Investment Fund Foundation to the Nutrition Science Program of the New York Academy of Sciences.

## Competing interests

The authors declare no competing interests.

## Peer review

The peer review history for this article is available at: <https://publons.com/publon/10.1111/nyas.14743>

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