Nutrition and Health
Series Editor: Adrianne Bendich

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Handbook of Food Fortification and Health

From Concepts to Public Health Applications
Volume 2

Humana Press

Nutrition and Health Series

Adrianne Bendich, PhD, FACN, FASN, Series Editor

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ISBN 978-1-4614-7109-7 ISBN 978-1-4614-7110-3 (eBook)
DOI 10.1007/978-1-4614-7110-3
Springer New York Heidelberg Dordrecht London

Library of Congress Control Number: 2013940062

Printed on acid-free paper

Humana Press is a brand of Springer
Springer is part of Springer Science+Business Media (www.springer.com)

Preface

In this book the Editors aim to disseminate important material pertaining to the fortification of foods from strategic initiatives to public health applications. It covers (in two volumes) policy, preclinical studies, clinical investigations, and the impact of fortification on the individual and whole communities. The importance of food fortification relates to the fact that optimal nutritional intake is an essential component of health and well-being. Unfortunately, situations arise on a local or national scale when nutrient supply or intake is deemed to be suboptimal. As a consequence, ill health occurs, for example, neural tube defects in the developing fetus, organ damage in adults, or increased rates of premature deaths. In terms of public health, malnutrition due to micronutrient deficiency can be quite profound, imposing economic and social burdens on individuals and whole communities. The complex inter-relationship between poor health outcomes and nutrient delivery can, however, be modulated by appropriate food fortification. Thereafter issues arise as to the efficacy of food fortification, what strategies should be employed and what nutrients to add. The food carrier is also important, as well as its stability. Ethical issues also arise, and the concept of potential harm also needs to be addressed in terms of cost-benefits. All of these aspects, and many others, are covered in *The Handbook of Food Fortification*: *From Concepts to Public Health Applications*.

This comprehensive text examines the broad spectrum of food fortification in all its manifestations.

The term *fortification* has multiple meanings and is often used synonymously in relation to the addition of any component to food to facilitate a nutritional advantage. In this book we cover fortification not only in terms of its more strict definitions, in terms of the addition of micronutrients, i.e., minerals and vitamins, but also within the context of its wider and holistic applicability. The book thus recognizes the international differences in definitions and usage of fortification. At the same time we also include chapters on novel fortificants that are contained within more complex food matrices. However, whilst some micronutrients are permitted fortificants in one country, their inclusion in some foods may be prohibited or at the "discussion" or pre-legislative stage in another country. These complexities in terminology are recognized by the Editors. In all there are two volumes with eight main parts, namely:

Volume 1

Volume 2

Part I: Novel Food Vehicles and Agents for Fortificants
Part II: Impact on Individuals
Part III: Public Health, Concepts and Issues
Part IV: International Perspectives

Key features within each chapter include key points and, where relevant, guidance on safe levels and recommendations.

The Handbook of Food Fortification: *From Concepts to Public Health Applications* represents a multidisciplinary approach to food fortification and is written by many authoritative individuals, from centers and institutions around the world. It is designed for nutritionists, dietitians, medical practitioners, educationalists, health experts, epidemiologists, and other health-related professionals. It is also suitable for students, graduates, postgraduates, researchers, lecturers, teachers, and professors.

London, UK

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Series Editor Page

The great success of the Nutrition and Health Series is the result of the consistent overriding mission of providing health professionals with texts that are essential because each includes (1) a synthesis of the state of the science, (2) timely, in-depth reviews by the leading researchers in their respective fields, (3) extensive, up-to-date fully annotated reference lists, (4) a detailed index, (5) relevant tables and figures, (6) identification of paradigm shifts and the consequences, (7) virtually no overlap of information between chapters, but targeted, inter-chapter referrals, (8) suggestions of areas for future research, and (9) balanced, data-driven answers to patient as well as health professionals' questions which are based upon the totality of evidence rather than the findings of any single study.

The Series volumes are not the outcome of a symposium. Rather, each editor has the potential to examine a chosen area with a broad perspective, both in subject matter as well as in the choice of chapter authors. The editor(s), whose training(s) is (are) both research and practice oriented, have the opportunity to develop a primary objective for their book, define the scope and focus, and then invite the leading authorities to be part of their initiative. The authors are encouraged to provide an overview of the field, discuss their own research, and relate the research findings to potential human health consequences. Because each book is developed de novo, the chapters are coordinated so that the resulting volume imparts greater knowledge than the sum of the information contained in the individual chapters.

The Handbook of Food Fortification: From Concepts to Public Health Applications edited by Professor Victor R. Preedy, PhD, DSc, FRIPH, FRSH, FIBiol, FRCPath, Professor Rajaventhan Srirajaskanthan, BSc (Hons), MD (Res), MRCP, and Vinood B. Patel, PhD, clearly exemplifies the goals of the Nutrition and Health Series. The major objective of this comprehensive two volume text is to review the growing evidence that food fortification has a major role in assuring adequate intake of the essential nutrients. Fortification also serves as an important vehicle for introducing newer bioactive components of food, such as carotenoids, into the diet of a population. This volume includes 63 up-to-date informative reviews of the current major fortification programs underway around the world and examines the consequences of the programs. Practicing health professionals, researchers, and academicians can rely on the chapters in this volume for objective data-driven sources about essential vitamins and minerals, proteins and fats as well as other dietary components that have been included in basic food sources. This new comprehensive review of the science behind the fortification strategies that help to assure the health of the populations at risk by providing essential nutrients is of great importance to the nutrition community as well as for health professionals who have to answer patient, client, or graduate student questions about the newest clinical research in the nutritional effects of food fortification.

The Handbook of Food Fortification: From Concepts to Public Health Applications represents the most comprehensive compilation of the recent data on the actions of specific essential nutrients

and bioactive dietary components in at risk populations around the globe. It is to the credit of Drs. Preedy, Srirajaskanthan, and Patel that they have organized this volume so that it provides an in-depth overview of the critical issues involved in the determination of the best fortification strategies for infants, toddlers, school-age children, and adult populations, whether they were born in developing nations or in developed nations. The volumes' editors provide their in-depth knowledge and expertise to help the reader to understand the value of food fortification. Professor Preedy is a senior member of King's College London where he is a Professor of Nutritional Biochemistry and is also a Professor of Clinical Biochemistry at King's College Hospital. He is also Director of the Genomics Centre and a member of the School of Medicine. He is a member of The Royal College of Pathologists, a Fellow to The Institute of Biology, The Royal College of Pathologists, The Royal Society for the Promotion of Health, The Royal Institute of Public Health, The Royal Society for Public Health, and in 2012 a Fellow of The Royal Society of Chemistry. Professor Srirajaskanthan is a consultant gastroenterologist at University Hospital Lewisham and Kings College Hospital, London. He trained at the prestigious Guy's, King's and St. Thomas' Medical School where he obtained his MD (MBBS) and a B.Sc. in Neuroscience. He is a member of the Royal College of Physicians. His specialist training includes Gastroenterology, Hepatology, and Internal Medicine. Dr. Patel is a Senior Lecturer in Clinical Biochemistry at the University of Westminster and honorary fellow at King's College London. Dr. Patel obtained his degree in Pharmacology from the University of Portsmouth, his PhD in protein metabolism from King's College London and completed postdoctoral research at Wake Forest University School of Medicine.

Each of the two volumes contains about 30 comprehensive chapters. The first volume contains four related parts. The first part provides an overview and perspective on national and international fortification strategies and policies. Five chapters examine the complexities of developing fortification initiatives and use examples of successful and not so successful fortification programs. The first chapter presents a new attempt to provide blueprint for global flour fortification with essential nutrients for countries with major population groups at risk for micronutrient deficiencies. The Flour Fortification Initiative (FFI) goal is to provide a flour fortification standard milling practice throughout the world. The focus is wheat and maize flours. The FFI model is based upon engaging partners in the public, private, and civic sectors. The chapter uses the example of how countries began fortifying flour with folic acid to prevent birth defects to illustrate the public-private-civic sector collaboration involved in flour fortification.

Micronutrient fortification of staple foods had been in place for decades before the understanding of the role of folic acid in birth defect prevention was confirmed in clinical trials. The historic perspective and current fortification programs in both developing and developed nations are examined in the next chapter. Clear definitions of terms, tables that include the countries with mandatory fortification programs, details concerning the concentration of micronutrients used in fortification, as well as a review of the positives and negatives of using food fortification to improve nutrient status are included. The following chapter examines the critical need for fortification of foods provided to the neediest populations that are consuming foods provided for humanitarian relief. Humanitarian food aid involving global food assistance utilizes organizations including the United Nations Food and Agricultural Organization (FAO), World Food Program (WFP), and the Food for Peace Act (FFP). WFP reported that over 5,000,000 metric tons of food aid was distributed to humanitarian relief efforts in 2010. The chapter includes data on diverse foods provided and the micronutrients included in the major humanitarian food programs. Food fortification programs in the Middle East are described in the next chapter that examines the vast diversity in the economic status of the 20+ countries included in the term "Middle East." The chapter examines the difficulties in reaching relatively small populations within wealthy nations that may benefit from foods fortified with micronutrients and uses national programs of flour fortification with iron as an example. Currently, ten countries in the Middle East do not have a mandatory flour fortification program in place. The last chapter in this part describes the historic development of fortification practices in Canada and begins with the establishment of mandatory

uniform iodine fortification in 1949 after more than 2 decades of voluntary fortification initiatives across the country that used different concentrations of iodine. In contrast to the United States that mandated flour fortification with certain B vitamins and Iron in the early 1940s, Canada did not have mandatory flour fortification until 1976. Canada continues to examine its fortification policies especially with regard to voluntary food fortification initiatives.

The second part contains six chapters on national programs to implement iron fortification of staple foods and water. The chapter authors remind us that there may not be one mechanism that can provide iron to all populations at risk. Food fortification is a major source of iron for millions of anemic infants, children, and women worldwide. The six chapters are devoted to reviewing the multiple foods and iron compounds that have been used to enhance iron status in at risk populations. Iron has a complex chemistry as well as metabolism and reactions are dependent upon pH, potential oxidation, competition for other minerals, and presence of food components that can block or enhance its absorption. Because of these issues, and the fact that population groups consume different staple foods, many foods have been considered for iron fortification. For example, soy sauce in China, tonyu in Japan, and Nuoc-mâm (fish sauce) in Vietnam has been successfully used to reduce iron deficiency. As dairy products are widely consumed in Europe and North America, the next chapter considers the pros and cons of fortifying dairy products and milk with a number of potential iron fortificants.

Another chapter describes the use of parboiled rice to deliver both iron and zinc. Fortification of iron and zinc in the parboiling process increased iron and zinc concentrations, especially in polished rice where most of iron and zinc is usually removed during milling. Parboiled rice is produced on industrial scale and traded globally. It is commonly consumed in South Asia and Africa where iron and zinc deficiencies are widespread. Iron and zinc deficiencies have been estimated to affect 70–95 % of the population in Asia. There is also a chapter that describes the benefits of fortifying millets with iron and zinc. Millets are used chiefly as food grains in Africa, Eastern Europe, China, India, and other Asiatic countries. Finger millet, sorghum, and pearl millet are widely grown and consumed as the staple in several parts of India. The beneficial effects of fortifying millets for Indian populations are described. There is also a chapter on the clinical finding from experiments with iron fortification of drinking water that seem quite promising.

Many iron compounds are used as food fortificants. These must meet the requirements of high iron bioavailability, inertness in relation to the sensorial properties of the fortified food, absence of toxicity, resistance during storing or processing of the fortified food, and have a bioavailability similar to that found with naturally occurring iron in food. Two chapters describe specific sources of iron. One of the possible newer salts of iron that has been used to fortify food is ferric pyrophosphate and is described in the next chapter. Ferric pyrophosphate is a poorly soluble iron compound that does not change organoleptic properties of foods even when used in many difficult-to-fortify food vehicles. Reduction in the particle size of this iron salt has greatly increased its bioavailability. The second chapter describes the benefits of heme iron. Heme is a biologically important source of dietary iron because of its significantly greater bioavailability compared to non-heme iron sources. Dietary heme sources include foods containing myoglobin and hemoglobin such as meats, fish, and poultry. The positives and negatives of using heme iron as a food fortificant are described. Several studies have evaluated fortification with heme iron in different foods using biscuits, cookie fillings, weaning foods, flour, and black beans.

The third part of this volume contains 11 chapters that describe fat-soluble and water-soluble essential nutrients that are used in a number of food matrices to enhance the dietary intakes of these nutrients. Fats used as vehicles for fortification include margarine, fat spreads, cooking oils, eggs, and dairy products. Dairy products are also used as vehicles for minerals and other water-soluble nutrients. Both cow milk and soy milk products are described; commonly used fortificants include calcium, zinc, iron, iodine, and selenium. The critical issues of maintaining safe doses of minerals and at the same time assuring the palatability of the fortified milk products are discussed in each chapter. Other dairy-based foods that have been fortified with fat-soluble vitamins and certain minerals include cheeses, yogurt, fermented milk products, butter, and cream.

Additionally, corn, rice, noodles, water, and salt are reviewed as sources of added nutrients for at risk populations. Corn fortification is reviewed and indicates that it is an excellent vehicle for delivering iron, folic acid, zinc, copper, vitamins B1 and B2, and calcium. Rice, flours, and noodles have also been used to fortify their levels of folic acid and other B vitamins, and at times, vitamin C. Salt has been used as a carrier for iodine for almost 100 years and continues to be a well-accepted food source for reducing iodine deficiency disorders in at risk nations. Two detailed chapters review the biological effects of deficiencies, the choices of each fortificant and types of salts used for fortification, the complexities of maintaining the concentrations of this fortificant, assessment of bioavailability, and clinical studies of efficacy.

Margarines and fat spreads have delivered vitamins A and D in certain developed nations for more than 50 years and have also been used successfully in underdeveloped countries. Vegetable oils are also suitable vehicles for fortification with the fat-soluble vitamins A, D, and E. The fat-soluble vitamins form a true solution and are uniformly distributed in vegetable oils. The stability of vitamin A is greater in oils than in any other food, and oil facilitates the absorption of vitamin A by the body. There is a separate chapter devoted to the value of fortifying vegetable oils with vitamin A and the other fat-soluble vitamins. Newer fortificants in fats include n-3 fatty acids, plant sterols, vitamin E, vitamin C, and carotenes as well as iodine. A new potential fortificant is also described in a separate chapter. Conjugated linoleic acid (CLA) is a mixture of isomers of the essential fatty acid, linoleic acid. CLA is mainly found in food products from ruminants such as dairy products and beef. CLA is a major fat in milk fat. The reason that CLA is considered as a fortificant for nondairy sources is that recent research studies have reported anticarcinogenic, antiatherogenic, antioxidative, and immune system enhancement as well as reduction of body fat. Preliminary clinical studies are reviewed.

Enhancing the efficiency of plants to concentrate nutrients essential for human life through selective breeding is called biofortification. The uptake of certain minerals by plants is usually controlled by several genes. Choosing plants that contain higher than normal levels of the desired mineral and using the seeds of these plants for cultivation of the next generation can result in more uniform higher levels of this mineral in subsequent generations. Biofortification through genetic engineering of staple foods is a new and important avenue for fortifying staple foods. Rice, corn, and carrots have been successfully enriched with essential nutrients and carotenes. Biofortification studies of the enhancement of selenium levels in lentils are described specifically in a unique chapter. A second unique chapter describes the biofortification of eggs with tocotrienol and tocopherol, vitamin E sources, by feeding the egg laying hens with a diet high in tocotrienols and tocopherols in rice bran oil.

The fourth part, entitled Biofortification: Biological Modes of Enhancing Nutrient Intake, contains seven chapters that include discussions of genetic modifications to rice, corn, wheat, and sweet potatoes. Traditional breeding methods alone may not be a valid option for grain biofortification due to low levels of genetic variability for mineral uptake into the edible portions of the plant. Gene technology can enhance micronutrient concentrations in many grains. These chapters include detailed descriptions of the genetic vectors used to deliver new codings for mineral binding proteins, transporters, and other mechanisms to significantly enhance the concentration as well as the bioavailability of the essential nutrient. Examples reviewed include the successful biofortification of rice with vitamin A, iron, zinc, folic acid. Unlike minerals that are available in the soil, plants must synthesize vitamins, and several genes are involved in the synthesis of any vitamin. Thus, the complexity of inserting all of the genes required for the synthesis of folate, as an example, and assuring that the biochemical reactions occur in the right order and in the right place within the plant cell, is monumental. Plant scientists have, in fact, been able to biofortify rice so that its concentration of folate is significantly greater than seen with traditional breeding programs. Biofortification of corn, using genetic engineering, has resulted in significantly increased concentrations of beta carotene (provitamin A), folate, vitamin E, and ascorbic acid (vitamin C) as described in another chapter. The chapter includes a detailed description of the newest genetically engineered multivitamin corn. Another chapter describes the processes used to affect the selenium content of wheat agronomic biofortification

which involves fertilizing the growing crop with the micronutrient, which the plant converts to several organic Se forms, notably selenomethionine, which are more suitable for human consumption. As exciting as the new genetic engineering research is to the scientific community, there may be many barriers to consumer and national acceptance of these new fortified products. The chapter on the potential benefits and risks of adopting folate fortified rice in Chinese communities is explored in a fully tabulated chapter. In a complementary chapter, there is an insightful discussion of the introduction of orange sweet potatoes in sub-Saharan Africa where the usual sweet potato was white and lacked any carotenoid content. The linking of agriculture to nutritional content of a staple food is reviewed. This part also contains a unique chapter that reviews the nutritional content of gluten-free foods and tabulates the essential micronutrient levels in many gluten-free products. The chapter includes a detailed discussion of the importance of biofortification in enhancing the micronutrient content of grains used in these products.

The second volume of *The Handbook of Food Fortification: From Concepts to Public Health Application* emphasizes the clinical and public health consequences of fortification programs. The four parts in this volume include chapters devoted to novel food vehicles and agents for fortification; the impact of fortification on different population groups and individuals; public health concepts and issues and finally, a critical part on international perspectives. The first part contains ten chapters that explore the potential for fortifying staple foods and commonly consumed foods including eggs, meats, yogurt, cheese, fish and fish sauces, and drink products with essential micronutrients as well as fiber and n-3 fatty acids. There is a strong rationale presented for using eggs as vehicles for fortification. Fortified eggs combine an important animal food that naturally contains high quality protein and amino acids, fats and essential fatty acids, and certain vitamins and minerals, with a unique capacity to be fortified with added essential nutrients and phytonutrients and effectively deliver these with high bioavailability. Nutrients discussed included iodine, selenium, zinc, iron, copper, manganese, chromium, fat and water-soluble vitamins and carotenoids, choline, and long chain n-3 fatty acids that are provided to the hens for transfer to the eggs. New fortificants for cheeses and new technologies to incorporate these into cheese products are included in another chapter. Microencapsulation, emulsions and gel particles, and immobilization on polymeric complexes have permitted the addition of probiotics, essential micronutrients, polyphenols, and carotenoids to be incorporated into cheeses. Other commonly consumed foods consumed daily (mainly in Asia) include fish sauce and soy sauce. In certain nations, there is mandatory fortification of these sauces with iodine. Currently, incentives are underway to also fortify with iron compounds to further enhance the nutritive value of these sauces as described in a separate chapter.

Ocean fish are an important source of long chain n-3 fatty acids; however farmed fish require sources of n-3 fatty acids in their diets to be able to incorporate these oils into their muscle tissues. New sources of n-3 fatty acids for farmed fish and enhanced production practices to assure decreased risk of oxidation are reviewed. Enhancement of long chain n-3 fatty acids in feeds has been undertaken with ruminants including beef cattle and lambs, pork, and poultry. The risks and benefits of these fortification strategies are discussed in a separate chapter. Another opportunity to deliver long chain n-3 fatty acids to the diet, especially for individuals who do not consume fish, is the development of n-3 fortified beverages. The technological issues as well as the clinical evidence of efficacy are discussed in a new meta-analysis of published data found in systematic review included in this part. Beverages that were fortified include cow and soy milks, fruit and vegetable juices, and drinks.

Four chapters describe the use of waste and/or by-products from commercial production of processed foods for the development of excellent sources of missing nutrients in relevant populations. Examples of novel source of nutrients, polyphenols, and fiber are discussed. The term “apple pomace” refers to the left-over solid biomass after extraction of juice from fresh apple fruits and this product is being used in baked goods and other applications. Bovine lung is the second example of a waste product from cattle slaughtering that is being used to successfully fortify iron levels in processed ready-to-eat foods for anemic children. Date fiber is a by-product of date syrup production and this has been

incorporated into yogurt to add further value to this important staple food. Evaporated sugarcane juice, unlike refined sugar, is an important source of bioavailable iron. This by-product has been used as a natural sweetener for fruit juices and preliminary clinical studies reviewed in the chapter suggest that this fortificant is beneficial in anemic children.

The second part in this volume contains seven chapters that examine the effects of fortified food in specific populations including pregnant and lactating women, preterm infants, preschool and school-age children, postmenopausal women, and elderly living in nursing homes. A well-referenced literature review confirms that micronutrient fortified foods and beverages provided during pregnancy improved micronutrient status and reduced anemia rates in women. Also, micronutrient fortified foods, when combined with energy and essential fatty acids, resulted in improved pregnancy outcomes including increased birth weight and length. In addition, multiple micronutrient fortified foods combined with additional calories and essential fatty acids modestly improved the growth of infants and improved iron and vitamin A, but not zinc status. The effects on child development were inconsistent. One study reported an unexpected negative impact of fortification on morbidity. Malnutrition is often seen in school-age children in undeveloped communities. Two controlled studies are reviewed and confirm that intervention with a fortified lunch meal can improve iron and riboflavin blood levels in one study, and improved blood levels of many micronutrients when a multiple micronutrient powder was mixed with the food before cooking. Iodine deficiency is associated with significant declines in cognitive function in growing children. Fortification of foods with iodine and other micronutrients including iron was shown to enhance cognitive functions and school testing results in studies reviewed in another chapter in this part.

An even more at risk population for inadequate nutrient intake than young children are those that are born preterm. Moreover, human milk is an inadequate source of protein and minerals for the growing preterm infant. Neither human milk from a woman who has given birth prematurely nor human milk from a woman who has had an infant born at term is sufficient to provide all the nutritional needs of the preterm infant. Thus, human milk for the preterm infant is usually supplemented or fortified with additional protein, sodium, phosphate, calcium, magnesium, copper, zinc, and many vitamins (B2, B6, C, D, E, K, folic acid).

Another population group at risk for malnutrition is the elderly who are hospitalized or in nursing homes. Fortified foods and beverages are helpful in providing opportunities to enhance the nutritional status of these populations who are capable of consuming oral diets. One of the key nutrients that is often low in serum of immobile elderly is vitamin D. With lack of exposure to sunlight, there is a reduced ability to produce cutaneous vitamin D. This chapter reviews the other factors that can result in low vitamin D status including limited dietary intake, intestinal absorption, and kidney capacity to convert vitamin D to its active form. Fortification of bread with calcium and vitamin D enhanced blood levels of vitamin D and improved markers of bone health. Similarly, in a separate study reviewed in the next chapter, fortification of milk with calcium and vitamin D significantly enhanced bone mineral densities in elderly women.

The third part in the second volume examines the rationales used to implement food fortification strategies as public health interventions. Six chapters examine global as well as recent national initiatives to fortify foods with folic acid, vitamin B12, vitamin D, and other key nutrients. The first chapter in this part provides a broad overview of the requirements for consideration of beginning a new fortification program and also includes an in-depth analysis of potential barriers to adopting a food fortification program nationally. There is a unique chapter that examines the complexities involved with development and monitoring of a fortification program from regulatory perspective. There is a comprehensive explanation of the risk analysis process. One chapter reviews the overall use of folic acid fortification globally: 66 countries (one-third of the world's nations) have recommended mandatory folic acid fortification. Wheat or maize flour, and/or flour products such as noodles or pasta, are the food vehicles of choice used by all countries. Additional vehicles include rice and milk. Two chapters discuss the national strategies undertaken to fortify flour and/or other grains and foods with

folic acid for prevention of neural tube birth defects. In Canada, the fortification program resulted in significant decreases in these birth defects as well as other benefits to infants as well as adults. It is well recognized that higher than normal folate status can block certain of the signs and symptoms of vitamin B12 deficiency. The chapter on vitamin B12 provides cogent, well-referenced arguments for consideration of food fortification with vitamin B12 in addition to folic acid fortification.

The final part of the second volume examines the current international perspectives concerning fortification and its consequences. Of great importance is the consistent lack of essential vitamins and minerals in infants, and young children under 2 years of age, women of childbearing potential and elderly populations. The following chapters provide perspectives of ways that nations cope with malnutrition and the timeframe of adoption of mandatory food fortification programs in developed as well as developing nations. Country programs from Oman, Vietnam, Pakistan, Australia, India, Brazil, and Nigeria are included. There is also a concluding chapter that summarizes the current state of food fortification programs. It is important to note, as described in the chapter, that Oman was the first country in the Middle East to make fortification of flour with folic acid a national compulsory legislation in 1996. Iron was also added to flour. Although there was an 80 % decrease in neural tube birth defects following fortification, anemia levels in preschool children remained above 10 %. However, a partial explanation may be that 9.5 % of the children suffered genetic hemoglobinopathies associated with anemia. Anemia is a serious problem for Brazilian children. In Brazil, mandatory addition of iron (30 % recommended nutritional intake (RNI) or 4.2 mg/100 g) and folic acid (70 % RNI or 150 μg) to milled wheat and corn flour was implemented in 2001. The core objective of increasing the accessibility of milled cereal grains with iron and folic acid is to reduce the prevalence of iron-deficiency and neural tube defects in Brazil. Iron-fortified water and orange juice are being explored as alternative vehicles to reduce iron deficiency in Brazil. Nigeria also has a significant issue of childhood anemia. Nigeria is one of ten countries in the world with the largest number of underweight children, with an estimated six million children under five who are underweight. Micronutrient deficiency is a direct cause of child morbidity and mortality. Micronutrients such as iron, iodine, vitamin A are missing in children's diets and 40 % of Nigerian pregnant mothers did not take any iron tablets, a recommended supplementation during pregnancy. It appears that a number of processed foods voluntarily add iron and other essential nutrients whereas mandatory programs do not appear to be in place.

A number of countries continue to have relatively high levels of childhood malnutrition. A recent survey on nutritional status of women and young children carried out in 2010 in randomly selected provinces in Vietnam confirmed that about 30 % of children under 2 years of age were stunted, 10 % were underweight, 2 % were wasted. In addition, about 15 % of children under 2 years of age had anemia. Moreover, 3 % had zinc deficiency, 12 % had vitamin A deficiency, and 50 % had marginal vitamin A status, almost 60 % had vitamin D deficiency and over 98 % had mild hypocalcaemia. Programs undertaken to provide a micronutrient fortified cereal as a complementary food for young children has resulted in a decrease in the number of malnourished children as well as increases in growth. Pakistan also has a significant number of children that suffer from iron and vitamin A deficiency. However, due to its geographical position, there is also a very high incidence of iodine deficiency and folate deficiency seen in women of childbearing potential. Salt fortification with iodine has increased over the past years and the hope is that 100 % of salt in Pakistan will contain iodine by 2013. New programs are being initiated with the primary goal of reducing the prevalence of iron-deficiency anemia among preschool children from 30 to 10 %, and in women of reproductive age from 50 to 18 %, and half the occurrence of neural tube defects among newborns from 0.4 to 0.2 % of live birth, through universal fortification of wheat flour with iron and folic acid. Indonesia also has a significant number of children with micronutrient deficiencies. Food fortification in Indonesia was initiated in 1994 with mandatory fortification of iodized salt. In 1997 trials on wheat flour fortification with iron, zinc, folate, vitamins B1 and B2 were started and as a result wheat flour fortification became mandatory in 2001. In 2011, vitamin A fortification in cooking oil was encouraged as a voluntary fortification.

Since 2001, India has implemented a school feeding program in all public primary schools. Under this program, commonly known as the "midday meal" scheme, primary schoolchildren receive free lunch meals, cooked and served at school for at least 200 days in a school year. In most States, the program has a standardized menu that consists mainly of rice and dhal (sauce prepared using pulse/lentils) or vegetables. A micronutrient intervention study in Himalayan villages in India was implemented and described in a separate chapter. The 8 month study of the intake of the micronutrient fortified food by schoolchildren was associated with significant improvements in vitamin A, folate, and vitamin B12 status as well as significant improvements in iron status but not hemoglobin levels or reductions in anemia. Although India is a sun-rich country, vitamin D deficiency has been reported in all age groups from pregnancy to late adolescence. Vitamin D deficiency is often accompanied by low calcium intakes. Reasons for this deficiency include cultural as well as genetic issues. Very few foods are fortified with vitamin D in the Indian market, and these are not commonly consumed. As there is very little vitamin D in Indian children's diet, providing vitamin D through supplementation or fortification to vulnerable groups may be the strategy of choice.

The historical account of the move from voluntary folic acid fortification of flour to mandatory fortification in 2007 in Australia is very informative, especially as Australian researchers provided seminal data showing the value of folic acid for neural tube birth defect prevention. The range of programs, initiatives, and resources available to nations and communities to help implement fortification programs is vast. The last chapter in the second volume provides readers with web addresses to hundreds of resources.

The logical sequence of the parts in each volume as well as the chapters within each part enhances the understanding of the latest information on the current standards of practice in food fortification in different countries around the world. This comprehensive two volume resource has great value for academicians involved in the education of graduate students and postdoctoral fellows, medical students, and allied health professionals and public health nutritionists who plan to interact with populations at risk for macro and/or micronutrient deficiencies.

The volume contains over 400 detailed tables and figures that assist the reader in comprehending the complexities of food technology, biological mechanisms of metabolism of essential nutrients, composition of human breast milk compared to the needs of the preterm infant, sources of infant and childhood nutrition as well as nutrition requirements through the lifespan for males and females. There are in-depth discussions of the biological significance of the microbiome and its importance in maintaining growth and health. The overriding goal of this volume is to provide the health professional with balanced documentation and awareness of the newest research and fortification approaches including an appreciation of the complexity of the interactions between genetics, maternal health, the critical role in term and preterm infants of nutrient deficiencies and new issues of bioavailability in this relatively new field of investigation. Hallmarks of the 63 chapters include key words and bulleted key points at the beginning of each chapter, complete definitions of terms with the abbreviations fully defined for the reader and consistent use of terms between chapters. There are over 2,600 up-to-date references; all chapters include a conclusion to highlight major findings. The volume also contains a highly annotated index.

This unique text provides practical, data-driven resources based upon the totality of the evidence to help the reader understand the basics of the effects of nutritional deficiencies, complexities involved in the fortification of foods with single as well as multiple micronutrients, new research using the novel sources of nutrients as well as new foods for fortification, and preventive strategies that are being implemented in the most at risk populations in developing nations across the world. Explanations are provided for the role dietary components may play in the early development of infants and the role of genetics, metabolic, or other effectors. Of equal importance, critical issues that involve, such as food preferences, nutrient interactions that affect absorption and regulatory and public health perspectives in developing and developed nations are included in well-referenced, informative chapters. The overarching goal of the editors is to provide fully referenced information to health professionals so

they may have a balanced perspective on the value of various food fortification options that are available today as well as in the foreseeable future.

In conclusion, *The Handbook of Food Fortification: From Concepts to Public Health Applications* edited by Professor Victor R. Preedy, PhD, DSc, FRIPH, FRSH, FIBiol, FRCPath, Professor Rajaventhan Srirajaskanthan, BSc (Hons), MD (Res), MRCP, and Vinood B. Patel, PhD provides health professionals in many areas of research and practice with the most up-to-date, well-referenced and comprehensive volume on the current state of the science and medical practice guidelines with regard to the value of food fortification programs. This volume will serve the reader as the most authoritative resource in the field to date and is a very welcome addition to the Nutrition and Health Series.

Morristown, NJ, USA

Adrianne Bendich, PhD, FACN, FASN
Series Editor

About Series Editor

Dr. Adrianne Bendich, **Ph.D.**, **FACN**, **FASN**. has successfully served as Series Editor for the Nutrition and Health book series for 15 years and continues to identify key areas of clinical nutrition research that can benefit from the development of targeted, objective volumes edited by the leading researchers in their fields of investigation.

Prior to retiring in September, 2010, Dr. Bendich held the position of Director of Medical Affairs at GlaxoSmithKline Consumer Healthcare where she was responsible for the Medical leadership for the Venture Group, and provided Medical support for well-known Brands including TUMS, FiberChoice, Os-Cal, Geritol and Citrucel. Additionally, she served as a member of GSK's successful Advisory Committee team in support of FDA's Rx to OTC switch of alli®.

Dr. Bendich is internationally recognized as an expert in Women's Health, calcium and vitamin D in bone health, folic acid and pregnancy outcomes, antioxidants and carotenoid effects on immune functions. She served as the GSK corporate representative to the Women's Health Initiative (WHI) for 9 years as GSK Consumer Healthcare provided all calcium and vitamin D supplements for the WHI study.

She has held memberships and professional positions (ongoing and former): Editorial Board, Journal of Nutrition in Gerontology and Geriatrics, Antioxidants, an e-journal, Journal of Women's Health and Gender-based Medicine; Associate Editor for Nutrition, the International Journal; Chair, Corporate Advisory Committee, Society for Women's Health Research; Member of the Program Advisory Committee for Helen Keller International; Advisor to the Nutrition Department, Montclair State University; Member: Advisory Board, Current Topics in Nutraceutical Research; member ASN's Industry Board and several RIS groups.

Dr. Bendich was a recipient of the Burroughs-Wellcome Fund Professorship and Roche Research Award; she is listed in Who's Who of American Women Scientists and many other Who's Who volumes; she is a recipient of the CRN Apple Award for contributions to the science of vitamin and mineral supplements. In 2012, Dr. Bendich was elected a Fellow of the American Society for Nutrition, the highest honor of the Society.

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Acknowledgments

The development of this book is built upon the foundation of the excellent work provided by the staff of Humana and we are very thankful to them. In particular we wish to acknowledge the outstanding support, advice, and patience of the Series Editor, Dr. Adrianne Bendich, Developmental Editor, Michael Griffin, and Editor, Amanda Quinn.

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Part I
Novel Food Vehicles and Agents for Fortificants

Chapter 1
Multiple Fortified Egg for Comprehensive Nutritional and Health Support

Niva Shapira and Ossie Sharon

Key Points

- Egg is a very effective vehicle for multiple "nutritional fortification," combining an innately high nutritional value with a unique capacity to concentrate added essential nutrients, increase bioavailability, and effectively deliver them.
- Egg can become/be used as a "strategic" food for general dietary enhancement, as well as for specific nutrients that may be scarce in the food supply.
- "Functional modification" can effectively address the presumed deleterious egg-related effects, i.e., LDL oxidation, postprandial lipemia, fasting blood glucose, and inflammation.
- Improvement of the egg composition may justify recommendations for its use—as is, and as a fortified vehicle—for general nutritional support and for specific requirements.
- Egg modification/fortification offers a highly environmental/sustainable and economic/affordable method of enhancing high-quality animal protein production that is well-accepted by wide-ranging populations.
- The combination of a nutritious vehicle as a basis for multiple fortification is especially relevant, because undernutrition and specific deficiencies are often clustered and mutually exacerbated.
- Fortified egg is especially relevant perinatally, for maternal-fetal-infant nutritional support, when future health and performance is programmed for life.
- Sustainable fortification can be achieved by organic forms of minerals that potentially attain high egg content with lower feed concentrations.

Keywords Egg • Functional modification • Fortification • Malnutrition • Micronutrient deficiencies • n-6/n-3 PUFA • n-9 MUFA • Vitamins • Minerals

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V.R. Preedy et al. (eds.), *Handbook of Food Fortification and Health: From Concepts to Public Health Applications Volume 2*, Nutrition and Health, DOI 10.1007/978-1-4614-7110-3_1,

Abbreviations

≈	Approximately
>	Greater than
≥	Greater than or equal to
<	Less than
≤	Less than or equal to
μg	Microgram
%	Percent
+	Plus
±	Plus/minus
AA	Arachidonic acid
AI	Adequate intake
ALA	Alpha-linolenic acid
Apo	Apolipoprotein
CDC	Centers for Disease Control
Cr	Chromium
Cu	Copper
CVD	Cardiovascular disease
DASH	Dietary Approaches to Stop Hypertension
DHA	Docosahexaenoic acid
dL	Deciliter
DNA	Deoxyribonucleic acid
DPA	Docosapentaenoic acid
DRI	Dietary reference intake
EAA	Essential amino acid
EFA	Essential fatty acid
EPA	Eicosapentaenoic acid
FSA	Food Standards Agency
Fe	Iron
g	Gram
HDL	High-density lipoprotein cholesterol
HAOX	High antioxidant
HN-6	High n-6 PUFA
HN-9	High n-9 MUFA
I	Iodine
IOM	Institute of Medicine
IU	International unit
K	Potassium
kcal	Kilocalorie
kg	Kilogram
LA	Linolenic acid
LCPUFA	Long-chain polyunsaturated fatty acid
LDL	Low-density lipoprotein cholesterol
mg	Milligram
min	Minute
Mn	Manganese
MUFA	Monounsaturated fatty acid
n-3	Omega-3

n-6	Omega-6
n-9	Omega-9
NRC	National Research Council
OA	Oleic acid
OD	Optical density
PC	Phosphatidyl choline
PUFA	Polyunsaturated fatty acid
RAE	Retinol activity equivalents
RDA	Recommended daily allowance
RNA	Ribonucleic acid
Se	Selenium
SEM	Standard error of the mean
SO_4	Sulfate
UN	United Nations
US$	United States dollar
USDA	United States Department of Agriculture
WHO	World Health Organization
Zn	Zinc

Introduction: Why Egg Fortification

Fortified egg has the unique advantage of combining a primary animal food—innately providing the highest quality protein and amino acid proportions [1], fats and essential fatty acids (EFA), vitamins, and minerals—with a unique capacity to concentrate added essential nutrients and phytonutrients and effectively deliver them with high bioavailability [2–9].

Such a combination has the advantage of synergistic interaction in support of bodily functions and is greatly needed, as specific micronutrient deficiencies and general malnutrition are often clustered in targeted populations and conditions [10].

Micronutrient malnutrition can affect all age groups, but young children and women of reproductive age are among those most at risk [10]. Many nutrients are highly expended during pregnancy and lactation and progressively depleted, especially in conditions of multiparity and dietary inadequacy [11]. Malnutrition has many adverse effects on human health—even when moderate and subclinical—with profound implications for the burden of disease, economic development, and productivity, as translated to public health costs and loss of human capital [10]. In turn, early nutritional support has demonstrated a significant positive impact (Fig. 1.1). Success of maternal nutrition interventions incorporating eggs for improving perinatal outcomes has also been documented [11].

United Nations (UN) data (2002–2009, 369 studies from 76 countries) have suggested considerably inadequate nutritional status of school-aged children, i.e., 20–30 % prevalence of deficiencies of iron, iodine, zinc, and vitamin A [12]. In resource-poor settings (1988–2008), over 50 % of studies revealed inadequate mean/median intakes and folate insufficiency in 91 %. This emphasizes the need to increase the quality of maternal-child diets [13], especially during the perinatal period, when early "programming" of future health [14, 15] and performance is established (Fig. 1.2). A recent study has shown that even some recommended diets are linked to a high likelihood of micronutrient deficiency, increasing the risk for many dangerous and debilitating health conditions and diseases [16].

Increasing nutritional density of diets is advised by the World Health Organization (WHO), with food fortification being one of the principal methods and considered to have much wider and more sustained impact than concentrated supplements with isolated nutrients [10]. An example of a fortified milk-based supplement intervention is provided in Fig. 1.3. The egg offers great potential for multiple fortification and for becoming a unique source of comprehensive nutritional support.

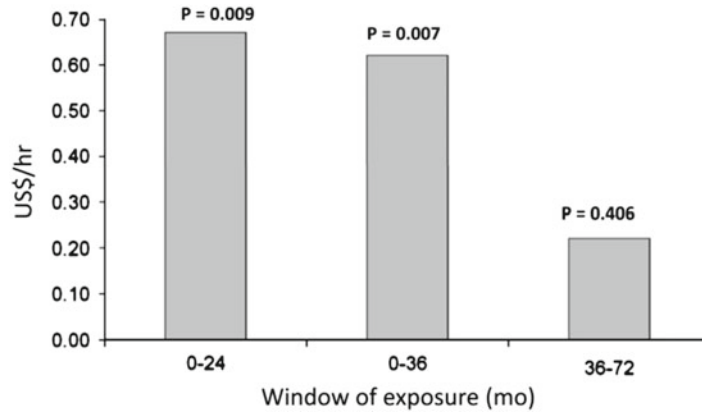

Fig. 1.1 Impact of timing of exposure to fortified (protein + micronutrients) sweetened beverage supplement during early life on later income earned per hour, compared with control sweetened beverage ($n = 602$ men, aged 26–42 years). In 2004 US dollars. Reprinted with permission from Martorell et al. J Nutr. 2010;140(2):411–4

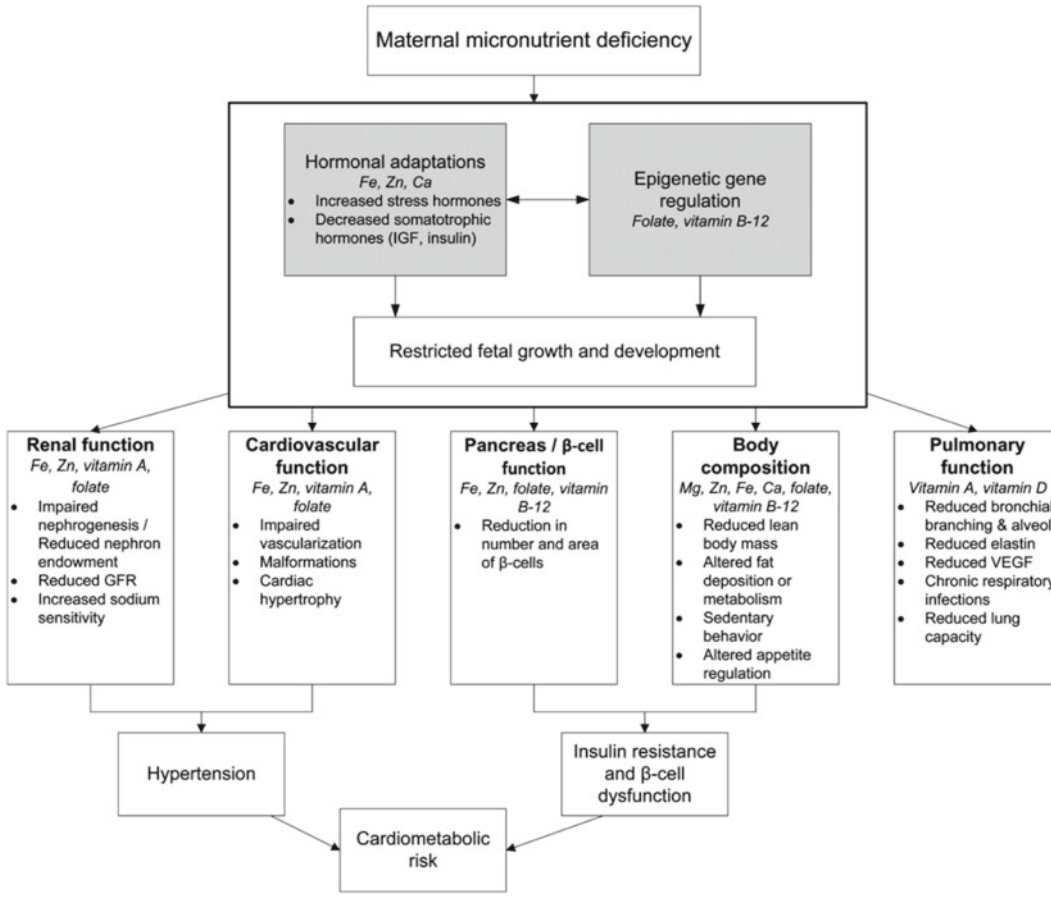

Fig. 1.2 Conceptual framework for how maternal diet and micronutrient status may affect future health and the development of chronic disease in the offspring. Reprinted with permission from Christian and Stewart. J Nutr. 2010;140:437–45

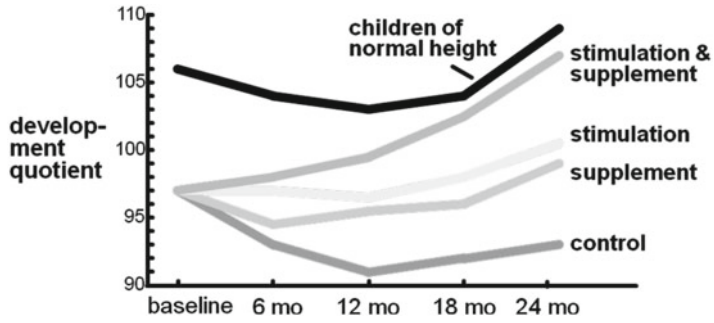

Fig. 1.3 Mental development quotient of growth-retarded children (low birth height-for-age) given fortified milk-based supplement, psychosocial stimulation, and/or both (9–24 months), compared to non-supplemented stunted and normal-height controls: the Jamaican Study. Reprinted with permission from: Grantham-McGregor et al. Lancet. 1991;338(8758):1–5

"Health-Oriented" Egg: Functional Modification Vis-à-Vis Medical Risk

Following the long debate regarding egg-associated cholesterol risks, the WHO concluded that there are more benefits than risks in regular egg consumption [17]. Egg itself has demonstrated significant contributions to nutritional status, even in western countries. Further, long years of research have suggested that dietary cholesterol in most cases does not influence blood cholesterol levels as much as the amount of saturated fat eaten, except in cholesterol "hyper-responders" (≈33 % of the population) [18], and some positive effects were shown, i.e., egg-induced increase in plasma high-density lipoprotein HDL; increased low-density lipoprotein (LDL) particle size, which reduces the atherogenicity [19]; and very high satiety index [20], contributing to appetite control and better weight loss/management, especially with low energy and low saturated fat intake, and with an active lifestyle [17].

However, a recent commentary that re-evaluated the permissive approach to egg intake has reiterated the concern regarding egg yolks for patients at risk of vascular disease, citing two recent large prospective epidemiological cohort studies that suggested a link between egg consumption and increased risk of new-onset diabetes mellitus and for increased health complications associated with preexisting diabetes [21].

In contrast, experimental and clinical studies have suggested that the egg's effect depends on its composition [22] and can be modified to improve metabolic responses such as LDL oxidation (Fig. 1.4), postprandial lipemia, inflammation, endothelial function, and fasting blood glucose, key measures associated with chronic disease risk. Research has shown the health advantages of eggs high in antioxidants and n-9 monounsaturated fatty acids (MUFA) or n-3 polyunsaturated fatty acids (PUFA), with lowered n-6 PUFA and n-6:n-3 ratio over regular eggs. Such health-oriented modified eggs may become increasingly important both as healthier "stand-alone" eggs and as a healthy vehicle for additional fortification, especially for populations at risk—i.e., hyper-responders to dietary cholesterol, hypercholesterolemics, diabetics, prediabetics, and those in highly inflammatory states [22, 23].

Micronutrients

While efforts to alleviate undernutrition had long focused on protein and energy, awareness has increased regarding widespread micronutrient insufficiencies, most common in developing countries, though also affecting industrialized nations due to food overprocessing, intensive agriculture [10],

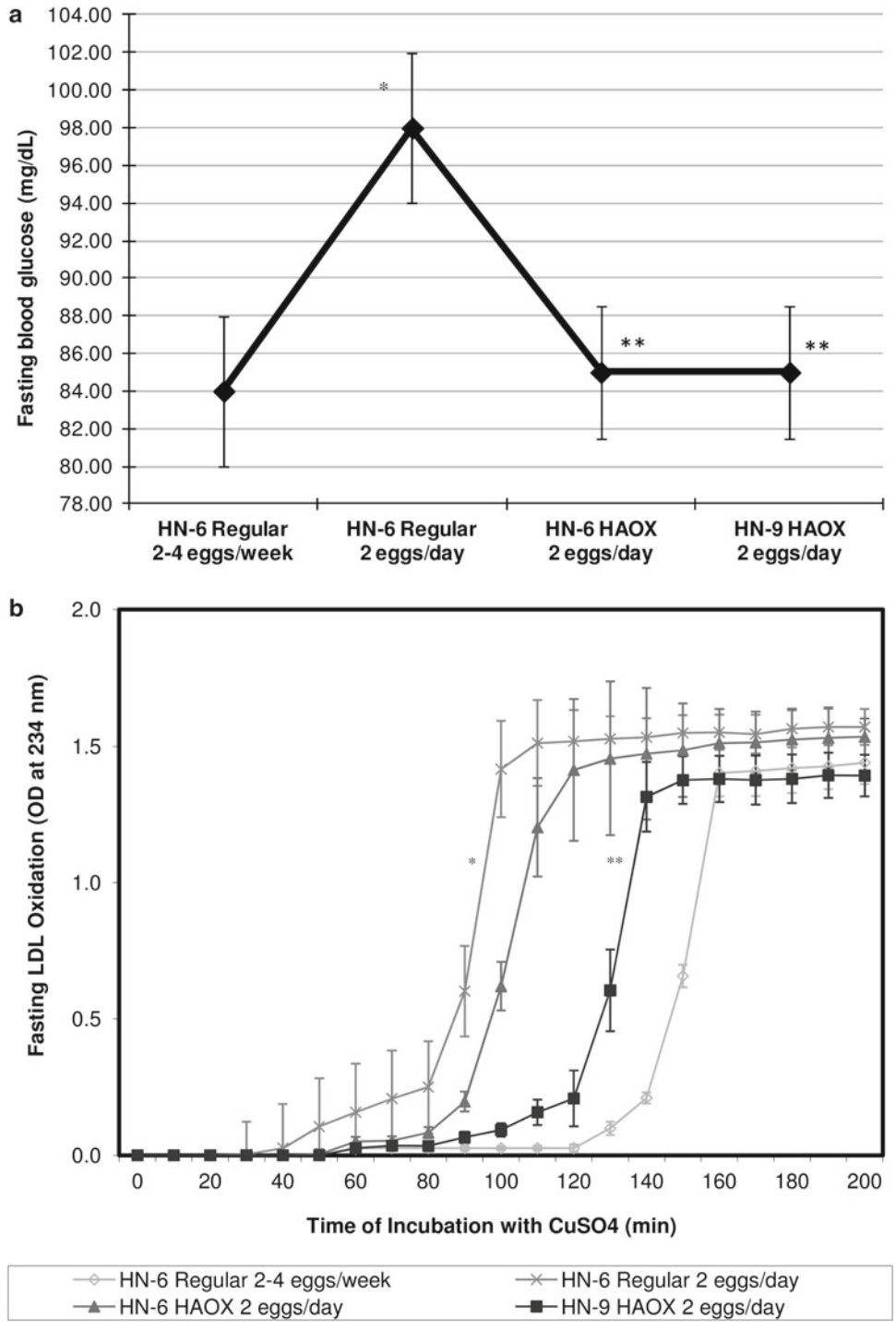

Fig. 1.4 Egg composition effects on glycemic and LDL oxidation (**a**) Fasting blood glucose concentrations (mg/dL), and (**b**) lag time (min) to LDL oxidation (by optical density, OD) following four (3-week) egg regimes (mean ± SEM, $n = 17$)(23) Authors' original illustration. (**a**) $^{*}P < 0.01$ (vs. HN-6 Regular 2–4 eggs/week), $^{**}P < 0.05$ (vs. HN-6 Regular 2 eggs/day). (**b**) *HN-6* high n-6 PUFA; *HN-9* high n-9 MUFA; *HAOX* high antioxidant. $^{*}P < 0.01$ (vs. HN-6 Regular 2–4 eggs/week); $^{**}P < 0.01$ (vs. HN-6 Regular 2 eggs/day)

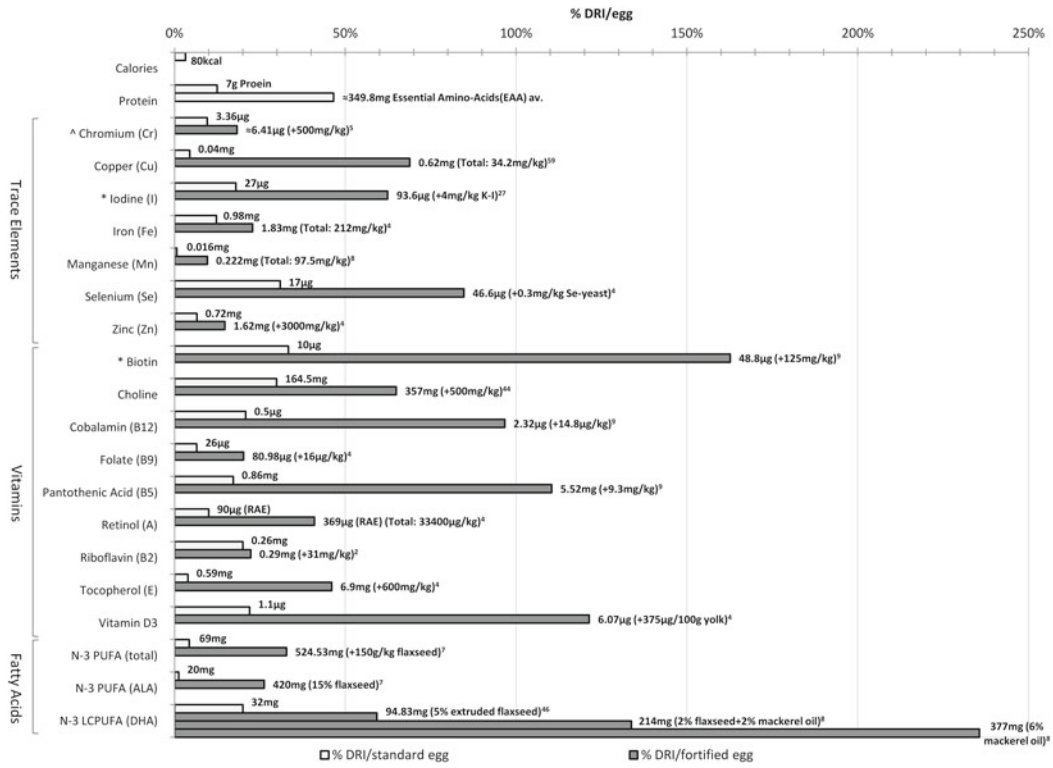

Fig. 1.5 Standard and fortified egg content and percent of daily recommendations (%DRI) for selected nutrients, based on representative studies of laying hen feed supplementation 2, 4, 7–9, 26, 43, 58–60. Authors' original illustration. Standard egg content per USDA except *Food Standards Agency (FSA), United Kingdom; ^Kirkpatrick & Coffin, 1975. + = amount added to basal feed level. Total = final feed content

and land degradation. Even recommended western diets, such as the Mediterranean-style or Dietary Approaches to Stop Hypertension (DASH), were shown to meet only 51.9 % of the Dietary Reference Intakes (DRI)[1] for 21 nutrients, even less for 6 (vitamins D and E, biotin, chromium, iodine, and molybdenum) [16].

Egg as a complete primary food contains most essential nutrients, including essential amino acids (EAA ≈ 17 % DRI, protein ≈ 13 % RDA), with some nutrients showing potential for fortification.

Research studies into micronutrient fortification of eggs were described by Naber [3] and Vilà [8], Leeson and Caston [2], and most recently by Suess-Baum et al. [6], Schiavone et al. [4], Sirri et al. [7], and Zang et al. [9]. Together, the evidence suggests there may be great potential for multiple fortification, though with highly variable transformation rates.

Representative results are presented here and compared to standard egg, as per their potential contribution to DRI (Fig. 1.5).

Trace Minerals in Egg Fortification

Limited access to high-quality food sources in developing countries, as well as consumption of processed foods in western countries, may cause dietary inadequacy of trace minerals. Young children

[1] Institute of Medicine (IOM), National Academy of Sciences.

and women of reproductive age are at highest risk, due to increased needs of growth and gestation/lactation [10]. Frail elderly patients have significantly lower mineral levels compared to elderly in the community, while being vulnerable to acute and unbalancing effects of isolated supplements [24], suggesting an advantage to natural and/or fortified food sources. Trace minerals most studied in egg modification have been iodine and selenium [4]; zinc, iron, copper, manganese, and chromium fortification have also been studied and reviewed [8].

Iodine

The NRC recommendation for iodine in the laying hen diet for white-shelled eggs is 0.35 mg/kg feed [25]. The amount in a standard western egg[2] (56 g) is 27 μg, and the DRI for males aged 19–50 years is 150 μg/day. Egg iodine content can potentially be increased ≈3–12-fold control with feed supplementation [4, 8] and is considered a highly bioavailable source [5].

Iodine is one of the three key micronutrients of global public health concern [10], with almost two billion people deficient or at risk. A community model of egg iodine fortification against a deficiency disorder showed a urinary iodine increase from ≈7.0 μg to 13.95–20.8 μg/dL in Thai women aged 20–63 years [26].

Iodine is critical to thyroid hormones and cellular oxidation, for growth and metabolic rates. Deficiency symptoms include depressed thyroid function, cretinism, lethargy, and mental dysfunction.

Selenium

NRC—0.06 mg/kg feed [25], standard egg—17 μg, DRI—55 μg.

Egg selenium increases progressively with feed supplementation and has high bioavailability, suggested to reach ≈78–82 % [5], as indicated by increased plasma levels (≈40 %) following two/day selenium-fortified eggs (28–32 μg/egg) [4]. Selenium fortification is relevant against low soil levels and depletion [27].

Selenium is critical for antioxidant enzymes, against inflammation, for thyroid hormone function, and in fat and vitamin E metabolism. Deficiency symptoms include cardiac myopathy, fragility of red blood cells, cataracts, and repeated infections.

Zinc

NRC—50 mg/kg feed, standard egg—0.72 mg, DRI—11 mg.

While standard egg zinc has moderate bioavailability, ≈30 %, zinc fortification (to 1 mg/egg) was shown to enhance bioavailability to 72–75 % [5]. Zinc deficiency is the fifth leading risk factor for disease in the developing world, with approximately one-third of the world's population at risk. Poor populations and/or individuals with infections and diarrhea or following low-zinc diets (i.e., vegetarian or low in animal foods) are especially at risk [28].

Zinc is critical for growth, mental development/function, appetite, taste/smell, fertility, and immunity. Deficiency symptoms include impaired wound healing and cognitive function, mild anemia, and growth failure.

[2] United States Department of Agriculture (USDA).

Iron

NRC—45 mg/kg feed, standard egg—0.98 mg, DRI—8 mg.

Egg iron content is not easily enhanced, though experimental increases ranged from 5 to 18 % following supplementation. Combining iron supplementation with zinc and copper synergistically increases egg accumulation. Iron-methionine was found to be the most effective source [4].

Egg iron (≈10 % DRI) is associated with phosvitin and ovotransferrin, which reduce its bioavailability (≈27 %) [29]. However, iron absorption enhancers, i.e., vitamin C from fresh vegetables and fruits, as well as animal protein and heme sources, i.e., fish/seafood, poultry, or meat/sauce, can improve egg iron utilization [6].

Iron is one of the three key micronutrients of global public health concern, the most common and widespread nutritional disorder in developing and industrialized countries, especially where animal food is scarce. Iron is critical for formation of hemoglobin and oxygen transport, mental function, resistance to infection, cellular respiration, and enzyme metabolism.

Clinical anemia and even subclinical depletion of iron stores have adverse consequences on health, child development, cognitive function, energetics, and productivity [10], whereas iron supplementation improved attention, concentration, and intelligence quotient [30].

Pregnancy/lactation DRI are approximately double, and iron deficiency anemia may increase risk of poor gestational outcomes [10].

Copper

Standard egg content—0.04 mg, DRI—0.9 mg.

Egg copper increases progressively with feed levels [31], with adverse effects (reduced egg production) shown only at >450 mg/kg [32]. At 125–150 mg/kg feed, a significant reduction in egg cholesterol was shown [33].

Strict vegetarians/vegans, pregnant/lactating women, premature and low-birthweight infants, with unfortified formula/milk (the already-low copper concentrations are bound to milk protein), and frail elderly [24] are most at risk for deficiency.

Copper is involved in antioxidant enzyme and neurotransmitter activity, and with connective tissue, bone, and blood vessel integrity. Deficiency symptoms include retarded growth, appetite loss, increased blood cholesterol, and depression.

Manganese

NRC—20 mg/kg feed, standard content—0.016 mg, DRI—2.3 mg.

Egg manganese is very low even after fortification (0.78 % DRI). However, the response to supplementation (>40 %) alone or synergistically with zinc and copper [8] may suggest fortification potential.

Manganese is critical for skeletal growth, antioxidant enzymes, macronutrient metabolism, and brain development. Deficiency symptoms include impaired growth, skeletal abnormalities, increased blood cholesterol, impaired glucose tolerance, and overweight.

Chromium

DRI is 35 μg.

Egg chromium concentration responds both to inorganic and organic feed sources [8]. Mean egg chromium level ≈60 μg/kg [34] (3.36 μg /56 g) was shown to initially increase (during the first few weeks of supplementation), and later decrease, leveling off at ≈2–3-fold the basal level [8, 35].

Chromium is critical for insulin function, against type 2 diabetes, and for glucose, fat, and protein metabolism. Deficiency symptoms include glucose intolerance, impaired growth, and neuropathy.

Vitamins in Egg Fortification

Feed vitamin supplementation has demonstrated improved laying hen productivity and egg quality and enhanced vitamin content, which is highly bioavailable for humans. The transfer efficiency of vitamins from hen feed to eggs varies greatly, i.e., from very high—vitamin A; high—riboflavin, pantothenic acid, biotin, and vitamin B12; medium—vitamins D3 and E; to low—vitamin K, thiamin, and folic acid [3].

Vitamin A

NRC—900 μg (Retinol) retinol activity equivalents (RAE) (3,000 IU)/kg feed, standard egg—90 μg RAE, DRI—900 μg RAE.

Vitamin A feed-to-egg transformation was found to be very high [3], progressive, and linear, with high availability, >95 % to humans [5]. Liver storage was initially increased much more than in the yolk (≈2-fold) [4] at feed levels of 780–6,600 μg RAE/kg, suggesting long-term liver reserves to ensure high egg A content.

Vitamin A is one of the WHO's three key micronutrients of global deficiency concern. Insufficiency is exacerbated by infections and diarrhea, most apparent during early childhood and pregnancy/lactation.

Vitamin A is critical for growth, metabolism, immune function, against anemia and infections, eye health and vision, epithelial cell integrity, and wound healing. Deficiency symptoms include night blindness, xerothalmia (the leading cause of childhood blindness in the developing world), poor bone growth, and susceptibility to infection.

Vitamin E (Tocopherol)

NRC—5.0 mg or IU/kg feed, standard egg—0.59 mg, DRI—15 mg.

Egg vitamin E content increased dose-dependently with feed content, i.e., doubling feed level (110 vs. 55 mg/kg) doubled egg content [36], but transfer efficiency declines with fortification levels, by 8.4 % for every 100 mg/kg feed [4]. Fortified egg is an excellent source of E, with bioavailability reaching >65 % [5] as shown by increased human plasma levels [37], i.e., by 46.1 % following egg increase of 500 % [23]. Vitamin E significantly reduced lipid oxidation and improved egg production [38]. An antioxidative/protective E:PUFA ratio of 0.4–0.8 is recommended in animals and humans [39].

E is critical for antioxidative defense, immunity, neuromuscular function, and against inflammation, including in CVD and cancer. Deficiency symptoms include hemolytic anemia in newborns, infertility, hyporeflexia, muscle wasting/weakness, and retinal degeneration.

Vitamin D3 (Cholecalciferol)

NRC—7.17 μg (300 IU)/kg feed, standard egg—1.1 μg (46 IU), DRI—5 μg (627 IU).

Egg D3 readily increases with feed content, i.e., between 25 % and 3.0-fold [8]—reaching 100 % DRI [4]—and has high availability [4, 5, 9].

Vitamin D deficiency is now recognized as a pandemic, due to reduced sun exposure, which is required for internal D synthesis, and low number of foods containing D naturally (i.e., fish) and/or fortified with adequate levels [40], especially during winter in northern countries [41].

D is critical for bone formation and strength, calcium and phosphorus absorption and metabolism, and for muscle, nerve, and immune function. Deficiency symptoms include rickets, osteopenia, impaired glucose regulation, and impaired immune function.

Vitamin B2 (Riboflavin)

NRC—2.5 mg/kg feed, standard egg—0.26 mg, DRI—1.3 mg.

Egg B2 increases progressively with increasing hen feed content, up to threefold with 5–10 mg/kg [4], and has very high bioavailability of ≈95 % [5].

B2 deficiency is among the most common subclinical nutrient deficiencies in America, affecting 28 million people [42] as part of multiple-nutrient deficiencies [43]. It is more common in developing countries, with diets low in animal foods and high in milled grains [10].

B2 is critical for growth, eye health, immune function, methylation (DNA/RNA), and energy and homocysteine metabolism. Deficiency symptoms include poor growth and digestion, abnormal respiratory quotient, and eye sensitivity.

Vitamin B5 (Pantothenic Acid)

NRC—7.45 mg/kg feed, standard egg—0.86 mg, DRI—5 mg.

Egg B5 is readily increased with feed levels, with very high feed-to-egg conversion rate [9] and high bioavailability, ≈95 % [5].

B5 is critical for nerve function, for cholesterol and steroid hormone metabolism, and for energy, fat, protein, and carbohydrate metabolism. Deficiency symptoms include fatigue, impaired coordination and sleep, and low antibody production.

Vitamin B12 (Cobalamin)

NRC—4 μg/kg feed, standard egg—0.50 μg, DRI—2.4 μg.

Egg B12 showed the most rapid response to feed changes and is more effective for increasing human levels than when administered in its crystalline form [44], with bioavailability of ≈24–36 % [5], strongly suggesting the egg's great potential for B12 fortification [8].

B12 deficiency is generally caused by inadequate intake and/or malabsorption. Pregnant/lactating women, vegetarians/vegans, and the elderly are at increased risk [27, 43].

B12 is critical for nerves/brain, cardiovascular, and red blood cell function and is active in folate and DNA metabolism. Deficiency symptoms include pernicious anemia, homocysteinemia, neurologic deterioration, and impaired appetite, memory, and cognitive function.

Folic Acid/Folate

NRC—0.25 mg/kg feed, standard egg—26 μg, DRI—400 μg.

Egg folate was found to be 43 times more concentrated than in hen plasma [4], suggesting the active potential for egg fortification, with high bioavailability of ≈70 % [5].

Folate fortification (mostly in flours) is one of the leading world projects, including in developed countries, aimed particularly at prevention of birth defects [10]. Combinations of folate with B12 and/or other nutrients demonstrated an advantage over folate alone.

Folate is critical for red blood cell formation, reproduction, immune and nervous system function, DNA/RNA production/protection, and homocysteine metabolism. Deficiency symptoms include birth defects, poor growth, anemia, and impaired immunity, digestion, and memory.

Biotin

NRC—0.1 mg/kg feed, standard egg—10 μg, DRI—30 μg.

Egg biotin is a major dietary source, with regular egg ≈30 % DRI, potentially increased to ≈70 % DRI [8]. The bioavailability of biotin is high in cooked or processed eggs, but low in raw eggs due to the presence of avidin, an absorption-inhibiting protein [6].

As the main sources of biotin are vegetables, nuts, and animal protein, diets based mostly on grains, i.e., in developing countries, may be at risk of insufficiency, suggesting high egg biotin fortification potential [10].

Biotin is critical for growth, muscular and neurologic function, and protein/amino acid, fatty acid, and carbohydrate metabolism. Deficiency symptoms include insomnia, poor appetite, and dermatitis.

Choline

NRC—1,050 mg/kg feed, standard egg—164.5 mg, DRI—550 mg.

Egg choline is innately a leading, highly available dietary source. Supplemental choline, particularly in combination with feed folic acid and vitamin B12, increased egg choline and phosphatidyl choline (PC) by 20–25 % [45].

As mean choline intakes in the population are far below adequate intake (AI)[3] levels, dietary guidance encourages the intake of choline-rich foods, including egg [46], which is a highly available source.

Choline is critical for brain function and development, metabolism of fat/cholesterol, and liver and gall bladder function. Deficiency symptoms include high blood pressure, impaired/fatty liver, and impaired growth, memory, and heart function.

Essential Fatty Acids (EFA)

Standard egg contains 20 mg ALA, 32 mg docosahexaenoic acid (DHA, 22:6 n-3), with essentially no eicosapentaenoic acid (EPA, 20:5 n-6); 857 mg LA and 105 mg arachidonic acid (AA, 20:4 n-6), with14:1 n-6:n-3 ratio.

ALA (n-3) and LA (n-6), the essential fatty acids, are transformed competitively by the same enzyme to LCPUFA—EPA (n-3), DHA (n-3), and AA (n-6)—and related eicosanoids. The DRI for total n-3 (0.6–1.2 % kcal) is based mostly on ALA; the DHA + EPA recommendation is 10 % of total (0.06–0.12 % kcal), though DHA and EPA are not considered essential.

Declining marine fish (primary n-3 LCPUFA source) consumption and increasing vegetable oil (high n-6 PUFA) in the western diet have resulted in a dramatic increase in dietary n-6:n-3 ratio, reflecting a relative and absolute deficiency in n-3, an imbalance linked to various western syndromes, and disease risks [47].

[3] IOM.

N-6 Polyunsaturated Fatty Acids (PUFA)

N-6 PUFA are essential for growth and development, brain and reproductive function, and balanced blood triglycerides and cholesterol. However, high intakes of n-6 (mostly LA), as typical to the western diet with increasing grain and vegetable oils, lead to increased AA and derivative proinflammatory/oxidative eicosanoids, associated with greater risk of atherogenesis thrombogenesis, carcinogenesis, and chronic inflammatory diseases [47], which are inversely associated with n-3 ALA and DHA and n-9 oleic acid (OA, 18:1) [48].

While egg fortification with n-3 has sometimes demonstrated a minor effect on decreased n-6 levels—though mostly not—proactive reduction of egg n-6 and increasing n-9 have contributed to a significant reduction in egg (regular, high n-6)-induced LDL oxidation in humans (Fig. 1.3) [23].

N-3 Polyunsaturated Fatty Acids (PUFA)

N-3 PUFA is essential for humans, primarily as a precursor to n-3 LCPUFA, critical for normal growth and development, including brain health and function. Many clinical and epidemiological studies have shown protective contributions of n-3 in infant development, against inflammatory disorders (i.e., arthritis, lupus erythematosus, and asthma), platelet aggregation, hypertension, and hyperlipidemia, CVD, cancer, and more recently, against various disordered mental states, including depression, attention-deficit hyperactivity disorder, and dementia, especially with the typical high n-6 intake western diet. N-3 LCPUFA are increasingly scarce in modern diets, as a result of diminishing supply, mostly from northern marine fish, and restriction on intake due to contamination [11].

Moreover, farmed fish were recently shown to have high n-6 PUFA/LCPUFA and very low n-3 LCPUFA [49]. The uniquely high efficiency of transformation of feed n-3 18:3 to egg 20:5 and 22:6 LCPUFA in laying hen is long-known [7], which may suggest fortified egg as an alternative and major source of n-3 LCPUFA.

There are two main approaches to increasing egg n-3 content: supplementation of laying hen feed with ALA from a land-based vegetable source (flaxseed, rapeseed) [50] or with n-3 EPA and/or DHA from a marine source (fish, algae).

Consumption of n-3-fortified eggs (≥1/day) has been associated with improved blood n-3 levels, lipid profiles, and/or glucose, without elevating total cholesterol [51–53], effects known to be associated with a reduced risk for cardiovascular mortality and diabetes. Perinatally, the n-3 content of fortified eggs can be applied for maternal and infant support, with significant contributions of %DRI, and has been previously shown to have favorable effects on blood parameters and outcomes [11]. The potential contribution of n-3-fortified eggs to a population's intake, i.e., the American diet, is illustrated in Fig. 1.6.

Carotenoids

Carotenoids tend to accumulate in egg yolk, with lutein and zeaxanthin attaining especially large amounts (≈282 μg per standard egg).

Adding carotenoids to hen feed can lead to a substantial increase in egg [8, 54], that can contribute significantly to reduced lipid oxidation and free radical formation and may act synergistically with other antioxidants, such as vitamin E and selenium compounds [7]. Egg consumption can enhance blood carotenoid levels [18, 37], i.e., by 55.0 % following egg lutein increase of 289 % [23], benefiting

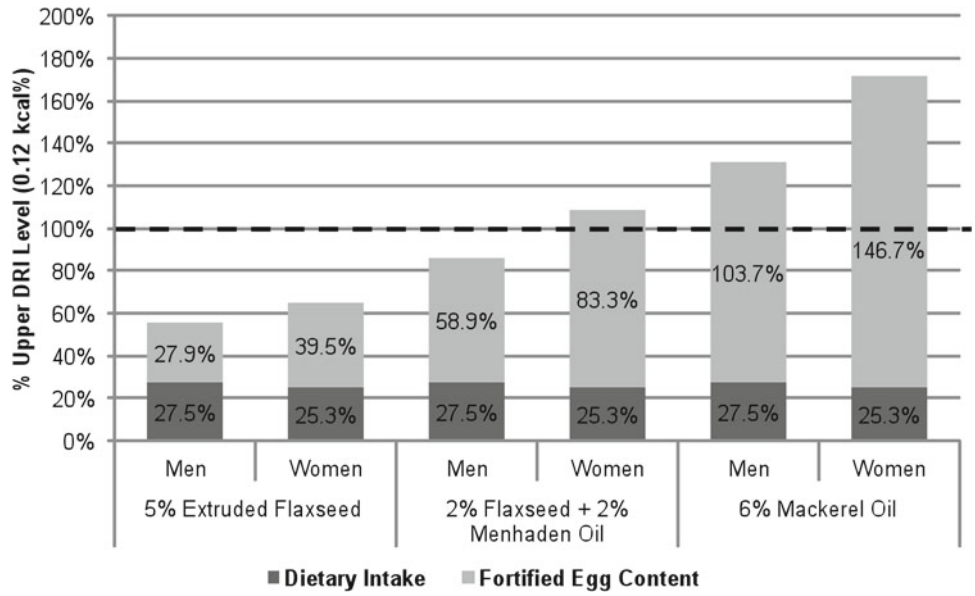

Fig. 1.6 Potential egg DHA contribution. Calculated contribution of one each of selected DHA-fortified eggs, from land-based n-3 PUFA59 and marine-based n-3 LCPUFA(8) feed supplementation, to daily DHA requirements (%DRI), and compared to intake among Americans (1999–2000, Centers for Disease Control [CDC]). Authors' original illustration

heart and eye health, specifically increasing macular concentration and pigment-related optical density [6], generally low in older adults, with related increased risk of blindness. Egg is considered to be a useful source of carotenoids due to their high bioavailability, attributed to the fatty acid composition [6].

Synergistic and Other Combinations

Egg represents a complete food with regard to composition and further demonstrates a unique synergistic effect. The "wild-type" egg represents the advantage of the natural (prehistoric) low n-6:n-3 ratio of 1:1 (vs. typical western 16–20:1) with enhanced antioxidants and iodine [55] and innate phospholipids, which enhance delivery effectiveness. Synergistic combinations of antioxidants—vitamin E, carotenoids, and selenium—in DHA-fortified eggs have been shown to significantly decrease oxidative stress, as reflected by reduced cholesterol oxidation [56] and malonyldialdehyde formation from lipid peroxidation [37].

Similarly, supplementation with flaxseed and other nutrients (vitamin E, selenium, iodine, and folic acid) and/or with an herbal mix containing phytochemicals (such as the phenols thymol and carvacrol, and the terpene p-cymene) resulted in multiple fortification, enhanced antioxidative value, and reduced egg cholesterol content; concurrent lowering of n-6 levels resulted in a notably lower n-6:n-3 ratio of 1.7–2.2:1 compared to control [57].

The combination of reduced n-6 and reciprocal increase in n-9 plus carotenoids, vitamin E, iodine, and selenium, was shown to reverse egg-induced LDL oxidation (≈37 % by 2 regular eggs/day) to that of a low-egg diet (2–4/week), and to normalize fasting blood glucose (Fig. 1.4) [23].

Feed supplementation with flaxseed, minerals, vitamins, and lutein produced "multiple-enriched" eggs yielding much greater nutritional density/value than standard eggs, including increases of 2.6-fold the n-3 ALA (to ≈20 % RDA), threefold DHA (≈100 %), threefold vitamin D (≈30 %), fourfold folic acid (≈70 %), sixfold vitamin E (≈66 %), 2.5-fold iodine (100 %), and fourfold selenium (≈45 %), sixfold lutein and zeaxanthin (≈70 % of international recommendation), with significantly lowered n-6:n-3 ratio [58]. Such multi-pronged approaches suggest flexible possibilities for synergistically designed eggs, for both nutritional fortification and medical/physiological protective aspects.

Discussion

The research evidence reviewed above presents the potential for multiple modification/fortification of the egg, which together with the high innate nutritional value, offers a unique opportunity for comprehensive supplementation and improved health benefits.

Most of the nutrients discussed have demonstrated a tendency to increase in the egg with feed supplementation, though feed-to-egg transformation efficiencies vary greatly. Certain compounds, i.e., organic mineral sources such as amino acid complexes, have often demonstrated higher bioavailability than inorganic forms [4]. This is in accordance with a recent study showing that organic manganese, zinc, and copper feed supplements could increase laying hen performance, production, and quality measures with much lower doses (50–75 % NRC recommendations) than inorganic forms [59].

Certain combinations, i.e., zinc, copper, and iron, and folic acid and choline, can synergistically increase transformation efficiencies, and others, i.e., carotenoids and vitamin A, may competitively reduce them [8]. All such modifications can be executed by the simple and low-cost method of laying hen feed supplementation, an approach that is well-known and accepted in most countries and cultures, including among socioeconomically disadvantaged populations.

The dramatic increases in n-3 LCPUFA exemplify how significantly egg composition can be adapted. High n-3 eggs could therefore become an alternative and increasingly important dietary source of n-3 LCPUFA, providing a sustainable answer to the growing scarcity of marine n-3 in the food chain [47]. As n-3 and n-6 compete for the same enzyme, reducing n-6 may be the first prerequisite to maximizing the n-3 potential in health-oriented/functionally modified eggs [60]. Using the egg as a vehicle for multiple fortification is a relevant nutritional answer for multiple insufficiencies in developing as well as in western populations, due either to undernutrition or to overprocessing of foods, intensive agriculture, and soil degradation. Egg fortification is especially relevant during critical periods, i.e., perinatally for maternal replenishment, well-being, and functioning; for infant/child growth, including optimal brain development [11]; and in the elderly in conditions of catabolic stress [24]. Egg fortification could be further tailored to meet specific needs [23, 56, 57].

Despite many years of health recommendations for egg restriction against CVD risk, and accumulating evidence suggesting daily consumption to generally be safe for healthy people, research increasingly suggests that egg-related health risk depends on the composition. It has been shown that egg modification can improve the physiological responses, potentially providing an answer to recently reiterated medical concerns regarding egg-related risks, especially for high-risk populations [22]. Health-oriented modified eggs would be especially relevant to deficient and at-risk individuals, and thus may expand the population for whom eggs are acceptable. Intensive enhancement of egg nutritional density could reduce the amount of egg intake required to attain consumers' desired nutrient levels [11], and thus the concerns of high egg risk.

The advantage of the egg as a vehicle is further emphasized in terms of environmental concerns, with the egg having one of the lowest "carbon footprints" among animal foods, and thus being highly relevant for the new "sustainable agricultural" concept; as a relevant tool against the obesity

pandemic, given its high satiety index [20]; and as a relatively low-cost animal protein food, highly important for food security, nutritional equality, and support for low-income populations in developing as well as in western countries.

Conclusions

Egg is a very effective vehicle for multiple modification/fortification, combining an innately high nutritional value with a unique capacity to concentrate added essential nutrients and phytonutrients, as shown by their increase in egg following feed supplementation. Egg can become a "strategic" food for comprehensive dietary enhancement, as well as for specific nutrients that may be scarce in the food supply (i.e., n-3 LCPUFA, vitamins B12 and D3, chromium, manganese, choline, and lutein, among others). Presumed egg-related risks can be effectively addressed by "functional modification" to improve their effects, i.e., on LDL oxidation, postprandial lipemia, increased fasting blood glucose, inflammation, and related factors; this can be attained by reducing n-6 and increasing n-3, n-9, antioxidants, and other selected micronutrients. Improving egg composition may justify its use—as is, and/or a fortified vehicle for specific needs. "Nutritional fortification" of eggs, combining the unique nutritious vehicle and nutrient concentration capacity with their effective delivery, offers a highly sustainable and affordable method of production that is well-accepted by wide-ranging populations.

Acknowledgements The authors thank Hagit Hershkowitz-Friedman, M.Sc. for her assistance in this paper.

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Chapter 2
Apple Pomace: Source of Dietary Fibre and Antioxidant for Food Fortification

Shashi Bhushan and Mahesh Gupta

Key Points

- Apple pomace is a renewable natural bioresource.
- It is highly nutritious especially in terms of dietary fibre and antipoxidants.
- Fruits and vegetables are better source of dietary fibres than cereals owing to presence of natural antioxidant.
- Dietary fibres and antioxidant component from apple pomace have demonstrated potential health benefits.
- Apple pomace is an ideal ingredient for food fortification.

Keywords Apple pomace • Dietary fibre • Antioxidant • Nutrition • Health

Abbreviations

%	Percentage
g	Gram
Mg	Milligram
CVD	Cardiovascular diseases
MI	Myocardial infarction
P	Phosphate
K	Potassium
Mn	Manganese
Ca	Calcium
Mg	Magnesium
Fe	Iron
DPPH	2, 2-Diphenyl-1-picrylhydrazyl

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V.R. Preedy et al. (eds.), *Handbook of Food Fortification and Health: From Concepts to Public Health Applications Volume 2*, Nutrition and Health, DOI 10.1007/978-1-4614-7110-3_2,

Introduction

In present context of depletion of natural renewable resources, management and profitable utilization of bioresources like apple pomace will be the key to development. The left-over solid biomass after extraction of juice from fresh apple fruits is called 'Apple Pomace'. It has high moisture content (75–85 %) and biodegradable organic matter. It is being generated in huge volume (25–30 % of the total fresh apple fruits processed) across the world [1–3]. Owing to presence of fermentable carbohydrates, it is prone to microbial degradation, thus generating foul smell causing environmental pollution around the dumping sites. Apple pomace is attracted a lot of attention from scientific as well as industrial community; however, still its beneficiations seems to be a dream too far. It also poses economic loss to the industry, as waste disposal cost is quite high [1]. Around the world efforts are being made for its utilization in many ways and forms (Fig. 2.1). Apple pomace is highly nutritious and contains variety of carbohydrates, protein, amino acids, polyphenols and some aromatic compounds. The extraction of these compounds from apple pomace is well studied [2]. Food enrichment or fortification using these compounds especially non-starch cell wall polysaccharides (dietary fibre) and antioxidants along with their specific role in human health will be discussed in this chapter. Food fortification is adding one or more essential nutrients to a food, whether or not usually present in the food, for the purpose of preventing or correcting a demonstrated deficiency of one or more nutrients in the population or specific population groups. The increasing awareness among the people about the natural food and its components, exhibiting health-promoting properties, led to development of dietary food supplements. In recent years, scientific communities are rapidly accumulating supportive evidence about the role of such food ingredients in both health promotion and disease prevention.

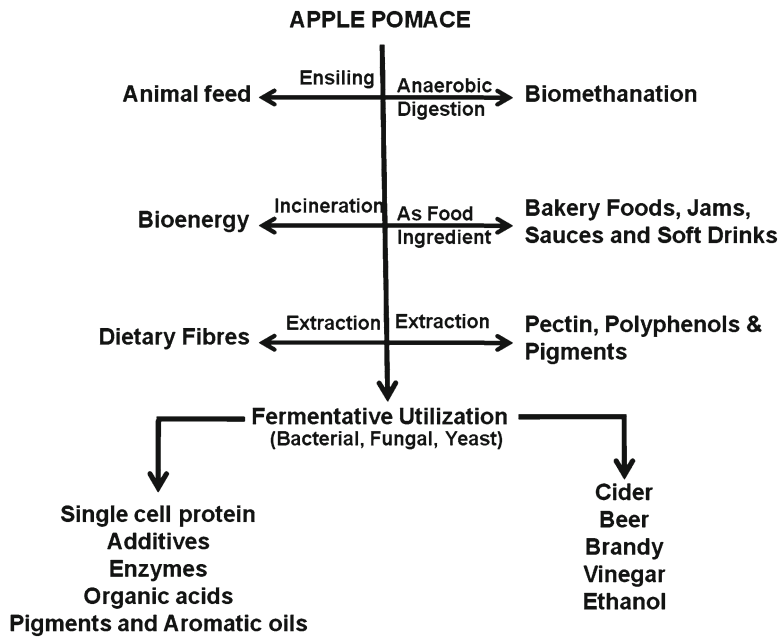

Fig. 2.1 Value addition of apple pomace

Component and Nutritional Composition of Apple Pomace

Apple pomace consists of apple skin/flesh and pulp, seeds and twigs/stems (Fig. 2.2). High moisture content, enzymatic/oxidative browning and presence of seed are three major bottlenecks in processing and utilization of apple pomace. The high water activity makes it vulnerable to microbial decomposition; exposure of pomace to oxygen results in browning by polyphenoloxidase and damaged seed release amygdaline (cynoglycosides), thus imparting bitterness. The dried apple pomace contains large amount of carbohydrates, proteins, pectin, total phenolics (Fig. 2.3) and minor quantities of minerals (P, K, Mn, Ca, Mg and Fe). The nutritional profile of apple pomace was studied comprehensively [4–12]. The major carbohydrates are galacturonic acid (upto 64 %), arabinose (upto 23 %) and galactose (upto15%), with minor amounts of rhamnose, xylose and glucose. Apple pomace is considered as a potential source of dietary fibre (about 45–60 %) which includes pectins (5.50–11.70 %), cellulose (20.20–43.60 %), hemicelluloses (4.26–24.40 %), lignins (15.30–23.50 %) and gums. Apple pomace consists of approx. 10–15 % pectin on dry weight basis. Apple pectin is widely used in food industry as gelling agent in preparation of variety of food products. The pectin-based drug carriers for oral administration of colon-specific delivery were found safe and compatible, thus pectin extracted from apple pomace has application as drug delivery system [13].

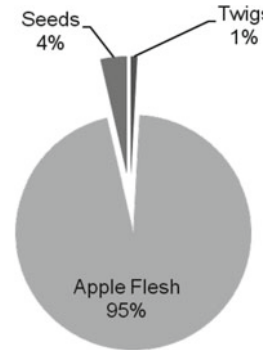

Fig. 2.2 Apple pomace component

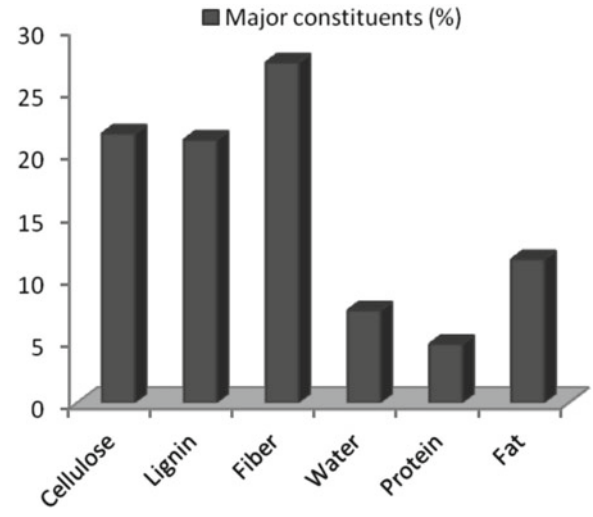

Fig. 2.3 Major constituents of apple pomace (dw %)

Table 2.1 Yield of major polyphenolic compounds in apple pomace

Compounds	Yield (mg/kg dw)
Flavonols	1,500–2500
Dihydrochalcones	1,500–1,700
Flavanols	550–1,200
Hydroxycinnamic acids	500–750

Reference(s): Lu and Foo [7], Schieber et al. [9], Lavelli and Corti [10], Garcia et al. [11], Cetkovic et al. [12]

Apple pomace is a very good source of natural antioxidants, having strong antioxidant activity [7–12]. The major phenolic compounds present in peel are flavonols (quercetin 3-O-rutinoside, quercetin 3-O-galactoside, quercetin 3-O-glucoside, quercetin 3-O-xyloside, quercetin 3-O-arabinoside and quercetin 3-O-rhamnoside), flavanols (epicatechin, procyanidin B2 and unidentified procyanidins), cinnamic acids (chlorogenic acid and p-coumaroylquinic acid) and dihydrochalcones (phloretin 2′-O-glucoside and phloretin 2′-O-xyloglucoside). The total phenolic contents yield in apple pomace ranged between 200 and 300 mg/100 g [7, 10–12], while the yield of the major polyphenolic compound is given in Table 2.1.

Bioactive Molecules and Supportive Health Benefits

The discussion under this subhead primarily focused on potential health benefits of dietary fibre and polyphenolic compounds.

Dietary Fibre

Dietary fibres are intrinsic and intact in plants mainly cellulose, hemicelluloses, pectic substances and lignins and resistant to hydrolysis by digestive enzymes in the human's body [14]. The dietary fibre consumption reported to have positive health effects, especially in management of gastric and cardiometabolic disorders. In addition to these disorders, dietary fibre showed to have preventive role in world's widely spreading diseases like obesity and diabetes [15–17]. Dietary fibre and antioxidants (vitamins C and E, carotenoids, polyphenols) are the two nutritional components with a recognized role in the prevention of chronic diseases. Dietary fibre is a matrix that contains phyto-components, including polyphenols and hence have potential in prevention or management of risk of cardiometabolic disorders and degenerative diseases [18–22]. Until now, the cereal-based dietary fibres were mostly used in food industry. However, recent reports advocated that presence of higher amount of bioactive molecules like antioxidants in fruits and vegetable dietary fibres seems to be a better option than cereal. These fibres have showed protective effect on cardiometabolic human health. In gastric disorder management, dietary fibre reported to bind excess hydrochloric acid, cholesterol and gastric juices, increase the faecal bulk and stimulate intestinal peristaltis. High dietary fibre intake is associated with lower risk of cardiovascular disease (CVD) and myocardial infarction (MI), yet the results supporting the fact of current dietary recommendations to increase the consumption of fibre-rich diet as a preventive measure against CVD [18]. The scientific literature supports the notion that high-fibre diets are important in prevention and management of obesity and chronic diseases including type 2 diabetes, heart disease, and cancer [16, 17, 19, 23].

Natural Antioxidant

Apple pomace is a potential source of polyphenols, known to have health-promoting properties. Several researches have determined that the antioxidant concentration and antioxidant capacity of the apple peel is higher than that of the pulp fractions or the whole fruit. [17, 24] The phenolic compounds from apple pomace showed strong antioxidant activities, and their DPPH-scavenging activities 2–3 times and superoxide anion radical scavenging activities were 10–30 times better than vitamins C and E [8]. The total phenolics, total flavonoids, total flavan-3-ols, and some individual phenolic compounds contributed significantly to the antiradical activities of apple pomace [8, 10, 12]. The phenolic-rich crude extract from pomace inhibited in vitro tumour-cell proliferation and showed beneficial effect on key stages of carcinogenesis in colon cells under in vitro conditions [21, 25].

Phloridzin, recognized as an anti-diabetic agent, is found to inhibit glucose transport competitively through the binding of its glucose moiety to the Na+/glucose co-transporter [26]. Phenolics from apples have the ability to inhibit protein glycation [27]. These properties are potentially relevant for the inhibition of oxidative- and glyco-oxidative-related diseases. Antiviral effects against the *Herpes simplex* virus types 1 and 2 [28] and inhibition of the growth of *Staphylococcus aureus, Pseudomonas aeruginosa* and *Bacillus cereus* [29] have been demonstrated by apple phenolics. The effect of apple and pear peel supplemented diets also showed positive effect on plasma lipids and plasma antioxidant capacity in laboratory animals [17].

The specific health-promoting activities or positive health effects under in vitro/in vivo model system of either extracted molecules or crude extracts of apple pomace are tabulated in Table 2.2. The studies revealed that the polyphenols responsible for the antioxidant activity and dietary fibre in apple are still present in the pomace, and therefore can be easily extracted for possible food fortification or nutraceutical product development. Apple pomace can become a cheap and readily available source of dietary fibre and natural antioxidants.

Application in Food Fortification

The use of apple pomace for development of nutritionally enriched food products has been investigated for variety of food products. Its application in food fortification can be gauzed from its deployment in preparation of variety of food products such as bakery products, jam, sauce, papad, etc., as reviewed [1–3, 30–32]. Apple-based dietary fibre is found to be superior water binder to wheat and oat bran [33]. As a natural source of dietary fibre and antioxidant, apple fruit skin powder was added in muffin preparation [34].

Conclusion

The bioactive constituents, especially dietary fibre and polyphenolics present in apple pomace, can potentially be used as a source of food fortification. It also has advantages being natural, renewable and well tested for centuries (as in human food consumption system for long). This not only helps in getting natural health-promoting food ingredient, but will also help in rescue of food industries involved in apple processing through monetary gain and reduction in environmental pollution.

Table 2.2 Health-promoting activity of various dietary fibre and polyphenolic constituents

Constituent	Model system	Health-promoting activity	Results	Reference (s)
Apple peel and pulp phenolics	In vivo	Influence on plasma lipids and Inhibition of lipid peroxidation	Significant high positive influence showed on plasma lipid levels and inhibition of lipid peroxidation by apple peel Diets as compared to pulp	Leontowicz et al. [17]
Dietary fibre-rich colloids and alcohol-insoluble substance isolated from apple pomace	In vivo	Effect on weight gain and cholesterol metabolism	Apple DF in diets led to lower weight gain in rats and beneficial physiological effects of dietary fibre-rich diet on cholesterol metabolism	Sembries et al. [35]
Apple pomace crude extract	In vitro	Colorectal cancer	Beneficially influence of phenolics-rich extract on key stages of carcinogenesis in colon cells was observed	McCann et al. [25]
Polyphenolic extract of apple juice	In vitro	Potential as cancer chemo-preventive compounds	Specific polyphenolic compounds found as potent Cyp1A and cyclooxygenase 1(Cox-1) inhibitors	Zessner et al. [36]
Different apple constituents (whole apples, apple juice, puree, pomace and apple pectin)	In vivo	Influence composition of the cecal microbial community	Apple pectin-rich diet helps in butyrate- and b-glucuronidase producing microorganism, thus producing more butyrate, which reported to induce apoptosis in cancer cell lines and functions as fuel for the enterocytes	Licht et al. [37]
Untreated and polyphenol-free apple pomace dietary ingredient	In vivo	Influence on intestinal fermentation and serum lipid	Dietary fibre-enriched diet found to increase ileal digesta hydration and cecal SCFA concentrations, and decrease in triacylglycerol, glucose levels along with atherogenic index of plasma, thus showing positive health benefits	Kosmala et al. [38]

Acknowledgement Authors acknowledge the pursuit of Himachal Pradesh Horticultural Produce Marketing and Processing Corporation Ltd. (HPMC), Shimla, regarding beneficiation of apple pomace, and logistic support and funding received from Council of Scientific and Industrial Research and Ministry of Food Processing and Industires, GOI, India. The authors are also grateful for the support and guidance received from Dr. P. S. Ahuja, Director, CSIR-Institute of Himalayan Bioresource Technology, Palampur (HP) India.

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Chapter 3
Fortified Food Made from Animal Products: From Product Design to Nutritional Intervention

José A.G. Arêas, Raquel de Andrade Cardoso-Santiago, and Regilda Saraiva dos Reis Moreira-Araújo

Key Points

- Fortification using bovine lung as a highly bioavailable iron source was proposed.
- A ready-to-eat, iron-rich texturized product using chickpea and corn as a matrix plus lung was developed.
- Extrusion cooking was employed to achieve the desired final texture.
- Processing maintained the high bioavailability of the iron sources.
- The developed product was highly acceptable and comparable to counterparts available in the marketplace.
- The product was tested in a poor area in Brazil with high anaemia prevalence.
- After 2 months of intervention, prevalence dropped from about 60–11 %.
- This intervention proved to be safe, effective and inexpensive.

Keywords Bovine lung • Bioavailable iron • Enriched food • Nutritional intervention • Anaemia

Abbreviations

Hb	Haemoglobin
W/A	Weight for age

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V.R. Preedy et al. (eds.), *Handbook of Food Fortification and Health: From Concepts to Public Health Applications Volume 2*, Nutrition and Health, DOI 10.1007/978-1-4614-7110-3_3,

Introduction

Animal products, especially meat, are rarely used in fortification because of their high intrinsic production costs. In general, meat production consumes 10 times more energy than grain production when the input/output energy balance is taken into consideration [1]. Consequently, there has been a shift from meat towards grain production, although there are several reasons why a significant amount of food will always be of animal origin. Meat presents great acceptability. It is highly nutritious and is the best source of bioavailable iron. Meat consumption is traditional in all cultures and plays an important social role. In addition, on rough and rocky terrain meat production is more viable from the economic and energy point of view than vegetable production, which depends on ploughing and mechanical harvesting [2]. Although it is the richest product in nutrient intake and thus could be used as a fortificant, meat is usually expensive and this has prevented its use as an alternative material for food enrichment. However, some offal like lung, aesthetically rejected because of its intrinsically unpleasant texture, and thus not very expensive, could be the raw material best suited for iron fortification and combating anaemia.

Lung would become the richest source of bioavailable iron if consumed as food and could be used to prevent anaemia in vulnerable populations around the world. For many years, our group has been involved in recovering and upgrading slaughterhouse waste for use as food. The initial approach was to texturize these materials to produce a protein ingredient usable in various food preparations. This approach has posed a great challenge. Complex processing techniques, especially extrusion, have been only partially successful in reorganizing the protein of these products into fibre-like products. More recently, we have focused on iron content and its biological sensitivity to processing, and we have managed to develop ready-to-eat extruded products based on lung and grains that provide bioavailable iron and are suitable for nutritional intervention to combat iron deficiency anaemia [3–5].

The Iron Deficiency Anaemia Problem

Anaemia is a worldwide problem, and 2–3.5 billion people have some form of iron deficiency [6–8]. Prevalence studies have shown that anaemia is a worldwide phenomenon affecting different groups of individuals irrespective of age, sex and even social class. It is the single most common and widespread nutritional deficiency, affecting mainly reproductive-age and pregnant women and pre-school and school children [6].

The spatial distribution of anaemia observed in developing countries reveals prevalence rates ranging between 40 and 50 % for women and children under 5 years old. Within this population more than 50 % of pregnant women have some sort of iron deficiency [9, 10] and 51 % of children under 4 years of age suffer from anaemia.

Although nutritional education is regarded as sufficient to combat iron deficiency anaemia, it takes a long time to be effective, and many years are needed when interruptions in the process are frequent. Childs et al. [11] sought to establish whether an educational program to reduce the incidence of iron deficiency anaemia could be carried out using existing health resources. They studied 1,000 children from Birmingham and concluded that the program resulted in no reduction in anaemia. This highlights how difficult it is to conduct such programs using normal local resources.

Supplementation is a form of early intervention before a nutritional education program takes place. However, it can also pose problems and does not always provide the expected results. To be effective, it is essential for the target population to cooperate, and most of the time this doesn't happen. Many programs fail because medications used to treat iron deficiency anaemia taste bad and often produce

undesirable side effects such as diarrhoea, flatulence, nausea and darkening of the teeth. All this leads the individual to stop taking the medication before the planned deadline.

The advantages of food fortification are that it is cheap, does not require the population to change habits and promotes the intake of the iron they need. A wealth of scientific and technological evidence shows that iron fortification can ensure adequate nutrient intake levels. Since iron deficiency is high in most developing countries, the risk of the population getting too much iron because of fortification is low [12–19]. Moreover, the fact that fortification usually provides no more than 30 % the daily requirement reduces the risk of excessive iron intake.

The disadvantage of fortification comes from the role of iron as an important catalyst of deleterious chemical reactions where the product is added. The main reactions involving iron are lipid oxidation and pigment degradation, and their oxidation products and off-flavour cause rapid deterioration of food acceptability [20]. This has brought about the need for iron complexation and microencapsulation to block iron's reactivity when it is free in the food environment. The challenge in the process of food fortification is to avoid these problems by choosing the right iron source [21].

Some foods and iron compounds have been tested in Brazil for suitability in nutritional intervention. The focus has been on iron sources that produce the least amount of sensorial change in the fortified foods, which, in turn, should be widely consumed by the target population, locally produced, and economically viable [22, 23].

Product Development with Animal By-Products

The Population to be Targeted and the Choice of an Appropriate Product

According to a report published by the World Health Organization [7], iron deficiency anaemia contributed significantly in 2002 to the global burden of disease. Estimates of the Pan American Health Organization point to Peru as the country with the highest prevalence of anaemia in all of Latin America and the Caribbean (57 %), followed by Brazil, where 35 % of children aged 1–4 years present this nutritional deficiency [18].

Anaemia prevalence numbers vary from region to region in each country, depending on the population under study and methodology employed for population sampling and haemoglobin analysis. In Brazil, a very wide disparity from region to region of results for pre-school children has been reported with anaemia prevalence varying from 33 to 91 % [24]. Because of these figures, and the burden on cognitive development which anaemia represents, school children are the main population targeted for anaemia control in Brazil and in other countries.

To control anaemia among school children, curative and preventive actions within feasible cost/effectiveness ratios were proposed. Due to the multiple causes and nature of the disease, it is often necessary to adopt an integrated approach to the treatment of anaemia. In places where anaemia is highly prevalent, supplemental iron intake with medicinal preparations of iron sulphate has been used for those most susceptible. However, repeated intervention programs based on iron sulphate have proven to be ineffective in reducing anaemia among school children. In poor areas of Brazil, only small, 70–60 % declines in anaemia prevalence following weekly iron sulphate intake have been reported [25].

The most effective way to prevent anaemia in this group is to promote consumption of a diversified diet and to use fortified foods. Therefore, the fortification of food products that are already part of the population's habitual diet, or that can be easily accepted, is of prime importance in anaemia control in this or any targeted group [12, 13, 26–29].

Developing an Acceptable Ready-to-Eat Product with Bovine Lung

The use of alternative raw materials and techniques that facilitate the development of new foods provides a sustainable solution to food-related problems. Brazil is among the top five producers and consumers of bovine meat, so meat products for fortifying other foods are permanently available. Although it is well known that animal-derived iron is highly bioavailable and favourably impacts iron absorption from other sources [30], meat has always been too expensive to use for iron fortification. However, offal from the meat industry, rejected for aesthetic and cultural reasons, is inexpensive enough to be considered a viable ingredient for iron fortification. These nutritionally sound products are underutilized in food and human nutrition. They also represent an environmental problem if not properly disposed of. Moreover, meat production expends the most energy of any food commodity and maximizing meat use will help to make better use of this investment [1, 31, 32].

Despite its great nutritional potential, bovine lung, a low-cost slaughterhouse waste, is strongly rejected due to its intrinsic lower texture quality. It has been shown that extrusion can improve this texture without damaging the product's high nutritive value [33–39]. Extrusion has thus been extensively used to substantially improve the nutritional quality of a series of products using selected raw materials. In this way, chickpea (*Cicer arietinum* L.), bovine lung, amaranth (*Amaranthus caudatus* L.) and corn (*Zea mays* L.) have been used separately or in mixtures to produce a wide range of products with high nutritive value [3, 4, 35, 36, 38, 40, 41]. Among these, a ready-to-eat, iron-, protein- and vitamin-rich product made of corn, chickpea and bovine lung has been especially developed to help control iron deficiency anaemia among pre-school children [3, 4]. This product represents an appropriate and economically viable way of using a raw material that would otherwise be an environment problem and that results in a ready-to-eat convenience food for pre-school children.

To obtain products with appropriate sensory characteristics, it is initially necessary to optimize the extrusion parameters. These include extrusion temperature, raw material moisture, screw speed, feed rate and diameter of the matrix and sub matrix, as well as the thread configuration used in processing. These parameters have been reported as highly important for final product characteristics, directly affecting density, expansion, rehydration properties, starch gelatinization, cell structure, texture and nutritional value.

After the optimization process, sensory and nutritional assessment should be conducted. Preference and acceptability studies have been used to evaluate flavour, texture, aroma, colour and overall assessment to assure in part that the product will be consumed [42, 43]. Chemical analyses followed by biological assays in animals or humans have been shown to be satisfactory for nutritional assessment. Extrusion has proven able not only to produce highly acceptable final products but also to maintain the original high nutritive value, especially the bioavailability of lung iron [3, 5, 38, 39].

The development of the product was based on previous work by Batistuti et al. [41], who optimized the extrusion conditions for chickpea texturization. Chickpeas are legume seeds with high nutritive value, particularly their iron content and the biological value of their protein. The product developed by these authors was highly acceptable and had a bland taste. It was the basis for the iron-fortified ready-to-eat product incorporating bovine lung. Optimal processing conditions were then employed and increasing amounts of previously freeze-dried and defatted lung were added. All quality parameters of the final products were monitored, and there was a slight decrease in product acceptability as lung concentration increased. Figure 3.1 shows a 9-point hedonic scale, and the grade distribution for the blends tested by an untrained 40-member panel. The hedonic scale ranged from 1, disliked extremely, to 9, liked extremely, and 5, neither liked nor disliked, was the neutral grade.

Although there was a reduction in the frequency of higher grades, the results indicated that the average acceptability of the products was not significantly different and that the product could consist of up to 10 % lung on a dry basis. The use of chickpeas admixed with lung for intervention purposes

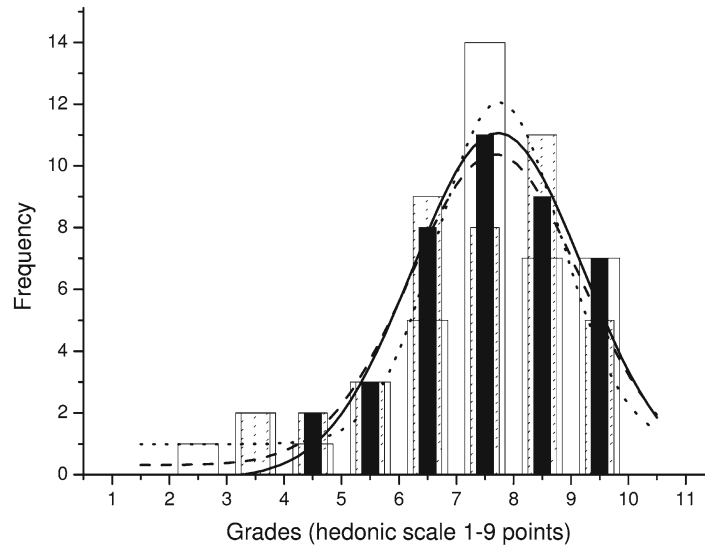

Fig. 3.1 Sensory evaluation grade distribution for pure and admixed ready-to-eat chickpea products. Pure chickpea (*black square*); 95:5 mixture with lung (*white square with a dot in the centre*); 90:10 mixture with lung (*white square*). Gaussian fitting for chickpea (*bold line*); 95:5 mixture with lung (*line with space*); 90:10 mixture with lung (*ellipse*) (Adapted from reference [3], Copyright Elsevier Publishers, reprinted with permission)

was also supported by the remarkable iron bioavailability observed for products made from these two raw materials [38, 39]. A 30 g pack of the 10 % lung final product eaten 3 times a week could provide school children between 2 and 6 years old with 30 % of their daily iron requirements.

Guidance on Safe Levels

Bovine lung can be incorporated in any proportion to the food matrix for iron fortification. Fresh bovine lung contains about 20 mg of highly bioavailable iron per hundred grams of tissue with freeze-dried lung containing about 100 mg/100 g [3, 4, 44, 45]. Therefore, the use of this iron source is safe since it is difficult to reach the tolerable upper intake level using this raw material. Moreover, incorporation of more than 10 % of lung on a dry basis into any product will impair its acceptability [4]. In the development of this product, we decided on a 10 % incorporation rate for sensory reasons since otherwise the product would be less acceptable.

Scaling Up for Large-Scale Production

To produce the volume of product needed for an intervention trial, an adaptation for industrial-scale production requiring the addition of corn grits was necessary [44, 45]. Corn was added to maintain superior production performance, expandability and ease of receiving new materials without significant changes in flavour and also due to its commercial importance. The inclusion of corn in the formulation thus resulted in a more nutritious version of this type of product, which is widely consumed and has a high potential as a vehicle for remedying nutrient deficiency in the population [46]. The proportion of chickpea, lung and corn that resulted in the best extrusion performance, acceptability and iron concentration was 72:8:20 [3, 44, 45].

The nutritional characteristics of this fortified product compared to the 90:10 blend and a commercial corn brand are shown in Table 3.1. The superior iron and protein content of the enriched products can be seen in this table. The ready-to-eat product developed for intervention with a 70:8:20 blend of

Table 3.1 Proximate composition (g/100 g), iron content (mg/100 g) and caloric content (KJ/100 g) of blends of chickpea, bovine lung and corn (70:8:20); chickpea and bovine lung (90:10), and corn-flavoured and unflavoured commercial snacks (dry basis)

	Protein	Ash	Lipids	Total carbohydrate[a]	Total iron	Caloric content
72:8:20 Blend						
Not flavoured	17.43	2.86	1.68	78.03	5.57	1661
Flavoured	18.69	5.13	14.85	61.33	7.41	1900
90:10 Blend						
Not flavoured	21.31	3.46	0.58	72.07	9.81	1586
Flavoured	19.92	3.96	20.24	55.88	9.18	2029
Corn (commercial)						
Corn flour	10.72	0.78	2.23	86.27	1.80	1707
Flavoured	6.67	0.74	25.09	67.5	1.22	2184

[a]By difference

Source: Adapted from reference [3], Copyright Elsevier Publishers, reprinted with permission

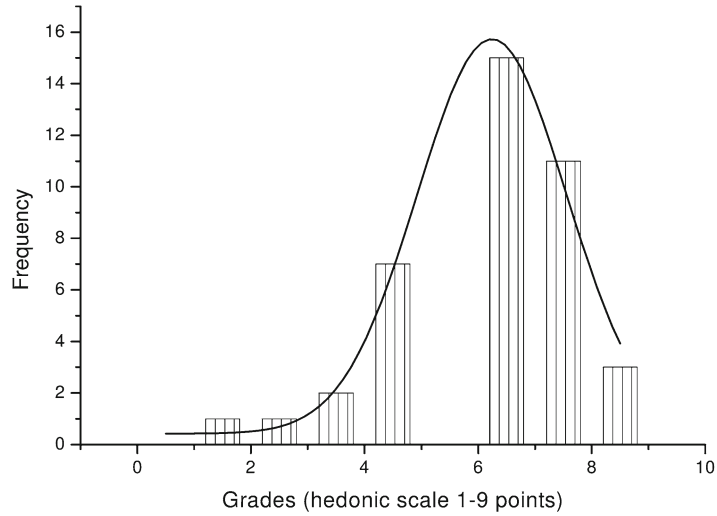

Fig. 3.2 Sensory evaluation grade distribution of the sensory evaluation for the ready-to-eat chickpea, lung and corn (70:8:20) products. (Adapted from reference [3], Copyright Elsevier Publishers, reprinted with permission)

chick pea, lung and corn was comparable to the commercial ones in terms of appearance and had slightly lower average acceptability (Fig. 3.2). The absence of grade 5 (neither liked nor disliked) from the panellists was peculiar. Nevertheless, the percentages of panellists giving a grade of 6 or above were high and similar in all cases. Therefore, the product was considered suitable for the planned intervention since its composition was appropriate for reducing iron deficiency anaemia in school children. It was then tested for control of anaemia prevalence in one of Brazil's poorest regions.

Testing the Developed Product

The Intervention Population

The developed fortified product was tested in the city of Teresina, state of Piauí, Brazil. The initial population under study consisted of 380 children, ranging in age from 32 to 72 months from two different local day nurseries. One group was randomly selected and used as control. By the end of

Table 3.2 Anaemia distribution in the two groups before and after the intervention period, according to age

Groups	Anaemia Indicator criteria Hb (g/dL)	Age (months)	Before		After	
			N	%	N	%
Experimental	<11	<72	28	21.5	0	0.0
	<12	≥72	52	40.0	15	11.5
	Anaemic		80	61.5	15	11.5
	Normal		50	38.5	115	88.5
Total			130	100.0	130	100.0
Control	<11	<72	32	24.6	34	26.2
	<12	≥72	50	38.5	41	31.5
	Anaemic		82	63.1	75	57.7
	Normal		48	36.9	55	42.3
Total			130	100.0	130	100.0
			$\chi 2 = 0.35$; $p = 0.840$		$\chi 2 = 67.25$; $p = 0.000$	

Source: Adapted from reference [44], Copyright Elsevier Publishers, reprinted with permission

the intervention period part of this original population had dropped out and 260 children in two groups of 130 each remained. The experimental group (130 children) received a 30 g pack of the enriched ready-to-eat product 3 days a week replacing part of the normal day nursery lunch. The control group (130 children) continued on the original diet. The product was offered in such a way that both the control and experimental group received the same energy intake. Table 3.1 shows the proximal composition and caloric content (1,900 kJ/100 g or 579 kJ/30 g portion) of the products used for intervention [3, 44].

The Baseline Anaemia

Anaemia prevalence at the 2-day-nurseries was similar before the intervention period, as shown in Table 3.2. Average haemoglobin content was 11.8 mg/dL in the experimental group, in which 80 of the examined children (61.5 %) were anaemic according to the criteria adopted (<11 mg/dL, for children up to 72 months, and <12 mg/dL, for children above 72 months [47, 48]). In the control group at the beginning of the intervention period average haemoglobin content was 11.6 mg/dL, and 82 (63.1 %) of the examined children were anaemic [3, 44].

The Effect of the Intervention

After the intervention period average haemoglobin content in the experimental group increased to 13.1, and only 15 children (11.5 % of total) in the higher age group (above 72 months old) were anaemic (Table 3.2). The average haemoglobin content in the control group at this time was 11.8, and there were 75 (57.7 % of total) anaemic children, 34 (26.2 % of the total) aged below 72 months and 41 (31.5 % of the total) above 72 months (Table 3.3).

The chi-square test showed that at the beginning of the intervention, there was no association between anaemia prevalence and control or experimental groups. After the intervention period, however, this association was significant.

Table 3.3 Iron consumption (mg/day), mean distribution and standard deviation of weight (kg) and blood haemoglobin concentration (g/dL) before and after the intervention period

	Before	After
	Mean ± SD	Mean ± SD
Experimental group		
Weight	17.2 ± 2.7	18.2 ± 2.8
Haemoglobin	11.8 ± 1.0	13.1 ± 1.0
Iron consumption	2.43 ± 1.1	6.96 ± 0.3
Control group		
Weight	16.9 ± 3.1	17.4 ± 3.2
Haemoglobin	11.6 ± 1.0	11.8 ± 1.2
Iron consumption	2.53 ± 1.2	2.54 ± 1.1
Weight	$t = 0.89$; $p = 0.380$	$t = 2.13$; $p = 0.035$
Haemoglobin	$t = 1.80$; $p = 0.075$	$t = 9.35$; $p < 0.001$
Iron consumption	$t = 1.37$; $p = 0.173$	$t = 44.4$; $p < 0.001$

Source: Adapted from reference [44], Copyright Elsevier Publishers, reprinted with permission

Table 3.4 Nutritional status of the children before and after intervention with a 30 g pack of the enriched snack 3 times per week

		Weight/age (W/A)								
		Before intervention				After intervention				
		<3	3–10	≥10	Total	<3	3–10	≥10	Total	Statistics
Experimental group	*N*	9	33	88	130	5	21	104	130	$\chi^2 = 5.14$; $p = 0.076$
	%	6.9	25.4	67.7	100	3.8	16.1	80.1	100	
Control group	*N*	13	22	95	130	13	20	97	130	$\chi^2 = 0.12$; $p = 0.943$
	%	10.0	16.9	73.1	100	10.0	15.4	74.6	100	

Source: Adapted from reference [45], Copyright Brazilian Society of Food Science and Nutrition, reprinted with permission

Tables 3.3 and 3.4 show the remarkable decrease observed in anaemia prevalence in the experimental day nursery after the intervention period. No children below 72 months old had a haemoglobin concentration lower than 11 g/dL and only 15 children aged above 72 months (11.5 % of the total) had a haemoglobin concentration lower than 12 g/dL (Table 3.3). On the other hand, the control group, which received the normal day nursery diet without any modification, showed no significant reduction in anaemia prevalence, although there were oscillations in the number of children below the haemoglobin cutoff value. There was a significant association between haemoglobin concentration and the groups after intervention. Mean values of blood haemoglobin concentration increased significantly after the intervention in the experimental group, whereas they remained constant in the control group (Table 3.3) [3, 44].

At the beginning of the experiment, the amount of ready-to-eat products actually eaten daily by the children averaged 95 % of the pack offered, increasing to 98 % as the end of the intervention period approached.

After the intervention period, both day nurseries returned to their habitual diet. Seven months after the end of the intervention, the experimental group was tested again for haemoglobin concentration. The results showed anaemia in 98 (75.4 %) children (Fig. 3.3). Spearman's correlation test showed a significant association among these three different moments [3, 44].

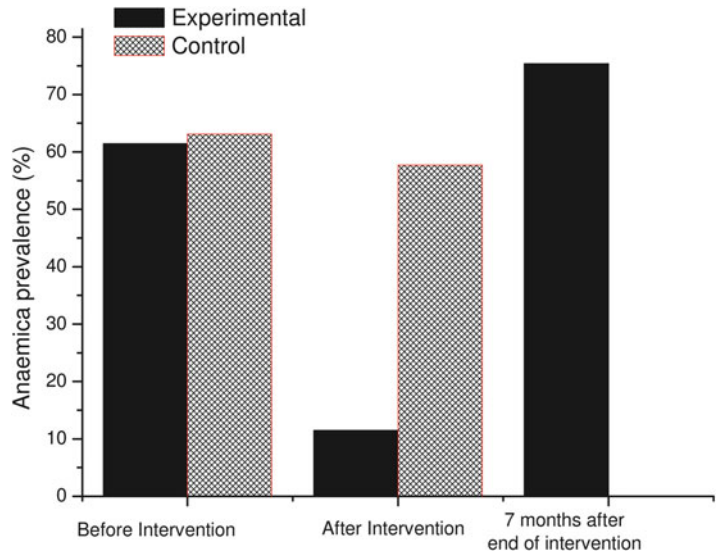

Fig. 3.3 Anaemia prevalence among children in the 2-day-nurseries before, after and 7 months after the end of the intervention. (Adapted from reference [3], Copyright Elsevier Publishers, reprinted with permission)

The Impact on Children's Anaemia and Nutritional Status

The high nutritive value of the developed product, especially the high iron content from an animal source, was ideal for an intervention program aimed at anaemia control. Besides nutritive value, production cost is another consideration for food fortification. The final product price of the newly developed ready-to-eat product made of chickpea, bovine lung and corn, estimated according to usual food-industry procedures for calculating the cost of this type of product [49], was only 20 % higher than those for commercial products made of pure corn, which have no nutritional benefits.

The major cost constraint comes from the cost of lung, which may drop in the future as production is scaled up. Another important factor for a successful intervention program with fortified food is product acceptability. Panellists showed similar preferences for the developed product compared to commercial brands and to the original product made only with chickpea and bovine lungs [3].

Besides all the benefits from anaemia control in this population, the children also exhibited better nutritional status, as assessed by their weight to age (W/A) ratio. Although the intervention lasted only 2 months, positive changes in this score were detected (Table 3.4). There was an important decrease in the 'light to moderate under-nutrition' (percentile between 3 and 10) and 'strong to moderate' nutritional status (percentile <3) from 32.3 to 19.9 % in the experimental group, and an increase in eutrophic children (percentile >10) from 67.7 to 80.1 % [45]. This can be explained because fortification of the product with bovine lung provided protein of high biological value, vitamins A, B1, B2, B6 and PP, and other minerals essential for children's growth and cognitive development in addition to the bioavailable iron.

Recommendations

Bovine lung as an iron source can be used in different products, depending on the habits of the population targeted to receive the fortified product. Its addition gives the final product a darker appearance, and this should be taken into consideration. Manufactured products with an original dark colour will accept lung incorporation well. Moreover, it is always possible to use this change in

appearance in a favourable way by including salty and sweet flavours such as barbecue, pizza, chocolate, etc., that are compatible with the final colour. This approach has been shown to increase product acceptability [5].

Conclusion

This chapter showed the feasibility of using lung, an underutilised product from the meat industry, in food fortification. The development of a fortified product depended on the successful texturization of lung by extrusion together with other raw materials that provided a suitable matrix for the product structure. The reported final ready-to-eat product was comparable to commercial brands and presented similar acceptability and superior mineral, especially iron, content, and vitamins and protein of high biological value. The developed product was efficient in reducing iron deficiency anaemia among school children when formulated to provide 30 % of the daily intake needs of this target population. The cost of this product was 20 % higher than its commercial counterpart, but the main constraint is the cost of bovine lung, which can be reduced as production scales up. Iron fortification with a meat product such as lung is a viable strategy that resulted in a stable, safe and efficient product with the purpose of reducing anaemia prevalence in school children.

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Chapter 4
Dietary Lipid Sources as a Means of Changing Fatty Acid Composition in Fish: Implications for Food Fortification

Jaume Pérez-Sánchez, Laura Benedito-Palos, and Gabriel F. Ballester-Lozano

Key Points

- Fish consumption is the most important source of n-3 long chain polyunsaturated fatty acids in the human diet.
- Marine fish have specific requirements for arachidonic, eicosapentaenoic and docosahexaenoic acids, while freshwater and salmonid fish can convert C_{18} polyunsaturated fatty acids of both n-6 and n-3 series to their longer chain.
- Fish species differences in fatty acid essentiality are highly related to fatty acid desaturase and elongase abilities.
- How the actual acknowledgement on fish lipid metabolism can be used for selecting fish strains that provide high levels of n-3 chain polyunsaturated fatty acids for human consumers remains still largely unexplored.
- Eicosapentaenoic acid and docosahexaenoic acid from fisheries are not enough to cover the theoretical needs of the human population.
- Strategies for improving the nutritional fatty acid profile of fish should exploit different means of dietary intervention.
- Changes in fillet fatty acid composition after switches in dietary fatty acid composition follow a simple dilution model.
- Multilinear regression approaches from dietary information and fish adiposity are highly valuable tools to predict the fillet fatty acid composition through all the productive cycle of farmed fish.
- Updated information on feedstuffs contaminants does not represent a serious concern with the advent of alternative diets.
- The challenge in the future is to assure the production of high quality fish according to the human nutritional guidelines and the concomitant policies for a sustainable utilization of marine resources as fish feed ingredients.

Keywords Fish • Lipid metabolism • Fish feeds • Fillet fatty acid composition • Fish oil finishing protocols • Simple dilution model • Regression equations • Predictive fatty acid modelling • Fish consumption

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V.R. Preedy et al. (eds.), *Handbook of Food Fortification and Health: From Concepts to Public Health Applications Volume 2*, Nutrition and Health, DOI 10.1007/978-1-4614-7110-3_4,

Abbreviations

ARA	Arachidonic acid
CLA	Conjugated linoleic acid
DHA	Docosahexaenoic acid
DL-PCB	Dioxine-like polychlorinated biphenyl
EFA	Essential fatty acid
EFSA	European Food Safety Authority
EPA	Eicosapentaenoic acid
FAME	Fatty acid methyl ester
LA	Linoleic acid
LC-PUFA	Long chain polyunsaturated fatty acid
LNA	α-Linolenic acid
OA	Oleic acid
OCP	Organochlorine pesticide
PCB	Polychlorinated biphenyl
PL	Phospholipid
PUFA	Polyunsaturated fatty acid
ROS	Reactive oxygen species
UCP	Uncoupling protein

Introduction

Organisms of vegetal and animal kingdoms can synthesize saturated and monounsaturated fatty acids using carbons from different sources. The major resulting products are palmitic acid (16:0), stearic acid (18:0) and their Δ9 desaturase products, palmitoleic acid (16:1n-7) and oleic acid (OA, 18:1n-9). However, due to the absence of Δ12/Δ15 desaturase enzymes, vertebrates require a dietary source of n-6 and n-3 polyunsaturated fatty acids (PUFAs) to meet their requirements in essential fatty acids (EFA), especially arachidonic acid (ARA, 20:4n-6), eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) (Fig. 4.1). Thus, once obtained from the diet, linoleic acid (LA, 18:2n-6) is metabolized by elongation and Δ6/Δ5 desaturation to ARA. The same desaturases operate for the conversion of α-linolenic acid (LNA, 18:3n-3) to EPA and DHA. However, the conversion of ALA to DHA is low in humans, particularly at the conversion of EPA to DHA [1]. Similarly, when evaluating changes in plasma phospholipid (PL) fatty composition, supplementation of LNA, up to 5 g per day, does not have a significant impact on the PL DHA content [2]. This reinforces the nutritional value of fish, in particular marine oily fish, as virtually the most important source of n-3 long chain PUFA (LC-PUFA) in the human diet [3, 4]. However, global fisheries are in decline, and farmed fish constitute an increasing proportion of fish in the human food basket. Thus, to assure the continuous growing of aquaculture production, the industry is obligated to find suitable alternatives to fish meal and fish oil in fish feeds. Plant products are the obvious choice, but vegetable oils are devoid of n-3 LC-PUFA, and fillet fatty acid composition of farmed fish points towards a reduction in EPA and DHA levels. The sustainable development of aquaculture and the preservation of health benefits of fish consumption represent, thereby, a complex trade-off, and the aim of this chapter is to review recent findings in fish nutrition as a means to assure the production of high quality fish according to the human nutrition guidelines and the concomitant policies for a sustainable utilization of finite marine resources as feed ingredients. Special attention is focused on salmonids and warm marine fish, in particular gilthead sea bream (*Sparus aurata*), which is now the most important farmed fish in the Mediterranean area.

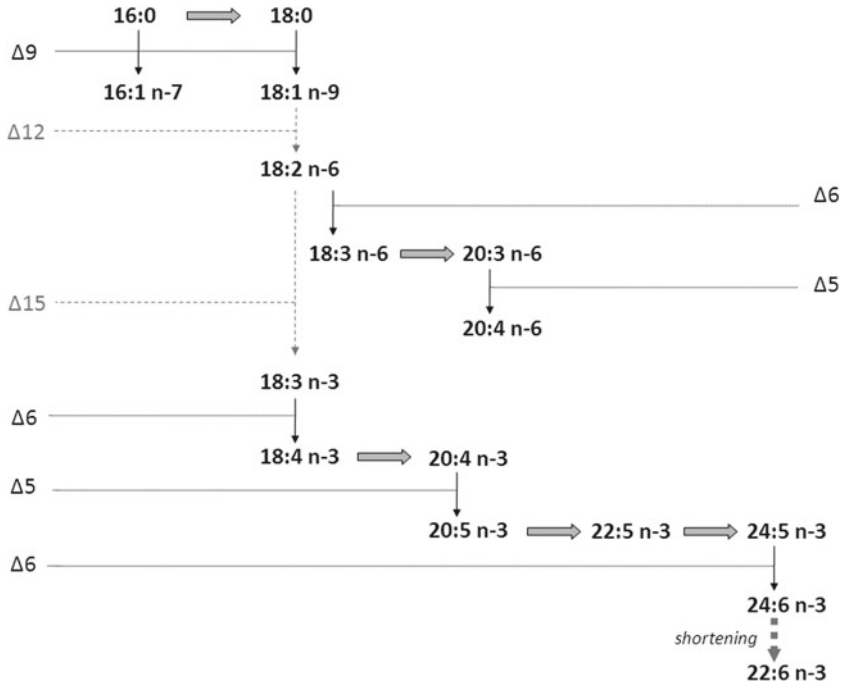

Fig. 4.1 Biosynthesis of LC-PUFA from fatty acid precursors. *Horizontal arrows* represent fatty acid chain elongation reactions. *Vertical arrows* represent fatty acid desaturations. The *dotted arrow* represents peroximal chain shortening. Δ12 and Δ15 desaturases are found only in plants and some invertebrates

Basic Aspects in Lipid Fish Nutrition

Fatty Acid Essentiality

Lipids are probably the most studied group of nutrients in fish nutrition, but most of the interest in lipid and fatty acid metabolism is due to the high abundance of n-3 LC-PUFA in marine ecosystems. Certainly, n-3 LC-PUFA play a key role in a wide range of metabolic pathways and biological process. However, both in humans and in fish, EFA requirements are more difficult to establish than for other nutrients because the intake and cellular availability of a given fatty acid affect that of the others [5]. For that reason, EFA requirements vary largely in fish with season, temperature, stage of development and reproductive cycle, although fatty acid essentiality is specially dependent on whether the species is freshwater, anadromous or marine [6]. Thus, it is generally accepted that marine fish have specific requirements for ARA, EPA and DHA, while freshwater and salmonid fish can convert C_{18} PUFA of both n-6 and n-3 series to their longer chain PUFA. Nevertheless, while the conversion of LNA to EPA is appreciable in salmonids, the final steps in the synthesis of DHA are relatively inefficient and cannot meet requirements over a long period of time if only LNA is present in the diet [7].

Fish species differences in fatty acid essentiality are, thereby, highly related to fatty acid desaturase and elongase abilities. Thus, as recently reviewed by Vagner and Santigosa [8], Δ5/Δ6 desaturases have been characterized in salmonids and freshwater fish species, including zebrafish (*Danio rerio*), Atlantic salmon (*Salmo salar*), rainbow trout (*Onchorynchus mykiss*), Nile tilapia (*Oreochromis niloticus*) and carp (*Cyprinus carpio*). Expression and nutritional regulation of Δ6 have also been

proved in marine fish species, such as gilthead sea bream, European sea bass (*Dicentrarchus labrax*), Atlantic cod (*Gadus morhua*), turbot (*Scophthalmus maximus*) and cobia (*Rachycentron canadum*). Nevertheless, it appears that there is a block at either the Δ5 desaturase and the C_{18}–C_{20} elongase step and, even for Δ6 desaturases, enzyme activities are very low and the flux through the LC-PUFA biosynthetic pathway is practically negligible [9]. For that reason, marine fish have an absolute requirement for n-3 LC-PUFA, especially EPA and DHA, which has been fixed in recent reviews in the range of 0.4–3.7 % of dry diet [10]. The assignment of EFA requirements is, thereby, ambiguous, although it is generally accepted that these requirements are higher in early life stages, probably due to the high demand for somatic growth and neural tissue development in a rapidly growing organism [11].

Symptoms of Fatty Acid Deficiencies

Absence of specific PUFAs from the diet lies to a wide range of deficiency symptoms that, in fish, includes reduced growth and survival, impaired reproductive function, fin erosion, lordosis, stressful behaviour, heart failure, shock syndrome, liver steatosis and intense accumulation of lipid droplets in the hindgut [10]. In mammals and humans in particular, a number of reviews have outlined the relationship of n-3 LC-PUFA deficiency with atopic diseases, food allergies, obesity, metabolic syndrome, cardiovascular health, vision and neurological dysfunction [3]. The importance of the desaturase programme also arises in mutant model organisms. For instance, *Caenorhabditis elegans* mutants that lack Δ6 desaturase activities have delayed growth, altered behaviour and reduced fertility at lower temperatures [12]. Mice lacking Δ6 desaturase activity also suffer from fertility problems with a large proportion of the mutants being sterile [13]. It appears, thereby, that data from various organisms highlight three main physiological abnormalities arising from unsaturated fatty acid deficiencies: (1) impaired neural development and function, (2) alterations in the skin barrier and (3) inability to adapt to low temperatures, which reflects a defective control of membrane lipid composition that is especially evident in response to nutritionally and environmental stresses [14].

There is also abundant information indicating how deregulated unsaturated fatty acids may influence adipose tissue expansion and obesity complications [15]. Such problems also develop in fish, and gilthead sea bream fed deficient diets in n-3 LC-PUFA have a reduced fat storage capacity. This metabolic defect is exacerbated overwintering, and concurrent changes in liver and adipose tissue mass strongly support an enhanced flux of fatty acids from adipose tissue towards liver in fish with EFA deficiencies (Fig. 4.2). This reallocation of body fat depots is accompanied by a robust down-regulated mRNA expression of Δ9 fatty acid desaturases in the adipose tissue, similarly to what has been observed in Δ9 knockout mice [16]. On the other hand, gilthead sea bream adipocytes become enlarged with high inclusion levels of vegetable oils in practical fish feeds, which is viewed as a risk-failure of the mechanisms involved on the recruitment of new adipocytes with the reduced intake of n-3 LC-PUFA [17]. This in turn allows non-adipogenic tissues (e.g., liver and muscle) to be exposed to an excessive fatty acid influx, a well-known phenomenon that has been described as lipotoxicity [18].

Lipotoxicity and Respiration Uncoupling

Highly unsaturated fatty acids are prone to oxidation by reactive oxygen species (ROS), resulting in the formation of cytotoxic and highly reactive lipid peroxides. Hence, accumulation of fatty acids near the highly oxidative mitochondrial matrix makes them prone to peroxidation, and aerobic organisms have developed a wide series of adaptive mechanisms to protect mitochondria against oxidative insults and tissue damage. One of the most sophisticated processes is mediated by uncoupling

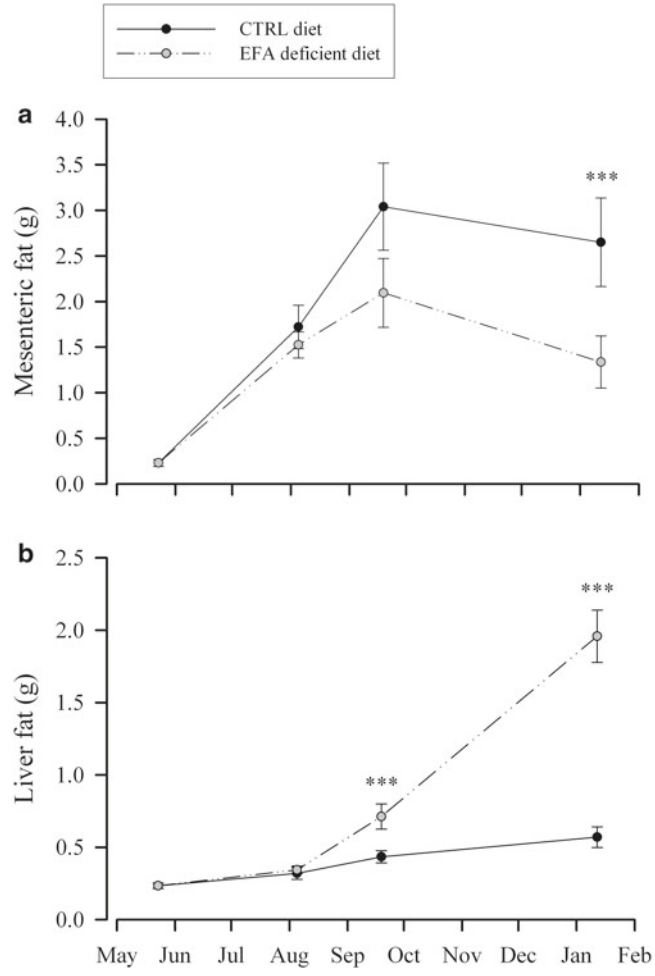

Fig. 4.2 Mesenteric (**a**) and liver fat content (**b**) in gilthead sea bream fed plant protein-based diets with fish oil (CTRL diet) or a blend of vegetal oils (EFA deficient diet) as the unique dietary lipid source. Values are the mean ± SEM of ten fish. Significant differences between dietary treatments were analyzed by Student's *t*-test (***$P < 0.001$)

proteins (UCP), a family of mitochondrial transporters that uncouple oxidative phosphorylation by the net discharge of proton gradient. How fatty acids activate the net protonophoric activity of UPCs is still debated, although it is generally accepted that UCPs enhance fatty acid oxidation and attenuate mitochondrial ROS production [19]. The archetype of the family is UCP1 with a well-documented role in non-shivering thermogenesis in the narrow adipose tissue of rodents, hibernators and newborns [20]. Less clear is the ancestral function of UCP1 and other members of the UCP family (UCP1-3), although studies in avian and ectothermic vertebrates, including fish [21, 22], support a primary role as redox-sensors to match both energy demand and antioxidant defence. Thus, a general statement is that UCP1 and UCP3 are up-regulated or high when the fatty acid supply to the mitochondria is likely to exceed the capacity to oxidize fatty acids, and down-regulated or low when oxidative capacity is high or improved. In that context, a strong negative correlation between mRNA UCP3 expression and the oxidative capacity of muscle tissues has been reported in gilthead sea bream (heart > red skeletal muscle > white skeletal muscle), and interestingly measurements of mitochondrial respiration uncoupling are positively correlated with circulating levels of free fatty acids [22]. Whether this knowledge can be used for selecting fish strains with lean phenotypes that still provide high levels of n-3 LC-PUFA for the human consumers remains to be explored. However, given that mice over-expressing UCP3 have a low incidence of obesity [23], this possibility cannot be ignored in large-scale fish breeding programmes.

Progress Towards Sustainable Fish Feeds

Fish Performance and Safety

Whereas global fisheries are in decline, aquaculture industry is still growing and several efforts have been directed towards the reduction of wild-fishery-derived raw materials in the feeds of farmed fish. Much attention has been focused on plant ingredients and there is now accumulating evidence for a large and combined replacement of fish meal and fish oil in fish feeds for salmonids and marine fish. Hence, the inclusion level of marine feedstuffs in fish feeds has been steadily declining over the course of the last decade, and current targets for fish meal and fish oil inclusion levels give Fish-In Fish-Out (FIFO) coefficients lower than 3.6 for most fish production systems [24]. In particular, fish oil can be totally replaced with vegetable oils in practical gilthead sea bream diets with a 35 % inclusion level of fish meal [25]. Alternatively, up to 65–70 % of fish oil can be replaced in diets with a 15–20 % of fish meal inclusion level with no, or minimal effects, on growth performance and fatty acid composition of polar lipids from tissues with different requirements in EFA [26–28]. More controversial are the results arising from studies analyzing the effect of nutritional background on fish diseases resistance, because both increased or decreased mortalities have been reported with the replacement of marine feedstuffs with plant ingredients in a wide range of fish. Thus, a general statement is that nutrient requirements for fish growth are different from those of immune function and health status. As an example, the maximized replacement of fish oil with vegetable oils allows a faster progression of diseases outcomes in gilthead sea bream challenged with the intestinal parasite *Enteromyxum leei* [29]. However, further research is needed to determine if the differences in diseases progression are due to changes in dietary fatty acid composition rather than to any other vitamin or micronutrient naturally associated to fish oil.

Food safety is also a major issue on the progress towards new and sustainable fish feeds, and important research efforts have been done over the course of the last decade to assess the carry-over from fish feeds to human consumers of polychlorinated biphenyls, dioxins and other harmful lipophilic organic chemicals that are now ubiquitous contaminants in marine ecosystems. Fortunately, vegetable oils are generally cleaner than fish oils, and overall the inclusion of vegetable oils in fish feeds decreases the contaminant loads of persistent organic pollutants into edible fish meat for human consumers. This has been proved in different toxic-kinetics studies conducted in a wide range of fish species [30, 31], as exemplified in Table 4.1 for gilthead sea bream, which certainly contributes to gain public acceptance for fish fed with alternative and environmentally safety diets.

Fish Oil Wash-Out

Of course a further and important consideration is the effect of alternative dietary oils on the nutritionally quality characteristics of edible fish portions. Certainly, high levels of n-3 LC-PUFA are important quality factors in human foods, and even salmonids and freshwater fish, which do not have specific n-3 LC-PUFA requirements, are facing increasing pressures to include EPA and DHA in their finishing diets. Hence, a common approach for improving or restoring the muscle fatty acid profile to what is characteristic of marine fish could be the implementation of a finishing period on a fish oil-based diet. However, fish oil wash-out protocols are fish species-specific and need to be adapted to each particular fish farming condition. Thus, as indicated by Turchini and Mailer [32], growth and lipid deposition rates have a major effect on the timing of the nutritionally mediated changes in muscle fatty acid composition. Other species-specific variables can be considered when analyzing the effectiveness of a fish oil wash-out protocol, although in all instances several weeks are needed to

Table 4.1 Concentration (ng/g fresh weight) of organochlorine compounds (polychlorinated biphenyls, PCBs; dioxin like PCB, DL-PCBs; organochlorine pesticides, OPCs) in experimental diets and gilthead sea bream fillets of marketable fish size

	Diet			Fillet		
Compound	FO	33VO	66VO	FO	33VO	66VO
PCB 28+31	0.2	0.1	<0.1	0.2	0.1	0.2
PCB 52	0.2	0.2	0.1	0.3	0.2	0.2
PCB 101	0.5	0.5	0.3	0.6	0.4	0.3
PCB 77*				<0.2	<0.2	<0.2
PCB 118	0.6	0.6	0.4	1.0	0.7	0.6
PCB 153	1.6	1.5	0.9	1.8	1.4	1.2
PCB 105*	<0.1	<0.1	<0.1	0.6	0.3	0.3
PCB 138	0.8	0.7	0.5	1.1	0.8	0.7
PCB 126*				<0.2	<0.2	<0.2
PCB 128	<0.1	<0.1	<0.1	0.3	0.2	0.2
PCB 156*	<0.1	<0.1	<0.1	<0.4	<0.4	<0.4
PCB 180	0.4	0.4	0.3	<0.1	<0.1	<0.1
PCB 169*				0.6	0.5	0.4
PCB 170	<0.2	<0.1	<0.1	0.2	0.2	0.2
SUM PCBs	4.4	4.1	2.8	6.5	4.8	4.1
SUM DL-PCBs	<0.1	<0.1	<0.1	1.3	1.0	0.8
HCB	0.2	0.2	0.5	0.2	<0.1	0.4
p,p-DDE	2.5	2.0	1.7	3.6	2.5	2.1
p,p-DDD	2.3	2.0	1.3	2.3	1.7	1.3
p,p-DDT	2.5	2.1	1.4	2.2	1.4	1.0
SUM OCPs	7.6	6.2	4.8	8.3	5.8	4.8

Source: Adapted from [31] Copyright 2009, with permission from Elsevier (license no. 2734830996508)
FO fish oil-based diet; 33VO: diet in which vegetable oil replaced 33 % of fish oil; 66VO: diet in which vegetable oil replaced 66 % of fish oil
DL PCBs are labelled by asterisk

have a significant impact on body fat stores and then on muscle fatty acid signatures. Scientific literature is, in fact, prolific on studies in which a complete restoration of the muscle fatty acid profile is not fully achieved after fish oil re-feeding: 32 days in red sea bream [33], 8 weeks in turbot and brown trout (*Salmo trutta*) [34], 12–25 weeks in Atlantic salmon [35, 36], 4 months in Murray cod (*Maccullochella peelii*) [37] and 5 months in European sea bass [38]. Despite this, most of the results point out towards a simple dilution model, which was firstly proposed and validated in brown trout and turbot [34].

The concept of a simple dilution model assumes that fatty acids are incorporated in a given tissue without any mobilization or turnover of pre-existing fatty acids. In other words, changes in the tissue fatty acid profile arise because the existing fat stores become diluted as fish grow and deposit increasing amounts of dietary-derived fatty acids, which can be modelled by the following equation:

$$P_T = P_{RT} + \left[(P_0 - P_{RT}) / (Q_T / Q_0)\right]$$

where P_T is the percentage at time t of a given fatty acid, P_0 is the fatty acid percentage at the start of the finishing period, and P_{RT} is the fatty acid percentage at time t in fish continuously fed the reference/finishing diet. Q_0 and Q_T are the initial and final (at time t) amount of tissue fat level, respectively.

In particular, the simple dilution model is a good general descriptor of fillet fatty acid composition in gilthead sea bream, and regression curves (predicted vs. observed values) give slopes nearby to the line of equality for practically all the analyzed fatty acids (Fig. 4.3). In Atlantic salmon, Jobling [39] tested three fatty acids (18:1 isomers, 18:2 n-6 and 18:3 n-3) and confirmed closely the predictions

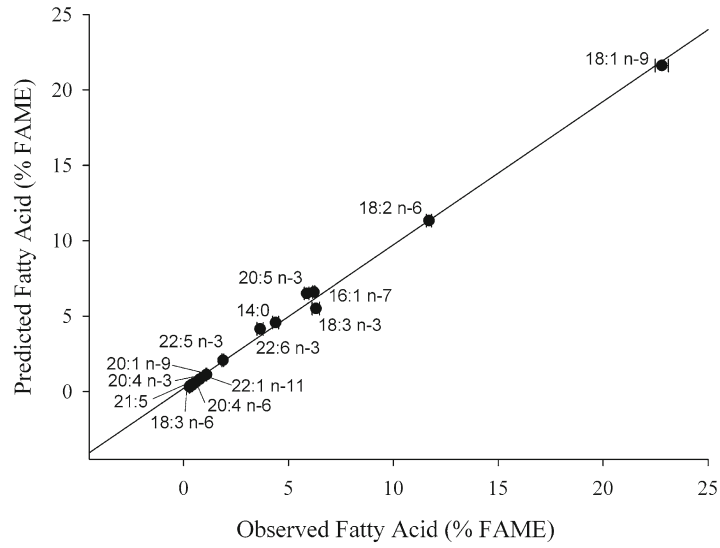

Fig. 4.3 Plot prediction (simple dilution model) of fillet fatty acid profile in gilthead sea bream of market size grow-out (11 months) with vegetable oils (dietary n-3 LC-PUFA: 0.9 % dry matter) followed by a fish oil wash-out period (12 weeks). Observed values are the mean ± SEM of 8 fish. The solid line is the plotted regression. Adapted from [42] Copyright 2008, with permission from Elsevier (license no. 2734831175916)

made with the dilution model. Jobling [40] again evaluated the dilution model with data from red sea bream studies [33] and a high degree of concordance was also found between the predicted and observed values. Similar results have been reported for Murray cod [37] and Atlantic cod [41], although the concordance with the model is higher in species with high lipid tissues. This is because the fatty acid composition of PL is highly regulated by the organism, whereas fatty acid signatures of neutral lipids highly reflect the fatty acid composition of the diet.

The apparent simplicity of fatty acid kinetics after changes in dietary lipids increases the robustness and predictive value of the model, but at the same time savings on fish oil resources are limited to a simple dilution. Hence, temporal changes on diet composition gave a minor effect on tissue fatty acid composition if the absolute intake for a given fatty acid becomes equal at the end of the production cycle. This notion has been proved in fast growing gilthead sea bream [42]. Thus, fish grow-out with practical diets with a 66 % of fish oil replacement (66VO diet, 0.9 % n-3 LC-PUFA) show after 12 weeks of fish oil re-feeding the same muscle fatty acid profile than fish continuously fed 33VO diet (1.8 % n-3 LC-PUFA). Nevertheless, additional research is needed to improve our understanding on the potential of restoration of fatty profiles by fish oil wash-out protocols. In particular, some authors suggested that fasting or partial feed restriction prior to the finishing wash-out period may serve to produce lean fish with still high levels of n-3 LC-PUFA [32]. Certainly, the restoration of a marine fish fatty profile in fish grow-out with vegetable diets is faster in fish strains of Atlantic salmon with lean phenotypes [43]. In any case, ration size by itself is the most important factor governing the effectiveness of the fish oil re-feeding period, which explains why the up-scaling of fish oil wash-out protocols is constrained by the pronounced growth seasonality of farmed fish reared in temperate areas. For instance, the active "feeding window" of gilthead sea bream varies from May to October in the Mediterranean region according to the information on daily and seasonal calendar that is provided by changing patterns of melatonin secretion [44]. Life-history decisions are, however, not fixed and depend on critical size and energy sufficiency at a specific stage "opportunity window" several months prior to transformation itself. The fine-tuning of these decisions is a

complex issue, but circumstantial evidence indicates that the growth hormone and prolactin family provides an integrated signal for growth, fattening and reproductive onset year-around [45].

Regression Approaches for Predictive Fatty Acid Modelling

Regardless of diet composition, the nature of lipid digestion has a substantial effect on the transfer of fatty acids from the diet into the animal product [46]. Thus, microbial enzymes in the rumen digestive system are responsible for the conversion of unsaturated fatty acids to various partially and fully saturated derivatives, such as stearic acid and conjugated linoleic acids (CLA). Unlike ruminants, dietary fatty acids in fish and terrestrial monogastrics are absorbed unchanged before their incorporation into the tissue lipid fraction. Therefore, dietary lipids have more direct and predictable effects on the fatty acid composition of fish, pigs and poultry products than that of beef and lambs. Besides, the tissue supply of PUFA, especially EPA and DHA, can be simply increased by increasing their proportion in the diet. This reinforces the interest for reliable fatty acid descriptors linking dietary- and muscle fatty acid composition in non-ruminants. In particular, the association between dietary and fillet FA composition is likely to be stronger in oily fish than in lean fish. Close associations between dietary and fillet FA composition are also more likely to be produced with non-endogenously synthesized fatty acids. However, regression formulas for the predictive modelling of muscle fatty acid composition from dietary information are practically reduced to Atlantic salmon [35] and Atlantic cod [47], and results accumulated so far remain insufficient and do not allow development of a proper strategy for increasing the muscle deposition rates of n-3 LC-PUFA.

Nutritional tailoring of fish fatty acid composition is thereby in an infancy state, although the situation becomes changing and research efforts in gilthead sea bream highlight the highly predictable effects of dietary lipids on the muscle fatty acid composition of a warm marine fish with either high or intermediated lipid deposition rates under intensive fish farming. Within this regard, data on muscle fatty acid composition from a given class of size (1-year-old gilthead sea bream) were compiled and analyzed [48]. Data were derived from asynchronous growth trials conducted with fish originating from three major European producers that assure a representative farmed fish population under the same standards of handling and maintenance. Partial and total replacement of either fish meal or fish oil was also considered in the experimental setup. Thus, the replacement strategy of marine raw materials with plant ingredients covered a wide range of changes in the fatty acid composition of diets containing 20–24 % crude lipid, which represents the normal lipid range in most commercially available fish feeds for carnivorous fish. All this ultimately translates into powerful linear regression equations that are specific for each fatty acid, being the correlation coefficients particularly high for monoenes, C_{18} PUFA and LC-PUFA. This is not surprising because marine fish have a very limited capacity to elongate and desaturate C_{18} fatty acids into long chain C_{20} and C_{22} PUFA, and most of them are entirely derived from the diet, which enables the mathematical modelling of fatty acid composition with a high level of confidence. By contrast, saturated fatty acids, especially 16:0 and 18:0, gives low correlation coefficients in a univariate regression model, probably due to the fact that these fatty acids are mainly the product of endogenous lipogenesis and interconversions between them limit the impact of dietary supply levels. Besides, most regression deviations are corrected by the use of multivariate regression equations that include fillet lipid level as a second independent variable for effectively mirroring year-around the fillet fatty acid composition of a marine and protandric marine fish,

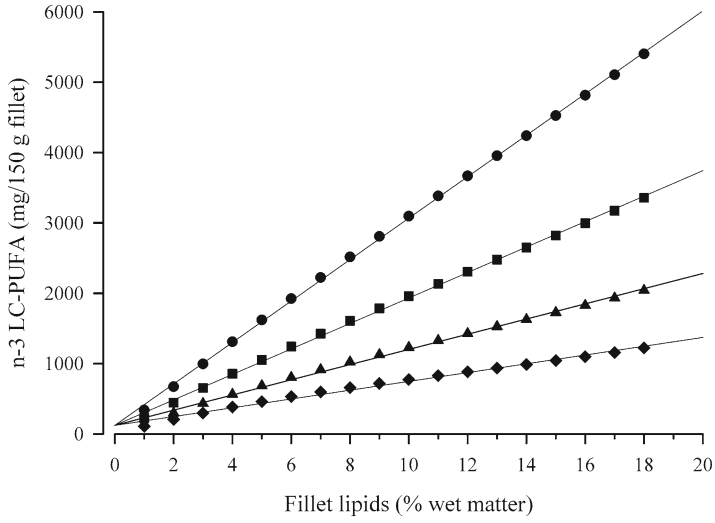

Fig. 4.4 Plot prediction for marine farmed fish of fillet content in n-3 LC-PUFA as a function of varying fillet lipid levels and dietary inputs in n-3 LC-PUFA (% dry matter: *filled circle* 3.7 %, *filled square* 2.7 %, *filled triangle* 1.8 %, *filled diamond* 0.9 %). Plots are made from the equation $Y = 61.38 + 0.10X - 3.21Z + 0.005X^2 + 0.09Z^2$, where Y is the fillet content in n-3 LC-PUFA (mg/g lipid), X is the dietary content in n-3 LC-PUFA (mg/g lipid), and Z is the fillet lipid content (% wet matter). The equation is constructed from data derived from gilthead sea bream, European sea bass, turbot and sole

such as gilthead sea bream [49]. The regression equations fit well from juvenile stages until male-sex reversal and, interestingly, switches in fillet lipid content significantly contribute to explain changes in ARA, DHA, monoenes and saturated fatty acids, whereas upstream (LA, LNA) or intermediate (EPA; DPA, 22:5n-3) fatty acids of n-3 and n-6 biosynthetic pathways become independent of fish adiposity.

Of note, further studies to determine the extent to which multilinear regression approaches for predictive fatty acid modelling are fish species-specific are now underway. The number of marine fish species included in the meta-analysis is still limited (gilthead sea bream, European sea bass, turbot and sole (*Solea senegalensis*)), but interestingly available results highly support a generic approach, especially for OA and C_{18} PUFA. Regardless of fish species the fillet content of n-3 LC-PUFA is also a highly predictable value in a wide dynamic range of dietary fatty composition (0.9–4 % n-3 LC-PUFA), although the ratio EPA:DPA:DHA is fish species-dependent due to the differences in elongation abilities from EPA to DPA (Fig. 4.4).

Guidance on Safe Fatty Acid Levels in Fish

Although a number of studies have been undertaken to compare sensory and nutritional aspects of farmed fish species with their wild counterparts, developed tools ensuring the nutritional value of fillet are a truly objective criterion that should be enforced irrespective of farming conditions. Within this context, the European Food Safety Authority (EFSA) reported that consumption of farmed fish twice a week, as rich source of EPA and DHA, can help to maintain cardiovascular health (www.efsa.europa.eu). In particular, for gilthead sea bream and European sea bass, the report assumes that EPA and DHA content is 1,200 mg per 100 g edible fillet, and thus the consumption of two 150 g portions slightly

exceeds the EFSA recommended weekly intake (3,000 mg) for EPA and DHA. However, even for fat fish (8–10 % lipids), the fillet content of n-3 LC-PUFA in fish grown-out with high levels of vegetable oils is far from providing 1,500 mg per 150 g portion, and thus more than two fish ration portions will be needed to meet the EFA requirements as shown in Fig. 4.4. Of note, from the plotted graph a fillet transfer efficiency of n-3 LC-PUFA of 16–21 % from a fillet yield of 40–45 % and a feed gain conversion ratio (feed/weight gain) of 1.2–1.3 also become evident. The input/output ratio of n-3 LC-PUFA for salmonids, and rainbow trout in particular, is of the same order of magnitude in fish fed fish oil-enriched diets, although the total replacement of fish oil with vegetable oils is an easily implemented tool to transform fish with LC-PUFA biosynthetic capabilities from a consumer into a net producer of health promoting n-3 LC-PUFA [50]. However, in that case, care must be taken on the absolute levels of n-3 LC-PUFA, because the derived fillet concentration on EPA and DHA (400–600 mg/100 g) is less than what is normally found (1,300–2,000 mg/100 g) in farmed salmonids or wild caught fatty fish (e.g. tuna, mackerel, sardine). Comparisons with other animal vectors are difficult due to differences on fat level, live weight or age at slaughter, but as indicated before, protected dietary lipid sources are required if substantial changes in fatty acid composition on meat and milk ruminant-products are desired [46].

Recommendations

An important challenge is to properly define the nutritional value of seafood products to reinforce the positive image of aquatic products for human health. Certainly, many marine fish species are known to be excellent dietary sources of n-3 LC-PUFA, but given the variations in lipid content of flesh from fatty, medium or lean fish, the total levels of n-3 LC-PUFA, especially EPA and DHA, can vary largely (www.nutraqua.com). In this way, all the initiatives contributing to improve our knowledge and understating on nutrient partitioning and composition charts are welcome in a scenario of global change. Meanwhile, the EPA and DHA sources coming from fisheries are not enough (70 % deficit) to cover the theoretical needs of the human population that are close to 3,000 T/day (Table 4.2). Further efforts are needed, therefore, to find new sources of n-3 LC-PUFA, in particular for intensive rearing of marine carnivorous fish. Whether new replacement sources will be derived from single cell-biomass, novel transgenic plants, krill, mesopelagic fish, fishery by-products or a combination of them is yet to be determined, although it will ultimately depend on scientific development, social acceptance, and governmental policy. However, in almost all the possible scenarios, dietary sources of n-3 LC-PUFA for fish feeds will be a limiting factor, and new efforts should be made for ensuring the healthful benefits of consuming farmed fish as the most important source of n-3 LC-PUFA in the human diet. Within this context, predictive regression approaches are highly promising tools to manipulate fillet fatty acid composition of marine farmed fish as an important source of n-3 LC-PUFA

Table 4.2 Global sources of EPA and DHA from fisheries

Human requirements for EPA + DHA	
Per individual	500 mg/day
World population requirement (six billions)	3,000 T/day
Annual need	1,095,000 T/year
Availability	
Global fisheries	100,000,000 T/year
Edible matter (50 %)	50,000,000 T/year
Fat content (5 %)	2,500,000 T/year
EPA + DHA	375,000 T/year
Deficit	>700,000 T/year

Source: Sadasivam Kaushik, INRA Saint-Pée-Sur-Nivelle (France)

in the human diet. Besides, given the close association between fish size and fillet adiposity, the information derived from multivariate regression approaches can be used to define for each fish species the most adequate marketable fish size. Alternatively, the salmonid industry that currently exploits over 50 % of global fish oil supplies could be transformed into a net producer of health promoting n-3 LC-PUFA from C_{18} PUFA of vegetable oils. On the other hand, we will be prone to consider fish as a whole nutritional package, in which n-3 LC-PUFA can interact with other nutrients, including trace elements, vitamins and amino acids. This opens new research opportunities that can modify the currently nutrient requirements for a given group of population (e.g., children, pregnant women, elderly people), which may be of special relevance in a global context of human food fortification due to the plasticity of cultured fish in lipid content and fatty acid composition.

Conclusion

Health benefits of fish consumption become generally well-documented, especially in relation to risk of dyslipidemia and cardiovascular diseases. Historically, attention has been focused on n-3 LC-PUFA because these fatty acids are almost exclusively found in seafood products, although it is likely that consumption of whole fish would have greater benefits than fish oil supplements. This reinforces the interest for fillet fatty acid kinetics and predictive modelling of fillet fatty acid composition by means of regression multivariate approaches. The proposed models fit well for gilthead sea bream and for other main species of the European aquaculture, helping to face the nutritional human recommendations and the concomitant policies advising the sustainable utilization of marine fisheries resources as feed ingredients for carnivorous fish. Of note, updated information on feedstuffs biocontaminants does not represent a serious concern for the future of aquaculture, and farmed fish today can be considered healthy and safe products for human consumers, although a major constrain is to find new sources of n-3 LC-PUFA to assure the health benefits of eating fish in the future.

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Chapter 5
Meat and Meat Products Enriched with n-3 Fatty Acids

María Teresa Díaz Díaz-Chirón, Ana Rivas-Cañedo, Jesús De La Fuente Vázquez, Concepción Pérez Marcos, and Sara Lauzurica Gómez

Key Points

- Meat and meat products enriched in n-3 PUFA can partially provide the daily recommended intake of n-3 PUFA, especially of long-chain n-3 PUFA.
- The fortification of meats with n-3 PUFA is interesting in ruminant meat, which is regarded as very saturated, and in pork for its high n-6 PUFA levels.
- Plant-derived sources lead to higher α-linolenic contents, whereas marine sources provide higher long-chain n-3 PUFA contents.
- The fortification of meat products can be achieved not only by dietary supplementation but also by totally or partially replacing the animal fat by other fat sources.
- Lipid oxidation is likely the main detrimental effect of the n-3 PUFA enrichment of meat and meat products; however, the use of antioxidants delays effectively lipid oxidation.

Keywords n-3 PUFA • α-Linolenic acid • Eicosapentaenoic acid • Docosahexaenoic acid • Meat • Meat products • Nutritional value

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V.R. Preedy et al. (eds.), *Handbook of Food Fortification and Health: From Concepts to Public Health Applications Volume 2*, Nutrition and Health, DOI 10.1007/978-1-4614-7110-3_5,

Abbreviations

AI	Adequate intake
BHA	Butylhydroxy anisole
BHT	Butylhydroxy toluene
C14:0	Myristic acid
C16:0	Palmitic acid
C18:0	Stearic acid
C18:1	Oleic acid
C18:2n-6	Linoleic acid
C18:3n-3	Linolenic acid
C20:5n-3	Eicosapentaenoic acid
C20:6n-3	Docosahexaenoic acid
COP	Cholesterol oxidation product
DHA	Docosahexaenoic acid
DM	Dry matter
DPA	Docosapentaenoic acid
DRI	Daily recommended intake
EL	Extruded linseed
EP	Extensive-pasture system
EPA	Eicosapentaenoic acid
EFSA	European Food Safety Authority
FAO/WHO	Food and Agriculture Organization of the United Nations/World Health Organization
IC	Intensive-concentrate system
INA	Information not available
MUFA	Monounsaturated fatty acid
n-3	Omega-3 fatty acid
n-6	Omega-6 fatty acid
PLS	Protected linseed and soybean
PSM	Protected sunflower meal
PR	Partially replaced
PUFA	Polyunsaturated fatty acid
SFA	Saturated fatty acid
TR	Totally replaced

Introduction

Meat is considered to be a good source of protein with high biological value as well as of micronutrients such as minerals (iron, zinc, selenium, …) and vitamins (B_6, B_{12}, A, D, …) with a high degree of bioavailability. These micronutrients are either not present in plant-derived food or have poor bioavailability. Consequently, consuming moderate amounts of lean meat as part of a balanced diet makes a valuable contribution to the intake of essential nutrients [1]. However, some constituents of meat, especially in red meat and meat products, have been proposed to be responsible for the development of cardiovascular disease and colon cancer. These elements include the fat content and the fatty acid composition. Meat and meat products are generally classified as “high-fat products”, although the various products available differ markedly in terms of their total fat content.

Meat is generally considered to have a fat content in the range 1–20 %, depending on the retail cut and the amount of fat trimmed. Both the fat content and fatty acid composition of meat are influenced by factors such as species, breed, sex, age/weight and diet [2]. Figure 5.1 shows the fat content and fatty acid composition of some meats and meat products obtained from different species. The fat content is usually higher in processed meat products (5–40 %), where large amounts of fatty tissue are used.

In relation to the nutritional value of meat fat, amongst 35–50 % of the fatty acids are saturated, with the major saturated fatty acids (SFA) being palmitic (C16:0) and stearic (C18:0) acids. Consumption of SFA, myristic acid (C14:0) and C16:0 but not C18:0, has been associated with increased plasma cholesterol and low-density lipoprotein levels, which have been linked to an increased risk of coronary heart disease [3]. Meat contains around 30–50 % of monounsaturated fatty acids (MUFA), with oleic acid (C18:1) being both the main MUFA and the fatty acid most frequently found in meat. Oleic acid is considered hypolipidaemic as it reduces cholesterol and triglycerides in plasma [4]. The proportion of polyunsaturated fatty acids (PUFA) in meat varies in the range 7–30 %. The PUFA, linoleic (C18:2n-6) and α-linolenic (C18:3n-3) acids are regarded as essential fatty acids for humans, and n-3 PUFA are considered relevant for normal growth and development, besides delaying the development of chronic diseases including cardiovascular disease, hypertension and diabetes [5]. The beneficial health effects of n-3 PUFA are mainly attributed to long-chain n-3 PUFA such as eicosapentaenoic acid (C20:5n-3, EPA) and docosahexaenoic acid (C22:6n-3, DHA). Indeed, numerous studies have demonstrated the beneficial effects of n-3 long-chain PUFA on recognized cardiovascular risk factors, such as a reduction of plasma triacylglycerol concentrations, platelet aggregation and blood pressure [4].

The daily intake of long-chain PUFA varies from country to country. Thus, the dietary intake of EPA + DHA in Europe is around 30–420 mg/day [4], whereas in countries with high fish consumption, such as Japan, the dietary intake is approximately 1,600 mg/day [6].

Since changing the eating habits of consumers is likely to be difficult, supplementing products that are already accepted with n-3 PUFA would appear to be a more successful strategy for improving the nutritional quality of food [7]. Modifying the fatty acid composition of animal products, such as meat and meat products, by increasing the n-3 PUFA content has been suggested [8, 9] as a good means of improving their dietary value. Likewise, supplementation of animal diets with n-3 rich sources has been shown to be an efficient method for increasing the n-3 PUFA content in animal muscles [8]. In meat products, the utilization of enriched meat or the inclusion of n-3 PUFA sources during the processing of meat products are the most common ways of improving their nutritional value.

Enhancing the n-3 PUFA Composition of Meat by Dietary Supplementation

Many authors have studied different ways of changing the fatty acid composition of meat, mainly increasing the proportion of n-3 PUFA, by dietary manipulation. Most of these methods have involved the addition of vegetables sources, particularly oil seeds, which are usually a good source of α-linolenic acid. However, the conversion of α-linolenic acid to its longer chain metabolites EPA and docosapentaenoic acid (DPA) seems to be limited, thus resulting in only a small increase in the deposition of EPA and DPA in muscle. Marine products such as fishmeal, fish oil and microalgae have therefore been used to supplement animal diets since they seem to stimulate deposition of both EPA and DHA to a greater extent [10]. In ruminants, dietary fats are hydrogenated in the rumen before intestinal absorption thus meaning that muscle fatty acids in ruminants are more saturated than those in non-ruminants.

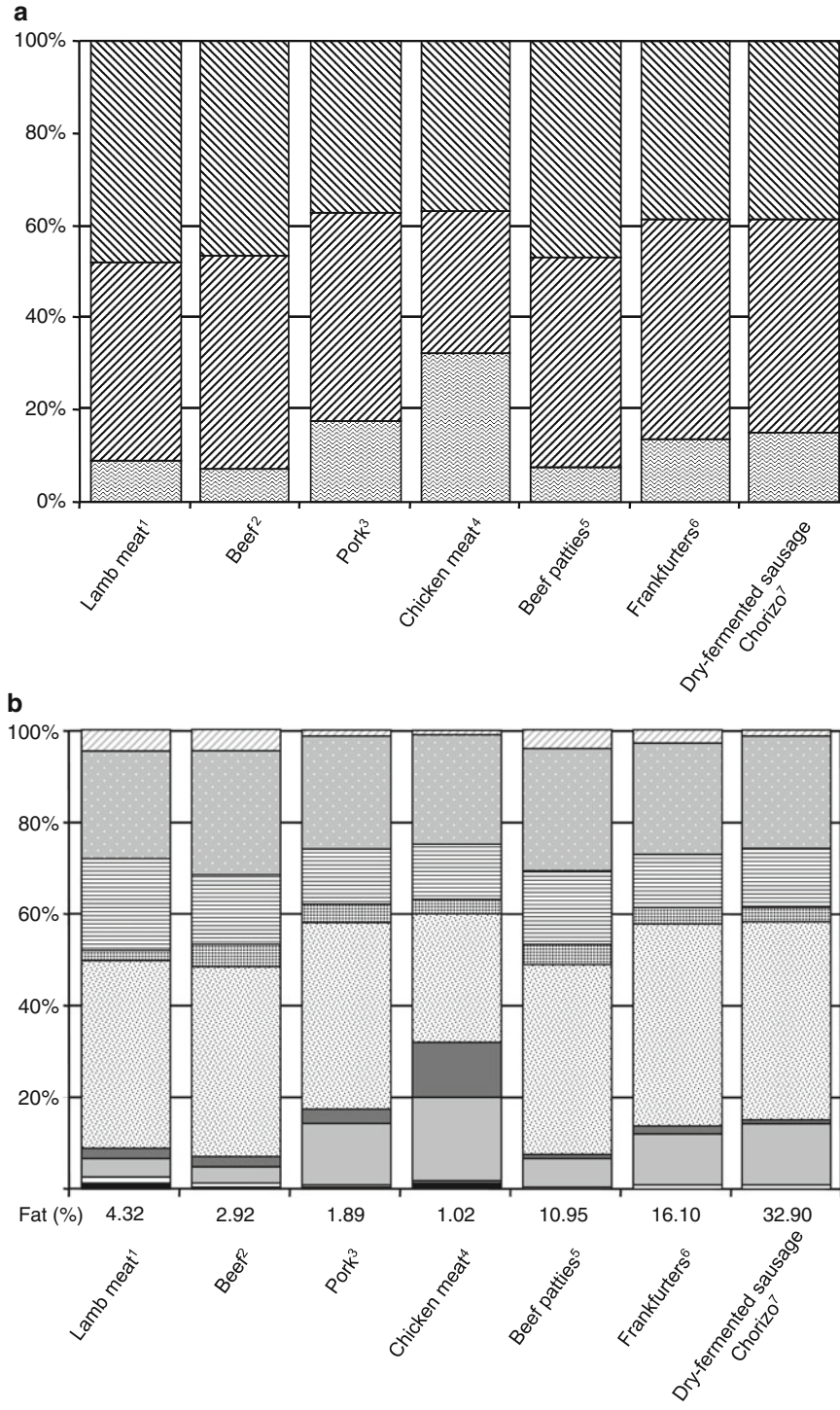

Fig. 5.1 Total fatty acid composition (**a**) and main fatty acid contents (**b**) of meats and meat products. *Legend*: *Bars* represent the contents (expressed as g/100 g of fatty acids) of: ▨ SFA, ▨ MUFA, ▨ PUFA, ▨ minor SFA, ▨ C16:0, ▨ C18:0, ▨ minor MUFA, ▨ C18:1, ▨ minor PUFA, □ C18:2n-6, □ C18:3n-3, ■ Long-chain n-3 PUFA. [1]Lamb and [2]Cattle raised at pasture and supplemented with concentrate; [3]Fattening pigs feeding *ad libitum*; [4]Cereal-based feed. Adapted from the following sources: [1][2], [2][13], [3][41], [4][18], [5][9], [6][42], [7][23]

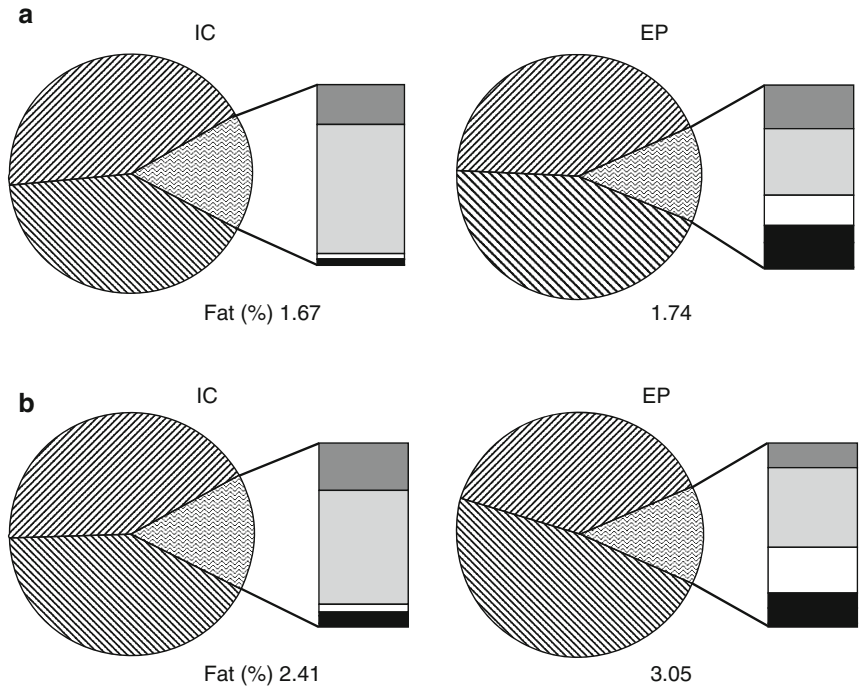

Fig. 5.2 Fatty acid composition in beef[1] (**a**) and lamb[2] (**b**) reared under intensive-concentrate (IC) or extensive-pasture (EP) systems. *Legend*: *Sectors* and *bars* represent the contents (expressed as mg/100 g of muscle) of: ▧ SFA, ▨ MUFA, ▩ PUFA, ▩ minor PUFA, □ C18:2n-6, □ C18:3n-3, ■ Long-chain n-3 PUFA. Adapted from the following sources: [1][13], [2][2]

Ruminant Meat

There is a marked difference between the dietary fatty acid composition and the absorbed fatty acids in ruminants. As noted above, unsaturated fatty acids are extensively metabolized in the rumen in successive steps. The first step in this process is the lipolysis of the dietary lipids, which releases free fatty acids that are subsequently isomerized and hydrogenated by bacterial enzymes [11]. De novo fatty acid synthesis by bacteria and protozoa in the rumen has also been described [11].

Different nutritional approaches to modifying the fatty acid composition of ruminant meat have been widely studied. The inclusion of forage in the diet of ruminants, for example, enhances the n-3 PUFA content as green forage is a good source of α-linolenic acid [12] with a linear increase in the proportion of C18:3n-3 in pasture-fed animals with increasing grazing periods being observed [12]. These authors also suggested a relationship between the duration of feeding on a diet rich in C18:3n-3 and the concentration of long-chain n-3 PUFA in muscle and adipose tissue.

Differences in the fatty acid composition of meat from animals reared predominantly on grain- and grass-based diets have been widely reported (Fig. 5.2). Thus, cattle grazing on pasture accumulated a four to five fold higher concentration of total n-3 PUFA (approximately 34.3 mg of long-chain PUFA/100 g of muscle) than those fed only on concentrate (approximately 7 mg of long-chain n-3 PUFA/100 g of muscle) [13]. In lambs, it was found that the muscle of lambs fed grass on an extensive system had 3 times more total n-3 PUFA, mainly C18:3n-3, than those reared intensively on concentrates [2]. However, although the long-chain n-3 PUFA content was twice as high in lambs reared on extensive-pasture compared to those reared on an intensive-concentrate system, this content was nevertheless low, at only 40 mg/100 g of muscle for the extensive-pasture animals [2]. Another way for increasing the n-3 PUFA content in ruminant meat is to include supplementary lipids in the animals' diet (Table 5.1). Thus, plant-based sources such as different oils and oilseeds, which mainly

Table 5.1 Literature examples of the use of different sources rich in n-3 PUFA in the diet of ruminants

			Increment with respect to control					
Species	Sources	Levels in diet	C18:3n-3	C20:5n-3 (EPA)	C22:5n-3 (DPA)	C22:6n-3 (DHA)	Total n-3 PUFA	Reference
Beef	Linseed	15.4 %	1.60-fold	1.14-fold	1.07-fold	1.31-fold	1.24-fold	[43][a]
	Linseed + fish oil	7.7 + 2.6 %	1.39-fold	1.39-fold	1.12-fold	2.57-fold	1.39-fold	
	Protected fish oil	6.9 %	1.33-fold[b,c]	2.45-fold[d,c]	INA	2.08-fold[d,e]	0.92-fold[d,c]	[44]
		13.8 %	1.21-fold[b,c]	2.57-fold[d,c]	INA	1.63-fold[d,e]	1.66-fold[d,c]	
		27.5 %	1.74-fold[b,c]	3.77-fold[d,c]	INA	1.60-fold[d,e]	2.67-fold[d,c]	
	Whole linseed	21.3 %	1.95-fold	1.45-fold	1.05-fold[f]	1.09-fold[f]	1.49-fold	[34]
	Fish oil	5.4 %	1.18-fold[f]	2.09-fold	1.20-fold[f]	2.09-fold	1.41-fold	
	Linseed + fish oil	10.6 + 2.7 %	1.36-fold[f]	1.36-fold[f]	1.05-fold[f]	2.23-fold	1.28-fold	
	Linseed	21.3 %	1.94-fold	1.50-fold	1.05-fold[f]	1.08-fold	1.85-fold	[14]
	Fish oil	5.4 %	1.38-fold	2.40-fold	1.26-fold[f]	2.12-fold	2.38-fold	
	Linseed + fish oil	10.6 + 2.7 %	1.44-fold	1.60-fold	1.05-fold[f]	2.20-fold	1.78-fold	
Lamb	Extruded linseed	3 % dry matter (DM)	1.98-fold	0.93-fold[f]	0.90-fold[f]	0.83-fold[f]	1.43-fold	[45][g]
		6 % DM	2.30-fold	1.06-fold[f]	0.90-fold[f]	0.83-fold[f]	1.64-fold	
		9 % DM	3.45-fold	1.30-fold[f]	1.13-fold[f]	1.00-fold[f]	2.32-fold	
	Linseed oil	4.3 %	91.80	24.30	22.80	7.30	142	[46][h]
	Fish oil	4.3 %	57.42	47.92	32.45	22.73	132	
	Protected linseed and soybean (PLS)	11.1 %	140.70	20.96	24.75	5.42	189	
	Fish oil + algae	2.1 + 15.5 %	30.86	85.26	45.26	93.80	254	
	PLS + algae	5.5 + 15.5 %	98.31	44.73	27.26	86.55	257	
	Extruded linseed (EL)	12.5 %	6.36-fold[d]	2.75-fold[d]	1.60-fold[d]	2.10-fold[d]	3.23-fold[i]	[8][j]
	Fish oil	3.3 %	0.47-fold[d,f]	7.06-fold[d]	1.74-fold[d]	6.34-fold[d]	5.09-fold[i]	
	EL + microalgae	10.7 + 4 %	4.74-fold[d]	2.45-fold[d]	1.39-fold[d]	2.44-fold[d]	2.76-fold[i]	
	Fish meal	9 % DM	0.71-fold	1.12-fold[f]	0.98-fold[f]	2.02-fold	0.87-fold[f]	[47][a]
	Fish oil	1.5 % DM	1.03-fold[f]	2.62-fold	1.23-fold	3.29-fold	1.42-fold	
	Protected sunflower meal (PSM)	10.5 % DM	0.68-fold	0.98-fold[f]	0.98-fold[f]	0.87-fold[f]	0.80-fold	
	Fish oil + PSM	1.5 + 9.02 % DM	0.74-fold	2.28-fold	1.13-fold[f]	3.11-fold	1.32-fold	

INA information not available
[a]Data referred to the analysis of phospholipids
[b]Data referred to the analysis of neutral lipid fraction
[c]Data are adjusted to a linear effect
[d]Data referred to the analysis of polar lipid fraction
[e]Data are adjusted to a quadratic effect
[f]Improvement statistically not significant
[g]Data referred to male lambs
[h]Data calculated from the sum of neutral and phospholipid fatty acids, expressed as mg/100 g of muscle. No control diet
[i]Data referred to total intramuscular lipids
[j]Mean values of days 0 and 7

provide α-linolenic acid, and marine products, which are major sources of EPA and DHA, have been added to the diets of ruminants. Thus, supplementation of ruminant diets with sources rich in n-3 PUFA produces an increase of 1.5–5 times more total n-3 PUFA in meat, linseed supplementation increases the C18:3n-3 content, whereas marine sources (algae and/or fish) supplementation leads to higher levels of EPA and DHA.

Supplemented steers with linseed or fish oil contained 82.4 and 77.6 mg of n-3 PUFA/100 g of meat and 18.4 and 27.6 mg long-chain n-3 PUFA/100 g of meat, respectively, whereas the corresponding values for non-supplemented steers were 55.2 and 13.2 mg/100 g of meat for n-3 PUFA and long-chain n-3 PUFA, respectively [14]. These contents represented the 3.7 and 5.5 % of the daily recommended intake (DRI) for long-chain n-3 PUFA for linseed and fish oil supplemented steers, respectively, whereas the values for control steers represented only 2.6 % of the DRI for these fatty acids.

In lambs, it has been reported that meat from animals fed on a non-n-3 enriched diet contained 38 mg of n-3 PUFA and 25 mg of long-chain n-3 PUFA/100 g of muscle [8], which represents only about 5 % of the DRI for long-chain n-3 PUFA (500 mg/day, according to [15]). However, when fish oil was added to the lambs' diet, values of 183 and 170 mg of n-3 PUFA and long-chain n-3 PUFA/100 g of muscle, respectively, were obtained. These latter values represented around 34 % of the DRI for long-chain n-3 PUFA. In the diets supplemented with linseed and microalgae, values of 99 and 116 mg of n-3 PUFA and 48 and 50 mg of long-chain n-3 PUFA/100 g of muscle were obtained, respectively, which represent nearly 10 % of the DRI for long-chain n-3 PUFA [8].

Non-Ruminant Meat

The fatty acid composition of muscle and fat tissues in non-ruminants can be significantly modified by incorporating the appropriate oil source in the feed, as dietary fatty acids are absorbed intact in the small intestine and then incorporated into tissue lipids in these species.

Various nutritional strategies for modifying the fatty acid composition of pork and poultry meat have been studied extensively. Pigs fed with fresh grass and herbs rather than with a more conventional feeding regime have a higher PUFA content in muscle and fat, including total n-3 PUFA and mainly C18:3n-3 [16].

In free-range broilers, it has been reported that the restriction on cereal-based feed intake produced an increment in pasture intake that raised 1.4 times more total n-3 PUFA in breast meat in comparison with meat from broilers that did not have any access to pasture or when feed was restricted [17].

Several studies involving the rearing of pigs and poultry on a diet rich in n-3 PUFA have been carried out, and the results have shown their subsequent incorporation into meat lipids (Table 5.2). Vegetable sources (linseed, flaxseed) and their oils mainly contain C18:3n-3. Although animals are able to synthesize long-chain PUFA from C18:3n-3, the literature shows that diets incorporating marine sources (algae and/or fish) result in significantly higher deposition of EPA and DHA than diets containing vegetable sources. The increased n-3 PUFA content in pork mainly arises upon supplementation with fish oil (Table 5.2). However, supplementation with linseed did not produce as marked an increase in n-3 PUFA content as that achieved with fish oil. Thus, [18] the meat from pigs fed a control diet contained 19.0 mg of n-3 PUFA and 11.5 mg of long-chain PUFA/100 g of muscle, representing only 2.3 % of the DRI for long-chain n-3 PUFA [16]. This content was doubled when crushed linseed was added to the animal diet (41 mg of n-3 PUFA and 22 mg of long-chain PUFA/100 g of muscle). However, when fish oil was supplemented to pigs' diet, 66 and 58 mg of n-3 PUFA and long-chain n-3 PUFA, respectively, were found per 100 g of muscle, thus representing 11.5 % of the DRI for long-chain n-3 PUFA.

In poultry, similarly to cattle, lamb and pig, the highest increment in C18:3n-3 content was achieved through linseed supplementation, whereas the highest increase in long-chain PUFA was obtained upon fish oil supplementation [11] (Table 5.2). However, due to the low amount of adipose tissue

Table 5.2 Literature examples of the use of different sources rich in n-3 PUFA in the diet of non-ruminants

Species	Sources	Levels in diet (%)	Increment with respect control					Reference
			C18:3n-3	C20:5n-3 (EPA)	C220:5n-3 (DPA)	C220:6n-3 (DHA)	Total n-3 PUFA	
Pig	Tuna oil	2	1.22-fold[a]	0–0.27[b]	INA	41.2-fold	2.64-fold	[48]
	Crushed linseed	3	2.25-fold	2.45-fold	1.60-fold	1.29-fold[a]	1.97-fold	[18]
	Fish oil	6	0.85-fold[a]	6.23-fold	1.74-fold	7.29-fold	4.64-fold	
	Extruded linseed	5	3.08-fold	3.08-fold	1.69-fold	2.00-fold	2.17-fold	[42]
	Marine algae	0.25[c]	0.92-fold[a]	1.04-fold[a]	1.20-fold[a]	3.43-fold	1.08-fold[a]	[49]
		0.25[d]	1.09-fold[a]	0.82-fold[a]	0.85-fold[a]	2.14-fold	0.93-fold[a]	
		0.5[d]	1.01-fold[a]	0.89-fold[a]	1.05-fold[a]	3.29-fold	0.97-fold[a]	
Poultry	Tallow	10	0.94-fold[a]	0.57-fold[a]	2.10-fold	2.40-fold	1.16-fold[a]	[50]
	Olive oil	10	0.83-fold[a]	0.57-fold[a]	0.86-fold[a]	1.45-fold[a]	0.85-fold[a]	
	Sunflower oil	10	0.67-fold[a]	0-fold	0-fold	1.64-fold[a]	0.45-fold	
	Linseed oil	10	29.20-fold	17.00-fold	3.55-fold	3.55-fold	24.50-fold	
	Fish oil	1	0.44-fold[a]	5.60-fold	0.66-fold	4.40-fold	3.27-fold	[51]
		2	1.36-fold	8.20-fold	1.30-fold	15.93-fold	4.29-fold	
		3	1.51-fold	10.13-fold	1.90-fold	25.30-fold	5.28-fold	
	Linseed oil	2	5.83-fold	1.10-fold[a]	1.42-fold	1.70-fold	4.52-fold	[52]
		4	8.55-fold	1.95-fold	2.00-fold	2.50-fold	7.19-fold	
	Soybean bean oil	3	3.65-fold	2.00-fold[a]	2.60-fold	2.20-fold[a]	3.25-fold	[19]
	Linseed oil	3	23.70-fold	14.30-fold	8.40-fold	5.20-fold	19.70-fold	
	Fish oil	3	1.65-fold[a]	85.00-fold	20.40-fold	41.20-fold	10.20-fold	
Duck	Dried microalgae (rich in DHA)	0.5	INA	INA	INA	2.84-fold	1.86-fold	[53]

INA information not available
[a]Improvement statistically not significant
[b]Non-detected values in control
[c]Dietary supplementation during finishing period
[d]Dietary supplementation during last half of finishing period

associated with poultry meat, the influence of dietary lipids on muscular fatty acid composition is especially important [11]. Indeed, C18:3n-3 increases between 5 and 30-fold have been achieved upon linseed oil supplementation (Table 5.2), and C22:6n-3 increases of between 4 and 41-fold have been obtained upon fish oil supplementation.

The reported findings [19] show values of 68 mg of n-3 PUFA and 13 mg of long-chain PUFA/100 g of chicken meat when the birds were fed a palm fat diet, and 221 and 30 mg of n-3 PUFA and long-chain PUFA, respectively, per 100 g of chicken meat when the birds were fed a soybean oil enriched diet. Similarly, meat from birds fed with a linseed oil enriched diet contained 1,339 mg and 111 mg of n-3 PUFA and long-chain PUFA, respectively, and that from those fed with a fish oil enriched diet contained 695 and 611 mg of n-3 PUFA and long-chain PUFA, respectively, per 100 g of chicken meat. These long-chain PUFA contents for palm fat, soybean oil, linseed oil and fish oil diets represent 2.6 %, 6 %, 22 % and 122 %, respectively, of the DRI for long-chain n-3 PUFA.

Enhancing the n-3 PUFA Composition of Meat Products by Technological Processes

The link between the consumption of some meat constituents, such as saturated fats, and the risk of several diseases, together with the consumption of convenience meat products, which are considered

Table 5.3 Literature examples of the improvement of the fatty acid profile of meat products by using raw materials from animal production practices

			Improvement with respect to control					
Meat product	Source	Levels in diet	C18:3n-3	C20:5n-3 (EPA)	C22:5n-3 (DPA)	C22:6n-3 (DHA)	Total n-3 PUFA	Reference
Dry-fermented sausage "Salami"	Rice bran	38 % (w)	2.51-fold	1.28-fold[a]	0.86-fold[a]	0.96-fold[a]	2.27-fold	[23]
Dry-cured ham[b]	Linseed oil	3 %	5.59-fold	3.15-fold	1.12-fold[a]	0.78-fold[a]	3.17-fold	[54]
	Linseed + olive oils (1/1, w/w)	3 %	4.17-fold	1.96-fold[a]	1.04-fold[a]	0.81-fold[a]	2.36-fold	
Dry-fermented sausage "Salchichón"	Linseed oil	3 %	7.81-fold	2.00-fold	1.75-fold	0.98-fold[a]	5.38-fold	[24]
	Linseed + olive oils (1/1, w/w)	3 %	6.78-fold	2.00-fold	1.50-fold	0.86-fold[a]	4.68-fold	
Chicken frankfurters	Fish oil	2 %	0.86-fold[a]	3.73-fold[a]	INA	1.61-fold[a]	1.33-fold	[25]
		4 %	0.81-fold[a]	7.54-fold	INA	2.47-fold	1.88-fold	

INA information not available
[a]Improvement statistically not significant
[b]Data from analysis performed on *Biceps femoris*

to be one of the leading sources of fat, in developed countries makes the modification of the fatty acid profile of these products especially interesting. Two main strategies are commonly used to improve the nutritional value of the fatty acid profile of meat products. The first involves the use of raw materials (meat and/or fat) from animals fed on n-3 enriched diets to manufacture different meat products (Table 5.3). Dietary supplementation and the sources commonly used for that purpose have been discussed above. The second strategy involves increasing the n-3 fatty acid content by modifying the lipid fraction during the formulation of meat products, as shown in Table 5.4. Thus, the animal fat is partially or totally replaced with another fat source with a healthier fatty acid composition. Vegetable oils, plants rich in n-3 fatty acids (corn, palm, peanut, walnuts, soybean, high-oleic acid sunflower, linseed, olive, etc.), and marine sources (fish oil and algae) have been used as animal fat substitutes [20]. The type of fat substitute greatly influences the fatty acid profile of the resulting product. Thus, the partial replacement (25 %) of pork backfat with fish oil during the manufacture of Spanish dry-fermented sausage "chorizo" led to a 6,300 and 4,500 % increase in the levels of EPA and DHA, respectively [21], whereas a similar treatment in the same type of product resulted in a 433 and 5,060 % increase in the EPA and DHA contents, respectively, when an algae-derived oil was used [22].

It should be noted that the number of meat products manufactured using partial substitution of animal fat is far greater than those elaborated from raw materials obtained from n-3 supplemented animals. The reason for this is mainly financial. Thus, it must be taken into account that, in supplemented animals, the proportion of fatty acids deposited within the muscle in relation to the ingested amount is generally low. The use of raw materials from supplemented animals is therefore usually limited to the manufacture of meat products made from identifiable pieces of meat, such as dry-cured ham, although some exceptions can be found in the literature [23, 24]. The direct addition of n-3 sources is far more profitable in meat products processed through structural breakdown procedures such as chopping, mincing or any kind of homogenization during their manufacture.

The resulting product may show some differences with respect to the traditional product depending on the fat used, especially as far as the sensory properties are concerned. For example, the use of fish oil might result in the perception of some unpleasant notes, described as "fishy" in the resulting product. Nevertheless, the results in this respect in the literature are diverse. Thus, some authors have found no detrimental effect of fish oil supplementation on the sensory properties of chicken frankfurters

Table 5.4 Literature examples of the improvement of the fatty acid profile of meat products by partial (PR) or total replacement (TR) of animal fat with other fat sources. Reformulation practices

				Improvement with respect to control					
Meat product	Source		Levels	C18:3n-3	C20:5n-3 (EPA)	C22:5n-3 (DPA)	C22:6n-3 (DHA)	Total n-3 PUFA	Reference
Low-salt (0.5 %), low-fat (10 %) cooked beef patties[a]	Olive oil emulsion		5 % (PR)	1.58-fold	1.23-fold[b]	1.00-fold[b]	0[c]	1.42-fold	[9]
			10 % (TR)	1.77-fold	0.89-fold[b]	0.87-fold[b]	0–2.44[c]	1.55-fold	
	Wakame (*Undaria pinnatifida*) seaweed		3.39 %	1.28-fold[b]	2.64-fold	0.86-fold[b]	0[c]	1.25-fold	
Dry-fermented sausage "Chorizo"	Pre-emulsified fish oil		6.25 %	1.1-fold	64.0-fold	INA	46.0-fold	INA	[21]
	Algae oil from *Schizochytrium* sp.		3.75 %	0.90-fold	4.33-fold	1.14-fold	39.20-fold	3.65-fold	[22]
			6.25 %	0.90-fold	5.33-fold	1.09-fold	51.60-fold	4.54-fold	
Frankfurters	Low-fat (6 %)	Walnut	25 %	11.81-fold	INA	INA	0.71-fold[b]	12.82-fold	[42]
	Normal-fat (16 %)			13.34-fold	INA	INA	0.83-fold[b]	12.68-fold	
Bologna-type sausages "mortadella"	Low-fat (10 %)	Fish oil	1, 2, 3, 4 and 6 %	INA	33–220-fold	INA	1.56–5.11-fold	3.13–9.13-fold	[26]
	Normal-fat (30 %)			INA	35–330-fold	INA	4.50–20.00-fold	1.63–5.52-fold	
Low-fat pork liver pâtés	Mixture of oils		7.5 % (PR)	15.89-fold	0–1.22-fold[c]	0–0.24-fold[c]	0–1.00-fold[c]	10.78-fold	[30]
	44.39 % olive oil 37.87 % linseed oil 17.74 % fish oil		15.0 % (TR)	32.35-fold	0–2.69-fold[c]	0–0.46-fold[c]	0–1.80-fold[c]	20.69-fold	

INA information not available
[a]Data from cooked sample
[b]Improvement statistically not significant
[c]Non-detected values in control

[25] whereas other studies have reported some fishy notes in low-fat bologna-type sausages [26] or in dry-fermented sausages formulated with fish oil [21], although the overall acceptability remained unaffected in both cases. Likewise, the use of seaweed as an n-3 source may result in an atypical flavour in the final product depending on both the type of seaweed and dose. The use of walnuts in meat products might also affect the resulting product. Hence, the sensory panel detected a walnut-like flavour after the addition of walnuts to restructured beefsteaks, although they showed better texture than the controls [27]. In any case, the effect of animal fat substitutes on the sensory quality of meat products might be masked or toned down by means of seasonings, when applicable.

The replacement of animal fat can also entail several technological problems. An increase in the PUFA content may lead to "soft" meat and meat products [28]. However, no significant differences with respect to control were observed in salami manufactured from pigs fed on diets composed of maize and rice bran [23] or in chorizo in which 25 % of pork backfat was replaced with fish oil [21]. In fact, the latter study reported better juiciness of the modified sausages. Another possible effect concerns emulsion stability in products such as pâtés. Thus, the substitution of animal fat by another more unsaturated source of fat (with lower melting points than those of SFA) may reduce the emulsion stability of these products [29]. However, this effect was not observed in pork liver pâtés formulated with partial or total replacement of pork backfat by a combination of oils rich in n-3 PUFA [30].

The main problem as far as dry-fermented sausages are concerned is that there appears to be a limit for backfat substitution above which a significant drip fat-loss occurs during ripening, thus leading to a decrease in the MUFA and/or PUFA contents [31].

Problems with n-3 PUFA-Enriched Meat and Meat Products

Modification of the fatty acid profile of meat can entail some negative effects, amongst which lipid oxidation is likely the most relevant. Indeed, lipid oxidation is one of the major degradation processes responsible for losses in food quality. The rate and extent of lipid oxidation in muscle-containing foods is affected by many factors, including exposure to light, oxygen, temperature or the presence of both anti- and pro-oxidants, as well as the lipid content, the degree of unsaturation of the fatty acids and the presence of enzymes in meat [32]. Besides these factors, any processes that cause cell-membrane rupture (such as deboning, mincing or cooking) facilitate the interaction of pro-oxidants with unsaturated fatty acids, thereby resulting in the formation of free radicals and propagation of the oxidative reaction [33].

As mentioned above, the degree of unsaturation of fatty acids greatly influences lipid oxidation with more unsaturated fatty acids being more susceptible to oxidation. Indeed, highly unsaturated fatty acids seem to form free radicals, which then promote the breakdown of other fatty acids [34]. Thus, high levels of fatty acids containing a large number of double bonds (such as DHA which has six) in meat seem to enhance rapid lipid oxidation processes.

Lipid oxidation causes some detrimental effects in terms of the quality of muscle-based foods. Thus, the deterioration of essential fatty acids, together with the loss of nutrients such as the liposoluble vitamins A and E, results in a decrease in the nutritional value of meats and meat products [20]. In addition, various products that may exert different biological effects depending on the conditions of oxidation at the time of eating might be formed. According to some authors [35], lipid oxidation products are cytotoxic and genotoxic.

The oxidation of fats, mainly PUFA, also affects the organoleptic quality of meats and meat products by producing a loss of colour uniformity and unpleasant tastes and odours, amongst other effects. Lipid oxidation is highly correlated with pigment oxidation and hence with the colour of muscle-containing foods [36], as the free radicals formed during lipid oxidation seem to act either directly by promoting the oxidation of meat pigments or indirectly by acting on the reduction systems of the pigments [37].

The oxidative breakdown of lipids during cooking or ripening usually contributes to development of desirable odours in meats and meats products by forming volatile compounds such as aldehydes, ketones and alcohols. However, an increase in the levels of some of these compounds, especially those with low-odour threshold such as aldehydes or ketones, may cause an imbalance in the volatile profile of meats and meat products. An increase in the PUFA content in meats and meat products may result in the development of unpleasant notes in the final product as a consequence of higher levels of lipo-oxidation volatile compounds.

Cholesterol present in meat can also be oxidized by many factors in similar manner to unsaturated fatty acids to produce cholesterol oxides that affect human health. Cholesterol oxidation products (COPs) exhibit specific deleterious effects such as cytotoxicity, mutagenicity, carcinogenicity, atherogenicity, inhibition of sterol biosynthesis and modulation of immune function [38]. The intake of processed foods of animal origin containing high levels of COPs seems to be the greatest source of these products found in the human body [39].

It should be noted that all these adverse effects might, of course, be enhanced under some storage conditions. Hence, storage under appropriate conditions may minimize the harmful effects of lipid oxidation. Moreover, the oxidation of meat and meat products rich in n-3 PUFA can be limited by including of synthetic or natural antioxidants in either the dietary treatment or in the formulation. For example, the antioxidant effects of vitamin E by animal diet [36] or a mixture of butylhydroxy anisole (BHA) and butylhydroxy toluene (BHT) [21] have been successfully proven in n-3 enriched meat and meat products, respectively.

Guidance on Safe Levels

Minor differences in the DRI of n-3 fatty acids amongst countries and/or international health organizations may be found. Previously, health organizations and government agencies used to recommend intakes of 0.6–1 g/day for n-3 PUFA, from which 100–200 mg/day corresponded to long-chain PUFA. These values were then readjusted. For example, Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO) or the American Health Association have suggested adequate intakes (AI) for total n-3 PUFA of 1.5–2.5 g/day and specifically for long-chain n-3 PUFA in the range 140–600 mg/day [40].

In Europe, the European Food Safety Authority (EFSA) has proposed DRI separately for C18:3n-3 and for long-chain n-3 PUFA (EPA and DHA), since the conversion of the former into the latter may be affected by numerous factors. Thus, an AI of 250 mg/day for C18:3n-3 was initially proposed [4], although soon after the recommended level was considered to be too low [15]. The AI for long-chain PUFA has been set on 500 mg/day [15]. It should be noted that all these levels are suggested for healthy individuals, but additional levels are recommended in some particular cases such as pregnancy or lactation.

In the United States, there are no official recommendations but some suggestions based on scientific research have been made. Thus, 1.6 and 1.1 g/day of AI for C18:3n-3 were recommended for males and females, respectively, aged between 31 and 50. Concerning Canada, the DRI has been made for total n-3 PUFA which should be between 1.2 and 1.6 g/day. Scientific research suggests that DRI should be reviewed in order to consider health promotion rather than avoid deficiency symptoms.

Conclusions

Fortification of meats and meat products with n-3 fatty acids is an effective way of assuring the DRI of these fatty acids, especially in western countries, in which changing the eating habits of consumers may be difficult. Pasture rearing systems provide meat with higher contents in C18:3n-3. Nevertheless,

dietary supplementation enhances the nutritional fatty acid profile of meat more efficiently than grazing systems. The inclusion of vegetable sources in the animal's diet leads to an increase in the levels of C18:3n-3, whereas the use of marine sources provides higher contents of both EPA and DHA. Meat products can be enriched by either supplementation or by using healthier fat sources through formulation. Some adverse effects might be observed in fortified meats and meat products, although there are promising technological alternatives to reduce them.

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Chapter 6
Cheese Fortification

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Key Points

- Functional dairy products represent an important market.
- Microencapsulation may be needed for cheese fortification with functional ingredients.
- Emulsions can be used to immobilize lipophilic and hydrophilic active compounds.
- There are at least eight challenges for the addition of probiotics in cheese.
- The best point of addition of probiotics during Cheddar cheese production is in milk prior to renetting.

Keywords Cheese fortification • Probiotic bacteria • Hydrophilic compounds • Hydrophobic compounds • Emulsion • Polymeric complexes

Abbreviations

ME	Microencapsulation
W/O/W	Double emulsions: water/oil/water
WP	Whey protein

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V.R. Preedy et al. (eds.), *Handbook of Food Fortification and Health: From Concepts to Public Health Applications Volume 2*, Nutrition and Health, DOI 10.1007/978-1-4614-7110-3_6,

GA Gum Arabic
CFU Colony forming unit

Introduction

Incorporation of bioactive ingredients in cheese confers several nutritional advantages.

However, cheese fortification should ensure the retention and the protection of the bioactivity of the bioactive ingredients in cheese matrix during the process and storage time.

Strategies to fortify cheese should be developed to incorporate and protect lipophilic, hydrophilic or insoluble ingredients.

Cheese fortification should not compromise the sensorial and the physico-chemical properties of cheese.

Recent research in nutrition demonstrates the physiological effects of food components and extracts, such as lactic acid bacteria and their metabolites, the omega-3 fatty acids, the antioxidants, etc. The popularity of these functional compounds results in a rapidly changing demand from consumers. Functional foods allow consumers to find the desired ingredients in food matrices.

Functional dairy product accounts for 42.9 % of the functional foods market [1]. Dairy products have been the most popular delivery vehicles for a number of functional and healthy ingredients, from vitamin and mineral fortification to addition of bioactives to promote the health benefits. Since dairy products are normal part of our diet, it is easy to understand that vitamins and minerals have been incorporated in these products. Omega-3 fatty acid and chito-oligosacharride fortification in milk are typical example of fortification [2].

According to Donnelli [3], functional cheese is the next goal for the dairy industry, and numerous compounds have been suggested for this purpose (Table 6.1).

The objective of this chapter is to present the challenges associated with cheese fortification with health-promoting ingredients as well as the methods used to address these challenges.

Challenges Linked to Cheese Enrichment

Cheese is a solid milk concentrate that consists principally of protein (primarily caseins) and fat. Cheese curd is composed of insoluble caseins coagulated by enzymatic conversion of κ-casein to para-κ-casein [4]. During the cheese-making process, curd is generally made via the rennet-induced coagulation of milk casein with or without heat, pressure, salt or fermentation treatment with selected

Table 6.1 Bioactive compounds which could be used to fortify cheese and their potential health attributes

Compound	Potential health benefits
Folic acid	Blood metabolism, neural tube development
Lycopene	Prostate cancer, cardiovascular health
Magnesium	Immune system, cardiovascular health
Polyphenols	Antioxidant, antimutagenic and antibacterial properties, reduced coronary disease
Probiotics	Various gastro-intestinal disorders, stimulation of the immune system, blood cholesterol reduction, lower colon cancer incidence
ω-3 and ω-6 oils	Cardiovascular health

microorganisms [5]. Dairy products are recognized for their high nutrient density and are considered privileged vehicles for the transport of active molecules [6].

During cheese making, milk is first coagulated and then phase separation occurs. The contraction of the para-casein network during cooking, draining or pressing steps is responsible for the concentration of caseins and also fat, which is entrapped within the protein matrix. Cheese whey is the aqueous phase released from the curd and contains milk soluble solids, such as lactose, globular serum proteins, soluble minerals and vitamins. Depending on cheese variety, the volume of cheese whey can represent up to 90 % of total milk used for cheese making. In these conditions, the first challenge in cheese fortification is to retain bioactive compounds within the cheese matrix. As an example, when adding milk enzymes to accelerate the ripening of cheese, high losses of enzymes (up to 95 %) are observed in the whey [7]. Immobilization techniques are then required to improve the retention of functional ingredients in cheese. The effectiveness of the integration of bioactive compounds depends on the characteristics of food matrix and compounds to be immobilized. The natural bioactive molecules are either of hydrophilic or hydrophobic nature.

With respect to recovery into cheese matrix, losses in whey are not the only problem encountered. Processes must be adapted to the nature of the compounds, whether hydrophilic, hydrophobic, if they are in the form of suspensions of bacteria or if they are insoluble particles. As a result, the development of techniques to increase the retention of bioactive ingredients in the cheese matrix is a challenge for the cheese-making industry.

In addition to the concern with the recovery of the bioactive compounds in the cheese matrix, three other objectives should be reached: (1) assure that bioactive compounds remain stable and maintain their activity during storage, (2) minimize the effect of bioactive compounds on cheese sensory properties and (3) assure the delivery of the active products to the target site in the gastrointestinal (GI) tract.

Ensuring the bioactivity of the molecules to various stresses associated with the cheese environment is critical. Low stability of bioactive compounds during storage can be attributed to external factors such as pH, oxygen, salt level, enzymes, microorganisms and so on. Consequently, the processes of fortification must also preserve the biological activity during manufacture, ripening and storage of cheese.

The effect of fortification on taste, appearance and texture of products should be negligible. Various supplements can affect flavour. As an example, oils from fish origin (rich in omega-3 fatty acids) and probiotic bacteria are the most noteworthy.

To address the various challenges of cheese fortification, microencapsulation (ME) has often been suggested, and an extensive portion of this chapter will therefore address the benefits of ME. Unfortunately there is no universal ME procedure for the various bioactive ingredients. Therefore three approaches have been used: simple emulsions, multiple emulsions multilayer emulsions and coacervates and gel particles. Emulsions have long been studied for their ability to stabilize the bioactivity of hydrophilic or hydrophobic molecules such as antioxidants and antimicrobials in processed foods. Bioactive compounds can be dissolved in the dispersed phase of emulsions, or adsorbed to the interface. The emulsification process induces deformation, breakage and stabilization of the newly formed oil droplets. The formation of polymeric complexes can also be used to immobilize bioactive compounds, which increases their retention in cheese matrices. Several studies have demonstrated the effectiveness of these complexes to protect the bioactivity and ensure high availability in food product throughout processing, distribution and storage [8–12].

Microencapsulation Methods for Cheese Fortification

The purposes of encapsulating food ingredients (vitamins, minerals, probiotics, functional fatty acids, peptides, enzymes, phytochemicals and others) are to protect the core materials from degradation during processing and storage and to control the release characteristics of the core, namely its delivery

to the desired site in the gastro-intestinal tract. Bioactives molecules such as vitamins, omega-3 fatty acids, carotenes, polyphenols need to be carefully encapsulated prior to their incorporation in food matrices to prevent degradation. Omega-3 fatty acids, for instance, are susceptible to oxidation, resulting in the development of off-flavour. Microencapsulation protects sensitive oils and vitamins from exposure to oxygen, light and metal ions [13] and also minimize the interactions with other food components during processing and storage. Additionally, microencapsulation improves the functionality and release characteristics of bioactive components. It is essential that both the bioactivity and bioavailability are maintained during incorporation of the microcapsules into the food matrices. When the bioactivity of the core materials is threatened by the food environment, appropriate microencapsulation technique is required to protect them. For instance, an appropriate protection against the acidic pH in the stomach during GI transit allows a superior delivery of the bioactive molecules to the target site and improves its health benefits.

Immobilization of Lipophilic Compounds by Simple Emulsion

Oil-in-water emulsions can be used to immobilize lipophilic compounds. Since milk fat globules are naturally retained within cheese matrix, oil-in-water emulsion is an obvious carrier. The emulsion allows a good distribution of active compounds in cheese, getting a better stability during storage and protects the sensory qualities. Lipophilic compounds can be dispersed in butter oil and then homogenized in skim milk to produce a "functional cream", used for cheese milk standardization. Alternatively, vegetable oils, with health-promoting attributes, can be used instead of butter oil. The quality of the emulsion depends on the size of the droplets and its stability [14]. Fat droplet size distribution has been shown to affect fat retention and also the texture of cheese. Average fat droplet size in raw milk is 3.4 μm and the relative width of the distribution (standard deviation in particle size divided by the average diameter) is 0.4 [15]. When a large amount of emulsion is used in cheese milk formulation, it is important to reproduce this size distribution in order to minimize fat losses during cheese making and reduce cheese texture changes. Various parameters such as energy input, temperature and fat volume fraction need to be optimized to produce appropriate emulsions for cheese milk standardization.

For some applications, only a small volume of emulsion might be required to reach the target concentration of bioactive compound in cheese. In these cases, the preparation of a fine emulsion is preferred. A fine emulsion was shown to significantly reduce the detection of oily taste [9]. The application of physical methods such as homogenization, microfiltration, the ultrasonic treatment allows obtaining a better distribution of particles in emulsions and to obtain fine droplets (<1 μm) depending on conditions used. For example, micro-emulsions produced under controlled pH conditions enabled the stabilization of phospholipids to heat [16]. However, several other parameters influence the stability of emulsions and bioactive components and should be optimized (viscosity, interfacial composition, chain length, conformation and molecular weight of surfactants, ionic strength, pH, phase density, droplet size distribution, etc.) [9, 17].

For all food products, choice of the emulsifier is a key to achieving the desired textural and sensory properties. The design of the interface will also play a controlling role in the stabilization and breakdown of emulsion structure. High molecular weight amphipathic biopolymers, such as milk proteins, are very effective at stabilizing o/w-type emulsions. The adsorption of casein and whey protein onto the oil droplets provides effective steric and electrostatic stabilization against coalescence and flocculation. The control of the type and concentration of emulsifiers at the droplet interface is known to affect the oxidation rate. The electrical charge of the interfacial layer is also an important factor. A negatively charged surface will attract positively charged metal ions and bring them close to the lipid, promoting oxidation reactions. On the contrary, a positively charged emulsifier will repel the metal ions from the surface and consequently stabilize the emulsion against the metal-catalyzed lipid oxidation.

Physical stability of the emulsion is an important parameter and depends upon the charge density of the emulsion droplets. Charge density determines the intensity of electrostatic repulsions which reduce collisions causing flocculation or coalescence [18]. Electrically charged droplet surfaces will also impact the ability of the droplet to interact with ions in the aqueous phase of the emulsion. The interactions between the emulsion droplets with aqueous phase ions can impact both the physical and chemical stability (e.g. oxidative rate).

Hu et al. [19] studied the oxidative stability of salmon-in-oil emulsions prepared with different whey proteins (whey protein isolate, a-lactalbumin, b-lactoglobulin). The oxidation of the oil was lowered at pH values below the isoelectric point (PI) of the different proteins due to electrostatic repulsion of the metal ions away from the charged emulsion droplets surface. Additionally, it was noted that at the pH 3.0 the oxidative stability decreased from β-lactaglobulin followed by α-lactabumin and Whey protein isolate. This is an indication that the magnitude of the positively charged droplets was not the only factor affecting the oxidative stability and that other membrane properties such as the thickness of the interfacial membrane can play a role. A thick membrane at the emulsion droplet interface may act as a physical barrier between the water-soluble pro-oxidants and lipids inside the emulsion droplets. For the lipophilic vitamins (e.g. vitamins A, D, E, K) emulsion-based delivery systems are often used [20]. Semo et al. [21] demonstrated that casein micelles were useful for delivery of vitamin D2.

The interfacial engineering of the emulsion has received a great deal of attention over the last few years and the bioavailability of the encapsulated bioactive ingredients remains a major aspect requiring further studies. For instance, for oil-in-water emulsions, the use of multiple layers of biopolymers on the oil droplets can alter the in-vitro digestibility. Multiple shells prepared by cross-linking and the use of slowly or non-digestible encapsulation matrix compounds will affect also the bioavailability. These observations confirm the possibility of target-oriented release of core material in vivo. Multilayered emulsions have the potential to decrease the lipid oxidation rate due to their ability to control droplet charge and membrane thickness, both factors being known to affect metal-induced hydroperoxide production [22]. The oxidative stability of emulsion droplets stabilized by a lecithin/chitosan multilayer was better than that of emulsion droplets formed with lecithin only. The difference was explained by the cationic nature of the droplets and the thicker interfacial region.

Immobilization of Hydrophilic Compounds in Double Emulsion

Being soluble in water, hydrophilic compounds are poorly retained in the cheese matrix and losses in cheese whey can be as high as 95 %. Furthermore, the "contamination" of cheese whey could reduce the quality of products derived from whey, such as protein concentrates or isolates. In order to increase the retention of hydrophilic compounds in cheese, various strategies were implemented. The concept of double emulsions (e.g. W/O/W) is relatively new and has been studied for immobilization of hydrophilic compounds. These types of emulsion allow the imprisonment of micro droplets of an aqueous suspension inside fat droplets. They were developed for pharmaceutical as well as nutraceuticals applications [23]. Double emulsions have been developed to stabilize proteins during heat treatment, in presence of salt, and in severe pH conditions [24]. Multiple emulsions were also used for the protection of bioactive substances during the GI transit (protection against gastric acid pH and the presence of pancreatic enzymes) [9, 23]. Double emulsions are versatile vehicle for the transport of active molecules. The water-soluble compounds are dispersed in the internal aqueous phase, while the fat-soluble compounds are incorporated into the lipid phase. Several bioactive compounds such as phytosterols, phospholipids and polyunsaturated fatty acids are surface active. They are stored at the interfaces and play an active role in ensuring the stability of double emulsions [25].

Double emulsions are produced according to a two step process. First, the aqueous dispersion containing the hydrophilic compounds is finely dispersed in oil in order to produce a primary

water-in-oil emulsion. To obtain a stable and finely dispersed emulsion, lipohilic emulsifier such as polyglycerol polyricinoleate must be added to the oil phase [26]. In the second step, the primary emulsion is dispersed in the outer aqueous phase, which contains hydrophilic emulsifier. The use of skim milk as the emulsion outer phase is most appropriate since milk proteins have excellent emulsifying properties and the whole composition of the emulsion will closely match that of cheese milk. In order to improve the stability of double emulsions, it is important to eliminate osmotic pressure gradient between inner and outer aqueous phases. It is common practice to add lactose to the inner aqueous phase when skim milk is used as the aqueous outer phase. Energy input is an important factor in the preparation of multiple emulsions. High pressure homogenization can be used for the preparation of the primary emulsion in order to produce fine droplets. However, for the second homogenization step, energy input should be reduced to prevent emulsion disruption. Low shear conditions were shown to produce double emulsions with good entrapment yield, but in such conditions large droplets are produced (>20 μm). When added to cheese milk, these large droplets are poorly retained in cheese matrix and lost in cheese whey. Reducing the size of emulsion droplets improves retention in cheese but reduce entrapment yield. Shearing conditions in the second step of double emulsions should then be chosen to reflect the compromise between retention in cheese and entrapment yield.

Fortification of food matrices with vitamins and minerals is hampered by technical difficulties since they are sensitive to moisture, light, pH, temperature and oxygen and their potency is altered by their reaction with other ingredients [27]. The encapsulation of a water-soluble vitamin (vitamin B12) in double emulsion has been proposed by Fechner et al. [28]. In this case, the release rate of the vitamin B12 in the outer phase of a W/O/W emulsion stabilized by a conjugate caseinate-dextran rather than a pure protein was reduced under acidic conditions. Double emulsion to protect vitamin B1 [29] and magnesium [30] is also an interesting technique to encapsulate both the minerals and vitamins.

Immobilization of Active Compounds by Polymeric Complexes

The formation of polymeric complexes can also be used to immobilize several types of bioactive compounds. Many studies have demonstrated the effectiveness of these complexes to protect the bioactivity and ensure high availability in the food product throughout processing distribution and storage [8–12, 31].

Among the polymers studied, pectin, alginate and whey protein were used several times for the inclusion of probiotic bacteria and minerals. All three polymers form gels in the presence of calcium ions, although specific attributes are required for pectin (low methoxyl) and whey proteins (denatured form). Therefore, ME of probiotics can simply be carried out by extruding droplets of the polymer-cell suspension into a calcium chloride solution.

The encapsulation of probiotics in beads composed of alginate alone does not improve their stability in the cheese matrix during ripening [6]. This is probably due to the inability of alginate-ME to protect against low pH when ripening and storage extend over months as well as to the anaerobic conditions prevailing in vacuum-packed cheese, as one of the major benefits of this type of encapsulation is to protect the bacteria against oxygen [32]. These results suggest that the protection of probiotics by alginate alone [32] would occur only in fresh cheeses packaged in air and over a short storage period. This has to be demonstrated, however, since ME by spray-coating has not improved stability of oxygen-sensitive bifidobacteria in fresh Cheddar [33].

However, it is possible to improve and functionalize the alginate prior to the formation of a network to increase the immobilization efficiency and to obtain a network less soluble, more hydropho-

bic with smaller-diameter pores [34]. Initial attempts to improve the functionality of alginate for the protection of probiotics have focused on the addition of starch and coating with chitosan or poly-L-lysine. Coating of the alginate beads has indeed improved the survival of probiotics, particularly in a GI environment [35]. This opens the door for ME to provide benefits not only during processing and storage but also following consumption of the products. In this aim, recent methods have improved alginate with hydrophobic compounds [12]. The functionalization of alginate allows a better resistance of probiotics in GI conditions and a continual release of bioactive molecules to sites of absorption [36]. Preliminary results also showed that functionalized alginate increases the rate of probiotic incorporation in cheese matrix (unpublished data). Stabilization of vitamins (carotene) is also observed under extreme conditions of humidity and storage temperature after encapsulation with functionalized alginate [31].

When the functionalized alginate is in the presence of milk proteins, there is also a better protection of the bioactivity of probiotics in the GI tract [12]. Milk proteins have also been shown useful for encapsulating iron [37] and the use of polymeric complex including these proteins is to explore. The self-assembly of basic and acidic proteins allowed the preparation of nanoscale gels, on which bioactive molecules can be attached by electrostatic or hydrophobic interactions [38]. These structures are consolidated by the formation of disulfide bonds under the influence of heat. The whey proteins could be used for these reactions of self-assembly. Functionalized alginate can also provide a better protection of vitamins and minerals [31], antimicrobial compounds and antioxidants during storage at 4 °C [8, 39] and bacteriocins [40].

Particles of alginate/pectin co-polymers have been used to encapsulate folic acid, a hydrophilic compound [41] for Cheddar cheese fortification [42]. This study shows that water-soluble compounds of low molecular weight can be efficiently introduced into cheese. Apparently, alginate particles interact with ionized groups of folic acid. This technique is certainly a good way to immobilize other hydrophilic bio-products such as minerals or peptides inside cheese matrix. According to Kailasapathy and Masondole [43], a slightly higher retention of probiotic bacteria in cheese matrix can be observed when they are encapsulated, but this remains to be quantified according to the industry practices.

Protein and polyelectrolyte associative phase separation has been extensively studied in recent years. When oppositively charged macromolecular colloids are used to form a coacervate, the process is referred to complex coacervation. Colloids such as gelatine, or agar, with positive charge and colloid with negative charge such as carboxymethylcellulose, gum arabic are often used for the development of coacervates. Depending upon colloids' isoelectric points, adjustment of pH and/or dilution with water may be necessary in order to control the effective charge of each colloid. The formation of complex is guided by the polymer parameter (charge density, concentration, chemical nature and ratio) and by the environmental conditions such as the ionic strength, pH and ion type. This technique can be used to adsorb and build up the complex coacervate on the core material particles.

Weinbreck et al. [44] encapsulated various essential oils using whey protein (WP) and gum arabic (GA) as microcapsule polymers. They first produced an emulsion of the essential oil in water in the presence of WP. Thereafter a concentrated solution of GA was added to the emulsion at a pH where both the WP and GA are negatively charged. Then, the pH was reduced to a value where the WP has a net positive charge and GA has a net negative charge inducing complexation of the polymers and their coacervation (phase separation). This complexation resulted in an insoluble protective membrane that prevents the emulsion droplets from coalescing. Cross linking agents such as the transglutaminase can also be used to strengthen this membrane. The encapsulated oil is easily separated using filtration/centrifugation. This technique can be used for the microencapsulation of omega-3 fatty acids [45]. Use of glycated proteins for the microencapsulation has been reported by Augustin et al. [46] since they possess antioxidant properties and have the potential to form stable film around the oil droplets.

Compounds Which can be Added Without the Need of Microencapsulation

Adding Phenolic Compounds to Cheese

Plant-derived polyphenolic compounds are known as one of the principal antioxidants of the human diet. Hundreds of molecules including polyphenol structures have been identified in berries, tea, culinary herbs and edible plants (Fig. 6.1). Due to their beneficial health effects, polyphenolic compounds have been proposed as bioactive or nutraceutical compounds in foods including dairy products [47]. The consumption of food and beverages rich in phenolics, such as tea and wine, is generally correlated with reduced mortality due to coronary heart disease [48]. Several other bioactive compounds including plant or fruit extracts have been applied to cheese and dairy products to improve the quality of final products.

Herbal extracts from beet, mint and ginger were used to fortify cottage cheese and showed antioxidant activity against lipid oxidation [49]. Natural pigments such as red sorghum extract, grape anthocyanins and beet root betalains were added to the cheeses and evaluated their physico-chemical properties [50]. Our previous results also evidenced that cheese product formulated with some polyphenolic compounds improved antioxidant property and a high rate of polyphenolic compounds recovery in cheese was observed (Table 6.2) [51]. According to the results of this study, hesperetin and flavone were recovered at a very high level in cheese curd, which was evidenced by retention coefficients in excess of 0.94 (Table 6.2). It can be explained by their low solubility in liquid whey as well as their hydrophobic interaction with side chains of casein proteins. These hydrophobic compounds were nearly insoluble in water and remained predominantly in cheese curds, as compared to catechin, EGCG, tannic acid and homovanillic acid, which are hydrophilic compounds; their retention coefficients range between 0.54 and 0.83. The crude polyphenolic compounds, which included whole grape and green tea extracts and cranberry juice powder, showed high retention coefficient values, ranging between 0.74 and 0.87. Because these were crude mixtures of a variety of hydrophilic and hydrophobic compounds with various compositions, their retention coefficients all fell somewhere within the continuum of retention coefficients of the pure phenolic compounds tested in this study. Crude extracts from grape, tea and cranberry are important sources of proanthocyanidins. Proanthocyanidins interact selectively with proteins. According to Hagerman and Butler [52], the relative affinities of proteins and polypeptides for proanthocyanidins is influenced by the size of the polymer and binding sites interactions involved. Generally, there are four potential types of interactions that can occur between phenolic molecules and proteins, including hydrogen bonding, hydrophobic, ionic and covalent interactions [53]. It has been proposed that the proanthocyanidin-protein interactions are strongest near the isoelectric pH where the protein-protein electrostatic repulsion is minimized [54]. Hydrogen bonding between phenolic hydroxyl and peptide carbonyl is a major force stabilizing proanthocyanidin-protein complexes [55]. It is also believed that proteins rich in proline have high affinity with proanthocyanidins [52]. Milk is rich in proline and its level in raw milk is 330 mg/100 mL [56]. Finally, the chemical interactions with milk fat must also be considered. Cheese containing polyphenols have good antioxidant properties [57]. The addition of crude polyphenolic compounds from whole grape extract showed the highest level of free radical-scavenging activity. According to Riedl and Hagerman [58], complex polyphenolic is a more potent antioxidant than simple phenolic compound. The estimated total capacity of polyphenolic-procyanidins complex is more than 25 times more active than Trolox at pH 3–7.4, corresponding to 1–3 electrons donated per flavonoid monomer. It seems that flavonoid polymers have multiple electron-donating sites and superior molar capacities as compared to monomeric flavonoids [59]. Hilario et al. [60] also showed that goat's cheese containing hydroxycinnamic acids and flavonoid has higher radical scavenging and the level of these compounds in cheese could be related to feeding conditions. Hilario et al. [60] also showed that cheeses containing polyphenols can maintain their antioxidant properties for more than 1 month.

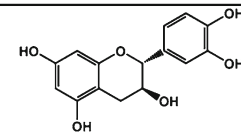
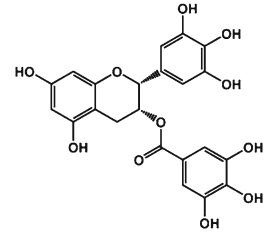
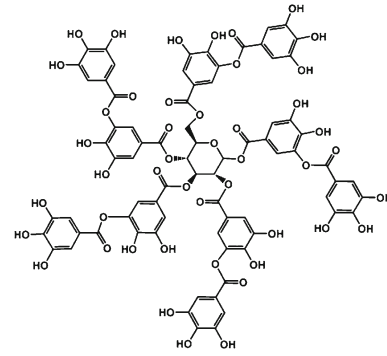
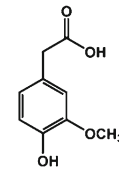
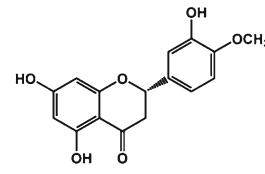
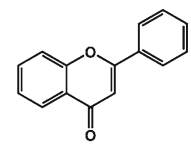
Compound	Structure	MW
Catechin		290.26
EGCG		458.37
Tannic acid		1701.20
Homovanillic acid		182.17
Hesperetin		302.27
Flavone		222.24

Fig. 6.1 Chemical structure and molecular weight of phenolic compound

Table 6.2 Retention coefficient of bioactive compounds and antiradical activity (free radical-scavenging capacity) in cheese curd

Compound	Retention of coefficient	Antiradical activity (nM Trolux equivalent/g curd)
Control		8.82 ± 0.70^{c}
Catechin	0.54 ± 0.04^{f}	30.63 ± 0.34^{a}
EGCG	0.73 ± 0.05^{d}	30.84 ± 1.23^{a}
Tannic acid	$0.83 \pm 0.04^{b,c}$	30.66 ± 1.30^{a}
Hormovanilic acid	0.64 ± 0.08^{e}	30.87 ± 2.88^{a}
Hesperetin	0.94 ± 0.02^{a}	26.94 ± 0.30^{b}
Flavone	0.95 ± 0.02^{a}	30.84 ± 0.18^{a}
Grape extract	0.80 ± 0.09^{c}	31.14 ± 0.20^{a}
Green tea extract	0.74 ± 0.04^{d}	29.70 ± 0.25^{a}
Cranberry powder	0.87 ± 0.03^{b}	27.42 ± 0.45^{b}

In the same column, means with the same lowercase letter are not significantly different
Source: From [51]
$P > 0.05$

The addition of polyphenolic compounds can however affect enzymatic gel-forming kinetics when rennet is used in the production of cheese curds. The pH change caused by polyphenolic addition affected the gel-formation kinetics [51]. The same authors have observed a sigmoidal trend in gel-forming kinetics (Fig. 6.2), but the parameters determining the protein coagulation patterns varied according to the different phenolic compounds added. This may be attributable to the different degrees of affinity between milk proteins and each of the phenolic compounds.

Cheese curds containing polyphenolic compounds at a concentration of 0.5 mg/mL resulted in a decrease of curd moisture content while the gel-strength was not affected [57]. Structural differences were observed when crude polyphenolic compounds were added to the cheese, resulting in rough and granular structures [57]. The addition of phenol to cheese can bring a desirable taste [47] but can also negatively affect the sensorial properties [61]. Development of pink-brown colour in cheese containing procyanidins can be observed. However, Prudencio et al. [50] were able to maintain the colour of the cheese containing anthocyanidin for more than 40 days at 6 °C. The colour development is due normally to enzymatic oxidation by tyrosinase and the activity of the enzyme is dependent on the pH, the moisture content, the redox potential, the presence of oxygen, free tyrosine and free fatty acid concentration [62]. The presence of polyphenols can also provoke proteins precipitation. This reaction can be prevented by encapsulation with polysaccharide-polyphenols complexes formation [47]. Ares et al. [61] were also able to reduce the bitterness or astringency that polyphenols can provide by adding several polysaccharides. Moreover, proanthocyanidins have antimicrobial properties [63]. This property can have beneficial impact on the preservation of microbiological quality of cheese. However, since polyphenols can inhibit proteases, a slower proteolysis is observed, increasing the time required for cheese maturation.

Probiotics

Probiotic bacteria are live microorganisms which, when added in sufficient quantity, confer health benefit to the host. There are eight challenges associated with the fortification of probiotic bacteria in cheese (Table 6.3).

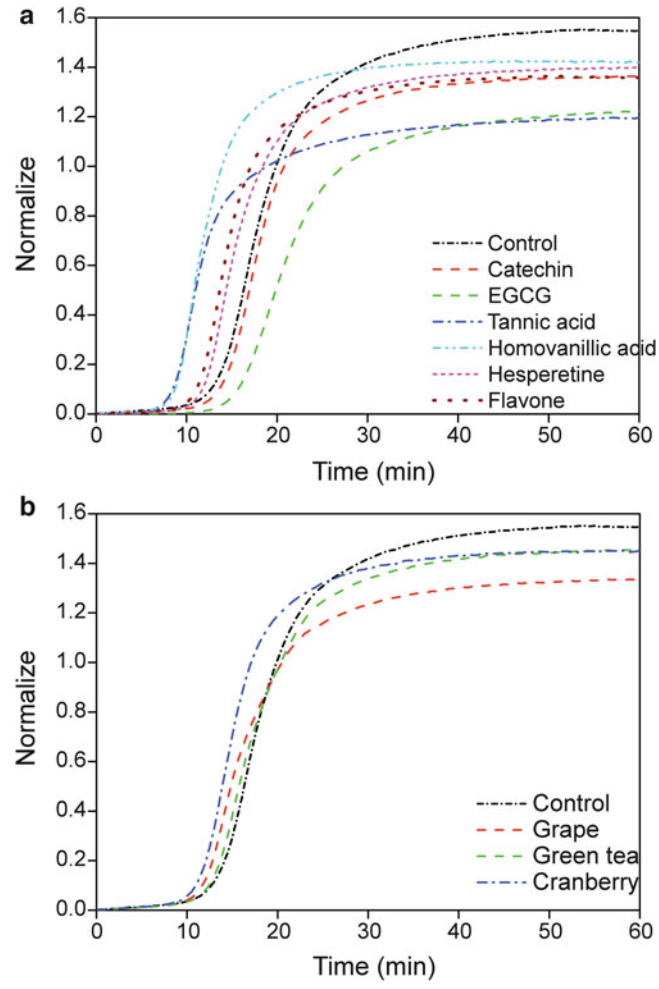


Fig. 6.2 Effect of phenolic compounds on gel-forming kinetics. (**a**) single phenolic compounds; (**b**) natural crude polyphenolic compounds. From [57]

Table 6.3 Challenges associated with the addition of probiotic bacteria in foods and some strategies to address them

Challenge	Potential solution
Strain selection	(1) List cultures with clinical data for the target health benefit (2) ascertain which strain would have the best chance of growing or surviving in the cheese matrix
Quantity (CFU) to add	Base decision on clinical trials. Required number must be viable at the "best before" date
Toxicity	Extensive record of safety. Nevertheless, ascertain if some sub-populations (e.g. patients with severely impaired immune system) could be affected.
How to add during processing	Direct inoculation into pasteurized milk before renetting
How to enumerate the viable probiotic in the presence of starter cultures	Ideally, PCR techniques based on strain-specific probes. Selective enumeration on agar media appropriate in many instances
Stability during storage	Salt level, strain selection, microencapsulation, low temperature, vacuum packaging
Prevent sensory problem	Strain selection, microencapsulation, control of cell population
Deliver in a viable form in the intestines	Buffering capacity of the cheese. Microencapsulation

Table 6.4 Effect of the point of inoculation on the viable counts in cheese and whey during Cheddar cheese production. Theoretical yield linked to inoculation: 6.0 log CFU/g of cheese

	Point of inoculation		
Cheddar production step or sample	Milk	Cheddaring	Salting
Curds after 30 min cheddaring	6.3	5.9[a]	
Residual whey at cheddaring	3.9[a]	6.6[b]	
Curds before salting	6.8[a]	5.9[b]	–
Salted curds	6.6[a]	5.4[b]	5.3[b]
Residual whey after pressing	4.6[a]	4.7[a,b]	5.7[c]
Cheese after pressing	6.6[a]	5.6[b]	5.0[b]

Source: Adapted from [33]

[a–c]In a given row, values which are followed by the same letter are not significantly different (P 0.05)

Numerous studies report viability losses of probiotics in cheese [64]. Thus, strain selection is critical to success in the development of foods with probiotics. Some species, for example *Lactobacillus casei*, can be selected on the basis of their ability to survive and even grow in a cheese matrix. Presumably, many strains of the *Lactobacillus rhamnosus* and *Lactobacillus plantarum* species would have the same property. If a non-strain-related allegation is hoped for, such a strain selection strategy is to consider. However, if a specific health benefit is desired, then a strain having clinical data is preferable. Unfortunately, the desired culture may be very sensitive to the hostile cheese environment. In this case, adaptation to the production process or microencapsulation of the cells is required. The probiotics field has traditionally focused on lactobacilli and bifidobacteria, but other cultures could specifically be used in cheese making. The propionibacteria are typically found in Swiss-type cheeses and there is mounting evidence for their health benefits. Cheese makers therefore have a variety of options with respect to strain selection

Once the strain is selected, the correct number must be added. The viable count claimed on the label is the one that needs to be found at the end of the storage period. Thus, the inoculation level must take into account the evolution of the culture during processing, ripening and storage. With some cultures, viability losses occur under these circumstances, and high inoculation rates are required. Cheese makers who are developing products must carry out tests to ascertain the correct number to inoculate.

The inoculation procedure strongly affects viable counts in the cheese. Probiotics are sensitive to heating and cannot be added to milk prior to pasteurization. When the bacteria are added to milk before renetting, between 80 and 90 % of the bacteria inoculated are recovered into the cheese [65]. Surprisingly, during Cheddar cheese manufacture, addition of the bacteria at later stages in production does not improve this recovery level (Table 6.4). In Cheddar cheese production, adding freeze-dried probiotic cultures at the cheddaring or salting steps result in high losses in whey or viability (Table 6.4).

Drops in viable counts during ripening and storage are difficult to prevent. Thankfully, probiotics are often more stable in cheese than in yoghurt, presumably because of the higher pH. Indeed, small decreases in pH during storage of yoghurt significantly reduce cell viability. Although the effect of pH has not as systematically been evaluated in cheeses as it has been in yoghurt, one can expect the same phenomenon to occur. Presumably, cheeses which show pH drops during storage (fresh cheeses) will be detrimental to viability, while products having increases in pH during ripening and storage (surface-ripened cheeses such as Brie, Camembert, Oka) will favour the stability of probiotics. High salting levels tend to reduce cell stability during storage [65]. Not surprisingly, cheeses which have low acid and salt levels, such as Cottage cheese, are popular carriers for probiotics [66]. Some probiotics are sensitive to oxygen [65]. Vacuum packaging, modified atmosphere packaging or the addition of antioxidants (processed cheeses) may be necessary. Unfortunately, although some of the parameters which affect the viability of

probiotics in cheese during storage are known (pH, temperature and salt), the range of technological adaptation is rather narrow. Indeed, these parameters are also critical to prevent the growth of undesirable bacteria in cheese, particularly the pathogens. At this point, ME is still considered for the protection of probiotics but, as mentioned previously, the technology is not an overwhelming success [33, 67].

As a function of strain and cell numbers, probiotic bacteria can affect the flavour of cheeses [64, 66]. Many probiotic lactobacilli carry out a homofermentation, where lactic acid is the main metabolite; this is not the case with bifidobacteria which synthesize acetic acid in addition to lactic acid. It is therefore understandable that flavour can be affected by the latter species. High probiotics CFU levels may generate these flavours. There is no recognized CFU threshold below which off-flavours do not occur. The literature suggests that when counts are below 10^7 CFU/g of product, there are no adverse effects. However, to attain what is considered a sufficient quantity of probiotics for a health effect, a population of about 10^8 CFU/g of cheese is recommendable. At these CFU levels, sensory differences might appear and investigations are strongly recommended for each product or strain. Microencapsulation is used in many products to prevent off-flavours, but not in cheese. This is a potential research area. In fact ME has been proposed to address many problems linked to the delivery of viable probiotics in cheese as addressed earlier, in the section pertaining on encapsulation in polymeric complexes.

Guidance on Safe Levels

Probiotic bacteria are widely considered as being safe, but there are nevertheless some safety issues [68]. Administration of probiotics to patients with severely reduced immune systems is currently controversial. The CFIA [69] recommends a billion cells of probiotics per portion for the use of non-strain-specific allegations, but some commercial products contain 50 billion cells per portion. Therefore there does not seem to be a toxic level of probiotic bacteria for generally healthy individuals.

Bioavailability can be defined as the fraction of an ingested nutrient that is available for use in normal physiological functions or for storage or the amount of nutrient transported through the digestive membranes to the serum. Many factors may interact with the absorption steps to modify the bioavailability of a specific bioactive compound, namely the particle size, e.g. tomatoes converted into tomato paste enhance the bioavailability of lycopene; the matrix in which the nutraceutical is added, e.g. the relative bioavailability of lutein is lower from the green leafy vegetables (45 % from spinach) than from other vegetables (67 % from other vegetables) due to the entrapment and complexing to proteins in chloroplasts and the molecular configuration (cis/trans), the *cis* isomer bioavailability is superior to trans to their greater solubility.

The effectiveness of nutraceutical compounds in the prevention of diseases depends on preserving the bioavailability of the active ingredients. Only a small proportion of these compounds remains bioavailable after the oral administration, due to insufficient gastric residence time, low permeability and/or solubility within the gastrointestinal tract and the instability under conditions encountered during food processing (light, oxygen, temperature) or in the gut (pH, enzymes, interactions with ingredients). All these factors limit the activity and health benefits of the nutraceutical compounds [70].

Recommendations

The enumeration of probiotic bacteria in foods is a difficult task and scientists as well as regulatory organizations could significantly underestimate the viable counts in cheese. Recommendations have recently been published on this aspect [71].

Conclusion

The development of new food applications is quite challenging, since product appearance, safety, ease of preparation, storage stability, sensory properties are not to be compromised by the incorporation of the bioactive ingredients that confer nutritional advantages. The strategies to fortify cheese require appropriate processes for lipophilic or hydrophilic or insoluble ingredients such as phytosterols. The different requirements for the microencapsulation result from the type of core material, the desired release profile as well as the properties of the cheese matrix.

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Chapter 7
Yogurt Fortified with Date Fiber

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Key Points

- Yogurt is considered a healthy food and incorporating dietary fiber will make it even healthier.
- Date fiber, a by-product of date syrup production, is a good source of dietary fiber.
- Consumer test results indicated that the appearance, color, and flavor ratings were significantly affected by fiber fortification.
- Yogurt fortified with up to 3 % date fiber had similar sourness, sweetness, firmness, smoothness, and overall acceptance ratings as the control yogurt.
- Fortifying yogurt with 3 % date fiber produced acceptable yogurt with beneficial health effects.

Keywords Yogurt • Date fiber • Sensory quality • Acceptability • Quality parameters

Abbreviations

a*	+ redness and – blueness
b*	Yellowness
L*	Lightness
UAE	The United Arab Emirates

Portions of this chapter used with permission from: J. Dairy Sci. 92:5403–5407, doi: 10.3168/jds.2009-2234

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Introduction

Yogurt is an important dairy product, particularly for consumers with lactose intolerance. Yogurt is considered a healthy food because it contains viable bacteria that are considered probiotics. Milk and dairy products do not contain fiber. Fiber is found in the cell wall of fruits, vegetables, and cereals [1, 2]. Fiber of different sources is added to products to increase cooking yield and water-holding capacity, reduce lipid retention, improve textural properties and structure, or reduce caloric content by acting as a bulking agent [3]. Consumption of foods containing fiber may prevent or decrease gastrointestinal disorders [4], hypertension, hypercholesterolemia, obesity [5], diabetes [6–8], coronary heart disease [9, 10], and cancer [11, 12]. Several researchers have studied the effect of dietary fiber on yogurt quality. Addition of 1.32 % oat fiber improved the body and texture of unsweetened yogurt and decreased the overall flavor quality [13]. The effect of wheat bran (natural and toasted) and flavor (pineapple and pi a colada) on yogurt quality were studied [14]. The pH increased and syneresis decreased with increasing fiber (1.5, 3.0, and 4.5 % by weight). Natural bran had a greater effect on consistency than did toasted bran, and yogurt flavored with pi˜a colada had higher viscosity than yogurt flavored with pineapple [14]. The effects of commercial fibers from apple, wheat, bamboo, or inulin on sensory and rheological properties of yogurt were studied [15]. Although some rheological characteristics were modified, the supplemented yogurts were acceptable to consumers. Yogurt fortified with apple fiber had a different color compared with unfortified yogurt. The reported findings showed that yogurt containing 1 % orange fiber had a lighter, more red and more yellow color [lower lightness (**L***), higher redness (**a***) and yellowness (**b***) values] in addition to having lower syneresis than control and yogurt containing 0.6 and 0.8 % orange fiber [16]. Fermented milk enriched with citrus fiber (orange and lemon) had good acceptability [17]. Addition of 0.5 % barley β-glucan or inulin and guar gum (>2 %) were effective in improving serum retention and viscoelastic properties of low-fat yogurt [18]. Incorporation of fiber obtained from asparagus shoots increased yogurt consistency and imparted a yellow-greenish color to the yogurt [19]. Dates are a good source of dietary fiber [20–22]. The dietary fiber content of dates ranges from 4.4 to 11.4 % depending on date variety and ripening stage [23–26]. A serving of dates (5–6 fruits) can provide 14 % of the recommended daily intake of dietary fiber [23]. The United Arab Emirates (*UAE*) is the fourth leading country worldwide for date production, producing 755,000 tons of dates annually, representing 12 % of the world's production [27]. The dates, one of the most important fruit crops in the UAE, are processed to produce date syrup. Date fiber, a by-product remaining after date syrup extraction, contains 51.57 % total dietary fiber [28]. Incorporation of date fiber into yogurt without affecting sensory quality and acceptability was investigated. The quality of fresh yogurt fortified with date fiber (acidity, pH, color, texture profile, sensory properties, and consumer acceptance) was studied [29].

Yogurt Making

Yogurt samples were prepared from fresh cow milk, purchased from a local supermarket, in the Food Preparation Laboratory of the Food Sciences Department, UAE University, following the procedure used at a local dairy company (Al Ain Dairy). Yogurt was made by dissolving milk solid nonfat (2.5 %) and stabilizer, Grindsted ES255 Emulsifier and Stabilizer system, Danisco Ingredients, Braband, Denmark, (0.6 %) in milk. The fiber was added according to the composition of the samples (0, 1.5, 3.0, and 4.5 %). The mixture was heated in a water bath at 85 °C for 30 min, cooled to approximately 42 °C, inoculated with commercial yogurt culture (YO-FAST-88, Chr. Hansen, H˝rsholm, Denmark), transferred to plastic cups, incubated at 43 °C for 4 h, and stored at 4 °C overnight before testing. Control yogurt without date fiber and yogurt containing 1.5 % wheat bran were also prepared.

Preliminary studies indicated that yogurt containing a high level of date fiber had a different flavor. Yogurt with the highest date fiber level was flavored with vanilla to mask the flavor that might arise from the high level of addition of date fiber.

Quality Parameters

The *pH* of the samples was determined using a digital pH meter (Thermo Orion pH meter, model 420, Waltham, MA). The measurements were done in triplicate. *Titratable acidity*, expressed as percentage of lactic acid, was determined by mixing 10 g of yogurt with 20 mL of distilled water and titrating with 0.1 N NaOH using phenolphthalein as an indicator to an end-point of faint pink color. The measurements were done in triplicate.

Texture Profile analysis of the yogurt samples was measured using QTS 20 texture analyzer (model QTS20, Brookfield Instruments, Harlow, UK) equipped with a 5-kg load cell. Texture profile analysis was carried out by a compression test that generated plot of force (g) vs. time (s). A 25-mm-diameter perplex cylindrical probe was used to measure textural profile of the yogurt samples at 10 ± 0.5 °C. In the first stage, the samples were compressed to 10 mm depth and the speed of the probe was fixed at 30 mm/min during the pretest, compression, and relaxation of the samples. The typical textural profile (force–time) curve was obtained with one complete run. Hardness, gumminess, adhesiveness, cohesiveness, and springiness of yogurt samples were calculated by the software program (TexturePro software, Brookfield Instruments). The data presented are average of five replications.

Color parameters L*, a*, b* values were measured by using a colorimeter (ColorFlex, HunterLab, Reston, VA). A white tile was used for standardization.

Three replications were conducted.

Thirty-three panelists consisting of students and staff of the university were recruited and instructed on how to perform *sensory Evaluation*. The evaluation was conducted in partitioned sensory evaluation booths at the Department of Food Science (UAE University). Yogurt samples were presented in white plastic cups under fluorescent light. All samples were marked with 3-digit codes, and the order of presentation of samples was randomized for each panelist. The panelists rated appearance, color, firmness (texture or body), smoothness, sweetness, sourness, flavor, and overall acceptance using a 9-point hedonic scale (1 = dislike extremely and 9 = like extremely).

Data was analyzed using SPSS Statistical Software (version 13.5, SPSS Inc., Chicago, IL). Sensory data were statistically tested using ANOVA to determine if a statistical difference existed ($P \leq 0.05$) and the least significance difference was used for means comparison.

Impact of Date Fiber on Yogurt Quality

Acidity, pH, and color values of fresh yogurt fortified with fiber are shown in Table 7.1. Yogurts fortified with date fiber or wheat bran had similar acidity (1.07–1.08) as control yogurt (1.04). Similar results were reported for yogurt fortified with oat fiber [13] and natural or toasted wheat bran [14]. Yogurt fortified with 4.5 % date fiber and flavored with vanilla had similar acidity (1.05) as the control and unflavored date fiber yogurts. The reported findings [14] show that yogurts fortified with natural or toasted wheat bran and flavored with pineapple or pi˜a colada had similar acidity. Acidity of yogurt was not significantly affected by fiber (date fiber and wheat bran) and flavor addition. The pH of yogurts fortified with date fiber ranged from 4.61 to 4.67, which is similar to the pH of yogurt fortified with wheat bran (4.64). Increasing the date fiber level had no effect on yogurt pH. Yogurts fortified with date fiber or wheat bran had significantly higher pH compared with that of control yogurt (4.47).

Table 7.1 Acidity, pH, and color[a] of yogurt fortified with date fiber (means ± SD)

Yogurt	Acidity, % lactic acid	pH	L*	a*	b*
Control	1.04 ± 1.0[b]	4.47 ± 0.06[c]	95.5 ± 0.3[b]	−0.8 ± 0.6[d]	9.1 ± 0.4[e]
1.5 % wheat bran	1.08 ± 1.8[b]	4.64 ± 0.03[b]	89.3 ± 0.3[c]	0.8 ± 0.7[e]	11.1 ± 0.2[c]
1.5 % date fiber	1.08 ± 1.8[b]	4.61 ± 0.02[b]	84.8 ± 1.1[e]	2.7 ± 0.4[c]	9.7 ± 0.4[e]
3.0 % date fiber	1.08 ± 1.8[b]	4.63 ± 0.04[b]	80.1 ± 1.2[d]	4.1 ± 0.6[b]	11.0 ± 0.3[c]
4.5 % date fiber	1.07 ± 1.8[b]	4.65 ± 0.02[b]	75.4 ± 0.9[f]	4.9 ± 0.8[b]	12.4 ± 0.3[b]
4.5 % date fiber flavored with vanilla	1.05 ± 1.8[b]	4.67 ± 0.02[b]	75.5 ± 1.1[f]	5.0 ± 0.4[b]	12.2 ± 0.4[b]

The columns and rows show the mean + SD of three replicates of the yogurt and yogurt containing wheat bran or date fiber

[a] L* (lightness), a* (+ redness and − blueness); b* (yellowness)

[b–f] Means within a column followed by different superscript letter differ ($P \leq 0.05$)

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Although addition of fiber had no effect on yogurt acidity and lactobacilli counts, it increased the pH. A similar result was reported for yogurt fortified with 1.32 % oat fiber [13]. Oat fiber yogurt had a significantly higher pH (4.31) compared with control yogurt (4.17), whereas other [15] reported that addition of commercial apple, wheat, bamboo, or inulin fibers had no effect on yogurt pH. We have no explanation for this effect other than to attribute it to the type of fiber. Flavoring yogurt fortified with 4.5 % date fiber with vanilla had no effect on pH. Flavored and unflavored yogurts fortified with 4.5 % date fiber had similar pH. Yogurt color was affected by the addition of date fiber or wheat bran. The date fiber had a brownish color, whereas the wheat bran had a yellowish color. Yogurts fortified with date fiber or wheat bran had significantly higher a* and b* values and lower L* values compared with the control yogurt. Increasing the date fiber level increased a* and b* values and decreased L* values significantly. Yogurts fortified with date fiber had significantly lower L* values and higher a* values compared with wheat bran yogurt. Yellowness of the yogurt depends on the level of date fiber. Yogurt fortified with 3 % date fiber had similar b* values as wheat bran yogurt. Yogurt fortified with 1.5 % date fiber had significantly lower b* values compared with wheat bran yogurt, whereas yogurt fortified with 4.6 % had significantly higher b* values. Similar results were reported for yogurts fortified with commercial apple fiber [15], orange fiber [16], and asparagus fiber [19]. Yogurt fortified with date fiber had a brownish color, whereas yogurt fortified with orange fiber or apple fiber had a yellowish color and that fortified with asparagus fiber had a yellow-greenish color. Findings [15] reported that fortification with commercial wheat, bamboo, or inulin fibers had no effect on yogurt color. This indicated that yogurt color is dependent on the color of the fiber source. Flavoring 4.5 % date fiber yogurt with vanilla had no effect on yogurt color. Flavored and unflavored yogurts fortified with 4.5 % date fiber had similar color values. Yogurt fortified with 1.5 % date fiber had similar textural properties (hardness, gumminess, adhesiveness, cohesiveness, and springiness) as control yogurts (Table 7.2). Although the addition of 1.5 % date fiber had no effect on yogurt texture, 1.5 % wheat bran yogurt had significantly higher hardness, gumminess, and springiness values and a significantly lower adhesiveness value compared with the control. Fortifying yogurt with 3.0 % date fiber had significant effect on the textural properties. Hardness, gumminess, and springiness increased and adhesiveness and cohesiveness decreased significantly. Increasing the hardness may be related to date fiber absorbing more moisture because of its higher water-holding capacity. Yogurt fortified with 3 or 4.5 % date fiber had similar textural properties showing that increasing date fiber level to 4.5 % had no significant effect on yogurt texture. The use of a 1:1 ratio of inulin to galactomannan produced yogurt with the highest curd tension [30], and addition of β-glucan (0.5 %), partially hydrolyzed guar gum, and inulin (2 %) improved the texture and rheological properties of low-fat yogurt [18]. Flavoring 4.5 % date fiber yogurt with vanilla (to improve yogurt flavor) decreased the hardness and increased adhesiveness significantly without affecting gumminess, cohesiveness, or springiness; we have no explanation for this effect. As reported previously [14] yogurt flavored with pi˜a colada was more viscous than that flavored with pineapple.

Table 7.2 Texture properties (means ± SD) of yogurt fortified with date fiber

Yogurt	Hardness g	Gumminess g	Adhesiveness g.s	Cohesiveness g.s	Springiness mm
Control	37.5 ± 3.1^{a}	20.8 ± 1.8^{a}	-76.1 ± 13.5^{b}	0.56 ± 0.01^{b}	7.4 ± 0.09^{c}
1.5 % wheat bran	47.6 ± 1.9^{c}	25.6 ± 1.4^{c}	-101.2 ± 10.8^{c}	$0.54 \pm 0.01^{b,c}$	7.7 ± 0.16^{b}
1.5 % date fiber	36.5 ± 3.5^{a}	20.9 ± 1.9^{a}	-64.0 ± 11.9^{b}	$0.55 \pm 0.01^{b,c}$	7.3 ± 0.26^{c}
3.0 % date fiber	55.0 ± 5.4^{b}	29.0 ± 2.6^{b}	-175.7 ± 39.2^{a}	0.53 ± 0.02^{c}	7.6 ± 0.16^{b}
4.5 % date fiber	57.0 ± 5.0^{b}	30.4 ± 2.5^{b}	-180.4 ± 29.1^{a}	0.53 ± 0.01^{c}	7.7 ± 0.18^{b}
4.5 % date fiber flavored with vanilla	49.2 ± 4.3^{c}	$26.4 \pm 2.7^{b,c}$	-134.4 ± 16.0^{c}	$0.54 \pm 0.01^{b,c}$	7.6 ± 0.17^{b}

The columns and rows show the mean + SD of five replicates of the yogurt and yogurt containing wheat bran or date fiber
[a–c] Means within a column followed by different superscript letter differ ($P \leq 0.05$)

Table 7.3 Sensory quality and acceptability[a] of yogurt fortified with date fiber ($n = 33$)

Yogurt	Appearance	Color	Firmness	Smoothness	Sweetness	Sourness	Flavor	Overall acceptance
Control	8.3 ± 0.7^{b}	8.5 ± 0.7^{b}	7.6 ± 1.4^{b}	7.5 ± 1.2^{b}	6.9 ± 1.1^{b}	7.4 ± 1.0^{b}	7.5 ± 1.2^{b}	7.4 ± 1.2^{b}
1.5 % wheat bran	6.2 ± 2.2^{c}	6.2 ± 1.9^{c}	6.0 ± 2.2^{c}	6.1 ± 1.9^{c}	3.4 ± 1.5^{c}	4.3 ± 1.8^{d}	3.3 ± 1.4^{d}	4.2 ± 1.6^{d}
1.5 % date fiber	6.2 ± 1.0^{c}	6.3 ± 1.3^{c}	$6.7 \pm 1.1^{b,c}$	$6.6 \pm 1.1^{b,c}$	5.9 ± 1.4^{b}	$6.5 \pm 1.2^{b,c}$	6.1 ± 1.2^{c}	$6.8 \pm 1.0^{b,c}$
3.0 % date fiber	6.4 ± 0.7^{c}	6.2 ± 1.1^{c}	$6.6 \pm 1.1^{b,c}$	$6.7 \pm 1.2^{b,c}$	6.2 ± 1.1^{b}	$6.5 \pm 1.4^{b,c}$	$5.9 \pm 1.2^{c,e}$	$6.8 \pm 0.9^{b,c}$
4.5 % date fiber	6.5 ± 0.7^{c}	6.2 ± 0.7^{c}	5.9 ± 1.0^{c}	6.2 ± 1.1^{c}	5.9 ± 1.1^{b}	5.4 ± 1.2^{e}	5.1 ± 1.2^{e}	$5.8 \pm 0.9^{c,e}$
4.5 % date fiber flavored with vanilla	6.0 ± 1.3^{c}	5.8 ± 1.4^{c}	5.9 ± 1.5^{c}	6.4 ± 1.2^{c}	5.9 ± 1.0^{b}	5.4 ± 1.4^{e}	5.0 ± 1.4^{e}	5.3 ± 1.1^{e}

The columns and rows show the mean + SD of 33 panelists/judges evaluated the yogurt and yogurt containing wheat bran or date fiber
[a] A 9-point hedonic scale was used with (1) = dislike extremely and (9) = like extremely; mean ± standard deviation
[b–e] Means within a column followed by different superscript letter differ ($P \leq 0.05$)

Sensory quality and consumer acceptance of yogurt fortified with fiber were presented on Table 7.3. Fortifying yogurt with date fiber had a significant effect on all sensory properties except sweetness. Yogurt fortified with date fiber had significantly lower ratings for appearance, color, and flavor. Firmness, smoothness, sourness, and overall acceptance ratings depended on the level of fortification. Yogurt fortified with up to 3 % date fiber had similar firmness, smoothness, sourness, and overall acceptance ratings as control yogurt. Increasing date fiber fortification level to 4.5 % decreased firmness, smoothness, sourness, and overall acceptance ratings significantly compared with control yogurt. Yogurt fortified with wheat bran was significantly different compared with control yogurt. Although wheat bran and date fiber fortified yogurts had similar ratings for appearance, color, firmness, and smoothness, the wheat bran fortified yogurts had significantly lower ratings for sweetness, sourness, flavor, and overall acceptance. As reported previously [13] addition of fiber improved the body and texture of unsweetened yogurt and decreased overall flavor quality. Yogurts fortified with wheat, bamboo, or inulin fibers were acceptable and had similar sensory properties as plain yogurt [15] and citrus fiber–enriched fermented milk was reported to be acceptable [17]. Flavoring yogurt fortified with 4.5 % date fiber had no effect on sensory quality and acceptability. Flavored and unflavored yogurts fortified with 4.5 % date fiber had similar sensory quality and acceptability ratings.

Guidance on Safe Levels or Guidance on Levels to be Added

Date fiber contains 52 % total dietary fiber, 20 % sugars and other nutrients. These nutrients are naturally found in the dates. Date fiber is a by-product of date syrup production. Addition of any amount of date fiber is safe.

Recommendation

Date fiber can be added to yogurt (up to 3 %) as a functional ingredient without affecting its quality.

Conclusions

Fortifying yogurt or dairy products with fiber is of great interest to improve the functionality and create functional foods with health benefits. The addition of dietary fiber to yogurt would complement its healthy characteristics. Fortifying yogurt with 3 % date fiber produced an acceptable product with potential beneficial health effects.

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Chapter 8
Convenience Drinks Fortified with n-3 Fatty Acids: A Systematic Review

Clemens von Schacky

Key Points

- A portion of the population does not eat fish, but needs to be supplied with the two essential omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). This opens the market for foods and drinks enriched with EPA + DHA.
- A systematic analysis of the literature was performed to assess the current state of the evidence on drinks enriched with EPA + DHA, and 13 publications reporting on 13 studies were found.
- In all publications, EPA + DHA in the respective drinks were reported to be bioavailable; a comparison of bioavailabilities was impossible due to differences in analytical methods.
- Parameters that were favorably affected were serum lipid parameters, and, in one study, pain-free walking distance in patients with peripheral atherosclerosis. Reports on other parameters were less consistent. Results were in keeping with results from studies using other sources of EPA + DHA.
- Except for one study in patients with malabsorption, safety and tolerability of the drinks studied appeared good, if reported.
- Study design, execution, and reporting were less than ideal in most studies.
- Recommendations for improved study design and reporting are given that will make studies more efficient and produce more robust data, if needed.

Keywords Eicosapentaenoic acid • Docosahexaenoic acid • Fortified drinks • Omega-3 Index • Systematic review

Abbreviations

CrP	C-reactive protein
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid
ICAM	Intercellular adhesion molecule
PAI-1	Plasminogen activator inhibitor-1
VCAM	Vascular cell adhesion molecule

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V.R. Preedy et al. (eds.), *Handbook of Food Fortification and Health: From Concepts to Public Health Applications Volume 2*, Nutrition and Health, DOI 10.1007/978-1-4614-7110-3_8,

Introduction

Omega-3 or n-3 fatty acids are a family of essential fatty acids comprising, among others, alpha-linolenic acid, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Plant-derived alpha-linolenic acid has little, if any, cardiovascular benefit, and is poorly, if at all, converted to the longer-chain EPA, which, in turn, is not converted to DHA [1, 2]. Therefore, this review focuses on EPA and DHA.

EPA and DHA have a number of biologic effects, among them beneficial effects like reductions in cardiovascular mortality and morbidity, demonstrated in most, but not all, studies [1, 2]. This is why increased ingestion of EPA and DHA is recommended by cardiac societies for patients after a myocardial infarction, with congestive heart failure, or to prevent cardiovascular disease [1, 2]. Also, scientific societies recommend increased ingestion of EPA and DHA for pregnant and lactating women [3]. Other areas currently actively investigated are prevention and treatment of some psychiatric diseases and some chronic inflammatory diseases.

The typical dietary source of EPA and DHA is fatty fish. However, fish is not ingested by some parts of the population: In Germany, for example, 35 % of young women and 32 % of young men do not ingest fish at all, according to a national survey [4]. This opens the field for alternative sources of EPA and DHA, by supplements or by fortification of food or drinks, the topic of this review.

Method: For the purpose of this review, "convenience drink" was defined as a nonalcoholic fluid produced to be consumed by and studied in humans. Medline searches were performed in the second half of October 2011 with "convenience drink," "drink," "fruit juice," "juice," and "Puleva" in combination with "omega-3," "n-3," "eicosapentaenoic," and "docosahexaenoic." The abstracts of the resulting publications were screened and the publications pertinent to this review were selected.

Milk

An open study was conducted in eight normolipidemic volunteers with 500 mL/day of partially skimmed milk, enriched with 300 mg EPA + DHA for 6 weeks [5]. In plasma fatty acids, EPA increased from 0.70 ± 0.06 to 0.92 ± 0.07 % and DHA from 2.21 ± 0.26 to 2.93 ± 0.29 % (both $p < 0.05$). Plasma triglycerides decreased by 19 % and HDL increased by 19 % at 6 weeks, while total cholesterol was not affected. Palatability or untoward effects were not reported. The authors concluded that the milk used increased omega-3 fatty acids in plasma [5].

A group in Spain performed an intervention study in 30 healthy volunteers (age 33.17 ± 7.2 years). Subjects consumed 500 mL semi-skimmed milk enriched in vitamins A and D for 4 weeks (largely saturated fatty acids, no EPA, no DHA), followed by 8 weeks of consumption of 500 mL semi-skimmed mild enriched with vitamins A, D, E, B6 and folic acid, and containing 5.17 g oleic acid, 0.2 g EPA and 0.13 g DHA [6]. After intake, EPA and DHA increased in the plasma fatty acids of the volunteers. EPA and DHA reduced total cholesterol by 6 % ($p < 0.05$), and LDL by 19 % ($p < 0.05$), while triglycerides and other lipids were not significantly changed. EPA and DHA reduced vascular cell adhesion molecule-1 (VCAM-1) by 16 % ($p < 0.05$), while intercellular adhesion molecule (ICAM) was not affected [6]. Since the milk enriched in EPA and DHA also contained substantial amounts of oleic acid, causality could not be established with certainty. The authors concluded that the milk provided has favorable effects on risk factors for cardiovascular disease [6].

In a follow-up study from the same group, an identical design was used in 30 volunteers aged 51.3 ± 5.3 years [7]. Again, EPA and DHA increased in plasma fatty acids of the volunteers. Plasma concentrations of triglycerides (24 %), total cholesterol (9 %), and low-density lipoprotein cholesterol (13 %) were significantly reduced. Significant decreases in plasma concentrations of vascular VCAM-1 (9 %) but no other significant changes were observed [7]. The 17 % reduction in

homocysteine can safely be ascribed to the supplementation with folic acid, which increased by 98 % increase in plasma. The authors concluded that the milk used may be useful for decreasing risk factors for cardiovascular disease [7].

The same group recruited 60 patients with peripheral arterial disease for a 1-year randomized intervention study, again comparing 500 mL/day of the two semi-skimmed milks already mentioned [8]. Pain-free walking distance increased in the intervention group by a factor of 3.5, and correlated with the increase of EPA in plasma fatty acids, while both remained stable in the control group. The ankle brachial index also increased somewhat in the intervention group, but remained stable in the control group. Total cholesterol decreased in the intervention group, while other blood lipids remained stable in both intervention and control groups, except high-density lipoprotein, which decreased in both groups. No changes occurred in C-reactive protein (CrP), plasminogen activator inhibitor-1 (PAI-1), ICAM-1, VCAM-1, and E-selectin. The authors concluded that the milk used improved clinical outcomes and reduced a variety of risk factors [8].

Again, this group recruited 40 patients after a myocardial infarction for a randomized intervention study, again comparing 500 mL/day of the two semi-skimmed milks already mentioned for 1 year [9]. All patients participated in a rehabilitation program including exercise training, and received lifestyle and dietary advice. The components given increased in plasma. Total cholesterol (from 4.89 6 0.21 to 4.37 6 0.14 mmol/L), low density lipoprotein-cholesterol (from 3.02 ± 0.17 to 2.62 ± 0.12 mmol/L), apolipoprotein B (from 0.98 ± 0.04 to 0.85 ± 0.02 g/L), and high-sensitivity CrP (from 3.90 ± 0.92 to 2.01 ± 0.21 mg/L) decreased significantly ($p < 0.05$) in plasma of the intervention group, but were unchanged in the control group. Homocysteine decreased in both groups, while other parameters measured remained unchanged in both groups (e.g., triglycerides). The authors concluded that the milk provided contributed to reducing a variety of risk factors [9].

In the four studies from Spain mentioned it was reported that "dairy products used were well accepted and compliance was good" (sic!).

More recently, parts of the same group used the same milks to conduct a 1-year randomized controlled trial in 72 hyperlipidemic adults [10]. All 33 patients in the control group, and 39 in the intervention group concluded the trial; the method of randomization, however, was not reported. EPA and DHA increased in plasma fatty acids of the intervention group, as did plasma biomarkers of bone formation, while these parameters remained stable in the control group. No adverse effects were reported, and "compliance with the study protocol was good." The authors concluded that the milk provided improved nutritional status and bone formation markers in adult hyperlipidemic patients [10].

In a 12-week randomized controlled trial, 157 patients with mildly elevated triglyceride levels received 125 mL/day of either a control soy bean milk containing no EPA or DHA (but 2.2 g olive oil), or a soy bean milk containing a fish oil, providing 0.86 g/day EPA + DHA [11]. For various reasons, 16 patients discontinued, but 141 patients completed the study. Although EPA, but not DHA, increased in red cell phospholipids fatty acids, no changes were noted in plasma levels of triglycerides, low-density and high-density lipoprotein cholesterol, high-sensitivity CrP, and soluble tumor necrosis factor-receptors 1 and 2. "Compliance of test foods" (sic) was 88 and 86 % on average in EPA and control group (n.s.); safety and palatability were not commented upon. The authors concluded that it is not likely that fish oil changes the inflammatory parameters studied in subjects without inflammation [11].

Except for one study [5], all studies mentioned reported approval from a local ethics committee, but no study reported an estimation of study size.

Fruit and Vegetable Juices

A soy-based fruit drink used two parallel 1-year randomized controlled trials: one in 396 well-nourished and one in 384 marginally nourished school-aged children [12]. The study was approved by local ethics committees, and study size was based on a conventional power calculation. In a factorial design,

the drink contained a micronutrient mix (iron, zinc, folate, and vitamins A, B-6, B-12, and C), with 110 mg EPA + DHA, or both or placebo 6 day/week. Of the well-nourished children 281, of the marginally nourished children 340 completed the trial. DHA + EPA treatment increased plasma DHA and total plasma omega-3 fatty acids in both substudies. Effects of DHA + EPA were not detected in test of cognition. Compliance in the well-nourished children was among 75 % and among 86 % in the marginally nourished children. Safety and palatability were not commented upon. The authors saw no effect of EPA + DHA on the tests of cognition used [12].

An orange juice (180 mL/day) containing either 50 or 100 mg of microencapsulated algae-derived DHA was randomly compared in healthy 4–12-year-old children for 6 weeks [13]. The study had the approval of the local ethics committee; an estimation of study size was not performed. Fifteen children received 15 mg/day and 16 children 100 mg/day DHA in orange juice. Dose-dependently, DHA increased in plasma phospholipid fatty acids. Compliance was excellent because the orange juice was consumed for 96 ± 6 % of the study days. Safety was not commented upon, but the taste was rated as "good" or "very good" by 93 % of the study participants. The authors concluded that the enriched orange juice increased plasma phospholipid DHA in children [13].

For a 2-week randomized trial with a tomato juice (500 mL/day), 22 healthy women were recruited [14]. The study compared a control tomato juice to an identical tomato juice containing 250 mg microencapsulated fish oil-derived EPA + DHA. Of the control population, two participants withdrew because of the taste of the juice, and two for other reasons, which left 7 controls, and 11 EPA + DHA participants to complete the study [14]. The study had the approval of the local ethics committee; an estimation of study size was not performed. Of 24 laboratory parameters reported (from cholesterol to CrP), ICAM, VCAM, and homocysteine were found to be significantly reduced by EPA + DHA ($p < 0.05$), with homocysteine also being reduced in the control group. No comments were made on compliance or safety. The authors felt that the enriched in EPA + DHA improved cardiovascular risk factors [14].

In a 7-week trial, 179 healthy participants were included, and randomized to receive approximately 1 g EPA + DHA in either a fish paté, a fruit juice, or a fish oil, which was compared to a control group [15]. The study was approved by the local ethics committee, and a conventional study size estimation was based on a null hypothesis. Hundred and fifty nine participants completed the study, 11 participants dropped out before the baseline visit, and 9 during the study (2 because they could not consume the fruit juice). Fish paté and fruit juice were reported to have been "safe, well tolerated and highly palatable." EPA, docosapentaenoic acid, and DHA increased similarly in the intervention groups, but not in the control group. No changes were observed in conventional blood lipids (e.g., low-density lipoprotein, Apolipoprotein A, or Apolipoprotein B), in a group of markers of inflammation (e.g., CrP, interleukin-6, or monocyte chemo-attractant protein-1), or in a marker of oxidative stress (urinary F_2 isoprostane). Interferon-gamma increased in the intervention groups, which could not be explained by the authors. The authors concluded that they saw no changes in serum lipids, markers of inflammation or oxidative stress, but that fish paté, a fruit juice, or a fish oil delivered EPA and DHA similarly, and was independent of the food matrix [15].

Other Drinks

Ten patients with chronic intestinal malabsorption, dependent on parenteral nutrition, agreed to participate in an open exploratory 12-week trial of daily oral ingestion of 337 mL of a drink, providing 1.09 g EPA and 0.46 g DHA [16]. Due to an unacceptable increase in stool frequency, five patients were dropped out. The other five completed the study, mostly ingesting 337 mL of the drink daily. In these five participants, EPA and DHA almost doubled in plasma phospholipid fatty acids, and more

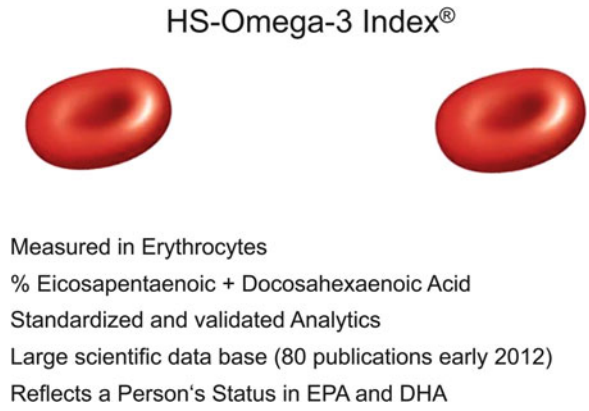

Fig. 8.1 Definition of the HS-Omega-3 Index

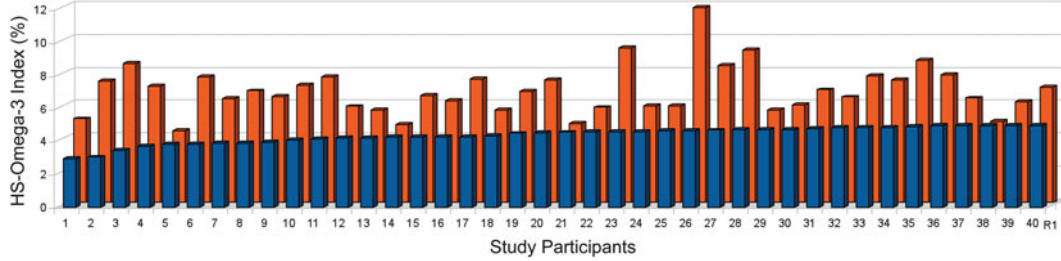

Fig. 8.2 HS-Omega-3 indices in 40 subjects before (*blue bars*) and after (*red bars*) intake of 0.5 g/day Omega-3 fatty acids for 8 weeks. Subjects were selected for an HS-Omega-3 Index below 5 %. The mean HS-Omega-3 Index increased from 4.37 + 0.51 to 6.80 + 1.45 % ($p < 0.0001$, paired *t*-test). Interindividual variability of the increase was large

than doubled in plasma triglyceride fatty acids. Markers of inflammation, like serum CrP, interleukin-6, tumor necrosis factor a, or soluble tumor necrosis factor receptor II did not change systematically. The authors concluded that some patients with chronic malabsorption can absorb EPA and DHA from the product tested, but side effects were common, and an anti-inflammtory effect was not noted [16].

Our own 8-week randomized double-blind intervention study compared a 200 mL convenience drink containing 500 mg EPA + DHA to an identical drink not containing EPA + DHA, but 1.1 g omega-6 fatty acids [17]. One hundred ninety patients with atherosclerotic disease were screened, and the 50 with an Omega-3 Index <5 % were recruited into the study. The Omega-3 Index represents the percentage of EPA + DHA in red cell fatty acids, as analyzed with a strictly standardized analytical method ([1], Fig. 8.1). The study had the approval of the local ethics committee, was registered at clinicaltrials.gov, and its size was calculated based on the primary endpoint, a mean increase of the Omega-3 Index of 1.65 (±1.2) %, and conventional assumptions. Secondary endpoints were palatability and safety of the convenience drink. Data analysis was by intention to treat. The Omega-3 Index increased from 4.37 (±0.51) to 6.80 (±1.45) % in the verum group ($p < 0.001$), but remained stable in the placebo group (Fig. 8.2). Palatability was 8.30 (±1.64) on a visual analog scale from 0 to 10, with 10 being the best value. Although the study was too small to detect rare side effects, the convenience drinks used were safe and well tolerated. The study was the first to recruit participants based on a given Omega-3 Index, and therefore was the first to note a large interindividual variability in response to a fixed dose of EPA + DHA, a phenomenon that impacts on study design and interpretation of results, as discussed in more detail in the publication (Fig. 8.2). We concluded that the convenience drink studied appeared to be a viable alternative to fish or fish oils [17].

Issues in Study Design

The publications discussed in this review span from the year 2000 to 2011 are all based on clinical trials. Although the Consort Guidelines on reporting on clinical trials were in vigor already in the 1990s [18], adherence to these guidelines was incomplete in the publications discussed. While almost all studies had a clearance of the local ethic's committee, basic requirements, like definition of a primary endpoint, and a study size estimate based on a defined primary study endpoint, were rarely fulfilled. Without a primary endpoint, and a study size estimation based on it, it is impossible to judge, whether a neutral result is due to an inadequate study size or due to ineffectiveness of the intervention. This makes basing conclusions on neutral results of a trial methodologically impossible and invalidates the entire trial. Besides the time, money, and effort lost, this poses ethical questions, such as whether it is ethical to recruit participants for a trial that may produce inconclusive results. Not all trials were conducted according to "Good Clinical Practice," and only one trial was registered at a trial registry. In the future, therefore, by respecting the current requirements for clinical trials, higher quality trials will be conducted and stronger data will emerge to base conclusions on.

Except for one trial, study participants were recruited irrespective of their baseline status in terms of EPA + DHA, i.e., the Omega-3 Index (see below). In Western countries, the mean Omega-3 Index is generally lower than, e.g., in Korea or Japan, but exceptions occur [1, 2, 19]. In every population studied so far, the Omega-3 Index had a statistically normal distribution, meaning that some persons have a low, some a high, and most persons an intermediate Omega-3 Index (Fig. 8.3). Therefore, at study end, after being exposed to EPA + DHA or control, the mean Omega-3 Index in the intervention group will likely be higher than the mean Omega-3 Index in the control group, but an overlap of levels is likely. This can be aggravated by the large variation in interindividual responses to a given dose of EPA + DHA ([17], Fig. 8.2). Moreover, study participants recruited were often healthy, while parameters measured, like inflammatory cytokines, tend to be elevated only in chronic inflammatory conditions, but not in healthy persons. Jointly, the problems discussed in this paragraph introduce a bias towards a neutral result of a trial. This bias could be minimized by selecting study participants according to a baseline Omega-3 Index, according to the parameters to be studied, and treating to a target Omega-3 Index with variable doses of EPA + DHA.

Some of the trials mentioned had a maximum duration of 1 year. The 1-year trials reported positively on compliance and palatability. Whether this means that long-term safety of the product

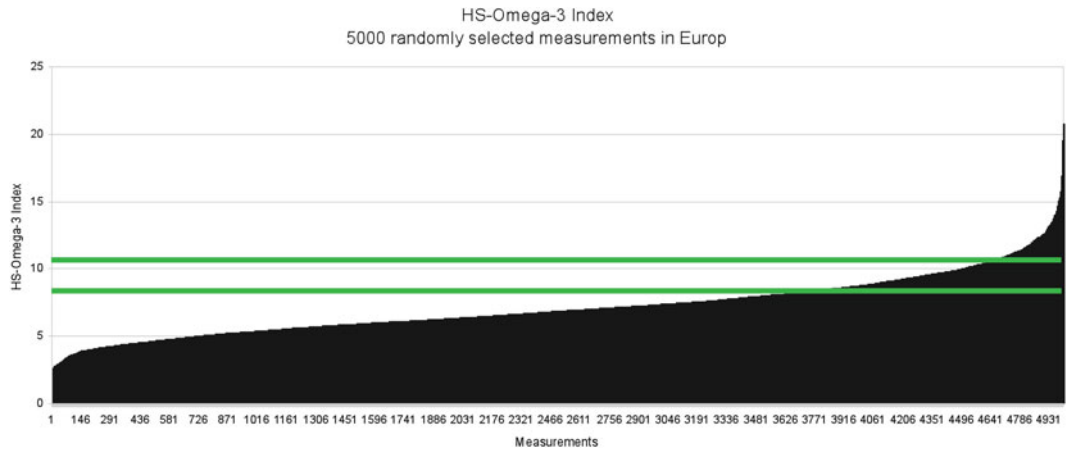

Fig. 8.3 HS-Omega-3 Index in 5,000 randomly selected European individuals. As expected, the HS-Omega-3 Index had a statistically normal distribution

was also investigated, is unclear. The same is true for the shorter trials that either did not comment on safety or palatability, or, if they did, can only provide information for the relatively short period studied. However, many food products reach the market without being subjected to scientific studies, or results of such studies are not published.

Bioavailability and Analytical Issues

Recently, it has become clear that different chemical forms of EPA and DHA differ in their bioavailability. Bioavailability of ethylesters < free fatty acids < triglycerides < recombined triglycerides < phospholipids [19–21]. This impacts on biological activity: In a randomized intervention study, 1.01 g EPA and 0.67 g DHA reduced plasma triglycerides significantly, only when given as recombined triglycerides, but not as ethylesters [22]. Moreover, bioavailability of EPA and DHA depends on other food ingested concomitantly, i.e., bioavailability is affected by matrix effects (e.g., [23]). Moreover, from person to person, absorption of EPA and DHA differs substantially ([17], Fig. 8.2). This argues for studies of sufficient size to study the effects of drinks fortified with omega-3 fatty acids. As mentioned, before every intervention trial, study size needs to be estimated based on a specific primary endpoint.

In almost all the trials mentioned, changes in fatty acid compositions, either of plasma or in red cells, were reported. The methods to analyze fatty acid composition, however, were specific to the analyzing laboratory. Results of fatty acid analyzes differ substantially from laboratory to laboratory [1, 2]. Therefore, results from one laboratory cannot be directly compared to results from another laboratory, which obviates comparing directly the bioavailability of the respective drinks. This could be remedied by a standardized method solution that had earlier been suggested for red blood cell fatty acid composition analyzes, i.e., the Omega-3 Index ([24], Fig. 8.1). The Omega-3 Index currently forms the basis for 97 publications, and many concluded and ongoing research projects, defining the Omega-3 Index as an emerging biomarker for cardiovascular risk, and other conditions [1, 2]. Among other characteristics, the Omega-3 Index correlates with tissue fatty acid compositions, and therefore reflects a person's status in terms of EPA + DHA [1, 2]. The Omega-3 Index changes in a matter of months, while the composition of plasma phospholipid fatty acids changes within hours to days [1, 2]. Therefore, in order to conduct short-term bioavailability studies, changes in plasma phospholipid fatty acid composition are often measured (e.g., [21]). These studies would become directly comparable, if the analytical method for plasma phospholipid fatty acid compositions would become standardized, similar to the Omega-3 Index [24].

Guidance on Safe Levels

In the form of menhaden oil, the FDA has ruled EPA + DHA as "generally regarded as safe" in a daily dose up to 3 g (Docket No. 1999P-5332). In large clinical trials, untoward effects of EPA + DHA were identical to placebo, e.g., at a daily dose of 1 g [1, 2]. A case can be made for other sources of EPA + DHA, similarly low in contaminants, to be similarly safe. Of the studies reviewed here, only one used a daily dose >500 mg, concentrations of EPA + DHA, however, were much lower. This raises the question of additional calories taken in with the respective drinks, as it argues for drinks with higher concentrations of EPA + DHA.

Recommendations

Any new product should be formally tested before marketing, and the results should be reported. For any new product enriched in EPA + DHA, the most important parameter is likely to be bioavailability. In case that other parameters than bioavailability are the focus of future studies, study participants should be recruited according to a low Omega-3 Index to make the studies more efficient, as discussed above, since the Omega-3 Index had a statistically normal distribution in all populations studied so far (e.g., Fig. 8.3). If parameters are to be measured that tend to be normal in a healthy study population, a study population with elevated baseline levels is more likely to demonstrate an effect of the intervention than a study population with normal baseline levels.

The quality of the studies reviewed here was quite heterogeneous, and, partly for that reason, not all studies could answer their respective study question. Improvements in the quality of design, execution, evaluation of data, and reporting, as discussed in this review are necessary in order to make a more convincing case for the use of drinks enriched in EPA + DHA. This includes, but is not limited to formulating a precise study question, defining a primary endpoint, and basing a study size estimate on it, defining secondary endpoints, conducting the trial according to the pertinent rules and regulation, including "Good Clinical Practice," and publishing the trial according to the Consort Guidelines. In order to make results comparable, the method of fatty acid analyzes will have to be standardized, as is already the case for red cell fatty acid composition by use of the HS-Omega-3 Index ([1], Fig. 8.1).

Conclusion

The drinks discussed in this review served as viable vehicles to provide EPA + DHA, because they increased EPA + DHA in plasma or red cell fatty acids. Compliance with the studies was generally "good." If assessed, palatability and safety of the drinks studied were also good, except in one study in patients with intestinal malabsorption. Untoward effects were not reported. Generally, blood lipids like triglycerides were lowered, and HDL generally increased, although the data reported were not entirely consistent. The picture is even less clear for cytokines studied, like CrP, PAI-1, sICAM-1, sVCAM-1, and E-selectin. Importantly, one study reported an increase in pain-free walking distance in patients with peripheral atherosclerosis, but in children parameters of cognition did not improve. All effects reported are in keeping with the results from trials using other sources of EPA + DHA. The quality of study design and reporting varied. Future improvements in study design, execution, and reporting, and especially a standardization of fatty acid analysis will produce more robust and clearer data that make comparisons among the drinks tested possible.

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Chapter 9
Evaporated Sugarcane Juice as a Food Fortificant

Francisco Plácido Nogueira Arcanjo, Olga Maria Silverio Amancio, and Josefina Aparecida Pellegrini Braga

Key Points

- Iron deficiency anemia (IDA) is the most widespread preventable nutritional problem in the world, despite continuous efforts for its control.
- The nutritional compositions of *rapadura* (evaporated sugarcane juice) and standard refined sugar are different in many aspects, especially iron content.
- The use of *rapadura* is extensive and differs according to the eating habits of each region where it is used.
- *Rapadura* has traditionally been used in prelacteal feeding of newborns in various regions of Asia.
- In Brazil and other countries, r*apadura* is used as a substitute for refined sugar or in direct consumption as a sweet.
- *Rapadura* is effective in increasing hemoglobin levels and reducing IDA in different populations.

Keywords Anemia • Iron • Hemoglobins • Evaporated sugarcane juice • Rapadura

Abbreviations

GDP	Gross domestic product
IDA	Iron deficiency anemia
WHO	World Health Organization

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V.R. Preedy et al. (eds.), *Handbook of Food Fortification and Health: From Concepts to Public Health Applications Volume 2*, Nutrition and Health, DOI 10.1007/978-1-4614-7110-3_9,

Introduction

Iron deficiency anemia (IDA) is the most widespread preventable nutritional problem in the world, despite continuous efforts for its control. The World Health Organization (WHO) estimates more than two billion people suffer from this condition worldwide [1]. The prevalence estimate of global anemia in preschoolers is 293.1 million cases, or 47.4 % of the total population in this age range [2]. More recent evidence from studies in animals and humans associates anemia in the first years of life to impaired cognitive development in later stages [3]. Estimates report that in developing countries annual losses due to reduction in physical productivity through anemia are US$ 3.54 per person or 0.81 % gross domestic product (GDP), and that median total losses (physical and cognitive) are US$ 16.78 per person or 4.05 % GDP [4]. To combat anemia WHO advocates three main methods: dietary diversification, to include foodstuffs rich in iron, with high bioavailability; fortification of staple food items; and iron supplements for at-risk populations [5]. Dietary diversification is probably the most sustainable means of addressing the problem of IDA. According to WHO, the most promising diversification strategies are those that include the use of locally consumed foodstuffs [5]. Popular locally consumed foodstuffs with high iron content and bioavailability are of key interest, as they can be used to tackle anemia in populations with low iron stocks and/or high iron requirements such as growing children and women of childbearing age [5, 6].

In South America, the Caribbean and parts of Asia, one regional foodstuff that is high in iron content, easily purchased, and of low-cost is *rapadura (or jaggery)*—an unbleached, unrefined sweetener. *Rapadura* is essentially pure, dried sugarcane juice, obtained from the sugarcane plant (*Saccharum officinarum*), prepared and distributed in tablet form (historical method still used today to facilitate transportation and storage). It is produced on a large scale at sugarcane plantations in tropical regions; it is used as a cheaper, more accessible substitute for refined or industrialized sugar (Figs. 9.1, 9.2, and 9.3). It contains more micronutrients than refined sugar as its preparation helps to retain most of its essential nutrients, vitamins, and minerals. The nutritional compositions of *rapadura* and standard refined sugar are different in many aspects, especially iron content: *rapadura* contains 6.43 mg iron per 100 g, whereas the amount of iron in standard refined sugar is minimal (0.02 mg per 100 g) (Table 9.1) [7].

The History and Use of *Rapadura*

The plant source of evaporated sugarcane juice is *Saccharum officinarum*, believed to have originated from New Guinea and still cultivated today in tropical and subtropical regions. Evaporated sugarcane juice is known as *panela* in countries like Colombia, Venezuela, Mexico, Equator, and Guatemala, as *piloncillo* in Mexico, *papelón* in Venezuela and Colombia, *chancaca* in Bolivia and Peru, *empanizao* in Bolivia or *tapa de dulce* in Costa Rica, and *jaggery* or *gur* in India. The name *rapadura* is used in Brazil as well as in Argentina, Guatemala, and Panama.

Man has long cultivated sugarcane, records indicate that it was already cultivated in India before 400 B.C. Attempts were first made to cultivate sugarcane in the New World by Columbus; however, he was not successful. Nevertheless, other explorers who followed were able to introduce cultivated sugarcane into the West Indies, Brazil, and Mexico [8].

The use of *rapadura* is extensive and differs according to the eating habits of each region where it is used. In Brazil, it is used as a substitute for refined sugar or in direct consumption as a sweet. Until the nineteenth Century, it was a foodstuff for slaves. In the northeast of Brazil, it was widely consumed by the low-income population, especially in rural areas, together with manioc flour as a meal consumed in the workplace; today, consumption is becoming popular again but in families with different levels of income, many children or elderly consider it a tasteful sweet [9].

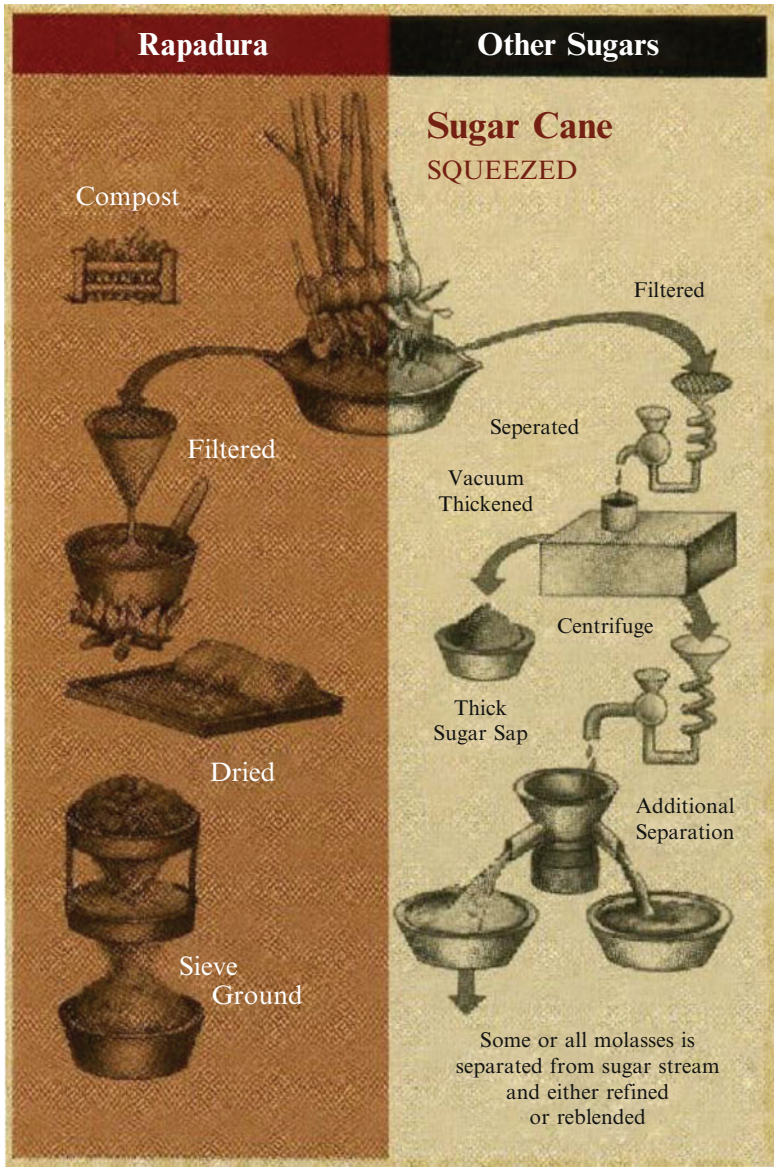

Fig. 9.1 Preparation of *rapadura* compared with refined sugar

Rapadura/jaggery has traditionally been used in prelacteal feeding of newborns in various regions of Asia. Even today, it is still common practice in India to delay breast-feeding of newborns for 48–72 h after birth, replacing it with inaugural feeds that include boiled water, tea, and diluted animal milk, with *jaggery*, sugar, or honey added [10–13]. In Ayurveda, the ancient system of Indian medicine, it has been associated with beneficial effects against several health conditions, including coughs, gastritis, diarrhea, and arthritis [8, 14]. In the northeast of Brazil, its consumption by breast-feeding women is traditionally associated to an increase in breast-milk production [9].

Fig. 9.2 Final processing of *rapadura*

Fig. 9.3 *Rapadura* as sold commercially for consumption

Table 9.1 Nutritional composition of *rapadura* and standard refined sugar per 100 g

	Calories (kcal)	Protein (g)	Total lipids (g)	Carbohydrates (g)	Fe (mg)	Mg (mg)	K (mg)	Zn (mg)	Mn (mg)	Se (mcg)	Vitamin C (mg)	Folic acid
Rapadura	430	5.6	12.8	73.8	6.43	52	346	0.45	1.26	5.6	0	89
Refined sugar	389	0	0.1	99.6	0.02	0	2	0	0	0.6	0	0

Source: USDA nutrient database for standard reference, release 14 (July 2001)

Rapadura as a Health Protector

Beneficial effects for the regular consumption of *rapadura* have been observed in Indian factory employees working in smoky and dusty environments, those consuming jaggery presented less respiratory illnesses than their non-*jaggery*-consuming counterparts. Sahu and Saxena reported on the use of *jaggery* in dust-exposed rats, results identified a preventive effect on smoke-induced pulmonary lesions in rats, providing more evidence for the potential use of the sweetener as a protective agent for workers in dusty and smoky environments [15].

Rapadura as a Food Fortificant

Despite the benefits of *rapadura* (or *jaggery*), there is little scientific evidence on the actions of systematic consumption of the sweetener. It has high iron content; however, only two clinical studies have been conducted on its use to improve hemoglobin levels and reduce anemia prevalence (Table 9.2).

Sood and Sharada used a locally produced supplement food (*laddoo*). *Laddoo* is composed of *jaggery*, processed rice flakes, garden cress seeds (100 mg% iron), and amaranth seeds (11 mg% iron) in the proportion (45:40:10:5), each *laddoo* contributing approximately 39 mg% iron. In the intervention group, 24 anemic children (aged 7–9 years) were given one *laddoo* per day for a period of 60 days, to investigate its effect on hemoglobin levels. The authors concluded that this intervention was effective in increasing hemoglobin and reducing anemia prevalence [16].

Arcanjo et al. [17], in a randomized, placebo-controlled, double-blind trial held in the northeast of Brazil, investigated whether regular consumption of *rapadura* as a natural sweetener in fruit juices (mixed with ascorbic acid) was capable of preventing and/or treating anemia in preschool children (2–3 years), during 12 weeks. Each participant in the experimental group ($n=75$) was given 200 mL cashew fruit juice sweetened with 25 g of *rapadura* mixed with 40 mg of ascorbic acid, while the individuals in the control group ($n=77$) received the same quantity of cashew fruit juice and ascorbic acid sweetened with refined sugar. The results of this study were as following: the group consuming the beverage with *rapadura*, mean Hb and hematocrit was 11.1 ± 1.09 g/dL and 36.6 ± 3.01 % at baseline and 11.6 ± 2.10 g/dL and 34.4 ± 3.01 % after intervention, $p=0.042$ and <0.0001, respectively. For the control group, mean Hb and hematocrit was 10.2 ± 1.20 g/dL and 33.8 ± 3.11 % at baseline and 10.3 ± 1.26 and 32.4 ± 2.61 % after the intervention, $p=0.44$ and 0.0035, respectively; anemia prevalence reduced significantly in the intervention group from 40 to 20 %, whereas there was

Table 9.2 Review of literature on evaporated sugarcane juice and its effects on hemoglobin levels and anemia prevalence

Authors, country, year	Title	Design, sample size	Outcomes	Results
Sood M, Sharada D, India, 2002	Iron food supplement	Before-and-after design, 60-day intervention in anemic children aged 7–9 years; intervention $n=24$, control $n=12$	Hemoglobin, height, weight	Increase in hemoglobin levels, no effect on height or weight
Arcanjo FP, Pinto VP, Arcanjo MR, Amici MR, Amâncio OM, Brazil, 2009	Effect of a beverage fortified with evaporated sugarcane juice on hemoglobin levels in preschool children	RCT, 12-week intervention in children aged 2–3 years; intervention $n=75$, control $n=77$	Hemoglobin, anemia prevalence	Increase in hemoglobin levels, 50 % reduction in anemia prevalence

Table 9.3 Intra-group comparison of the effects of consuming beverage sweetened either with *rapadura* (evaporated sugarcane juice) or refined sugar on hemoglobin levels and anemia prevalence among preschool children

	Group A (*rapadura*) ($n=75$)			Group B (refined sugar) ($n=77$)		
Mean age of study participants in years (SD)	2.6 (0.6)			2.7 (0.6)		
Estimated mean daily consumption of beverage per child (SD)	129.9 mL (53.6)			151.7 mL (31.9)		
Estimated mean daily consumption of iron per child from beverage sweetener	1.04 mg			0 mg		
	Before	After	*P*-value[a]	Before	After	*P*-value[a]
Mean Hb (g/dL)	11.1	11.6	0.042	10.2	10.3	0.44
(SD; Cl)	(1.09; 10.68–11.45)	(2.10; 11.24–12.01)		(1.20; 9.88–10.44)	(1.26; 10.04–10.59)	
Hematocrit (%)	36.6	34.4	<0.0001	33.8	32.4	0.0035
(SD; Cl)	(3.01; 35.96–37.33)	(3.01; 33.74–35.11)		(3.11; 33.11–34.40)	(2.61; 31.74–33.03)	
	Before	After	Reduction	Before	After	Reduction
Iron deficiency anemia[b] (%)	40	20	50	72.7	72.7	0

SD standard deviation; *Cl* confidence interval
[a]Based on paired Student's *t*-tests (considered significant at $p<0.05$)
[b]Defined as Hb concentration <11.0 g/dL (as per World Health Organization criteria for children <5 years old)

no decrease in the control group. The mean daily consumption of iron with the fortified beverage was 1.04 mg per child, which produced a mean increase of 0.5 g/dL in hemoglobin in the intervention group. The authors of the study concluded that *rapadura* is effective in increasing hemoglobin levels, and reducing IDA in preschool children aged 2–3 years (Table 9.3).

Despite the evidence from these studies, this is a need for more scientific investigation to evaluate its effects on hemoglobin and anemia prevalence. More research studies that contemplate other at-risk populations, especially children and women of child-bearing age, during longer intervention periods, with larger samples, which evaluate iron stocks, and studies with and without the use of iron absorption enhancers should be conducted.

Guidance on Safe Levels and/or Guidance on Levels to be Added

There are no currently available published reports from clinical studies that establish maximum levels of consumption.

Conclusions

The studies that investigate the effectiveness of foodstuffs fortified with evaporated sugarcane juice (*rapadura, jaggery, gur*), with high iron content, on hemoglobin levels and anemia are few, but their results witnessed an increase in hemoglobin levels and a reduction of anemia prevalence [16, 17]. Indicating that, these foodstuffs may be useful in the prevention or treatment of anemia in populations with high IDA prevalence. Their consumption should be stimulated within the perspective from WHO on diet diversification, which is to increase the consumption of foodstuffs with high iron content and good bioavailability [5, 6].

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Chapter 10
Fortification of Fish Sauce and Soy Sauce

Visith Chavasit, Siriporn Tuntipopipat, and Ratana Watanapaisantrakul

Key Points

- Fish sauce and soy sauce are the most popular seasoning sauces in the Southeast and East Asian regions.
- Both sauces are now mandated as vehicles for iodine in Thailand.
- In Vietnam and Cambodia, fish sauces are fortified with iron using sodium iron ethylenediaminetetraacetic acid (NaFeEDTA).
- Soy sauce in China is fortified with NaFeEDTA.
- Fish sauce in Thailand is voluntarily double-fortified with ferrous sulfate ($FeSO_4$) and potassium iodate (KIO_3); however, citric acid is required as a chelator to prevent precipitation.
- Human studies on the bioavailabilities of NaFeEDTA and $FeSO_4$ in these sauces using radio and stable isotope techniques and in populations mainly report no significant difference.
- In terms of cost, use of $FeSO_4$ with citric acid is more economical than NaFeEDTA.

Keywords Fish sauce • Soy sauce • Fortification • Iron • Iodine

Abbreviations

Fe	Iron
$FeSO_4$	Ferrous sulfate
g	Gram
hb	Hemoglobin
l	Liter
ml	Milliliter
NaFeEDTA	Sodium iron ethylenediaminetetraacetic acid
Ppm	Part per million
RDI	Recommended daily intake

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V.R. Preedy et al. (eds.), *Handbook of Food Fortification and Health: From Concepts to Public Health Applications Volume 2*, Nutrition and Health, DOI 10.1007/978-1-4614-7110-3_10,

SF	Serum ferritin
TfR	Transferrin
USI	Universal salt iodization
μg	Microgram

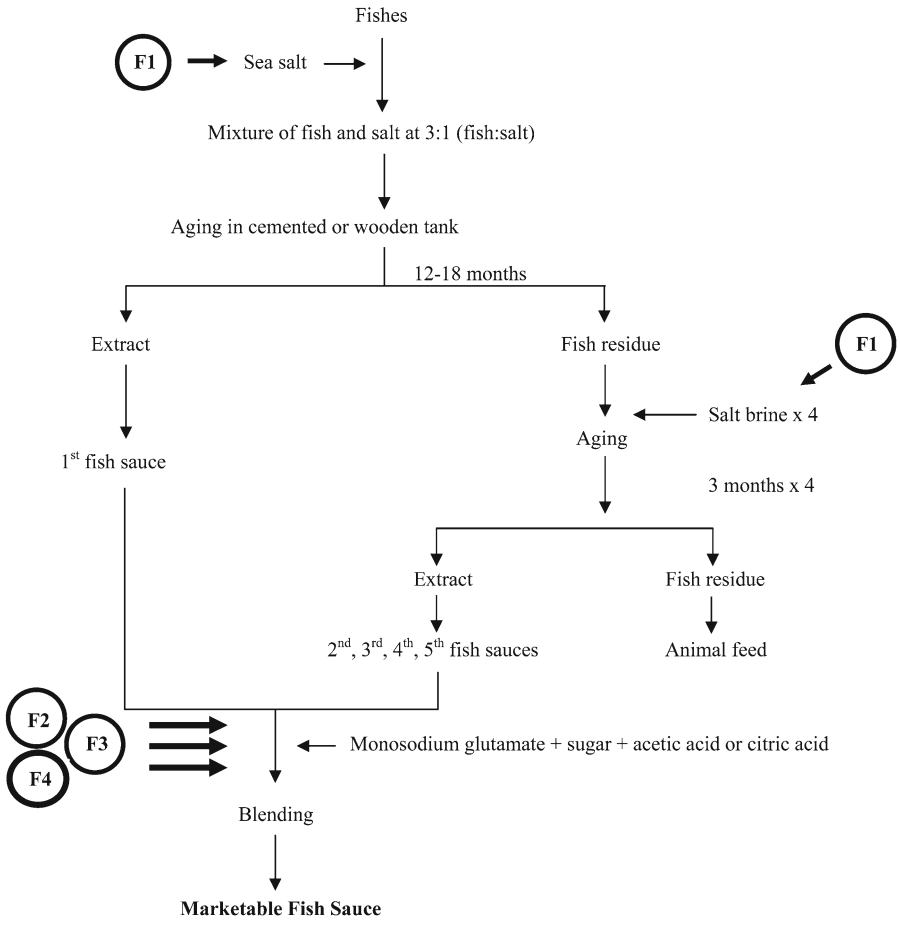
Introduction

Fish sauce is produced in many parts of the world, including Europe and Asia, though using different production methods. Fish sauces produced in China and Southeast Asian countries use a similar production technique [1]. Soy sauce originated in China, though from different parts of the country. Both sauces are necessary condiments for the cuisines of many countries in Southeast and East Asia and have been so for hundreds of years [1, 2]. Although these sauces are made from different components, both plant and animal, both sauces are protein-hydrolyzed condiments which provide salty and umami tastes [3] as well as unique aromas. The sauces' complicated flavor profiles derive from peptides, amino acids, products from browning reaction, and halophilic microorganisms, as well as volatile compounds developed from biochemical reactions.

Fish sauce is mainly consumed in Thailand, Vietnam, South China, Cambodia, Myanmar, and Indonesia [1], while soy sauce is consumed in most East Asian countries, especially China, Japan, and Korea [2]. Soy sauce is the second most popular condiment—after fish sauce—in Southeast Asian countries. The average daily consumption of fish sauce among people in Asian countries is estimated to be 20 mL per person, while average daily soy sauce consumption is 13 g per person [1, 4]. As condiments for creating a salty taste, fish sauce and soy sauce can totally replace the use of salt in some Asian countries, which can cause inadequate iodine intake from iodized salt in these populations. Since every household in the East Asian region normally has either a bottle of soy sauce or fish sauce or both, these sauces are potentially effective vehicles for micronutrient fortification, especially for iron and iodine. Iron deficiency in this region is the highest in the world [5]. Consequently, fortification of fish sauce and soy sauce with either iron or iodine, or both, is implemented on mandatory and voluntary bases in many countries of these regions. As food vehicles, fish sauce and soy sauce have advantages over other vehicles. For instance, their dark color can mask color changes due to iron fortification. Moreover, since they are in liquid form, a simple mixing technology can be used, which solves the non-homogeneity problem commonly found in the fortification of solid vehicles.

Fish Sauce Production

Different species of ocean fish, such as *Stolephorus spp., Clupea spp., and Sardinella spp*., are usually the main ingredient in fish sauce production [1]. However, fresh-water fish of related species are also used in some areas, though not to a great extent. After fresh fish are collected, they are immediately mixed with salt at a ratio of 3:1 (fish: salt). Traditionally, sea salt is used for fish sauce production. The mixture is then stored in either closed cement or wooden tanks, which are then placed in the sunlight or under shade, depending upon the production techniques of different localities. During storage, lysozyme in the fish plays an important role in protein hydrolysis, while halophilic microorganisms provide flavor. After 12 months, the liquid is collected as the first fish sauce. Salt brine can again be added into the solid residue, which is left for an additional 3 months before the liquid is collected as the second fish sauce. The same processes are later performed to produce the third, the fourth, and the fifth fish sauces. The first fish sauce is the best quality and is always used for mixing with lower grades, or even salt brine, to improve their sensory quality and standard. Fish sauce producers normally blend different fish sauces at different ratios to produce sauces of different grades and prices in the market



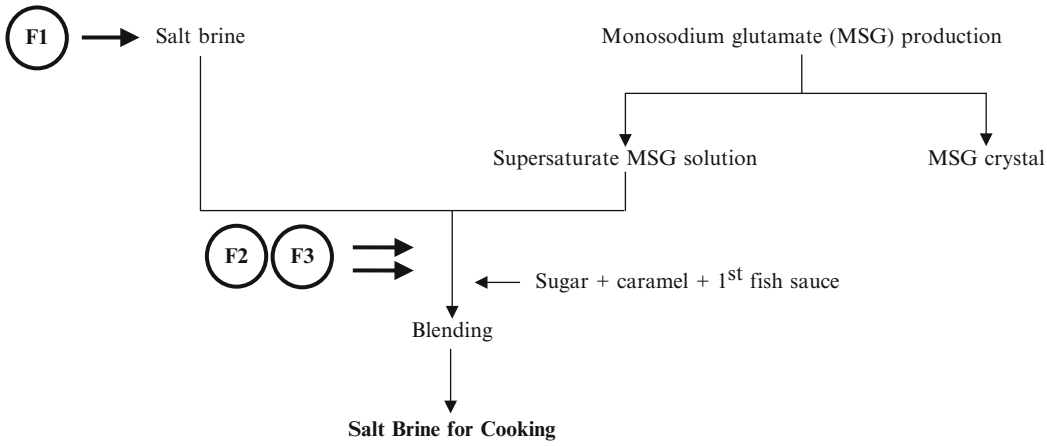
Fortification processes within the fish sauce production steps used in different countries
F1: Iodine fortification in various countries by using iodized salt (via Universal Salt Iodization, USI policy) in the production process
F2: Iodine fortification in Thailand by adding KIO_3 with other ingredients during blending process
F3: Iron fortification in Vietnam and Cambodia by adding NaFeEDTA, which requires additional batch mixing period for > 30 minutes
F4: Iron and iodine fortification in Thailand by blending $FeSO_4$ + KIO_3 + citric acid (as chelator) with other ingredients

Source: Unpublished

Fig. 10.1 Production and fortification processes of fish sauce. Production of fish sauce begins by mixing fish (normally anchovies) with salt before being aged for 12–18 months. The flavor of the fish sauce develops during the aging process. During fermentation, the fish meat decomposes using its own enzymes and other environmental microorganisms. Fortification of fish sauce can be performed by either using fortified salt for mixing and preparing a salt brine or by blending fortificants into the finished product

(Yongsawasdikul K, Plant manager, Rayong Fish Sauce Industry Co., Ltd. Rayong, Thailand, personnel communication, May 15, 2009). In the blending process, other ingredients—sugar, monosodium glutamate, and sometimes citric or acetic acid—are also added to improve flavor [6, 7] (Fig. 10.1).

Among low income populations, good quality fish sauce is too expensive to purchase. Consequently, an imitation product, often called "salt brine for cooking," is more readily available in low income markets, such as in Thailand. Imitation fish sauce or salt brine for cooking is produced by mixing salt brine with a by-product from monosodium glutamate production (supersaturated noncrystallized monosodium glutamate), caramel, and flavor. A trace amount of real fish sauce may be added to improve flavor [8, 9] (Fig. 10.2).



Fortification processes within the production steps of salt brine for cooking used in Thailand
F1: Iodine fortification by using iodized salt (via Universal Salt Iodization, USI policy) to prepare salt brine
F2: Iodine fortification by adding potassium iodate with other ingredients during blending process
F3: Iron and iodine fortification by blending $FeSO_4$+ KIO_3 + citric acid (as chelator) with other ingredients

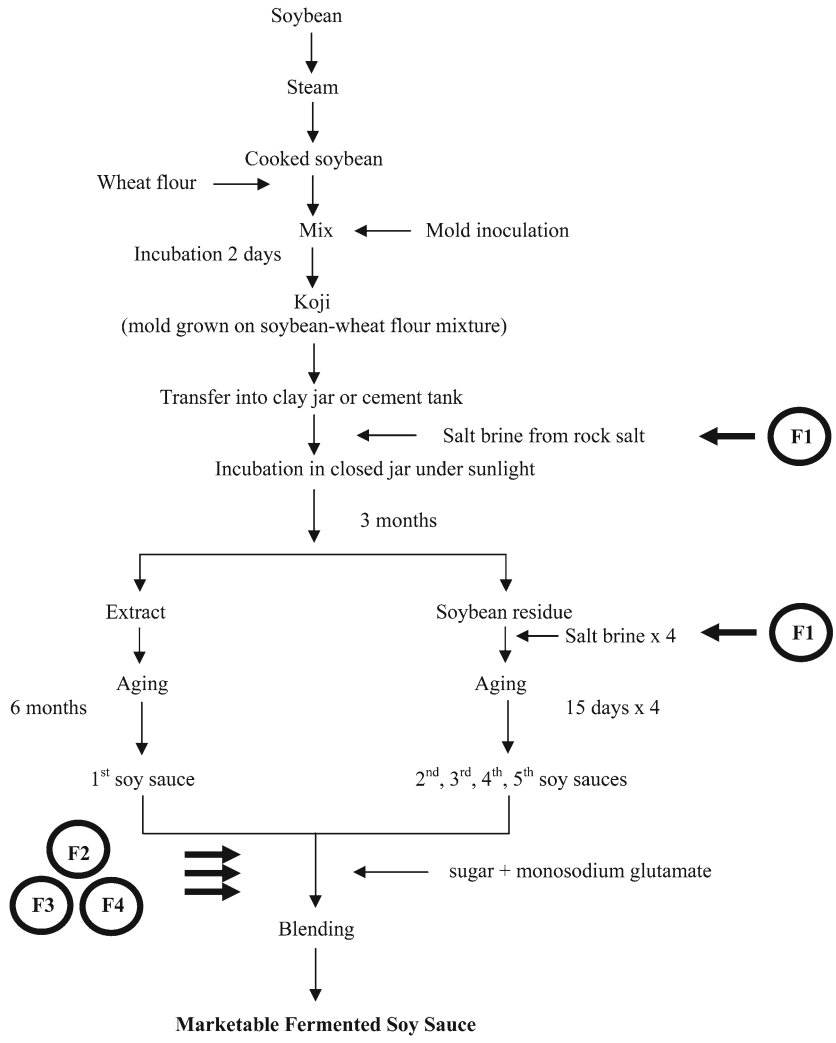
Source: Unpublished

Fig. 10.2 Production and fortification processes of salt brine for cooking. Salt brine for cooking (imitated fish sauce) is produced by mixing salt brine with an MSG solution as well as sugar and caramel. Real fish sauce also may be added in small amounts to improve flavor. Similar to fish sauce, fortification can be performed by either using fortified salt for a salt brine preparation or by blending fortificants into the finished product

Soy Sauce Production

Soybeans are the basic ingredient for soy sauce. Traditional soy sauce production begins with mixing cooked steamed soybeans with wheat flour to create a naturally selecting condition for mold growth by adjusting for the right moisture content. Thereafter, mold, such as *Aspergillus oryzae* and *Aspergillus soyae*, either from nature or inoculation, is grown on the surface of the soy bean-wheat flour mixture for 2 days, after which a white and green mold mycelium can be observed. During this period, enzymatic hydrolysis of carbohydrates, protein, and lipid by mold begins. The mold mixture, also called "koji," is then transferred into 22–25 % salt brine in a clay jar or cement tank. The container is usually closed to protect against rain water and left in the sunlight. Rock salt usually is used for preparing the salt brine. Even in salt brine where oxygen is limited, the mold dies, but mold enzymatic hydrolysis persists. After 3 months, the liquid part is collected as the first soy sauce, which may be aged for another 6 months before distribution. At the cottage industry level, this first soy sauce can now be sold. In large industries, a new salt brine is again added into the solid residue, left for 15 days, and collected as the second soy sauce. The third, fourth, and fifth soy sauces are produced following the same process. The blending of different soy sauces at different ratios results in soy sauces of different grades and prices in the market. Home-made soy sauce, which is found all over East Asian countries, is produced mainly using this traditional fermentation method [10–12] (Sumetha-aksorn P, Plant manager, Tawantip Soy Sauce Co., Ltd. Samutsakorn, Thailand, personnel communication, August 5, 2011) (Fig. 10.3).

Another type of soy sauce that is also popular among Asian people is chemically hydrolyzed soy sauce, sometimes called seasoning sauce. The production process is based on the hydrolysis of carbohydrates and protein in defatted soybeans using concentrated acid. Concentrated hydrochloric acid (HCl) is used for hydrolysis and sodium hydroxide (NaOH) is used to neutralize the hydrolysate. There is no need to add salt into this kind of soy sauce, since sodium chloride forms from the reaction

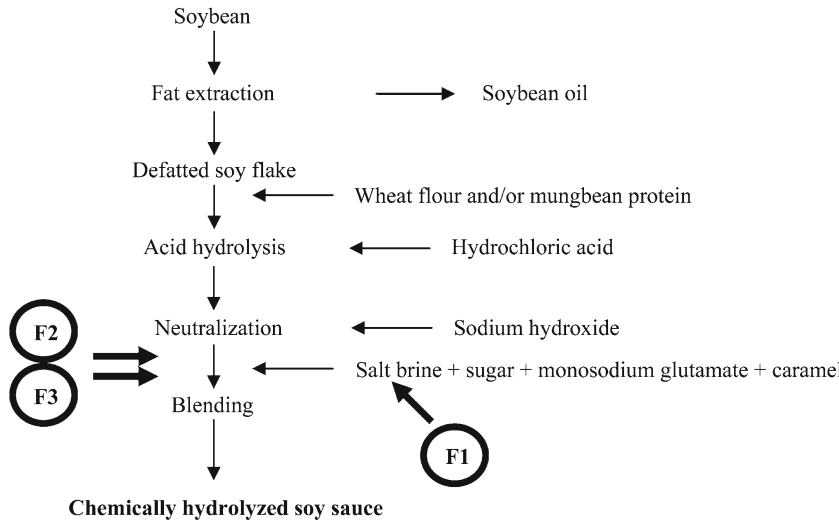


Fortification processes within the soy sauce production steps used in different countries
F1: Iodine fortification in various countries by using iodized salt (via Universal Salt Iodization, USI policy) in the production process
F2: Iodine fortification in Thailand by adding KIO_3 with other ingredients during blending process
F3: Iron fortification in China and Vietnam by adding NaFeEDTA, which requires additional mixing period for > 30 minutes
F4: Iron and iodine fortification in Thailand by blending $FeSO_4$ + KIO_3 + citric acid (as chelator) with other ingredients

Source: Unpublished

Fig. 10.3 Production and fortification processes of fermented soy sauce. The main ingredients for producing fermented soy sauce are soybean and wheat flour. The growth of the inoculated mold-produced enzymes plays an important role in developing the unique flavor of the fermented soy sauce during aging in salt brine. Fortification can be performed by either using fortified salt brine at the beginning or by blending fortificants into the finished product

between the added acid and base. Other sources of protein, such as wheat flour or mungbean protein, are also used in addition to defatted soybeans. The entire production period takes only 24 h (Fig. 10.4). Small industries usually do not produce this soy sauce because of the high investment cost of equipment, which needs to be highly resistant to acid corrosion. The chemically hydrolyzed soy sauce has a lower production cost and a shorter production period. However, the product lacks a natural fermentation



Fortification processes within the production steps of chemically hydrolyzed soy sauce used in Thailand
F1: Iodine fortification by using iodized salt (via Universal Salt Iodization, USI policy) to prepare salt brine
F2: Iodine fortification by adding KIO_3 with other ingredients during blending process
F3: Iron fortification by blending $FeSO_4$ and citric acid (as chelator) with other ingredients

Source: Unpublished

Fig. 10.4 Production and fortification processes of chemically hydrolyzed soy sauce. Chemically hydrolyzed soy sauce imitates the production process of fermented soy sauce by digesting soy protein (no fat) with strong acid instead of using natural enzymes from mold. The acid-hydrolyzed product cannot yet be consumed due to a high acid concentration; therefore, a base is needed to neutralize the acid. From this chemical reaction, sodium chloride (salt) is formed. The product is then diluted with salt brine and blended with other ingredients to improve flavor. Fortification can be performed by either using fortified salt for preparing salt brine or by blending fortificants into the finished product

flavor. In addition, another problem with chemically hydrolyzed soy sauce is the emerging carcinogen 3 MCPD (3 monochloropropane-1,2 diol/3-chloro-1,2-propanediol), which makes the product prohibited in many countries. Compared to traditionally fermented soy sauce, chemically hydrolyzed soy sauce is consumed more among urban poor populations [9, 12].

Differences in soy sauce production processes result in different peptide chain lengths. Compared to chemically hydrolyzed soy sauce, which is produced using a more severe hydrolysis process, traditionally fermented soy sauce contains more peptide and less nonprotein nitrogen (from free amino acid). This factor can affect the bioavailability of the fortified mineral. As mentioned in many previous studies, a shorter peptide chain can enhance iron absorption [13–15].

Iodine Fortification of Fish Sauce and Soy Sauce

Potassium iodate (KIO_3) is normally used as an iodine fortificant in the Southeast Asian region due to its stability under the hot and humid conditions; even potassium iodide is also allowed. Mandatory salt iodization began in Thailand in 1994 [9], however, only for table salt, which is seldom used among many population groups. Most urban Thai households, for example, prefer to use fish sauce and soy sauce to create a salty taste rather than table salt, which limits their consumption of fortified iodine in

table salt. Universal salt iodization (USI), in which salt for food processing and feed must be iodized, should be an efficient strategy for solving iodine deficiency [16]. However, USI is not practical in Thailand. Sea salt used for fish sauce production is normally not pure as it is contaminated with different kinds of hygroscopic salt. Fortified iodine is lost due to the migration of the iodine solution from the salt surface, especially during storage (piling) in factory warehouses [17]. The stocked salt, which is later (may be up to 1 year later) used for fish sauce production, contains no iodine. On the other end of the spectrum, if well-controlled, good quality iodized salt (containing 30 ppm iodine) is used for fish sauce production, the residual iodine content in the sauce can be much too high (>100 μg per serving) [18]. To better control the iodine content in fish sauce, Thailand's Food and Drug Administration in 2010 allowed fish sauce producers to fortify their finished products with 2–3 ppm iodine (30–45 μg iodine per serving of 15 mL fish sauce) during the fish sauce blending step, instead of using fortified sea salt at the beginning [9]. This approach is more practical, controllable, and economical (Fig. 10.1).

For fermented soy sauce, rock salt also has the hygroscopic problem. In Thailand, therefore, iodization of the finished product is allowed for fermented soy sauce as well [9] (Fig. 10.3), which can be done during the blending process instead of using iodized salt for fermentation. For chemically hydrolyzed soy sauce, there is no choice other than fortifying iodine into the finished product during the blending process, since most salt in the product occurs from a chemical process and not by addition (Fig. 10.4). An imprecise amount of salt is normally added with salt brine to standardize the total nitrogen content in the final product, which can result in uncontrolled iodine content in the finished product (Sumethaaksorn P, Plant manager, Tawantip Soy Sauce Co., Ltd. Samutsakorn, Thailand, personnel communication, August 5, 2011).

Iron Fortification of Fish Sauce

Garby and Areekul (1974) conducted a study on fish sauce fortification, where the fortificant used was NaFeEDTA [19]. Other iron fortificant types caused precipitation due to their interaction with protein in the fish sauce. In 1997, Suwanik et al. also investigated the double fortification of fish and soy sauces using NaFeEDTA and KIO_3 [20]. The method developed by Garby and Areekul, however, was not used, since food-grade NaFeEDTA was not yet commercially available. In 1998, fish sauce fortification with iron in Vietnam was conducted using food-grade NaFeEDTA developed by Akza Nobel Functional Chemicals Co., Arnhem, the Netherlands. The fortification dosage was 1 mg Fe per mL or 10 mg Fe per serving [21]. Although NaFeEDTA does not cause precipitation in fish sauce, its solubility is not acceptable. Consequently, an efficient motor-driven mixing tank, a mixing time of at least 30 min, and a filter are required to ensure homogeneity of the fortificant in the product [22] (Fig. 10.1). Solubility is not the only reason for using NaFeEDTA. Many studies have shown that iron chelated in EDTA (as NaFeEDTA) can have greater bioavailability in the human body than iron from other fortificants if the foods contain high absorption inhibitors, such as phytate [23]. Fish sauce fortified with NaFeEDTA is now commercially produced in Vietnam and Combodia with support from international organizations [24]. However, the use of NaFeEDTA in actual business practice can be limited due to its much higher cost compared to other iron fortificants and the limited number of global suppliers [25] (Table 10.1). The FAO/WHO Expert Committee on Food Additives (JECFA) also limits the amount of NaFeEDTA consumed by children aged 6 months—2 years to be lower than 2 mg per kg body weight [26]. Furthermore, the mixing step, which requires an efficient mixing tank, can be a bottleneck for large industry.

Another technique for iron fortification in fish sauce was developed in Thailand in 2001 [8]. This technique uses ferrous sulfate ($FeSO_4$), which is much lower in cost than NaFeEDTA and is produced in many countries all over the world. To prevent precipitation, 0.2–0.3 % food-grade citric

Table 10.1 Percentages of iron and relative costs (price, iron content, and % bioavailability) of different, normally used iron fortificants

Fortificant	% Iron	Relative cost per unit of iron	% Bioavailability	Relative cost after being adjusted by % bioavailability
H-reduced elemental iron	98.0	1	20	0.05
Electrolytic elemental iron	98.0	3	50	0.06
Ferrous sulfate	31.6	2.6	100	0.026
Ferrous fumarate	30.6	3.4	101	0.034
Ferric orthophosphate	26.0	5.9	31	0.19
Ferrous lactate	20.5	22.8	106	0.22
Ferrous gluconate	12.5	26.2	89	0.29
NaFeEDTA	12.5	50.4	250	0.20

Source: Nilson A, Piza J. Food fortification: a tool for fighting hidden hunger. Food Nutr Bull 1998;19(1):49–60
Iron fortificants are good examples of commercial fortificants that have various chemical forms and are available in the market. To choose the appropriate form for use, cost cannot be the only factor used in decision-making. Information on iron content and % bioavailability also are required, and the consideration should be based on relative cost

acid must be added along with $FeSO_4$. Citric acid acts as a chelating agent, which can prevent iron from interacting with peptide and protein in fish sauce and causing precipitation. Fortification can be performed during the normal blending process. The fortificant and citric acid can be mixed with sugar and monosodium glutamate and dissolved in the fish sauce in the mixing pond without the need for extra equipment, such as a special mixing tank (Fig. 10.1). In fact, citric acid is one ingredient generally used for improving the sensory quality of fish sauce in Thailand. As a result, fish sauce producers easily accept it. They also voluntarily employ this technique using their own funds as part of double fortification with iron and iodine.

Iron Fortification of Soy Sauce

Soy sauce fortification began in China in 1998 with NaFeEDTA being used as the iron fortificant [4, 27]. Since China can produce NaFeEDTA, the use of this fortificant may not be too costly. Iron-fortified soy sauce is now commercially produced by large industries, but it is not yet widespread throughout the country. Similar to fish sauce, $FeSO_4$ can also be used with the addition of either citric acid or sodium citrate as a chelator. As mentioned above, soy sauce is produced by at least two methods: traditional fermentation with mold or chemical hydrolysis. Each method results in products of different peptide chain lengths, which can cause precipitation. Chemical hydrolysis, which is a more severe process, results in a product that contains a shorter chain peptide and more amino acid. The iron-fortified chemically hydrolyzed soy sauce, therefore, is quite stable without precipitate. In fact, citric acid is not required for preventing precipitation in this product; however, it can enhance fortificant solubility during fortification, especially with $FeSO_4$. Traditional fermentation of soy sauce results in a product that contains a more long chain peptide, which makes it easily precipitated. To use $FeSO_4$ as an iron fortificant, citric acid is required for the fermented soy sauce to prevent precipitation as well as to enhance fortificant solubility. In some soy sauce industries, traditionally fermented and chemically hydrolyzed soy sauces may be mixed in order to reduce the cost. To fortify such mixed soy sauce with $FeSO_4$, sodium citrate is needed as a chelator to prevent precipitation [28] (Fig. 10.3). Benefits in using $FeSO_4$ with citric acid or sodium citrate to fortify soy sauce are the same as for fish sauce fortification [25].

Absorption of the Fortified Iron

As mentioned above, $FeSO_4$ and NaFeEDTA are the only two fortificants used to fortify fish sauce and soy sauce. During the 2003–2008 period, at least four studies on iron absorption of fortified fish sauce were conducted in Switzerland, Thailand, Vietnam, and Cambodia [21, 22, 29, 30]. In addition, studies on the iron absorption of fortified soy sauce were conducted in Switzerland and China as well as South Africa since 1990 [27, 29, 31] (Table 10.2).

Table 10.2 Studies and results on absorption of fortified iron in fish and soy sauces

Product	Fortificant	Type	Result	Reference
Fish sauce, Soy sauce	NaFeEDTA, $FeSO_4$	Stable isotope (^{57}Fe or ^{58}Fe)	No significant difference in iron absorption between both fortificants and the same fortificant of both sauces	Fidler et al. [29]
Fish sauce	Ferric ammonium citrate, Ferrous lactate, $FeSO_4$ with citric acid	Stable isotope (^{57}Fe or ^{58}Fe) in normal women	The highest absorption is $FeSO_4$ with citric acid	Walczyk et al. [30]
Fish sauce	NaFeEDTA	Randomized-double masked in anemic women for 6 months	The prevalence of iron deficiency and iron deficiency anemia were lower in the iron-fortified group than the control group after 6-month intervention	Thuy et al. [21]
Fish sauce	NaFeEDTA, $FeSO_4$ with citric acid	Randomized, double blinded, placebo-controlled trial in iron deficiency anemic students for 21 weeks	Both fortificants significantly and similarly improved the iron statuses of the subjects	Longfils et al. [22]
Soy sauce	NaFeEDTA, $FeSO_4$	Stable isotope (^{54}Fe and ^{58}Fe)	Iron absorption rate of NaFeEDTA was 2.2 times higher than that of $FeSO_4$ in adult women consuming a typical plant-based Chinese diet	Huo et al. [27]
Soy sauce	NaFeEDTA	Effectiveness of marketed fortified product	The adoption rate increased from 8.9 to 36.6 % and the hemoglobin levels of adult women and young children increased by 9.0 g/L and 7.7 g/L, respectively, after 2 years of the product's launch	Wang et al. [31]
Soy sauce with soy peptides	$FeSO_4 \cdot 7H_2O$	Radio isotope (^{59}Fe and ^{55}Fe)	The fermented products in soy sauce promote absorption	Baynes et al. [14]
Food iron with traditional oriental unfermented and fermented soy products	$FeSO_4 \cdot 7H_2O$	Radio isotope (^{59}Fe and ^{55}Fe)	There was an inverse relationship between food iron absorption and the high-molecular-weight fraction of the soy products	Macfarlane et al. [15]

Source: Unpublished

Most iron-fortified fish and soy sauces have been studied for absorption of the fortified iron in the human body. These studies usually were performed in anemic persons; hence, NaFeEDTA and $FeSO_4$ did not show a significant difference in iron absorption

In 2003, Fidler et al. evaluated iron absorption from NaFeEDTA which was added into Vietnamese fish sauce and Chinese soy sauce as compared to $FeSO_4$ as a reference fortificant [29]. The study was performed in Switzerland by using the double stable isotope technique in adult women. Iron absorption was evaluated by erythrocyte incorporation of double iron isotopes (^{57}Fe or ^{58}Fe) after intake of the labeled test meals for 14 days. The test meals consisted of: (1) rice + sauce + vegetables; (2) rice + sauce, and (3) rice. Each test meal had the same content of phytic acid (25–27 mg per serving) and added iron (5 mg per serving), which was at a molar ratio 0.4:1 of phytic acid to iron. Results showed that iron absorptions from NaFeEDTA and $FeSO_4$ of each sauce were not significantly different, as well as the absorptions of iron from NaFeEDTA in both sauces. Meanwhile, soy sauce had an inhibitory effect on iron absorption of rice-based meals (8.5 % without vs. 6.0 % with soy sauce).

Previous studies on iron absorption from fish sauce were performed by directly adding fortificant with meal and sauce [13, 29]. However, later studies using industrially iron-fortified products were performed in Thailand, Vietnam, and Cambodia [21, 22, 30]. The Thailand study was reported in 2005 and used a double stable isotope technique similar to the study in Switzerland [29]. Iron absorption from Thai fish sauces, which were fortified with $FeSO_4$, ferrous lactate, or ferric ammonium citrate with 0.3 % citric acid as a chelator, was evaluated and compared with $FeSO_4$ as a reference fortificant. Among the three iron fortificants, iron from the Thai fish sauce fortified with $FeSO_4$ with citric acid was absorbed the most [30].

Population studies in Vietnam and Cambodia, however, were conducted to evaluate the effect of fortified iron on improving iron status and reducing the prevalence of anemia. In 2003, Thuy et al. conducted a randomized, double masked study to assess the efficacy of NaFeEDTA-fortified Vietnamese fish sauce in 152 anemic women served with rice or noodle-based meals containing 10 mL of fish sauces with or without 10 mg iron addition 6 days per week for 6 months. The prevalence of iron deficiency (SF < 12 μg/L or TfR > 8.5 mg/L) and iron deficiency anemia (hb < 120 g/L) were lower in the iron-fortified group than the control group (32.8 % vs. 62.5 %, and 20.3 % vs. 58.3 %, respectively) after 6 months intervention [21].

A Cambodian study was conducted as a randomized, double blinded, placebo-controlled intervention trial among 140 iron deficiency anemic students (6–21 years old). The trial lasted for 21 weeks. During this time, the students consumed 114 school meals containing 10 mL Khmer fish sauce with or without 10 mg Fe per meal. The study compared changes in hemoglobin, serum ferritin (SF), C-reactive protein, body weight and height, as well as prevalences of vomiting, diarrhea, and acute respiratory infections due to the consumption of fish sauces fortified with NaFeEDTA and $FeSO_4$ with citric acid. Both fortificants significantly and similarly improved the iron statuses of the subjects, which led to the conclusion that Khmer fish sauce is a suitable vehicle for iron fortification and $FeSO_4$ + citric acid and NaFeEDTA were equivalent in efficacy and safety [22].

Fidler et al. found no difference in iron absorption of $FeSO_4$ and NaFeEDTA in soy sauce [29]. However, a study on iron absorption in iron-fortified soy sauce conducted in China by using ^{54}Fe and ^{58}Fe for $FeSO_4$ and NaFeEDTA, respectively, indicated that the iron absorption rate of NaFeEDTA was 2.2 times higher than that of $FeSO_4$ in adult women who consumed a typical plant-based Chinese diet. Plant-based diets, however, might contain high iron absorption inhibitors, which could be overcome by NaFeEDTA [27]. A study was performed to evaluate the effect of the marketed fortified soy sauce on the iron status of a Chinese iron-deficient population. Results showed that after 2 years of the product's launch, the adoption rate increased from 8.9 to 36.6 %, and the hemoglobin levels of adult women and young children increased by 9.0 g/L and 7.7 g/L, respectively [31].

In addition to the type of fortificant used, differences in peptide size in the food vehicle can also have a significant impact on iron absorption. A study on the absorption of iron ($FeSO_4 \cdot 7H_2O$) added into rice-based meals with soy flour, soy sauce, or lactic acid was performed in South Africa using radioisotopes (^{59}Fe and ^{55}Fe). The results indicated that soy protein might inhibit iron absorption; however, the fermented products in soy sauce can promote absorption [14]. Macfarlane et al. (1990) also used the same radioisotopes to study the effect of nine types of traditional oriental unfermented

and fermented soy products on dietary iron absorption. Results showed that there was an inverse relationship between food iron absorption and the high-molecular-weight fraction of the soy products ($r = 0.66$, $p = 0.01$) [15]. In addition, an in-vitro study on iron dialyzabilities in iron-fortified fermented and chemically hydrolyzed soy sauces indicated that more dialyzable iron was found from the chemically hydrolyzed soy sauce, which consisted of shorter peptide chain and more amino acid [32]. Further studies in humans, however, are needed to verify the in-vitro finding.

Guidance on Fortification Levels

One advantage to fortifying fish and soy sauces, which are liquids, is the solubility and homogeneity of the fortificant in these products. Normally, the fortification level is established based on the serving sizes of these sauces. The maximum fortification level per serving should not be more than 1/3 of the recommended daily intake (RDI). Since the bodily requirements for micronutrients are very small in amount, the volumetric household measurement of the fortificant powder (e.g., measuring spoon) is absolutely unacceptable. Only appropriate, precise scales or balances should be used. For iodine fortification, 2–3 ppm of iodine is added in order to attain a maximum level of 30–45 μg per serving, which is 60–90 % of the requirement. In small-scale production where only a trace amount of iodine fortificant is required, such as 5 g KIO_3 per 1,000 L of sauce, it may not be possible for a small producer to afford a costly balance or scale of such precision. Consequently, preparation of a fortificant stock solution is recommended. For example, a scale of 25 g precision is widely available at an affordable price for small industries. Hence, a stock solution containing 50 g KIO_3 in 1,000 mL can be easily prepared. Once being used, 100 mL of the iodine stock solution is mixed into 1,000 L of sauce. Compared to iodine, the fortification dose of iron is much higher; however, appropriate weighing is still required. Since iron can be more naturally found in foods than iodine, the maximum fortification level is normally lower than 1/3 of RDI per serving. However, nutrient bioavailability from the fortificant, as well as the presence of inhibitors in the diets of vulnerable populations, can also affect the fortification dose, especially for iron. The fortification dose of iron in fish sauce in Thailand is 3 mg Fe per serving, while the fortification doses of iron in fish and soy sauces in Vietnam, China, and Cambodia are 10 mg per 10 mL. Even though the total safe daily intakes of micronutrients, such as iron and iodine, are 4–6 times beyond the RDI, accurate quality control of the fortified products is still required, since long-time exposure to a very high fortification dose can affect a population's health.

Recommendations

The selection of a fortificant for a national food fortification program can directly affect program cost and consequently product price. A partnership between government and industry may not be possible if the fortification cost is too high, especially for food products with low profit margins, such as fish sauce and soy sauce. Hence, the fortificant selected should not cause problems in terms of nutrient cost and the production process; for instance, the fortification process should not lead to a bottleneck in the production process.

In a country where salt is not the main ingredient for achieving a salty taste, fortification of fish and soy sauces can be a better strategy for combating certain micronutrient deficiencies, such as iodine and iron. The fortification process is also much easier for these vehicles. However, in-line quality control for these fortified products should be properly conducted during processing in order to minimize complications in the finished product analysis, especially for the iodine-fortified products which need to be analyzed by using inductively coupled plasma mass spectroscopy (ICP-MS).

Conclusion

Fish sauce and soy sauce are good vehicles for micronutrient fortification, especially for iodine and iron. Available fortification techniques differ by country, but they have been shown to be feasible and have been practiced at an industrial level.

Policy makers, however, should look at both sides of the coin when considering fortification, as the fortified sauces can have benefits and drawbacks. For instance, though they make good vehicles for micronutrient fortification, both sauces are high in sodium which can aggravate rates of hypertension and other non-communicable diseases in populations. A campaign on reducing sodium consumption, therefore, could have a negative effect on micronutrient intake, especially in countries that are experiencing a double burden in terms of micronutrient deficiencies (such as in iron and iodine). Reaching a balance between sodium reduction and an increase in the fortification dosage, as well as the implementation of effective monitoring systems, should be one major goal for improving the health and well-being of vulnerable populations.

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Part II
Impact on Individuals

Chapter 11
Targeting Pregnant and Lactating Women and Young Children with Fortified Foods

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Key Points

- Untargeted fortified foods contain micronutrients in quantities too low to meet the high micronutrient needs of women during pregnancy and lactation and children during the complementary feeding period (6–23 months of age).
- Fortified complementary foods are effective to improve micronutrient status, child health, growth, and possibly development outcomes.
- Recommendations exist for the nutrient composition (quantity and formulation) of fortified complementary foods; for best outcomes, these should be followed and attention paid to the quality of the foods.
- Fortified complementary foods should be delivered as part of integrated nutrition strategies to ensure promotion of their utilization according to recommendations in the context of overall appropriate breast and complementary feeding practices.
- Evidence for the impact of fortified foods during pregnancy on pregnancy outcomes is also strong, but further research is needed to refine the guidelines used for formulations of fortified foods in different contexts.
- For all targeted food fortification programs, strong communication and promotion strategies are required to overcome barriers to regular utilization by the intended groups in quantities sufficient to improve micronutrient intake, and ultimately health and nutrition outcomes.

Keywords Birthweight • Birth outcomes • Targeted fortified foods • Complementary foods • Growth • Development

Abbreviations

CI	Confidence interval
cm	Centimeters
Hb	Hemoglobin
LAZ	Length for age Z-score

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V.R. Preedy et al. (eds.), *Handbook of Food Fortification and Health: From Concepts to Public Health Applications Volume 2*, Nutrition and Health, DOI 10.1007/978-1-4614-7110-3_11,

LBW Low birth weight
LNS Lipid-based nutritional supplements
mo Months
OR Odds ratio
RNI Recommended nutrient intakes
WAZ Weight for age Z-score

Introduction

Micronutrient deficiencies are highly prevalent among pregnant women, infants, and young children because of their high nutrient needs relative to energy intake. In resource poor settings, limited dietary diversity, particularly low consumption of micronutrient rich foods, and the effects of frequent infection on appetite, nutrient absorption, and nutrient losses exacerbate the problem of low intake.

Fetal and child malnutrition impacts health trajectory throughout the life course and is an important predictor of survival, growth, health and cognitive, motor, and behavioral development during childhood [1]. Micronutrient deficiencies during pregnancy and the first 2 years of life cause irreversible damage, particularly on brain development and other functional outcomes [2] and put women at risk of a number of health effects, including anemia. Early malnutrition may also impact adult health. Follow-up studies of historic cohorts from transitional countries have found associations among low birth weight (LBW), underweight, and stunting (i.e., being short for age) in young children and later occurrence of central obesity, insulin resistance, type 2 diabetes, hypertension, and cardiovascular disease [3]. Moreover, women who had been malnourished themselves during gestation and early childhood are more likely to give birth to infants with LBW [1]. An estimated 2.9 million newborns die in their first month of life every year, largely due to small size at birth [4].

The 2008 Lancet series on nutrition concluded that micronutrient interventions are among the proven efficacious interventions to reduce maternal and childhood malnutrition [5]. In addition, the Copenhagen Consensus 2012 list micronutrient interventions as part of bundled interventions to reduce malnutrition in young children as some of the best development investments [6]. A number of interventions exist to improve the micronutrient intakes of populations during pregnancy and early life, including supplementation and what is sometimes referred to as home fortification. Home fortification refers to the addition of micronutrients in powder or nutrient dense paste form to a food immediately before consumption. A number of these interventions have been shown efficacious to improve micronutrient status, reduce anemia, and to improve some functional outcomes [7]. For the purposes of this chapter we will limit our discussion to fortified foods developed specifically to meet the needs of pregnant women and young children, reporting the results for home fortificants, supplements, or other interventions only where these have been compared to targeted fortified foods.

Reaching Pregnant Women and Young Children with Fortified Foods

Fortification of staple foods and condiments is one of the strategies promoted by the World Health Organization and Food and Agricultural Organization of the United Nations to increase the intake of micronutrients in populations [8]. Although proven effective for this purpose, fortified staples and condiments will not ensure adequate micronutrient intakes of pregnant and lactating women and infants and young children. Micronutrient requirements during these critical life stages with rapid growth are substantially increased, and thus, the quantity of nutrients per given amount of food (the micronutrient density) must be higher. Particularly for children during the complementary feeding period, stomach capacity is limited and they would be unable to eat sufficient

quantities of fortified staple foods to cover their higher micronutrient requirements. Increasing the amounts of micronutrients in fortified foods intended for the general population to cover the higher needs of pregnant women and infants would pose a safety risk for excessive intakes of some micronutrients given the larger amounts of food that they consume, particularly adult men [9]. Therefore, if fortification is used to address inadequate micronutrient intakes in pregnant women, infants, and young children special, targeted fortified foods formulated with high micronutrient density are required.

In this chapter we will review the evidence for the impact of targeted fortified foods for pregnant women and young children on micronutrient status, pregnancy outcomes, growth, and development and present a case study from Mexico exploring some of the logistical considerations in the design and implementation of programs that distribute such products to populations living in poverty.

Evidence of the Efficacy of Targeted Food Fortification for Pregnant Women and Lactating Women

A recent review examined the impact of vitamin- and mineral-fortified products developed specifically for pregnant and lactating women on maternal nutritional status and growth, birth outcomes, and development of the offspring [10]. The review included findings from 16 studies in low- and high-income countries and included interventions with micronutrient-fortified beverages with or without milk, fortified supplementary foods, and fortified high-fat supplements. Fortification levels in the studies ranged generally from 50 to 100 % of the recommended nutrient intake (RNI) and commonly contained iron, zinc, copper, iodine, selenium, vitamins A, D, E, C, B1, B2, B6, and B12, folic acid, niacin, and pathothenic acid. In some studies the fortified product was evaluated against the unfortified product (as control group) keeping energy the same, whereas in other studies the control group received a micronutrient supplement only, a lower energy placebo drink, or no intervention at all, making it difficult to disentangle effects of micronutrients alone.

Despite these limitations, the authors of the review concluded that both micronutrient-fortified beverages and fortified supplementary foods, when given during pregnancy, have positive effects on prevention of maternal anemia and iron deficiency. For example, anemia prevalence was reduced by approximately one-third in fortified beverage studies in Tanzania [11] and haemoglobin concentration increased by 4–7 g/L in the Tanzania study [11] and in a fortified milk powder study in Chile [12].

Fortified supplementary foods during pregnancy, especially those containing milk and/or essential fatty acids, increased mean birthweight by around 60–73 g [10]. Under certain circumstances, for example during times of food shortages in food insecure regions of the Gambia, the impact on birthweight was much larger, as high as 115–330 g [13, 14]. The authors of the review conclude that both the macro- and micronutrients contribute to the positive effects of fortified foods on birthweight.

Some more recent studies have been designed specifically to explore the impact of fortified foods with high essential fat content on birth outcomes. Results show positive impact of supplementation on birth length, placental weight, very-early preterm delivery, preeclampsia and intrauterine growth retardation, stillbirth, and neonatal mortality. For example, in two studies where essential fatty acids were provided, birth length was 0.4–0.5 cm greater in the treatment group than in the control group [13, 15].

In some studies, the effect of fortified targeted foods on pregnant outcome was dependent on maternal nutritional status prior to and/or in early pregnancy. For instance, fortified food supplementation during pregnancy was not related to maternal weight gain during pregnancy in the majority of studies, but was so when maternal weights were low at start. Similarly, increasing intake of energy was related to improvements in birthweight, but primarily in women who were more malnourished prior to or early in pregnancy.

In summary, micronutrient-fortified foods and beverages provided during pregnancy have the potential to improve micronutrient status and reduce anemia rates in women. In addition, micronutrient-fortified foods, when combined with energy and essential fatty acids, may result in improved pregnancy outcomes, including increased birth weight and length but further research is needed to identify optimal content of fortified foods in specific contexts. Evidence suggests that the effects will be larger in women who are less well nourished prior to or in early pregnancy.

Evidence of the Efficacy of Targeted Food Fortification for Infants and Young Children

Complementary feeding interventions have been shown efficacious to reduce the prevalence of malnutrition among children 6–23 months of age. A recent systematic review evaluated the effect of different complementary food interventions on growth, morbidity, micronutrient status, and development [16]. Although this review did not use a formal meta-analysis, mean treatment effect sizes were calculated. The effect size refers to the difference in mean for each outcome in the intervention and the comparison groups, taking the expected variation in those outcomes (standard deviation) into account. An effect size of 0.2 is considered a small impact and 0.8 is considered a large impact. Interventions were grouped into categories depending on the main strategies used and mean effect sizes were calculated for each category of intervention to obtain a rough estimate of overall impact. Table 11.1 provides a summary of the evidence of targeted fortified foods for young children.

Child Growth

The review includes seven efficacy trials and one program evaluation (effectiveness study) in which the only intervention strategy was provision of complementary food (fortified or unfortified). In seven studies the comparison group received no intervention; in one a very low-energy food was included as comparison. The results of the review were somewhat inconsistent: there was a positive impact on either weight gain or length gain or both in Ghana [17, 18], Nigeria [19], Zambia [20], and Malawi [21], but no impact was observed in South Africa [22], Indonesia [23], or Brazil [24]. These apparently conflicting results may be explained, at least in part, by methodological differences.

The studies in Malawi and Nigeria included malnourished children only, and the Nigerian study provided high energy, which was not the case for the studies in South Africa, Indonesia, or Brazil. The overall mean effect size was 0.60 (range −0.02, 2.99) for weight and 0.47 (range −0.04, 1.81) for linear growth, but these effects are inflated by the results from Nigeria [19] (effect sizes: weight = 2.99, length = 1.81). Excluding that study, the mean effect size was modest, 0.26 (range −0.02, 0.57) for weight and 0.28 (range −0.04, 0.69) for length.

The effect of fortification of complementary foods, i.e., compared to a non-fortified food with similar energy content, was evaluated in six efficacy trials. Three of these studies involved home fortification using lipid-based nutrient supplements providing micronutrients and energy [18, 25, 26]. The others used cereal/legume mixes [17, 27] or a fortified milk formulation [28]. Only in the fortified milk study was there a significant impact on growth. The average effect size for all 6 studies was 0.11 (range −0.22, 0.73) for weight and 0.12 (range −0.02, 0.45) for length.

Table 11.1 Overview of studies using fortified foods in infants 6–23 months of age assessing impact on growth, morbidity, and/or iron status

Reference (year of publication)	Country	Age at baseline (months)	Intervention	Control	*N*	Impact on growth	Impact on morbidity	Impact on iron status and/or anemia
Lartey et al. (1999) [17]	Ghana	6	Fortified cereal/legume	No intervention	190	Positive effect on WAZ and LAZ	No impact	No impact on anemia; positive effect on iron deficiency prevalence
Beckett et al. (2000) [23]	Indonesia	12 and 18	Fortified food	Very low-energy food	78	No impact	Not reported	No impact
Obatolu (2003) [19]	Nigeria	4	Fortified food	No intervention	90	Positive effect on weight and height gain	Not reported	Not reported
Oelofse et al. (2003) [22]	South Africa	6	Fortified food	No intervention	30	No impact	Not reported	No impact on Hb concentration
Owino et al. (2007) [20]	Zambia	5	Fortified food	No intervention	116	Improved length/height	Not reported	Positive effect on mean Hb and anemia prevalence
Kuusipalo et al. (2006) [21]	Malawi	6–17	Fortified spread	No intervention	112	Positive effect on WAZ and LAZ	Not reported	No impact on Hb concentration
Smuts et al. (2005) [25]	South Africa	6–12	Multiple micronutrient foodlet	Placebo	99	No impact	No impact	Increased Hb and serum ferritin concentration
Faber et al. (2005) [27]	South Africa	6–12	Food + micronutrients	Food	292	No impact	Not reported	Increased Hb and serum ferritin concentration, reduced anemia prevalence
Dhingra et al. (2004) [28]; Sazawal et al. (2007) [30]	India	12–36	Milk powder + micronutrients	Milk powder	570	Positive effect on WAZ and LAZ	Lower incidence of diarrhea and acute lower respiratory infections	Increased Hb and serum ferritin concentration, reduced anemia prevalence

(continued)

Table 11.1 (continued)

Reference (year of publication)	Country	Age at baseline (months)	Intervention	Control	*N*	Impact on growth	Impact on morbidity	Impact on iron status and/or anemia
CIGNIS Study team (2010) [29]	Zambia	6	Infant food with high level of micronutrients	Infant food with low level of micro-nutrients	743	No impact. In subgroup with HIV infected mothers improved LAZ, and reduced stunting	Increased episodes of respiratory infections/ pneumonia	Improved iron status (Hb, ferritin, serum transferrin receptors)
Phu et al. (2010) [37]	Vietnam	5–11	Fortified infant complementary food	No intervention	246	Not reported	Not reported	Improved iron status and decreased prevalence of anemia, iron deficiency and iron deficiency anemia
Ouedraogo et al. (2010) [38]	Burkina Faso	6–23, anemic	Fortified gruel	Unfortified gruel	131	Not reported	Not reported	No impact

WAZ weight for age *Z*-score; *LAZ* length for age *Z*-score; *Hb* hemoglobin

Since the systematic review was published, two additional studies were found that evaluated the impact of locally produced high or low micronutrient-fortified foods on infant growth in the Zambia. Overall, the studies found no impact of the fortified foods on growth, except among infants of HIV infected women who had stopped breastfeeding [29]. where the high micronutrient fortified food improved linear growth and reduced stunting (adjusted Odds Ratio 0.17; 95% CI 0.04;0.84).

Morbidity

The systematic review reported on three efficacy studies evaluating the impact of fortified complementary foods on child morbidity. In two of these [17, 24], fortification had no significant impact on morbidity. In a study in India [30] using micronutrient-fortified milk, an 18 % lower incidence of diarrhea (odds ratio, OR = 0.82; 95 % confidence interval, CI 0.77, 0.89) and a 26 % lower incidence of acute lower respiratory illness (OR = 0.74; 95 % CI 0.62, 0.89) was observed.

In contrast, the recently published study from Zambia found evidence for *increased* symptoms of morbidity [31]. Compared with infants consuming the low micronutrient diet, infants consuming the high micronutrient-fortified food had more episodes of lower respiratory infections/pneumonia (OR = 1.65; 95 % CI −1.06, 2.59) and a higher incidence in hospital referral for pneumonia. Increased morbidity has also been observed in three other complementary feeding intervention studies, involving food supplementation interventions without fortification [32–34]. Unhygienic preparation and storage of complementary foods could be one of the possible explanations for these observed adverse effects on morbidity.

Child Development

Only five studies included results on behavioral, cognitive, and/or motor development. The provision of a fat-based fortified food product improved gross motor development in Ghana [18], but the impact was no greater than that observed by providing micronutrients alone. Fortified maize meal porridge was shown to improve motor development scores in one study [27] but not others [22, 28]. Positive results were found in Indonesia, but only in those who had low length-for-age *Z*-scores at baseline [35]. Positive effects were found on gross and fine motor development and on cognitive development in China [36], but this was not a randomized trial so difficult to attribute this difference to the fortified food.

Micronutrient Status

The systematic review [16] reported on 12 studies, comparing the impact of fortified complementary foods to either no additional food (five trials), or an unfortified complementary food (seven trials) on iron and anemia (and status of other nutrients in some cases). For the five studies compared to no intervention, the average impact was an increase of 4 g/L in mean hemoglobin and a reduction in the prevalence of anemia of 13 % points. For the latter group of seven studies, the average effect was an increase of 6 g/L in mean hemoglobin and a reduction in the prevalence of anemia of 17 % points.

A similar effect on improved iron status and reduced anemia was observed in a more recent study in Vietnam, where infants were fed a micronutrient-fortified complementary food (instant flour) [37]. In contrast, no impact on hemoglobin concentrations was observed after feeding children in Burkina Faso with an improved local ingredient-based gruel fortified with selected multiple micronutrients [38], but this study assessed no other iron indicators.

The systematic review mentioned previously concluded that overall, complementary foods fortified with multiple micronutrients including zinc have little impact on plasma zinc concentration. A similar finding was observed in a more recent trial in young children (9–17 months of age) in Senegal where plasma zinc concentration increased in children who received daily zinc supplementation for 15 days, but not in those who received a zinc-fortified complementary food containing a similar amount of zinc [39]. The lack of impact on zinc in these studies could perhaps be due to the relatively low bioavailability of zinc when consumed with cereal-based foods.

As part of that same review, a significant impact on mean serum retinol concentration was found in four of the five interventions using fortified complementary foods, and an average reduction of 13 % points was observed in the prevalence of vitamin A deficiency in the two studies that evaluated this outcome.

In summary, multiple micronutrient-fortified foods when combined with extra energy and essential fatty acids have the potential to modestly improve growth of infants and improve iron and vitamin A, but not zinc status. The effects on child development are less consistent and warrant further investigation. More research is also needed on the possible negative impact on morbidity of fortified food interventions, ensuring effective education messages on proper hygiene and storage conditions.

Guidance on Levels to be Added

Recommended Micronutrient Composition of Fortified Foods for Pregnant Women

For most micronutrients, the RNIs during pregnancy are higher than those for nonpregnant women of the same age (see Table 11.2) [40], to meet the physiologic changes and increased nutritional needs of pregnancy [10]. The percent of the RNI needed from fortified foods during pregnancy will depend on the nutritional status of the population and consumption from regular home diet. In resource-poor societies, multiple micronutrient deficiencies are likely among pregnant women and where the diet is

Table 11.2 Recommended nutrient intakes during pregnancy [40]

	First trimester	Second trimester	Third trimester
Vitamin A (μg RE)	800	800	800
Vitamin D (μg)	5	5	5
Vitamin E (mg)	15 (22.5 IU)	15	15
Vitamin K (μg)	90	90	90
Vitamin B1/Thiamin (mg)	1.4	1.4	1.4
Vitamin B2/Riboflavin (mg)	1.4	1.4	1.4
Vitamin B3/Niacin (mg)	18	18	18
Vitamin B5/Pantothenic acid (mg)	6	6	6
Vitamin B6/Pyridoxine (mg)	1.9	1.9	1.9
Vitamin B9/Folic acid (μg)	600	600	600
Vitamin B12/Cobalamin (μg)	2.6	2.6	2.6
Vitamin C (mg)	55	55	55
Calcium (mg)	ND	ND	1200
Iodine (μg)	200	200	200
Iron (mg)	27	27	27
Zinc (mg)[a]	5.5	7.0	010

[a]Based on medium bioavailability

based mainly on staple foods with low dietary diversity, fortified foods may need to provide most of the RNI for a number of nutrients. Iron requirements are significantly increased during pregnancy and difficult to fulfill for most pregnant women, irrespective of their nutritional status. Iron therefore needs to be provided through supplementation or fortification.

Although normal embryonic and fetal development require sufficient maternal vitamin A intake, consumption of excess preformed vitamin A during pregnancy may cause birth defects. No increased risk of vitamin A-associated birth defects has been observed at supplemental doses below 3,000 μg (10,000 IU)/day of preformed vitamin A, well within the level that would be expected in fortified targeted foods [41].

Recommended Micronutrient Composition of Fortified Complementary Foods for Infants 6–23 Months of Age

The World Health Organization/Food and Agriculture Organization of the United Nations has established levels of RNI [40] and upper limits (ULs) for micronutrients, based on nutritional needs of healthy children. The October 2008 Consultation on moderate malnutrition reported that "the nutritional requirements of moderately malnourished children probably fall somewhere between the nutritional requirements for healthy children and those of children with severe acute malnutrition during the catch up growth phase." [42] In resource-poor countries a high proportion of children in the complementary feeding period may be moderately malnourished or are at-risk of becoming so. For these infants, nutrient standards based on the RNIs might be too low, but specific guidance of appropriate nutrient levels in complementary foods for these children is not yet available. A study of malnourished children 3–6 years of age that showed positive impacts on linear growth used between 1.5 and 3 times the RNI [43]. National and Codex regulations may not permit levels greater than the RNIs in complementary foods, so again alternatives for this group require further exploration.

The amounts of energy and nutrients required from complementary foods have been extensively reviewed, taking into account the average nutrient intakes from breast milk [44–46]. The percentage of the RNI which should be delivered by fortified complementary foods varies widely, depending on the concentration of each nutrient in breast milk. The micronutrients for which most of the RNI needs to come from complementary foods are iron, zinc, and vitamin B_6 [47]. Recommended fortification levels for complementary foods have been published recently [48] and are summarized in Table 11.3.

Case Study of Micronutrient-Fortified Complementary Foods Delivered in a Programmatic Setting: The *Oportunidades* Program in Mexico

Many programs in countries around the world distribute free or at subsidized cost fortified complementary foods to low income families with young children and/or to women during pregnancy and early postpartum. Few, however, have been subjected to rigorous evaluations that permit measurement of the effects of such supplementation on child growth, development, and other outcomes. This is reflected in the dominance of randomized controlled trials in the evidence review presented in the previous section of this chapter.

An important limitation to measuring the impact of programs that distribute targeted fortified foods is that the foods are often not the only intervention and specific effects cannot be attributed to the food and/or other components of the program. Such is the case for the *Oportunidades* (formerly *Progresa*) conditional cash transfer program in Mexico. As part of an integrated package of attention to health, education, and nutrition (see Table 11.4), pregnant and lactating women, children 6–23 months of age,

Table 11.3 Desired micronutrient density of consumed complementary foods (per 100 kcal) per age group, assuming an average intake and composition of breast milk [48]

	Age		
Nutrient	6–8 months	9–11 months	12–23 months
Vitamin A (μg RE)	31	30	23
Vitamin D (μg)	2.5	1.5	0.9
Vitamin E (mg)	–	–	–
Vitamin K (μg)	3.3	2	1.2
Vit B1/Thiamin (mg)	0.08	0.06	0.07
Vit B2/Riboflavin (mg)	0.08	0.06	0.06
Vit B3/Niacin (mg)	1.5	1	0.9
Vit B5/Pantothenic acid (mg)	0.2	0.1	0.1
Vit B6/Pyridoxine (mg)	0.12	0.08	0.08
Vit B9/Folic acid (μg)	11	9	21
Vit B12/Cobalamin (μg)	0.07	0.08	0.12
Vitamin C (mg)	1.5	1.7	1.5
Calcium (mg)	105	74	63
Iodine (μg)	45	30	16.4
Iron (mg)[a]	4.5	3	1
Zinc (mg)[a]	1.6	1.1	0.6
Copper (mg)	0.04	0.02	0.04
Selenium (μg)	0	0	0.5
Choline (mg)	81	53	76
Magnesium (mg)	19	13	9
Manganese (μg)	4	3	2
Phosphorus (mg)	114	70	26
Potassium (mg)	129	84	69
Sodium (mg)	74	53	54

[a]Based on medium bioavailability

Table 11.4 Benefits and co-responsibilities of Mexico's conditional cash transfer program, *Oportunidades*

Benefits
Cash transfer to each beneficiary family (provided to the female head of household)
Facilitated access to curative public health-care services
Fortified complementary food for children 6–23 months of age, and 2–4 years of age with low weight-for-age
Fortified beverage for pregnant and lactating women from first prenatal control to 1-year postpartum
Cash transfers to families with children in school as of third grade (higher amount for higher grades and for females)
Co-responsibilities
Attendance at monthly health workshops on diverse themes, directed to different family members required to attend according to relevant themes
Regular attendance at prenatal control
Healthy child clinics with growth monitoring, vaccinations, and so on
Enrollment and regular attendance (<80 %) of children in school

and those 2–4 years of age with low weight receive a fortified milk-based food free of cost. The dry product (much like powdered milk) is distributed monthly or twice monthly and intended for use as a beverage for women and pap for children.

The *Oportunidades* program has had a positive impact on child nutritional status [49, 50], but because of the fact that the program provides multiple interventions, impacts cannot be attributed to the food supplement specifically. One of the unique features of the evaluation, however, is that it includes not only final outcomes such as micronutrient status and child growth, but also in-depth

Table 11.5 Nutritional content of the fortified complementary foods provided to pregnant and lactating women and young children as part of the *Oportunidades* program in Mexico

	Quantity in one portion	
Nutrient	*Nutrisano* for children (44 g powder)	Nutrivida for women (52 g powder)
Energy (kcal)	194.0	250.0
Protein (g)	5.8	12.0
Total fat (g)	6.6	11.2
Carbohydrates (g)	27.9	25.3
Sodium (mg)	24.5	81.2
Iron (mg)	10.0	15.0
Zinc (mg)	10.0	15.0
Retinol (μg)	400.0	0
Vitamin E (mg)	6.0	10.0
Vitamin C (mg)	50.0	100.0
Thiamine (mg)	0.8	0
Vitamin B_{12} (μg)	0.7	2.6
Folic acid (μg)	50.0	400.0

process evaluation and information on the intermediate outcomes that might link the program benefits to outcomes. These include the acceptance and utilization patterns of the fortified foods and breast and complementary feeding patterns among many others. The rest of this chapter will focus on that information and implications of the experience in Mexico that might be relevant to other programs that distribute targeted fortified foods. We will describe the development, testing, and refinement of the formulation for the food in the first section, then focus on issues related to acceptance and utilization by the target population in the following.

Formulation and Testing of the Targeted Fortified Foods

Nutrivida (beverage for women) and *Nutrisano* (pap for children), the foods distributed by *Oportunidades*, were developed by local experts in Mexico in the mid-1990s in response to knowledge about the specific micronutrient deficiencies prevalent in the country [51]. Acceptability and sensory testing were done as part of that development process and showed that the fortified foods had promise for acceptance by the beneficiary populations. Similar types of foods had been found to be effective to improve multiple nutritional outcomes in women and children, including growth [52] and development [53].

When the first impact evaluation was completed however, the results seemed at odds with the very high potential of the food to influence nutritional status; children had grown better and were less likely to be anemic, but the magnitude of impact was smaller than hoped. This led to some additional studies related to two aspects of the supplement, first bioavailability of the iron form used, and second, patterns of utilization of the supplement and more general information on feeding patterns, particularly among young children.

The product originally contained reduced iron, and it was quickly shown that the bioavailability of this form was insufficient to ensure absorption of an acceptable proportion of the iron contained in the fortified food. Additional studies then compared the bioavailability of alternative iron forms [54], their acceptability, and the influence of the change in iron on sensory properties [55] and on their efficacy to reduce iron deficiency and anemia in children [56]. As a result of this research the formulation of both foods, for women and children, was switched to one with higher bioavailability. The current formulation (Table 11.5) also contains a higher quantity of vitamin C, in recognition of the important role that this might have to aid iron absorption.

Utilization of the Fortified Foods and Contribution to Dietary Intake

As part of the original external evaluation of *Oportunidades* in rural areas, women were asked whether their child consumed *Nutrisano* regularly, but no information was collected to quantify the amount consumed or to describe the pattern of consumption by women. An in-depth study of utilization and dietary intake of the fortified food by pregnant and lactating women and children was later undertaken in small urban areas. Data collection included in-depth information on breast and complementary feeding practices and patterns of utilization of *Nutrisano* and quantified usual dietary intake of children and women. The results of these studies were published in reports to the *Oportunidades* program [57].

When asked about consumption during the previous 2 weeks prior to the interview, 33 % of women reported that their child within the target age range (6–23 months of age or 2–4 years of age with low weight-for-age) had consumed the fortified food 4 or more times during the week, and an additional 17 % reported that their children had consumed it between 1 and 3 times per week. Among the 50 % remaining, mothers were asked why the child had not consumed the supplement. Seven percent reported that they had not picked it up, don't like it, or multiple other reasons. The 43 % remained stated that the child had not consumed the supplement because they had none left; that they had already finished the quantity that should have lasted until their next allotment. Upon further exploration, the conclusion was drawn that this was mainly due to sharing the food with other household members (older children) and that the actual amount consumed by the targeted child (6–23 months of age) was on average only about 1/2 of the intended amount (22 g on days when the supplement was consumed vs. the recommended 44 g) [57].

This information clearly confirmed that *Nutrisano* was highly acceptable, but presented an important issue related to effective targeting within the household. Similarly, most women reported preparing the food as a liquid beverage and not as a pap for young children, with important implications for nutrient density and possibly the displacement of breast milk.

In the case of pregnant women and those in the first year postpartum, even fewer reported consuming the fortified food (*Nutrivida*); only 23 % had consumed any during the previous 2 weeks prior to the interview [57]. Similarly, it was concluded that this was less due to problems of access to the supplement or acceptance, but rather to sharing the food among other members of the household.

Twenty-four hour dietary recalls were done on 2 nonconsecutive days, 4 times over the course of 1 year (total of eight recalls per child) on a subset of children 6–36 months of age participating in the urban evaluation. No overall difference was found in dietary intake of energy, protein, fat, or micronutrients between beneficiary children and matched controls. However, among those children whose mothers reported that the child had consumed any amount of the fortified food on the previous day, total dietary intakes of zinc, iron, and folate were significantly higher among consumers than all other children (beneficiary children who had not consumed Nutrisano and non-beneficiary children) [57]. However, total intake of these nutrients, even among those who consumed the fortified food, still fell below age appropriate estimated requirements, likely due to the small quantity consumed. There was no difference between consumers and nonconsumers of the fortified food for energy, protein, fat, or vitamin A in urban areas.

Efforts to Improve the Pattern of Fortified Food Utilization

Based on the results of these studies, the *Oportunidades* program recognized the need to invest in further education about the fortified food and to promote its utilization according to recommendations among the beneficiary families. These efforts focused on the fortified food for children and less on the

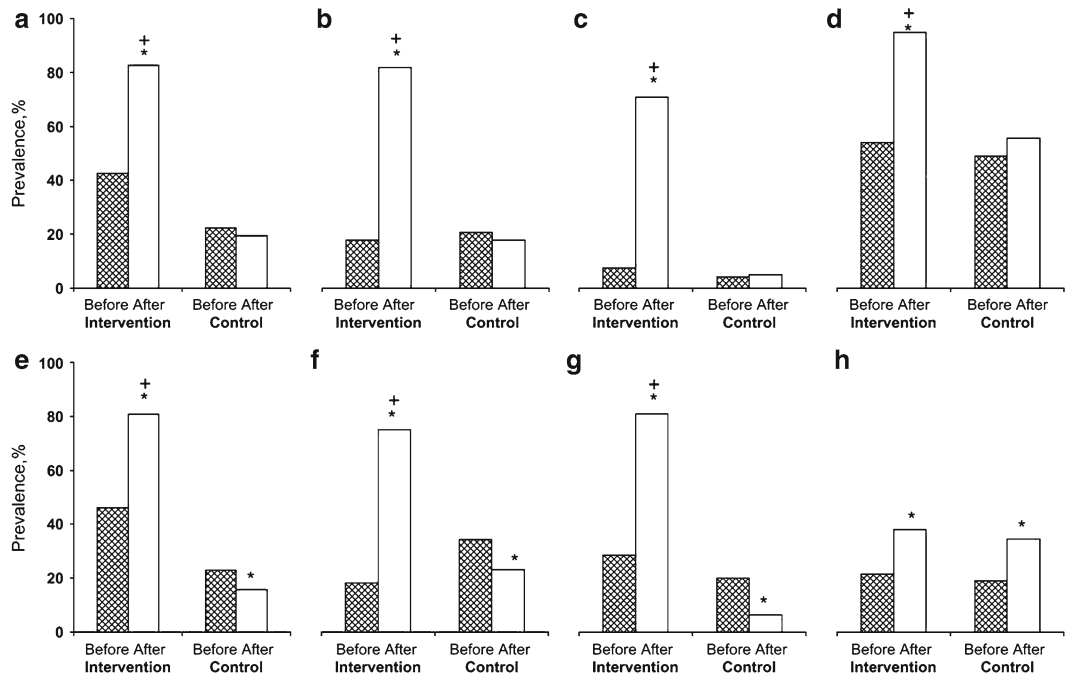

Fig. 11.1 Effect of the improved communication strategy on behaviors related to the use of the fortified complementary food in a controlled trial in two Mexican states. Veracruz **(a–d)** or Chiapas **(e–h)**. Prepared papilla correctly (**a**); Gave papilla every day (**b**); Gave papilla between breakfast and dinner (**c**); Gave papilla to the target child (**d**); Prepared papilla correctly (**e**); Gave papilla every day (**f**); Gave papilla between breakfast and dinner (**g**); Gave papilla to the target child (**h**). *Asterisk* different from before, $P<0.05$. *Plus* different from control, $P<0.05$. Reproduced with permission from Bonvecchio et al. [59]

food for pregnant and lactating women. Intensive qualitative research was done to identify barriers to appropriate utilization and the factors that might facilitate overcoming these [58]. A communications strategy focusing on four key messages related to the utilization of the fortified food for children was developed. These messages were meant to be those most likely to overcome the barriers to utilization, specifically, give every day; give between breakfast and dinner; give to the targeted child; prepare correctly as pap. The communication strategy was pilot-tested in two states and showed excellent promise to improve the pattern of utilization among beneficiary women under controlled conditions (Fig. 11.1) [59].

Unfortunately, the communication strategy was never fully rolled out and the pattern of utilization described above persisted in both urban and rural areas [60]. In subsequent qualitative research, mothers expressed disagreement with the recommendation to give the food only to the targeted child (National Institute of Public Health, Mexico unpublished).

As a result of this series of studies and others beyond the scope of this chapter, the *Oportunidades* program decided to pilot test a new and comprehensive nutrition strategy, continuing to provide the fortified foods to children but adapting the package of supplements in urban vs. rural contexts. The new Integrated Strategy of Attention to Nutrition includes intense behavior change communication to promote exclusive and extended breastfeeding, complementary feeding according to international guidelines and healthy eating habits, recognizing the limitations that the program may have to modify some traditions, such as intra-household sharing of the targeted fortified foods for small children. Rather than presuming to modify these traditions, the program is testing adaptations to ensure that children 6–23 months of age can still receive a daily dose of the fortified food, without forcing

change to family food utilization patterns that are difficult to modify. This includes provision of alternative, less expensive products for older children in the household, with strong education as to why *Nutrisano* should be reserved for the complementary feeding period. The new strategy is currently being evaluated on a pilot scale in urban and rural Mexico.

Conclusions and Recommendations

This review and case study from Mexico highlights a number of key issues related to the development and utilization of fortified foods to reach women and young children. In the case of foods for children the evidence is strong that fortified complementary foods can improve nutritional outcomes, and although there is always room for improving the formulation of such products based on better knowledge of nutrient requirements of children, the principal challenges lie in effective strategies to improve the utilization of these products, in the context of appropriate breast and complementary feeding practices.

Attention to the quality and formulation of fortified complementary foods is vital, but alone is insufficient to ensure that they will have the intended impact on nutrition outcomes in a population setting. Acceptance is certainly a first step to achieving appropriate utilization, but a number of potential barriers to utilization must be overcome. Beliefs and traditions related to feeding of young children may hinder efforts to ensure high utilization of such foods; the preparation of special foods for children, for example, is not customary in all populations and the sharing or not of foods within the household present a particular challenge to programs. Strong behavior change communication designed with knowledge of barriers to adequate utilization and factors that might facilitate this may be helpful; such knowledge would also allow identification of positive traditions in child feeding that should be encouraged. It is also possible, as was the case in Mexico, that programs may not be able to modify traditional feeding practices with education alone and may need to adapt the program to work within these practices to achieve their goals.

In the case of women, there is also strong evidence that where food intake is insufficient (i.e., the prevalence of low body mass index before or in early pregnancy is high), regular consumption of fortified supplementary foods will provide benefits for the mother and fetus. Similarly as with children, ensuring adequate consumption among women in need is an important challenge. There is also much to be learnt still about the appropriate formulation of fortified foods targeted to pregnant women. The field would benefit from a coordinated research agenda designed to answer essential questions and determine contextual factors that might guide decisions related to the most appropriate formulation. For example in Mexico, where low BMI is essentially absent in women and the prevalence of overweight and obesity is high, micronutrient supplements alone may be sufficient to improve maternal and newborn outcomes. Further research is needed, however, to guide programs on the selection of appropriate targeted products for pregnant women.

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Chapter 12
Fortification of Human Milk for Preterm Infants

Luigi Corvaglia, Elena Legnani, Arianna Aceti, Elisa Mariani, and Giacomo Faldella

Key Points

- Optimal early nutrition is critical for improving growth in preterm infants.
- The goal is to achieve a growth rate similar to in utero foetal growth.
- Human milk does not always meet the preterm infant's nutrients needs.
- Standard fortification cannot guarantee an adequate growth rate because of the low protein intake.
- Tailored/targeted fortification is based on HM analysis, so that each infant always receives the amount of nutrient that he/she needs.
- In adjustable fortification protein intake is "adjusted" on the basis of the infant's metabolic response.

Keywords Preterm infants • Nutrition • Human milk • Fortification • Protein intake • Growth

Abbreviations

AAP	American Academy of Pediatrics
AGA	Appropriate for gestational age
ARA	Arachidonic acid
BUN	Blood urea nitrogen
DHA	Docosahexaenoic acid
ESPGHAN	European Society of Pediatric Gastroenterology, Hepatology and Nutrition
GA	Gestational age
GER	Gastroesophageal reflux
HMF	Human milk fortifier
LBW	Low birth weight
LC-PUFA	Long-chain polyunsatured fatty acid
MCT	Medium-chain triglyceride
NEC	Necrotizing enterocolitis
NICU	Neonatal intensive care unit

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V.R. Preedy et al. (eds.), *Handbook of Food Fortification and Health: From Concepts to Public Health Applications Volume 2*, Nutrition and Health, DOI 10.1007/978-1-4614-7110-3_12,

NIRA	Near-infrared-reflectance-analysis
RCT	Randomized controlled trial
SGA	Small for gestational age
VLBW	Very low birth weight

Introduction

Human milk is the best feeding choice for preterm infants, as it offers protection against sepsis and other infections (particularly diarrhoea and acute otitis media) [1] and against necrotizing enterocolitis (NEC) [2], thanks to its various bioactive and immunomodulatory components. Furthermore, human milk feeding is related to long-term improved neurocognitive development [3]. It has a beneficial role in facilitating and improving adaptation to extrauterine life; its components also have the effect of promoting psychomotor and behavioural development [4].

The advantages of human milk in the short term are a better feeding tolerance, a shorter time to achieve full enteral feeding, reduced risk of NEC, good defence against infection and positive modulation of the immune response [5]. In the long term, human milk feeding reduces the incidence of hypertension, cardiovascular risks and insulin-dependent diabetes in adolescence and adult life [6].

Leucocytes are the predominant and most well-understood cellular compartment of human milk: several studies have shown that maternal leucocytes can survive and remain active despite gastric acidity, and that the colonization of the infant gut with mature leucocytes complements the infant's immunologic system with the mother immune experience via passive response pathway [7]. Human milk also contains other immune-related components such as IgA, oligosaccharides, lysozyme, lactoferrin, interferon-γ, nucleotides and cytokines; these elements give passive protection in the gastrointestinal tract and the upper respiratory tract, preventing adherence of pathogens to the mucosa [8]. The American Academy of Paediatrics (AAP) recommends the use of human milk in preterm infant's nutrition [4]. In addition, the Vermont Oxford Network has recently affirmed that the highest priority in management of preterm infants should be early feeding with human milk among efforts to reduce the occurrence of nosocomial bacteremia [9].

In conclusion, human milk represents the best choice for enteral preterm nutrition, because it is associated with many beneficial effects related to improvements in host defence, digestion and absorption of nutrients, neurodevelopment, gastrointestinal function as well as the mother's psychological well-being [10].

Preterm mother's milk has a different composition from term mother's milk, as nutrient concentration progressively drops with time and becomes too low to meet the requirements of growing preterm infants [11]. In addition, maternal milk composition changes greatly depending on maternal age, nutrition and period of lactation; therefore, human milk does not always meet the preterm infant's increased nutrient and protein need [12]. In particular, preterm mother's milk has a higher concentration of protein, non-protein nitrogen, total lipid, long-chain polyunsaturated fatty acids (LCPUFAs), energy, sodium, chloride, magnesium, zinc, copper, iron and vitamins. In recent years, the potential role of the LCPUFAs docosahexaenoic acid (DHA) and arachidonic acid (ARA) in infant growth, development and health has been extensively studied : LCPUFAs are the most abundant fatty acids in the brain and are necessary for growth and maturation of the brain and retina [13]. Their importance in infant nutrition was suggested by the rapid accretion of these fatty acids in the brain during the first postnatal year and the recognition of the importance of DHA in visual and neural systems in animal models [14].

DHA and ARA are present in human milk: their mean levels are 0.32 and 0.47 % of total fatty acids, respectively. Human milk always contains DHA and ARA, although DHA levels vary widely depending on maternal diet [15].

Recommendations

For term infants, human milk provides adequate nutrition to facilitate growth, as well as potential beneficial effects on immunity and on maternal–infant emotional state. However, the role of human milk in preterm infants is less well defined, as it contains insufficient quantities of some nutrients to meet the estimated needs. Optimal early nutrition is critical for improving growth and long-term outcome and decreasing morbidities in low and very low birth weight (VLBW) infants. The goal is to achieve a growth rate similar to in utero foetal growth (15–20 g kg^{-1} day^{-1}, approximately 120 kcal kg^{-1} day^{-1} of enteral feeding). It is very important to begin enteral feeding as soon as possible, and minimal enteral nutrition (trophic nutrition) is strongly recommended [16]. In fact, very low volumes of feeding stimulate gut hormones and promote structural and functional intestinal maturation, decreasing the risk of hyperbilirubinemia and cholestatic jaundice. Moreover, minimal enteral nutrition can improve feeding tolerance, permit a higher weight gain and improve bone mineralization. Even sick VLBW infants can benefit from trophic nutrition as early as at 1–8 days of life: advancement to full enteral feeding depends on tolerance and clinical condition of infants.

The early postnatal period represents a critical time for brain development: chronic undernutrition in early life can have prolonged and often permanent effects on subsequent central nervous system development [17]. The provision of optimal nutrition to VLBW infants in the neonatal intensive care unit (NICU) has become a priority. It should be noted that different nutritional outcomes depend not only on the way infants are fed but also on physiological and pathological conditions: in particular, it has been demonstrated that small for gestational age (SGA) infants have higher nitrogen losses in stools than appropriate for gestational age (AGA) infants, reflecting some deficit in protein digestion, transport or adsorption [18]. It should be noted that 99 % of extremely low birth weight (LBW) infants are below tenth percentile at 36 weeks post-menstrual age. Furthermore, preterm infants affected by chronic disease, such as bronchopulmonary dysplasia, require 20–40 % more calories than healthy babies [19].

During the first weeks of life, when preterm infant's clinical conditions are unstable, the acute and severe lack of a nutrient, particularly protein, may result in delays in establishing and maintaining adequate nutritional intake.

Human Milk Fortification

Rationale of Fortification

Recommended enteral macronutrient intakes for VLBW infants have been recently stated by the European Society of Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) [20] (Table 12.1). VLBW infants need higher calories, proteins and minerals than term infants to achieve adequate growth and development [21]. Human milk is an inadequate source of protein and minerals for the growing preterm infant, and despite its benefits, human milk is not sufficient to cover all the needs of preterm infant and to ensure a growth similar to intrauterine growth, because it provides insufficient amounts of many nutrients. Thus, human milk needs to be supplemented or fortified [3, 22]. Fortification of human milk is generally recommended for preterm infants born below 34 weeks gestation with birth weight <1,500 g, or infants with the need to limit volume intake or with a growth deficit [16].

According to the American Society of Nutrition—Life Sciences Research Office [23, 24], human milk does not ensure sufficient quantities of protein, sodium, phosphate, calcium, magnesium, copper,

Table 12.1 Recommended enteral macronutrient intakes for very low birth weight infants, as stated by the recent ESPGHAN guidelines [5]

Nutrient	Per kg day^{-1}	Per 100 kcal
Fluid (mL)	135–200	
Energy (kcal)	110–135	
Protein (g) (<1 kg body weight)	4–4.5	3.3–4.1
Protein (g) (1–1.8 body weight)	3.5–4	3.2–3.6
Lipids (g) (of which MCT < 40 %)	4.8–6.6	4.4–6
Linoleic acid (mg)	385–1,540	350–1,400
α-linolenic acid (mg)	>55 (0.9 % of fatty acids)	>50
DHA (mg)	12–30	11–27
AA (mg)	18–42	16–39
Carbohydrate (g)	11.6–13.2	10.5–12

ESPGHAN guidelines of macronutrient intakes for VLBW

zinc and many vitamins (B2, B6, C, D, E, K, folic acid) for VLBW infants [25]. Feeding VLBW infants with unsupplemented maternal milk has been associated with delays in growth and nutritional deficits and the minimum target for growth rate of 15 g kg^{-1} day^{-1} cannot be reached with unfortified human milk, even when providing high volumes of milk (>200 mL kg^{-1} day^{-1}) [6].

The main responsible for undernutrition and unsatisfactory growth is the low content of protein, which may cause a growth deficit and a negative nitrogen balance. Energy intake seems to play a secondary role in retarding growth [22]. An inadequate dietary intake of calcium and phosphate in preterm human milk may cause a poor radiological bone mineralization, rickets and fractures at 4–5 months of age and osteopenia in the long term [26]. Other metabolic deficiencies associated with long-term feeding with unfortified human milk are hyponatremia at 4–5 weeks [27], hypoprotidemia at 8–12 weeks [28] and zinc deficiency at 2–6 months [3].

Human milk always contains DHA and ARA, which are essential for brain and visual development, although DHA levels vary widely depending on maternal diet. The need to supplement human milk by LCPUFA is still debated: in a recent study, Henriksen et al. showed that supplementation with DHA and ARA for preterm infants fed with their mothers milk in the early neonatal period was associated with better recognition memory and higher problem-solving scores at 6 months [29].

Differents Types of Fortifications

Standard Fortification

Standard human milk fortifiers (HMFs) contain varying amounts of proteins, carbohydrates, calcium, phosphate, other minerals (zinc, manganese, magnesium and copper), vitamins and electrolytes (Table 12.2). These liquid and powder formulations are mixed with milk to achieve approximately 5–10 % nutrient enrichment [30].

A recent Cochrane review demonstrated that feeding with standard fortified human milk compared with unsupplemented human milk improved short-term and long-term growth, while it had no long-term advantages on neurodevelopmental outcomes and increased bone mineral content. It was also not associated with clinical adverse effects [31]. In this chapter, only one eligible trial was identified: the study randomly allocated 39 breastfed preterm infants (<33 weeks GA) to receive either multinutrient fortification or unfortified human milk feeding for 12 weeks post-discharge. The results showed that, in the intervention group, length and head circumference were higher after 12 weeks and weight, length and head circumference were higher at 12 months corrected age. No difference in Bayley II

Table 12.2 Composition of currently available human milk fortifiers [8]

Nutrient per gram of fortifier	APTAMIL BMF (Milupa)	FM 85 (Nestlè)	Enfamil HMF (Mead Johnson)	Similac (Abbott)
Energy (kcal)	14.5	18	14	14
Protein (g)	0.8	1	1.1	1
Fat (g)	Negligible	Negligible	1	0.36
Carbohydrate (g)	2.9	3.4	<0.4	1.8

Table 12.3 Different types of human milk fortification: standard fortification

Type of fortification	Method	Advantages	Disadvantages	Risks
Standard	Fixed components of HMFs added to maternal milk (2.5–2.9 g kg^{-1} day^{-1} of protein)	Easy and not expensive	The dose not always corresponds to the individual requirement	Under- or overnutrition

mental and psychomotor development index scores at 18 months corrected age was found. Bayley assessment consists of a mental scale, including items which assess memory, problem solving, discrimination, classification and language and social skills; a motor scale, which assesses control of gross and fine motor muscle group; and a behaviour rating scale, which includes orientation and engagement towards tasks and emotional regulation. Moreover, O'Connor reported that bone mineral content was statistically higher in the intervention group at 4 and 12 months corrected age [32].

Human milk fortification is usually started when enteral feeding exceeds 100 mL kg^{-1} day^{-1} or the milk enteral feeding exceeds 80 % of all feeding, and it is continued until infant weight is less than 2 kg [12].

At present, fortification of HM can be performed in three different ways: standard, targeted, and adjustable.

Standard fortification (Table 12.3), which is the method commonly used in most NICUs, consists in adding fixed components of HMFs: an empirical dose of the different components is administered, but this does not always correspond to the individual requirement. Although this method is easy to perform, growth rate is not always satisfactory, particularly compared with babies fed with formula, whose growth rates, both in the short and long term, are higher [33]. Thus, despite fortification, human milk fed preterm infants continue to grow at a slower rate than formula fed infants. In a recent study, Henrikson et al. [34] showed that 58 % of VLBW infants fed with fortified HM had extrauterine growth restriction at discharge.

There are different reasons of the limited success of standard fortification, but the most important is the low protein intake that is the primary limiting factor for growth of VLBW infants [35]. According to the AAP, recommended enteral protein intake for preterm infants ranges from 3.5 to 4 g kg^{-1} day^{-1} [36]; recently ESPGHAN has recommended 4–4.5 g kg^{-1} day^{-1} of proteins for infants up to 1,000 g and 3.5–4 g kg^{-1} day^{-1} for infants from 1,000 to 1,800 g [37].

Standard fortification with a protein intake of 2.5–2.9 g kg^{-1} day^{-1} cannot guarantee an adequate growth rate (which is estimated as about 15 g kg^{-1} day^{-1}). Several studies demonstrated that protein intake is the primary factor limiting the growth of VLBW. Carlson and Ziegler showed that in two groups (fortified human milk vs. preterm formula) energy intakes were similar, but protein intake of the group fed fortified human milk was significantly lower and never met the requirements, being far below current recommendations [35]. Embleton et al. found a severe protein-energy deficit in these infants during the first weeks of life, resulting in poor growth [18].

This lack in protein intake is also related to the fact that the protein concentration of expressed maternal milk is variable, decreases with the duration of lactation and also varies from sample to sample. This means that the exact content in protein of human milk is unknown and fortification must be made on the basis of an assumed protein concentration.

Table 12.4 Different types of human milk fortification: targeted fortification

Type of fortification	Method	Advantages	Disadvantages	Risks
Targeted/tailored	Periodically human milk analysis by infrared spectroscopy	Each infants receives the amount that he/she needs	The analysis requires sophisticated equipment	/

Table 12.5 Different types of human milk fortification: adjustable fortification

Type of fortification	Method	Advantages	Disadvantages	Risks
Adjustable	Protein intake adjusted on the basis of the infant's metabolic response (BUN)	Consider the actual protein status of infant; avoid over-intake of protein; easy method	BUN monitoring is reliable if renal function is adequate; BUN values in preterm infants can have confounding factors	/

New Concepts and Recommendations for Individualized Fortification: "Targeted" and "Adjustable"

The risk of protein deficit is related not only to the specific HMF but also to the wide inter-individual variability of human milk protein content. To overcome protein undernutrition related to standard fortification, individualized fortification seems to be a better approach. Currently, two methods have been proposed: the first one based on milk analysis (targeted or tailored) and the second one based on the metabolic response of each infant (adjustable).

1. Targeted/tailored fortification (Table 12.4)

This method is based on human milk analysis, so that each infant always receives the amount of nutrient that he/she needs: actually, the absence of any information on human milk composition before fortification can cause a potential risk of both under- and overnutrition when a fixed amount of HMF is added [37].

This method has been proposed and studied by Polberger et al. [38]. The authors analysed periodically expressed milk and added to it a variable amount of protein fortifier, in order to reach a predefined requirement (3.5 g kg^{-1} day^{-1} in this study).

The analysis of maternal milk is performed by an infrared spectroscopy equipment, providing a qualitative (carbohydrates, proteins and lipids) and quantitative evaluation of the milk sample. Ten millilitres of milk (from a 24 h pool) are sufficient for a complete analysis in a short time [3]. This method is very accurate but requires highly sophisticated equipment, which is not always available in NICU due to its high cost.

Tailored fortification has been used for the first time in 1999, and it has been recently proposed by Halleux et al. in a clinical trial in which ten VLBW infants fed with human milk with tailored fortification were evaluated [39].

Near-infrared-reflectance analysis (NIRA) has been recently validated for the evaluation of human milk protein and fat content in our NICU [40]. This device, which requires a 5 mL sample and takes only few minutes, has shown to offer excellent precision, accuracy and easy application. It is based on the relation between the reflectance intensity spectrum of milk sample at near infrared wavelengths and the composition of the sample. Each milk component has specific adsorption bands in the near infrared range.

Also Halleux et al. have proposed to tailor milk fortification with an infrared technique (Milkoscan): in this study, targeted fortification improved growth rate to levels closer to formula fed infants [39].

2. Adjustable fortification (Table 12.5)

By this method, protein intake is adjusted on the basis of the infant's metabolic response, determining periodically blood urea nitrogen (BUN). Normal BUN values are thought to be between 9 and 14 mg dL^{-1} (3.2–5 mmol L^{-1}). This procedure has many advantages: first of all, it does not make any assumption regarding the infant's protein requirement, but it monitors the metabolic response considering the actual protein status of each infant. It also avoids possible excessive intake of protein; finally, it does not need frequent milk analysis with high-cost equipment and it is practical for routine use. This method is easy to apply, but it is based on the BUN assay, which does not always completely reflect protein input, especially in the first week of life [41].

BUN monitoring reflects protein intake when renal function is adequate, with very low levels indicating inadequate intake and very high levels indicating possibly excessive protein intake. Therefore, BUN can avoid inadequate or excessive protein intake when it is not possible to determine human milk composition.

In a recent study Arslanoglu et al. demonstrated that BUN values give a good index for monitoring adequate protein intake in clinically stable preterm infants [42]. However, it should be noted that BUN values in preterm infants can have multiple confounding factors; BUN is influenced by hydration status, renal function, energy quality and quantity and patient's degree of illness. Ridout et al. have shown that in preterm infants with a birth weight <1,250 g, the relationship between BUN values and amino acid intake is lacking [43].

Guidance on Safe Levels

At present, human milk is mainly fortified with multicomponent powdered products [44]. The majority of HMFs used in clinical practice are derived from cow's milk: in order to minimize the risk of allergies, the protein component is represented by hydrolysed proteins. The solubility and miscibility of newest HMFs have been improved with the addition of lipids, reducing the formation of soaps with calcium and phosphorus [45]. Human milk is mixed with these fortifiers to achieve approximately 5–10 % nutrient enrichment [30].

It is important to note that the addition of HMFs to milk produces an increase in osmolality [32]. This increase is also related to the way in which fortification is performed: in fact, if fortification is not performed just before the administration of milk, osmolality increases in proportion to the time elapsed from its preparation. This happens because the complex carbohydrates, such as maltodextrines, contained in HMFs are broken down by human milk enzymes into osmotically active mono or disaccharides [46]. Also a human-milk-based HMF is available and under investigation. A recent multicentre randomized controlled trial (RCT) showed a low incidence of NEC and death in the VLBW infants fed with this product [47].

When to Start Human Milk Fortification

Usually, fortification of maternal milk is started when enteral feeds exceed 100 mL kg^{-1} day^{-1} and is continued until infant weight is 2 kg. In addition, experimental data suggest that it is safe to start fortification between 100 and 150 mL kg^{-1} day^{-1} of feeding volume. Early provision of adequate nutrition for preterm infants may improve nutritional status, growth and neurodevelopmental outcomes [48]. In a recent study, Tillman et al. evaluated the introduction of maternal milk fortification from the time of the first feeding in preterm infants with a GA less than 31 weeks: the results showed no difference in weight gain between the group in which fortification started from the first feeding and the group in which it was started after (50–80 mL kg^{-1} day^{-1}), but early fortification was related to a lower incidence

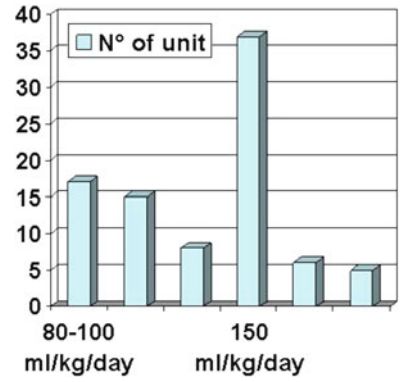

Fig. 12.1 Human milk fortification in different enteral volume tolerated in Australia, Canada, New Zealand, UK and Ireland, Scandinavia [49] (Unpublished)

of elevated alkaline phosphate levels (an early predictor of metabolic bone disease in premature infants) and was not related to any feeding intolerance. A recent survey of enteral feeding practice in Australia, Canada, New Zealand and some European countries showed a large variability in the required enteral volume tolerated (mL kg^{-1} day^{-1}) before adding maternal milk fortification: most of the units start fortification when infants tolerate 150 mL kg^{-1} day^{-1} of enteral feeding; 15–20 % of the units starts at 80–120 mL kg^{-1} day^{-1}; 5–7 % at 140 and 160 mL kg^{-1} day^{-1} and 5% starts at 170 mL kg^{-1} day^{-1} [49] (Fig. 12.1).

Fortification of Human Milk After Hospital Discharge

The majority of VLBW infants is discharged before they reach 40 weeks' postmenstrual age and at the time of discharge most preterm infants accumulate a significant deficit of energy, protein, minerals and other nutrients that might lead to a growth deficit, the risk increasing with lower gestational age and birth weight. Furthermore, this growth deficits can persist in infancy and beyond [50]. After hospital discharge, most of milk-fed preterm infants usually receive their milk directly from their mother's breast. For this reason, current clinical practice often cease multinutrient fortification when breast feeding has been established. After hospital discharge, multinutrient fortification might be easier if the infants are fed expressed breast milk rather than directly breast fed; although mothers could express breast milk and give at least some fortified milk with bottle, this might be unwelcome for women and interfere with the continuation of exclusive breast milk feeding. A recent RCT of multinutrient fortification of breast milk for preterm infants after hospital discharge shows that fortified breast milk increases growth rates during infancy: multinutrient fortification for preterm infants for 12 weeks post-discharge results in higher rates of growth, particularly fortified fed infants had larger head circumference; furthermore, follow-up at 12 months suggested that this trajectory was maintained during all infancy.

Long-Term Benefits of Human Milk Fortification: Growth and Development

A recent 1-year follow-up study performed by Aimone et al. evaluated the impact of maternal milk fortification on bone mineralization and body composition up to 1 year [51]. Total body bone mineral content, bone mineral density, fat mass and lean mass were measured by dual energy X-ray absorption at 4 and 12 months corrected age. Furthermore, Bayley Scale of Infant Development was administered

at 18 months corrected age. The data suggested that LBW infants who received fortified maternal milk early after discharge had greater whole-body bone mineral content during their first year of life compared with those discharged with human milk alone. Furthermore, the nutritional intervention did not promote an increase in body fat mass or trunk fat mass; no statistically significant difference was found between the two groups in the mental, motor or behaviour rating scales scores assessed at 18 month, but infants in the intervention group tended to have a greater number of successfully completed tasks in the language and motor facets of the mental and motor scale than those in the control group. This supports the general principle that preterm infants who grow well during infancy generally have better developmental outcomes [52].

In an Indian study, in which babies fed unfortified milk were compared with babies fed fortified milk, a SGA subgroup analysis was performed [21], showing that SGA preterm babies fed fortified milk had significantly better growth than those fed unfortified milk. Hence, fortification may be more useful if used in this subgroup.

Disadvantages of Human Milk Fortification

HMFs have been proven to be useful and safe for preterm infants and their accretion. Poor milk tolerance is an important problem for preterm babies due to various factors, of which gastric emptying is one of the most important. Breast milk shows a faster gastric emptying, and feed tolerance is improved if compared with formula milk. Many studies were performed to establish if fortified human milk given to preterm babies would also delay gastric emptying, because HMFs not only increase the caloric density of the feeds but also change their composition towards that of formula milk. The results were conflicting: Gathwala et al. [53] and McClure et al. [54] reported that fortification of breast milk does not alter gastric emptying in preterm infants. On the contrary, Ewer et al. demonstrated that the addition of HMFs could significantly slow gastric emptying [55]. This contradiction could be explained partially by different composition of HMFs used, because the major factor influencing gastric emptying is the caloric density of feed. In a study performed on healthy babies, De Curtis et al. showed a linear relationship between caloric density and the rate of gastric emptying [56]. The authors have also shown that maternal milk fortification leads to a rapid increase in osmolality. This can be related to the dextrin content of this milk; in fact, polysaccharides present in HMFs are broken down by amylase into mono- and disaccharides, thus increasing osmolality and leading to abdominal distention, reduced gastric emptying and frequent stool. Furthermore, it has been demonstrated that acid gastroesophageal reflux (GER) is worsened by an increase in the osmolality of milk. In a recent study it has also been shown that non-acid GER indexes are worsened [57].

Despite the documented increase in osmolality [58], clinical trials report that feeding tolerance is well maintained in the fortified human milk fed babies compared to controls. In the USA, in order to lower osmolality, carbohydrates have been partially substituted with fat as energy source.

In one RCT a higher incidence of infections in preterm babies fed fortified maternal milk was found [59]. This may be explained by the fact that HMFs alter some human milk anti-infective properties (i.e. reduction of lysozyme activity). The inclusion of iron in HMFs has been questioned, as a higher iron content might theoretically increase the risk of infection through its direct effect on lactoferrin, the major iron binding glycoprotein. The antibacterial activity of lactoferrin is related to its higher affinity for iron that can lead to saturation of this glycoprotein and to abolition of its bactericidal activity. Different studies have reported different results: Chan et al. concluded that the antimicrobial activity of preterm human milk can be affected by the addition of an iron-containing HMF; Santiago et al., instead, demonstrated that differences in iron content do not affect bacterial growth in maternal milk [60]. However, it has been found that the concentration of IgA, the major immunoglobulin in human milk, is not affected by fortification.

Conclusions

It is well established that human milk plays an essential role in the nutrition of preterm infants due its various bioactive and immunomodulatory components. However, human milk content is unsatisfactory for growth of preterm infants, providing insufficient quantities of protein, sodium, phosphate and calcium. In this perspective, fortification of human milk becomes crucial. Standard fortification is related to inadequate protein intakes, resulting in slower growth rate compared with preterm formulas. Additional protein supplementation is required for the majority of VLBW infants fed fortified maternal milk in a standard way. The main reason of chronic protein undernutrition with standard fortification is that this method is based on assumptions about nutrient content maternal milk; in fact HMF manufacturers have presumed that expressed preterm milk has protein content of 1.4–1.5 g dL^{-1} and usually add a fixed amount of HMFs to maternal milk. Furthermore, a standard fortification of human milk may not meet the recommended protein intake with deficit in growth and development of infants, or rarely overcome the recommended intake. Individualized fortification, considering milk composition or metabolic infants status, is the best choice to obtain adequate protein intake and growth and to meet the nutritional requirement and needs of preterm babies.

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Chapter 13
Fortification of School Meals

Malavika Vinod Kumar

Key Points

- Multiple micronutrient deficiencies exist in the poor in developing countries with the women and children being the most vulnerable. In children, one way to address this problem is through fortification of school meals. Two school meal fortification studies are discussed here, one using ferrous glycine phosphate and riboflavin as the fortificants and another study using a multiple micronutrient food fortificant.
- *Fortification of school meals using ferrous glycine phosphate and riboflavin as fortificants*: The study had a pre- and posttest design, with experimental and control groups. The experimental school had 65 children and the control school had 71 children, all of whom consumed a noon meal at the school daily.
- The children in the experimental school received a powder containing ferrous glycine phosphate and riboflavin, which was added to the meal during cooking every day for 6 months. The dosage was 28 mg of elemental iron and 1 mg of riboflavin per child per day. The children attended school for 5 days each week from Monday to Friday, except for holidays; they received the fortificants on 100 days during the 6-month period. There was no intervention in the control school. Hemoglobin was measured by the cynamethemoglobin method at baseline and endline.
- Binary logistic regression showed a significant ($p < 0.001$) time × group interaction for anemia. The prevalence of anemia in the experimental school was 69.0 % at baseline and 32.8 % after 100 days of intervention over 6 months, a statistically significant change ($p < 0.001$). The prevalence of anemia in the control school was 91.5 % at baseline and increased to 97.2 % at endline; the increase was not statistically significant. The prevalence of angular stomatitis was reduced from 21 % at baseline to 0 % at endline in the experimental school, whereas it was 23 % at baseline and 20 % at endline in the control school.
- *Fortification of school meals using a multiple micronutrient food fortificant*: A pre- and posttest design was used to study children 5–15 years of age with an experimental and a control group. The experimental group ($n = 211$) consisted of children from two residential schools and the control group ($n = 202$) consisted of children from three residential schools. The experimental group received a multiple micronutrient fortificant containing vitamin A, vitamin B2, vitamin B6, vitamin B 12, folic acid, niacin, calcium pantothenate, vitamin C, vitamin E, iron, lysine, and calcium

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V.R. Preedy et al. (eds.), *Handbook of Food Fortification and Health: From Concepts to Public Health Applications Volume 2*, Nutrition and Health, DOI 10.1007/978-1-4614-7110-3_13,

which was added to all the meals cooked for them daily for 9 months. There was no nutritional intervention in the control group.

- Biochemical measurements (hemoglobin, serum vitamin A, serum vitamin E, serum vitamin B12, and serum folic acid) were measured at baseline, after 4 months, and at the endpoint (after 9 months). The heights and weights of the children were also measured at baseline and endpoint. Serum vitamins A and E were measured in a subsample of 50 % and vitamin B12 and serum folic acid measured in a subsample of 25 % of the children. The mean gain in all biochemical measurements was significantly higher ($p < 0.05$) in the experimental group than in the control group at the end of the study.

Keywords Fortification • School meals • Children • Multiple micronutrient food fortificant • Micronutrient deficiencies

Introduction

In developing countries, the poor suffer from multiple micronutrient deficiencies mainly due to the plant-based cereal diets consumed wherein dietary phytate inhibits the absorption of many micronutrients like iron and zinc [1, 2]. Women and children are the most vulnerable in the family. One very important way to address the problem in children is through fortification of school meals. In India, many states have the noon meal program, where lunch is served to the school children. Fortification of this school meal is a very effective way to tackle micronutrient deficiencies in children. The type of the noon meal varies in different states in India. In Tamilnadu, the children are given a rice-based meal with lentil soup which has some vegetables. These meals are cooked in every school; therefore it is a decentralized process. The children are also additionally given an egg, twice a week. In Karnataka, the children are given a dry ration which is to be cooked at home. They also receive fortified sweet balls containing jaggery and cereals which are made centrally and distributed throughout the state. Here the fortification with micronutrients is done centrally in the factories where the balls are manufactured. In several states in the country, there are no noon meal programs at all. Fortification of a traditional culturally acceptable sweet such as groundnut balls or products which the children are used to consuming such as biscuits is a good way to tackle micronutrient deficiencies in school going children in states where there is no noon meal scheme. The number of micronutrients needed for the fortification depends on situations and the type of micronutrient deficiencies observed. It is a need-based situation.

Apart from schools, another way to reach out to children is through residential schools or orphanages which cater to underprivileged children. Here all the meals provided can be fortified. Since micronutrients are delivered in small doses throughout the day, absorption of the micronutrients is very efficient.

A study was undertaken to examine the feasibility of adding micronutrient fortificants to a noon rice meal during cooking. Rice is the staple cereal in South India, whereas wheat is the staple cereal in North India. Fortification of wheat flour with iron and folic acid is feasible and has been tried in many states in North India, but fortification of rice has remained a challenge. This study was therefore undertaken to find out the feasibility of adding the fortificants at the cooking stage itself in noon meal centers in Tamilnadu, where the government provides a rice-based noon meal to all children attending government and government-aided schools. If successful, this method would be a way to deliver the micronutrients through the cooked rice meal as an alternative to fortification of rice.

Fortification of Noon Meals in School with Ferrous Glycine Phosphate and Riboflavin

The study had a pre- and posttest design, with experimental and control groups. The study was approved by the institutional review board of the Sundar Serendipity Foundation. The two schools designated as the experimental and control schools were randomly selected from a list of schools in Chennai that provided the noon meal. After the two schools had been selected, we verified that the children in the schools were matched for age, dietary patterns, and socioeconomic status. The experimental school was then provided with a powder containing riboflavin and ferrous glycine phosphate. It was decided to add these two fortificants because clinical examination of these children showed the prevalence of angular stomatitis, a condition caused because of riboflavin deficiency, and anemia. There was no intervention in the control group except deworming.

For ethical reasons, it was decided that children with severe anemia (hemoglobin < 8 g/dL) in both the experimental and the control groups would be treated with ferrous sulfate tablets for 3 months and excluded from the study. The children in both groups were dewormed at baseline and endline. The experimental and control groups of children were homogeneous in terms of age and socioeconomic status; the families of all the children had a monthly income of less than Rs 2,000 (US$50). We chose an alpha of 0.05 and a power of 80 % with a two-tailed test for calculations of sample size. An assumption of 0.5 g/dL increase in hemoglobin with a standard deviation of 1 as in our earlier studies [3] yielded a required sample size of 63 children in each group.

The fortificants were added to the noon meal prepared for the children 5 days a week. Since the number of children eating the noon meal was fixed, we measured the quantities so that each packet provided a dosage of 28 mg of elemental iron from ferrous glycine phosphate and 1 mg of riboflavin per child per day. One packet was added to the food during cooking each day. The monthly requirement was delivered to the school, and the packets were counted at the end of the month to verify that their contents had been added to the food on each school day. The cooking staff of the experimental school certified that the micronutrient powder did not change the color or taste of the food. Each school has a central kitchen where the food is prepared and a central dining room where the children eat. It was generally observed that there was no wastage of the food prepared in the schools; all prepared food was consumed. The children were served the quantity of food required by them, and there was no food left over on the plate. In both the schools, the average attendance of the children during the 6 months of the study was more than 90 %.

Samples of venous blood (1 mL) were drawn from each child at the school and transferred into vials with ethylenediaminetetraacetate (EDTA) as an anticoagulant. The hemoglobin measurements were performed on these samples in the laboratory within a few hours after blood collection. Hemoglobin was measured by the cyanmethemoglobin method [4] with a spectrophotometer (UV double-beam model, Shimadzu, Japan) at baseline and after 6 months at endline. Measurements were performed in duplicate in all the samples, and the analysis was repeated if the results differed by more than 5 %. Anemia in children aged 5–11 years is defined as a hemoglobin level less than 11.5 g/dL. Since the children in this study were 5–9 years of age, 11.5 g/dL was used as the cutoff point for defining anemia. Both the experimental and the control children were given a tablet of albendazole (400 mg) at baseline and endline. Deworming was done to ensure that there were no worms competing for the micronutrients and that the intestinal tract was clear for absorption of the micronutrients [5]. Clinical assessment of angular stomatitis, a condition caused by deficiencies of B-complex vitamins, was conducted by a physician at baseline and endline. Repeated-measures analysis of variance was used to compare the effects of group x time for hemoglobin. If the interaction effect of group x time was significant ($p < 0.05$), *t*-tests between groups and paired *t*-tests within groups were performed. Proportions were compared by chi-squared tests. Binary logistic regression was performed to compare the effects of group x time for the binary variable of anemia. Significance was set at $p < 0.05$.

Table 13.1 Stability of ferrous glycine phosphate and riboflavin during cooking and storage in ferrous glycine phosphate and riboflavin fortification school study

Ingredient	Initial amount	Amount after cooking[a]	Loss after cooking (%)	Amount after 1 year storage	Loss after 1 year storage (%)
Iron in ferrous glycine phosphate (elemental iron mg per gram of ferrous glycine phosphate)	183 mg elemental iron per gram of ferrous glycine phosphate	183 mg elemental iron per gram of ferrous glycine phosphate	0	182 mg elemental iron per gram of ferrous glycine phosphate	0.55
Riboflavin	1 mg	0.997 mg	0.3	0.985 mg	1.5

[a]Cooking is done for 20 min at 95 °C

Table 13.2 Hemoglobin levels in the experimental and control groups at baseline and at endline after 6 months (mean ± SD g/dL) in ferrous glycine phosphate and riboflavin fortification school study

Time	Experimental group ($n=65$)	Control group ($n=71$)
Baseline	11.02 ± 1.03^{ab}	10.68 ± 0.5^{bc}
Endline	11.71 ± 0.94^{ad}	10.40 ± 0.49^{cd}

There was a significant group × time interaction, $p<0.001$ (ANOVA repeated measures) for hemoglobin
[a]Significant increase $p<0.001$ from baseline to endline in the experimental group
[b]Hemoglobin level was significantly greater in the experimental than in the control group at baseline
[c]Significant decrease $p<0.001$ from baseline to endline in the control group
[d]Hemoglobin level was significantly greater in the experimental than in the control group at endline

Table 13.3 Prevalence of anemia in the experimental and control groups at baseline and at endline after 6 months in ferrous glycine phosphate and riboflavin fortification school study

Time	Experimental group ($n=65$)	Control group ($n=71$)
Baseline	69.0[a]	91.5[b]
Endline	32.8[a]	97.2[b]

There was a significant group x time interaction, $p<0.001$ (binary logistic regression) for anemia
[a]Significant decrease ($p<0.001$) in prevalence of anemia in the experimental group
[b]No significant change in prevalence of anemia in the control group

The Impact of Ferrous Glucine Phosphate and Riboflavin on the Children

The stability of ferrous glycine phosphate and riboflavin for 12 months at 30 °C and 45 % relative humidity was measured. The stability of riboflavin and ferrous glycine phosphate during cooking at 95 °C for 20 min was also estimated. The results are given in Table 13.1. Both ferrous glycine phosphate and riboflavin were very stable during cooking and storage. Sixty-five children in the experimental school and 71 children in the control school completed the study. At baseline, the mean hemoglobin level was significantly higher in the experimental group than in the control group (11.02 and 10.68 g/dL, respectively; $p<0.05$), despite the fact that the children in both groups belonged to the same socioeconomic group, had similar dietary habits, and lived in the same community. At endline, the hemoglobin level had increased to 11.71 g/dL in the experimental group, a statistically significant change ($p<0.001$), and decreased to 10.40 g/dL in the control group, which was also a significant change ($p<0.001$) (Table 13.2). The prevalence of anemia (hemoglobin < 11.5 g/dL) at baseline was 69.0 % in the experimental group and 91.5 % in the control group. At 6 months, after 100 days of intervention, the prevalence of anemia in the experimental group had decreased to 32.8 %, a statistically significant change ($p<0.001$); in the control group, the prevalence had increased to

97.2 %, although this increase was not statistically significant (Table 13.3). At baseline, 21 % of the children in the experimental group and 23 % of the children in the control group had angular stomatitis. At the end of the study, angular stomatitis had completely disappeared in the experimental group, a statistical significant change ($p<0.001$) and had decreased in prevalence to 20 % in the control school, though this change had no statistical significance.

Organoleptic Properties of the Fortificants

There are many iron fortificants available in the market. Some fortificants, such as ferrous sulfate, have high bioavailability, but they have several problems associated with fortification, such as discoloration of the food during cooking or imparting of a distinct metallic iron taste to the food. Other fortificants, such as carbonyl iron, electrolytic iron, or ferric pyrophosphate, have lower bioavailability but do not cause coloration problems when added to food products. We decided to chelate ferrous glycine sulfate with phosphoric acid to produce ferrous glycine phosphate, which has a high bioavailability but does not have any organoleptic problems and does not discolor or impart any iron taste to the food during cooking. Phosphoric acid, in this case, acts as a sequestering agent that prevents the adverse effects of the oxidative breakdown of foods catalyzed by iron and thus averts discoloration or imparting of taste or rancidity to foods during cooking. Since ferrous glycine phosphate did not impart any taste or smell or change the color of the food during cooking, it was decided to administer 1 Recommended Dietary Allowance (RDA) of iron per child per day and test the bioavailability in a relatively shorter period of 100 days of intervention, rather than the more standard 6+ months of most fortification studies. In the present study, micronutrients had to be delivered in a single noon meal. We therefore devised a strategy of delivering a rather larger dose of approximately 1 RDA [6] of iron (28 mg) and riboflavin (1 mg) 5 days a week for a short period of 100 days of intervention. The RDAs of iron and riboflavin for Indian children 7–9 years of age are 26 mg/day and 1.2 mg/day, respectively [6].

We included riboflavin because of the presence of angular stomatitis at baseline in 21 % of the children in the experimental group and 23 % of the children in the control group. Riboflavin supplementation has been shown to improve hemoglobin in earlier studies [7]. It has been shown that riboflavin has a direct role in the release of iron from ferritin [8, 9]. Animal studies have shown that riboflavin deficiency impairs iron absorption [10, 11]. Moreover, other studies have shown [12–15] better hematological response when riboflavin was given along with iron than when iron was given alone. A study in Bangalore, Karnataka, a neighboring state, on 100 nonpregnant, nonlactating women [16] showed good correlations between blood hemoglobin and serum ferritin and dietary intakes of riboflavin. We felt that if the high prevalence of angular stomatitis was due to riboflavin deficiency, then iron absorption might be impaired if iron alone was given as a fortificant. Therefore we included both riboflavin and iron as fortificants in this study. Angular stomatitis may be due to infection when it responds to topical applications of antibiotics or gentian violet, as in earlier studies [17], or it may be due to micronutrient deficiencies [18]. Since angular stomatitis disappeared when riboflavin was used as a fortificant in cooking, it may be concluded that the cause of angular stomatitis in our study was micronutrient deficiency and not infection.

The bioavailability of vitamins and minerals given in the form of supplements as tablets or syrups has been extensively studied. A difference of our study was that the vitamins and mineral used as fortificants had to withstand the high temperatures of cooking and be stable during storage. We find that both ferrous glycine phosphate and riboflavin were extremely stable during cooking and storage and were able to improve hemoglobin status and eliminate angular stomatitis, respectively; hence, they were bioavailable.

Fortification of School Meals Using a Multiple Micronutrient Food Fortificant

A multiple micronutrient food fortificant is a powder containing multiple micronutrients which is to be added to the food during cooking. If this is used in noon meal schemes, it can deliver multiple micronutrients to the school children. For this method to be successful, the micronutrients should not change the color, odor, or taste of the food, should be stable at cooking temperatures, and should be bioavailable. This study tested the acceptability of the fortificant during cooking and storage and tested for the bioavailability of five important micronutrients: iron, vitamin A, vitamin E, vitamin B12, and folic acid, as well as other B-complex vitamins, in the target group of children in residential schools. The goal was to test the stability of the fortificant during cooking and storage and then to test its bioefficacy and bioavailability in residential schoolchildren 5–15 years of age.

The study had a pre- and posttest design with experimental and control groups. Two residential schools (A and B) were randomly selected as the experimental schools and three other residential schools (C, D, and E) as the controls from residential schools in the city of Chennai, Tamilnadu, South India. Schools A through E were chosen randomly from a computer-generated list and a random table. The study was approved by the institutional review board of the Sundar Serendipity Foundation. Children in the experimental schools were supplied with the multiple-micronutrient food fortificant. Children in the control group, except those with severe anemia and serum vitamin A deficiency, who were treated for ethical reasons and excluded from the study, received no intervention other than deworming. The study began when the schools reopened after the summer vacation and continued for 9 months until the schools closed again for the next summer vacation. There were 211 children in the experimental group (169 in school A and 42 in school B) and 202 children in the control group (90 in school C, 70 in school D, and 42 in school E). The experimental and control groups of children were selected after establishing their homogeneity in terms of age and socioeconomic status; the families of all the children had a monthly income of less than Rs 1,500 ($US30). The fortificant was provided to the experimental schools every month, and its use was monitored by counting the number of packets remaining in the school every week.

The dosage was 1 g of fortificant per child per day. The required daily quantity for all the children in a specific school was premeasured, packed, sealed, and delivered to the schools, so that one packet could be cut open each day and added to the food during cooking, throughout the day. The fortificant was dissolved in water and added to liquid food in the final stages of cooking, and it was sprinkled onto solid foods. The cooking staff of both the experimental schools certified that the fortificant did not change the color or taste of any food.

The heights and weights of the children in the experimental and control groups were recorded at baseline and 9 months. Blood samples (5 mL) were drawn from each child at the schools and transferred to the laboratory within 2 h of collection. During collection of these samples, 500 μL was transferred into vials with EDTA as an anticoagulant. The hemoglobin measurements were performed on these samples within a few hours of blood collection. The remaining 4.5 mL of blood was transferred into vials covered with black paper to prevent exposure to light, and the blood was allowed to clot. Serum separation was performed and the samples were frozen at −20 °C within a few hours after collection of blood. Analysis of serum for folic acid and vitamins B12, A, and E was completed within a month after blood collection. The samples were processed in a dark room with yellow lighting to prevent retinol isomerization.

The concentrations of blood hemoglobin and serum vitamin A, vitamin E, vitamin B12, and folic acid were measured. Hemoglobin was measured in all the children in both groups. Serum vitamin A was measured by high-performance liquid chromatography (HPLC) only in those children who were identified as having vitamin A deficiency by physicians who checked the eyes of the children for clinical signs of vitamin A deficiency, such as Bitot's spots or xerosis. Eighty-two children in the experimental group and 84 children in the control group had clinical signs of vitamin A deficiency. At baseline, only eight children in the experimental group had serum vitamin A levels under 20 μg/dL. Seven children in

the control group had serum vitamin A levels under 20 μg/dL, which is defined as biochemical vitamin A deficiency. For ethical reasons, these 7 children in the control group were given therapeutic tablets of vitamin A to combat the deficiency and were excluded from the study. Analysis of data for serum vitamin A and E was performed only for the remaining 77 children in the control group.

Serum folic acid and vitamin B12 were measured in the 44 children in the experimental group and the 54 children in the control group who had the lowest hemoglobin levels. All biochemical measurements were carried out before the start of the study (baseline), 4 months after the start of the study (midpoint), and 9 months after the start of the study (endpoint). Hemoglobin was estimated by the cyanmethemoglobin method [4] with a spectrophotometer. Serum vitamin A and E were measured by a rapid, reverse phase HPLC method for simultaneous determination of retinol and α tocopherol (vitamin E) [19]. Vitamin B12 and folic acid assays were performed with a paramagnetic particle, chemiluminescent immunoassay for the quantitative determination of levels in human serum using the Access immunoassay system (Beckman Instruments, Brea, CA, USA). Both the experimental and the control children were given a tablet of albendazole (400 mg) at baseline and at 4 and 9 months. Clinical assessment of angular stomatitis, a condition caused by deficiencies of B-complex vitamins, was conducted by physicians before the start of the study and after 9 months of intervention.

Stability of the Multiple Micronutrient Food Fortificant

To determine the stability of the fortificant, its composition was analyzed initially, after adding it to an Indian dish-sambar (lentil soup) and cooking it for 30 min, and after storage for 10 months at 30 °C and 45 % humidity. The required aliquots from the lentil soup were taken for the analysis of micronutrients. Six samples were taken before and after cooking. Micronutrient composition was analyzed by methods described in the Indian Pharmacopoeia.

Impact of the Multiple Micronutrient Food Fortificant: Stability of the Fortificant

All micronutrients except vitamin A were very stable after 30 min of cooking and 10 months of storage (Table 13.4). A drop of up to 20 % in the potency of vitamin A was observed during cooking, so the product was formulated to compensate for this loss of vitamin A.

Biochemical and Clinical Effects of the Intervention

At baseline, the experimental and control groups had similar serum levels of vitamin E, vitamin B12, and folic acid, but the hemoglobin level was significantly($p<0.05$) lower and the serum vitamin A level was significantly higher in the experimental group than in the control group. The differences between the groups could be due to differences between the schools in the diets consumed or in other, unknown, factors. Therefore all the biochemical measurements were analyzed school wise to study the impact of the fortification. At the end of 9 months of intervention there were significant ($p<0.05$) improvements in the mean values of all of the biochemical measurements in the experimental group (Table 13.5). In the control group, there were significant decreases from baseline to 9 months in mean hemoglobin and vitamin A levels, significant improvements in vitamins E and B12 levels, and no significant change in folic acid levels (Table 13.5). At 9 months, the mean values of all the biochemical

Table 13.4 Composition of the multiple-micronutrient food fortificant and its stability during cooking and storage

	Amount in the fortificant		
Micronutrient	Initial amount	After 30 min cooking	After 10 months storage
Vitamin A (IU/g)	3,000.00	2,425.00	2,326.00
Vitamin B_2 (mg/g)	1.00	1.00	1.00
Calcium pantothenate (mg/g)	1.00	1.00	1.00
Niacin (mg/g)	15.00	15.00	14.91
Vitamin B_6 (mg/g)	1.00	1.00	1.00
Folic acid (μg/g)	100.00	99.49	99.27
Vitamin B_{12} (μg/g)	1.00	1.00	1.00
Vitamin E (IU/g)	30.00	29.85	30.00
Vitamin C (mg/g)	30.00	28.01	23.96
Iron (mg/g)	10.00	10.00	10.00
Lysine (mg/g)	250.00	243.80	246.51
Calcium (% of weight)	13.75	13.75	13.75

Table 13.5 Biochemical and clinical measurements of children at baseline and 9-month endpoint when school meals are fortified with the multiple micronutrient food fortificant (mean ± SD)

	Experimental group			Control group		
Measurement	*N*	Baseline	Endpoint	*N*	Baseline	Endpoint
Hemoglobin (g/dL)	211	10.99 ± 1.41^{ab}	11.38 ± 1.06^{bc}	202	11.32 ± 1.13^{ad}	10.35 ± 0.99^{cd}
Serum vitamin A (μg/dL)	82	47.18 ± 19.66^{ab}	53.33 ± 25.82^{bc}	77	43.62 ± 13.83^{ad}	32.08 ± 10.74^{cd}
Serum vitamin E (μg/dL)	82	910.90 ± 269.00^{b}	1960.00 ± 400.80^{b}	77	973.90 ± 314.00^{e}	1932.55 ± 575.80^{e}
Serum vitamin B_{12} (pg/mL)	44	193.66 ± 84.90^{b}	1030.60 ± 1232.00^{bc}	54	217.29 ± 155.60^{e}	440.24 ± 269.00^{ce}
Serum folic acid (ng/mL)	44	4.87 ± 1.31^{b}	6.75 ± 2.17^{bc}	54	4.92 ± 1.09	5.08 ± 1.69^{c}
Angular stomatitis (% of subjects)	211	16.20	0	202	22.56	25.40

[a]Values at baseline differed significantly ($p < 0.05$) between experimental and control groups
[b]Values increased significantly ($p < 0.05$) in experimental group from baseline to endpoint
[c]Values in experimental group were significantly ($p < 0.05$) higher than in control group at endpoint
[d]Values decreased significantly ($p < 0.05$) in control group from baseline to endpoint
[e]Values increased significantly ($p < 0.05$) in control group from baseline to endpoint

Table 13.6 Changes in biochemical measurements from baseline to 9-month endpoint when school meals are fortified with the multiple micronutrient food fortificant (mean ± SD)[a]

	Experimental group		Control group	
Measurement	*N*	Change	*N*	Change
Hemoglobin (g/dL)	211	0.393 ± 1.12	202	−0.956 ± 1.33
Serum vitamin A (μg/dL)	82	6.038 ± 25.43	77	−10.064 ± 17.82
Serum vitamin E (μg/dL)	82	1037.450 ± 393.00	77	903.520 ± 618.50
Serum vitamin B_{12} (pg/mL)	44	687.604 ± 1110.00	54	233.283 ± 195.20
Serum folic acid (ng/mL)	44	1.864 ± 1.99	54	0.028 ± 1.60

[a]The change was significantly ($p < 0.05$) better in the experimental group than the control group for all measurements

measurements except vitamin E were significantly higher in the experimental group than in the control group (Table 13.5).

To determine whether the intervention had any effect on biochemical measurements, the changes in all of the biochemical measurements from baseline to 9 months were compared between the experimental and control groups (Table 13.6). The increase was significantly greater ($p < 0.05$) in the experimental group than the control group for all measurements. This result demonstrated the bioabsorption of iron,

vitamin A, vitamin E, vitamin B12, and folic acid from the fortificant. The prevalence of angular stomatitis at the start of the study was 16.2 % in the experimental group and 22.56 % in the control group. At the end of the study, angular stomatitis had completely disappeared in the experimental group, whereas the prevalence remained at 25.40 % in the control group (Table 13.5).

Anthropometric Effects

The heights and weights of 197 children in the experimental group and 196 children in the control group were measured at baseline and at 9 months. There were no significant differences between the groups in changes in height or weight from baseline to 9 months. Therefore, we analyzed the data from children 5–10 years of age and the data from children 11–15 years of age separately. Among children 5–10 years of age, the mean weight at the endpoint was significantly greater ($p < 0.05$) in the experimental group than in the control group, although at baseline there was no significant difference between the mean weights of the two groups (Table 13.7). The mean increase in height was 3.525 cm in the experimental group and 2.93 cm in the control group ($p < 0.05$), but the mean increase in weight did not differ between the groups (Table 13.8).

Enhancement of Stability and Shelf Life of the Micronutrients

The fortificant did not change the odor or color of any foods during cooking. The micronutrients in the fortificant were also stable during storage and cooking. This was achieved by encapsulating the heat labile vitamins, such as vitamin C, with food-grade cellulose acetate phthalate to provide a resistant coat. Similarly, the B-complex vitamins were coated with glyceryl stearate or other edible waxes or gums to protect them and to mask the unpleasant taste of some B-complex vitamins. Vitamin A is already encapsulated in gum acacia and sugar by the manufacturers. Lysine, vitamin E, and calcium pantothenate were left uncoated. The iron source was ferrous sulfate, with chelating agents and biopromoter added to enhance its bioavailability even in the presence of dietary phytates.

Table 13.7 Anthropometric measurements of children 5–10 years of age at baseline and 9-month endpoint when school meals are fortified with the multiple micronutrient food fortificant (mean ± SD)

	Experimental group			Control group		
Measurement	*N*	Baseline	Endpoint	*N*	Baseline	Endpoint
Height (cm)	59	118.63 ± 7.07	122.15 ± 7.19	100	117.06 ± 10.59	119.99 ± 10.46
Weight (kg)	59	20.71 ± 3.28	22.53 ± 3.42[a]	100	19.57 ± 4.38	21.22 ± 4.97[a]

[a]Values were significantly ($p < 0.05$) higher in the experimental group than in the control group at endpoint

Table 13.8 Changes in anthropometric measurements of children 5–10 years of age from baseline to 9-month endpoint when school meals are fortified with the multiple micronutrient food fortificant (mean ± SD)

Measurement	*N*	Experimental group	*N*	Control group
Height (cm)	59	3.53 ± 1.14[a]	100	2.93 ± 1.38[a]
Weight (kg)	59	1.82 ± 0.93	100	1.65 ± 1.11

[a]Values were significantly ($p < 0.05$) higher in the experimental group than in the control group at endpoint

The aim of this study was to test the efficacy of the delivery of multiple micronutrients in cooked food in residential schools. If the multiple-micronutrient food fortificant remained stable during cooking and storage and was efficacious in the improvement of serum biochemical measurements, this study would provide basic evidence that this method of delivery works and should be considered for larger field community trials.

Cost

Another reason for the development of the multiple micronutrient food fortificant is the cost factor. The cost of the fortificant is about 45 paise (1US¢) per person per day. Without lysine, the cost would be only 0.5US¢ per person per day. This is extremely low compared with the cost of multiple-micronutrient tablets.

Absorbtion of the Micronutrients

The use of the fortificant for 9 months resulted in a significant ($p < 0.05$) improvement in all biochemical measurements. Clinical signs of angular stomatitis, a condition caused by a deficiency of B-complex vitamins, especially vitamin B2, completely disappeared in the experimental group, whereas the prevalence of the condition remained the same in the control group. It could be inferred that the B-complex vitamins in general and vitamin B2 in particular, were absorbed from the fortificant and that the higher level of ingestion of these micronutrients was a factor in the disappearance of angular stomatitis in the experimental group.

The hemoglobin level was significantly ($p < 0.05$) lower in the experimental group than in the control group at baseline, but at the endpoint it was significantly higher in the experimental group than in the control group. Over the 9-month period, there was a significant increase in hemoglobin level in the experimental group and a significant decline in the control group. This same pattern was seen when each of the schools was individually analyzed. The mean increase in hemoglobin level in the experimental group was 0.393 g/dL, and the mean decrease in hemoglobin level in the control group was 0.9556 g/dL. A statistically significant decline in hemoglobin in children aged 5–15 years has been observed elsewhere [20]. It may be due to the insufficient bioavailability of iron from the predominantly vegetarian cereal-based diets of these children or the diversion of iron to myoglobin in the muscles as the children grow. This, however, needs to be verified by conducting further prevalence studies on anemia over a period of 10 months to 1 year in a similar population. The serum vitamin A level at baseline was significantly ($p < 0.05$) higher in the experimental group than in the control group. However, over the 9-month period, vitamin A values significantly increased in the experimental group and significantly decreased in the control group. At endpoint, the mean serum vitamin A level in the experimental group was significantly higher than in the control group. This trend was also seen when each school was individually analyzed.

The reason for the decline in serum vitamin A in the control schools is unknown, and similar prevalence studies in similar children are warranted. The normal ranges of serum vitamin B12 and E levels are 200–950 pg/mL and 500–1,800 μg/dL, respectively. The same method (HPLC) was used to measure both vitamins A and E; the times of elution of both of these compounds are a few minutes apart. There was a significant increase in serum vitamins E and B12 in both groups. The same trend was seen when the schools were individually analyzed. The increases in the levels of these two vitamins were significantly ($p < 0.05$) higher in the experimental group than in the control group. The normal range of serum folic acid levels is 3–17 ng/mL. In the experimental group, there were three children

with serum folic acid level <3 ng/mL at baseline, and there were none in the experimental group at endpoint. In the control group, there were three children with serum folic acid <3 ng/mL at baseline, and at endpoint there were two children with serum folic acid <3 ng/mL. There was no significant difference between the experimental and control groups in serum folic acid at baseline. There was no change in folic acid levels in the control group, whereas there was a significant increase ($p<0.05$) in the experimental group at endpoint. The same pattern was seen when the data from each school were individually analyzed.

These trends in biochemical measurements demonstrate the bioavailability of the micronutrients in the fortificant. Similar improvements in vitamin A and hemoglobin levels have been seen in other trials in which multiple-micronutrient tablets have been administered to schoolchildren [21–23]. Thus, it can be concluded that the delivery of multiple micronutrients by the method of fortification described in this study, i.e., through the food route, is as efficient as the conventional method of supplementation through tablets.

Conclusion

Fortification of school meals with micronutrients is an effective way of overcoming micronutrient deficiencies in school children.

Acknowledgement Tables 13.1, 13.2 and 13.3 were published in the Food and nutrition bulletin from the study "Efficacy of fortification of school meals with ferrous glycine phosphate and riboflavin against anemia and angular stomatitis in schoolchildren" by Malavika Vinod Kumar and Rajagopalan S, Food and Nutrition Bulletin, vol. 30, no. 3 © 2009, The United Nations University, pages 260–264.

Tables 13.4, 13.5, 13.6, 13.7, and 13.8 were published in the Food and nutrition bulletin from the study "Impact of a multiple-micronutrient food supplement on the nutritional status of schoolchildren" by Malavika Vinod Kumar and Rajagopalan S, Food and Nutrition Bulletin, vol. 27, no. 3 © 2006, The United Nations University, pages 203–210.

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Chapter 14
Food Fortification and Frail Elderly Nursing Home Residents

Kristina Norman and Matthias Pirlich

Key Points

- Food intake is often compromised in nursing home residents.
- Malnutrition is frequent in the institutionalized elderly.
- Food fortification with macronutrients is the least obtrusive way of enhancing food intake.
- Food fortification with macronutrients increases energy intake.
- Food fortification with macronutrients has no impact on functional measures.
- Food fortification with micronutrients is not well investigated in nursing home residents.

Keywords Malnutrition • Elderly • Nursing homes • Food fortification • Feeding assistance • Micronutrients

Abbreviations

ADL Activities of daily living
BMI Body mass index
ONS Oral nutritional supplements

Introduction

In higher age, several factors may lead to decreased intake of food. Physiological age-related changes such as decrease in taste and smell and reduced sensation of thirst may reduce the drive to eat, while physical disability on the other hand may impair the capacity of providing, cooking, or eating meals. Besides, high morbidity and multiple medication which are frequently present in the elderly, as well

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V.R. Preedy et al. (eds.), *Handbook of Food Fortification and Health: From Concepts to Public Health Applications Volume 2*, Nutrition and Health, DOI 10.1007/978-1-4614-7110-3_14,

as sociopsychological factors such as depression, isolation, poverty, or mental disorders may further contribute to insufficient nutritional intake (see Table 14.1). Recently, several studies have furthermore demonstrated an altered, decreased muscle protein synthesis following protein ingestion in the elderly [1, 2], placing them at high risk of developing sarcopenia, an age-associated phenomenon, where muscle mass and functional capacity are reduced [3]. The functional consequences of malnutrition very often lead to increasing isolation and a greater dependency that ultimately impair quality of life [4]. And vice versa, elderly nursing residents who exhibit various degrees of dependence are therefore at increased risk of malnutrition. An analysis combining data from 12 countries revealed a prevalence of malnutrition of 22.8 % in elderly subjects, with the highest occurrence in rehabilitation units, followed by hospitals and nursing homes, where nearly 14 % of residents were moderately or severely malnourished [5]. The prevalence of malnutrition in nursing homes can be considered an indicator of the quality of nutritional care, although it still remains unclear to what extent malnutrition is preventable in elderly nursing home residents.

Ways to Increase Nutritional Intake

Subjects who cannot ingest, digest, or absorb food at all must either be fed enterally by tube or intravenously, but less invasive options to enhance nutritional intake in the elderly exist (see Table 14.2). Oral nutritional supplements (ONS) which belong to medical nutrition therapy is one simple way of increasing nutritional intake. Although effective for short-term therapy, for long-term use, ONS frequently induces flavor fatigue and is associated with lower compliance but higher costs. Frequency of meals can, e.g., be increased by offering in-between meals or snacks, which results in more meals being eaten over the day [6]. Although this eating pattern is more similar to the eating behavior observed in free-living elderly [7, 8] it has not been shown to increase energy intake [9] in institutionalized elderly. Likewise, serving additional evening meals in elderly did not impact on energy intake nor health-related quality of life [10].

A large survey of 76 nursing homes in Germany reported that more than half of the institutionalized elderly required feeding assistance [11]. A study [12] showed that optimal feeding assistance provided during or between regularly scheduled meals, twice per day, 5 days per week, for 24 weeks increased energy intake and body weight in institutionalized elderly. Although associated with increased staff times (42 min per meal and 14 min per snack per patient), it is essential in patients who cannot eat properly themselves that they receive adequate assistance during meal times [13].

Food fortification is the least intrusive way of increasing nutrient intake. In contrast to the community, where food fortification is commonly and effectively used to supply higher amounts of single deficient micronutrients such as vitamin D, B12, folate acid (mostly in milk or bread) for free-living elderly, in elderly nursing home residents food enrichment is more often employed to compensate the lack of macronutrients or energy. By adding cream, butter or oil to gravy, soups or potato and vegetable mash, energy intake can be raised substantially. Maltodextrin as source of easily digestible carbohydrates can also be used to increase energy intake. Protein powder which can be added to gravy, soups, or mash is frequently used to improve protein intake.

Food Fortification Increases Nutritional Intake

Food fortification has shown to effectively increase nutritional intake in hospital patients who frequently do not entirely consume the served portions. In geriatric rehabilitation wards, serving smaller but energy-dense meals has shown to increase energy intake by 25 % [14] to 37 % [15] in elderly

Table 14.1 Reasons for nutritional deficiencies in elderly subjects

Factors	Age-related physiologic changes	Age-related physical changes	Health-related factors	Gerontopsychiatric factors	Nutrition-related factors	Socioeconomic factors
Examples	Sensory loss: loss of taste, smell, and eye sight	Poor dental state	Polymorbidity	Dementia	One sided nutrition	Isolation
	Loss of appetite	Chewing difficulties	Polypharmacy	Confusion, disorientation	Restrictive diets (low in fat, low in sodium)	Neglect
	Gastrointestinal alterations: maldigestion, malabsorption, obstipation, nausea	Swallowing problems	Frailty, sarcopenia	Depression	Alcoholism	Poverty

Many different factors can lead to decreased food intake and malnutrition in the elderly as detailed in the table

Table 14.2 How to improve nutritional intake in elderly?

Methods	Effectiveness regarding nutritional intake	Costs
Medical nutritional therapy including oral nutritional supplements	↑↑	↑↑
Increased frequency of meals or snacks	↑	↑
Feeding assistance	↑	↑
Food enrichment (either smaller or normal sized meals)	↑	↑
Family style meals	↑	–

Different methods to ensure nutritional intake are associated with varying degrees of effectiveness and costs

Table 14.3 Studies on food enrichment in elderly subjects

Author	*N*	Population	Intervention	Effect
Barton [14]	35	Hospital	Food enrichment	Increased energy intake
Gall [16]	144	Hospital	Food enrichment	Increased energy intake
Lorefalt [15]	10	Hospital	Food enrichment	Increased energy intake
Smoliner [18]	65	Nursing homes	Food enrichment	Improved nutritional status but no improvement in functional parameters
Ödlund Olin [17]	35	Nursing homes	Food enrichment	Increased energy intake
Manders [26]	176	Hospital	Fortification with micronutrients	Positive effect on plasma vitamin status
Manders [25]	176	Hospital	Fortification with micronutrients	Cognitive function (subscale of Alzheimer's Disease Assessment Scale) in patients with low BMI
Keane [28]	78	Nursing homes	Fortification with vitamin D	Raised serum levels of vitamin D

The table shows a list of studies on food fortification in institutionalized elderly and the investigated outcome
BMI body mass index

patients who did not meet their requirements with the standard food. Particularly in hospital patients with insufficient nutritional intake, enriched meals have shown to reduce the percentage of malnourished subjects [16]. Although the enriched meals increased protein and micronutrient intake, patients did still frequently not meet their protein requirements [14, 16].

What interferes with nutritional intake in hospital is not always easy to ascertain. Both acute disease and treatment have the potential to reduce nutritional intake, but most studies suggest that provision of food in hospital do not meet nutritional requirements either through lack of awareness or inadequate care. Therefore, when comparing studies in different settings, it has to be taken into account that interventions in the hospital setting are different from those in long-term care (see Table 14.3).

One small study investigating the effect of food enrichment in nursing homes fortified the standard diet with natural energy-dense ingredients such as cream, starch, and cheese and thus supplied around 500 kcal more per day in the intervention group during 15 weeks. Energy intake as well as activities of daily living (ADL) was used as outcome parameters. While energy intake was effectively increased, ADL did not change in the intervention patients, but decreased further in the control patients [17]. Another study in a bigger number of nursing home residents with imminent or apparent malnutrition used enrichment of food with natural ingredients as well as protein powder. Energy requirements were met in all patients during the study period but the intervention increased protein intake significantly in the intervention patients. Whereas nutritional status improved in standard and intervention group, functional capacities measured by Barthel ADL score and quality of life scales declined in both groups [18].

There seems to be an upper limit of the effect of food fortification. In patients with inadequate nutritional intake, it has proven to increase food intake thus ensuring sufficient energy intake. In patients with adequate energy intake from the beginning, protein intake can be enhanced by provision of enriched food. However, the increased energy and protein intake in the elderly did not affect functional status. Most likely, physical exercise together with increased nutritional intake is needed in order to improve physical capacities.

Food Fortification with Micronutrients

Elderly subjects are frequently deficient in micronutrients, particularly vitamin D and vitamin B12 [19, 20]. The easiest way of providing micronutrients is by supplementation which is however still controversially discussed [21, 22]. Half of the US American older adults are believed to consume multivitamins [23, 24] although data on efficacy are scarce and tolerable upper levels are sometimes even exceeded. Since the food served in nursing homes as well as the nutritional intake frequently do not meet requirements, fortification of food with micronutrients would be an interesting option as a safe and cost-effective method in institutionalized elderly. It has therefore also been investigated in nursing home residents, albeit only by a very small number of studies. One study showed that providing micronutrient enriched dairy drinks twice a day significantly increased intake of vitamins and minerals and therefore had a positive effect on plasma vitamin status (vitamin D, homocysteine, folate, vitamin B2, vitamin B6) [25] and body weight. In patients with a low BMI, a beneficial effect on cognitive function was observed [26]. One small study demonstrated the effect of vitamin fortification of puréed food which raised folate and vitamin D levels while B12 remained unchanged [27].

Another placebo-controlled randomized study examined the effect of vitamin D fortified milk on serum concentrations in elderly nursing home residents [28]. In phase I of the study, the patients were encouraged to drink an increased quantity of fortified or ordinary milk for 3 months and in phase II, the patients continued to drink either fortified or ordinary milk for 6 months in normal amounts. Despite elevating the vitamin D levels from 2.4 to 14.8 ng/mL after phase I and stabilizing the levels at 10 ng/mL, the serum levels did not reach the preventive level of 30 ng/mL associated with, e.g., fall reduction [19] or with additional health benefits such as reduction of common cancers, autoimmune diseases, type 2 diabetes, cardiovascular disease, and infectious diseases. In order to achieve sufficient vitamin D levels, supplementation in supra-physiological amounts are needed which are not feasible with food fortification. The Endocrine Society Clinical Practice Guideline suggests supplementation of at least 600 and 800 IU/day [29].

Guidance on Safe Levels

At present, there is not enough evidence to provide safe levels regarding food fortification with micronutrients for the institutionalized elderly. Further research is necessary in order to investigate this issue in this vulnerable population.

Recommendations

More research is needed before recommendations for the institutionalized elderly can be issued. Every effort has to be taken to ensure adequate nutritional intake so that malnutrition is prevented.

Conclusions

Food fortification in the institutionalized elderly is mainly used to supply macronutrients by adding natural ingredients. While it increases energy intake it does not always lead to adequate protein or micronutrient intake. Food fortification with micronutrients has hitherto not often been investigated in nursing home residents. While it constitutes an interesting option to provide micronutrients at risk, the main problem in this population is insufficient food intake in general.

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Chapter 15
Bread as a Vehicle Vitamin D Fortification: Application to Nursing Home Residents

Veronica Mocanu, Corina Galesanu, and Reinhold Vieth

Key Points

- Vitamin D has become recognized by policy makers as a nutrient that needs to be provided to institutionalized seniors, because it prevents osteoporotic fractures.
- Since older adults receive many medications, it may be preferable to fortify their food with vitamin D, rather than provide it as yet another pill.
- In a clinical trial in Eastern Europe, nursing-home residents had average serum levels of 25-hydroxyvitamin D (the measure of vitamin D status) that were at the threshold of severe osteomalacia, and after 12 months of receiving bread daily that was fortified with 320 mg elemental calcium and 5,000 IU (125 μg) vitamin D, they exhibited substantial gains in bone mineral density at both the hip and spine.
- Since bread is baked at high temperatures, it is a suitable option for fortification with heat-stable nutrients such as minerals and vitamin D.
- The vitamin D_3 is the most potent vitamin D and it should be used for food fortification.
- Food fortification policy for vitamin D should provide bread that contributes a suitable amount of vitamin D per serving, which would typically be about 20 % of the recommended daily vitamin D intake, i.e., about 3 μg (120 IU) per serving of bread.
- To ensure the serum 25-hydroxyvitamin D levels recommended by medical groups for bone health in the older nursing residents, the practical experience shows that much higher amounts of vitamin D_3 are required. Fortification of bread and cereals is a feasible way to improve vitamin D nutrition and a dose of 10 μg (400 IU) vitamin D_3 per 100 g serving from any sources is safe.

Keywords Bread • Fortification • Older adults • Nursing home • Vitamin D deficiency • Osteoporosis

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Abbreviations

$1,25(OH)_2D$	1,25-Dihydroxyvitamin D
25(OH)D	25-Hydroxyvitamin D
IGF-1	Insulin-like growth factor
IU	International unit
QoL	Quality of life
QUALEFFO 41	Questionnaire especially developed for measuring quality of life in patients with vertebral deformities
RDA	Recommended dietary allowance
UL	Tolerable upper intake level
UVB	Ultraviolet B rays

Introduction

Vitamin D deficiency is extremely prevalent in the older adults. Most often the first symptoms are caused by myopathy with muscle pain, fatigue, muscular weakness, and gait disturbances. More severe deficiency causes osteomalacia with deep bone pain, reduced mineralization of bone matrix and fractures. The conditions suggested to be associated with vitamin D insufficiency include total mortality, osteoporosis, muscle weakness, pain, cancer, cardiovascular disease, infections, and falls [1].

Circulating 25-hydroxyvitamin D [25(OH)D] is the recognized measure of overall vitamin D status, and represents the combined contributions of vitamin D synthesis in skin and intake from food. Adequate sunlight exposure may prevent and cure vitamin D insufficiency. However, the older adults often do not have access to the outdoors and the aging skin has a reduced ability to synthesize vitamin D. Moreover, the ultraviolet B rays (UVB)-containing sunshine suitable for vitamin D production is often not available outside, because of weather and seasonal limitations. The most rational approach to reducing vitamin D insufficiency is supplementation. The recommended dietary allowance (RDA) for older people (65 years or over) is 10 μg (400 IU) daily in Europe and 20 μg (800 IU) in US. The widespread use of vitamin D supplements and fortification has virtually eliminated rickets, but these approaches to vitamin D depletion often fail to target older adults effectively. Bread is very widely consumed in nursing homes, and may serve as an ideal vehicle for fortification. Vitamin D_3 is stable in bread, and is effective in raising serum 25(OH)D levels in adults [2].

Severe Vitamin D Deficiency in Institutionalized Older Adults

International comparison studies have shown that among adults serum concentrations of 25(OH)D vary between countries, but are often higher in the countries with food fortification strategies for vitamin D (USA, Canada, Belgium, Scandinavia) [3]. The prevalence of vitamin D deficiency is high and is rising in the elderly [4]. Depending on nutrition, country of residence, food fortification practices, and the definition of vitamin D depletion estimates of vitamin D depletion vary from 5 to 35 % in free-living older adults to 5–98 % in institutionalized older adults (Table 15.1).

Table 15.1 Studies on vitamin D status in older nursing home residents

Country	Study	25(OH)D (nmol/L) mean	Lower reference limit (nmol/L)	Vitamin D deficiency (%)
Canada, Toronto	[5]	39.9	25	5
USA, Wisconsin	[6]	71.5	40	6.6
USA, Omaha	[7]	67.5	30	8
Belgium	[8]	58.5	30	9.6
Norway	[9]	49.3	30	29
Turkey	[10]	24.8	37.5	40.1
Netherlands	[11]	25.0	25	53.6
Japan	[12]	29.9	30	57.9
Switzerland	[13]	12.5	30	82.5
Australia	[14]	16.9	28	86
Romania	[2]	28.8	40	87
France	[15]	7.9	25	98

Unpublished table

Vitamin D Status in Institutionalized Older Adults

The US Institute of Medicine (IOM) recently defined four categories of vitamin D status based on serum 25-hydroxyvitamin D (25OHD): (1) risk of deficiency (<30 nmol/L), (2) risk of inadequacy (30–49 nmol/L), (3) Sufficiency (50–125 nmol/L), and (4) above which there may be reason for concern (>125 nmol/L) [21].

Concentrations of 25(OH)D have been repeatedly found to be higher in independent healthy subjects compared with patients in hospitals and residents of nursing homes, indicating the increased reliance of the institutionalized older adults on vitamin D in food. A recent study assessing the serum 25(OH)D in institutionalized, osteoporotic women from nine countries (Australia, Belgium, France, Germany, Hungary, Italy, Poland, Spain, and UK) has shown that institutionalized women of the same age have the lowest level of vitamin D level compared with community dwelling women [8]. In institutionalized women (without vitamin D supplements), the prevalence of 25(OH)D inadequacy was 10.4, 41.2, 80.3, and 84.2 % when considering cutoffs of 80, 75, 50, and 30 nmol/L, respectively.

When results were subsequently grouped according to geographical regions serum concentrations of 25(OH)D were found to be markedly higher in the North America, Scandinavia, and Belgium compared with the rest of the world (Table 15.1), a difference that could be explained, at least partly, by the higher intakes of vitamin D from fortification and supplements in these countries [16].

Factors Contributing to Vitamin D Deficiency in Older Adults

Senior residents of nursing homes not only have a reduced ability to produce cutaneous vitamin D, but also have limited dietary intake, intestinal absorption, and kidney capacity to convert vitamin D to its active form (Table 15.2). The menus planned in the traditional way were not meeting residents' vitamin D requirements. The menus relied heavily on milk to provide adequate vitamin D, but milk intake tends to decline with age because of lactose intolerance and reducing caloric intake.

Table 15.2 Factors that contribute to inadequate serum vitamin D levels among older adults

Dietary deficiencies
Limited number of vitamin D-rich foods
Vitamin D fortified milk drinking is unreliable
Reduced intestinal absorption of calcium
Decreased $1,25(OH)_2D$
Decreased renal function
Increased body mass index and body fat
Decreased levels of IGF-1, estrogens
Low endogenous vitamin D synthesis
Reduced exposure to sunlight
Decreased efficiency of 7-dehydrocholesterol conversion in aging skin
Vitamin D resistance (decrease vitamin D receptor expression)
Decreased levels of $1,25(OH)_2D$, IGF-1, estrogens

1,25(OH)$_2$D 1,25-dihydroxycholecalciferol; *IGF-1* insulin-like growth factor 1 (unpublished table)

Estimates of Vitamin D Intake in Institutionalized Older Adults

Sunlight

Most populations require regular sun exposure during the summer to build up sufficient stores to ensure adequate vitamin D status during winter and spring. In the older adults, a sufficient serum level of 25(OH)D can be achieved with regular sunlight exposure for 15 ± 30 min daily. The British National Diet and Nutrition Survey of subjects aged >65 years found that in the noninstitutionalized subjects there was a strong association between 25(OH)D concentrations and vitamin D intake in autumn, winter, and spring, but not in the summer season [17]. This pattern suggests a higher dependency on dietary vitamin D during seasons with low solar exposure.

Dietary Intake

Natural dietary source of vitamin D are limited, and their contribution to vitamin D status becomes important only when sunlight exposure is restricted. The main dietary sources of vitamin D are fatty fish (salmon, sardines, tuna) and oils derived from them, some meat products (liver), eggs and wild mushrooms.

Data on dietary vitamin D intake are not available in many countries. Nevertheless, it is generally considered that the vitamin D intake is low in older adults, particularly in high risk populations, such as institutionalized people. The residents' intake is between 2 and 7.5 μg (80–300 IU) per day vitamin D from the food [18].

In the United States and Canada, 50 % of total vitamin D intake came from fortified milk. In older adults, the low vitamin D intake has been considered to be due to a decreased intake of milk, the principal dietary source of the vitamin D in those nations.

Supplements

Few older institutionalized adults received vitamin D supplements (9–30 %) [19]. Suominen et al. found that among 2,114 residents of nursing homes in Finland, only 21.3 % received vitamin D in the therapeutic dose of 10 μg (400 IU) or more, and 3.6 % in the recommended dose of 20 μg (800 IU) or more.

Vitamin RDAs for Older Adults

The most rational approach to reducing vitamin D insufficiency is supplementation. The requirement for dietary vitamin D, depends on the amount of sunshine exposure, according to a range where the higher limit is the estimated dietary requirement of an individual with minimal endogenous synthesis, whereas the lower limit indicates the intake of an individual able to produce adequate vitamin D.

In Europe, the RDA for vitamin D have been proposed in 1998 by the report on osteoporosis-action on prevention [20]. The RDA is 10 μg (400 IU) daily for people aged 65 years or over.

In the United States and in Canada, the IOM 2010 report on Dietary Reference Intakes for calcium and vitamin D [21] established dietary reference intakes for vitamin D. The IOM committee found that average blood levels of vitamin D above 20 ng/mL (50 nmol/L) are needed to ensure good bone health. In adults over age 70, regardless of exposure to sunlight and stores, the RDA value is 20 μg (800 IU). In addition, the tolerable upper intake level (UL) of 100 μg (4,000 IU)/day in United States and of 50 μg (2,000 IU)/day in Europe [22]. The UL refers to the highest level of daily nutrient intake that is likely to pose no risk of adverse health effects to almost all individuals in the general population.

Optimal Use of Vitamin D in Older Institutionalized Adults

The older adults are particularly susceptible to vitamin D insufficiency and the analyses suggest that the optimal 25-OH-D concentration lies between 75 and 100 nmol/L [23].

Vitamin D Supplementation and Bone Health

The report of Cranney et al. [24] on a systematic review of the scientific literature, found that vitamin D_3 at doses ≥12.5 μg (500 IU) combined with calcium (500–1,200 μg/day) increased BMD of the lumbar spine, femoral neck, and total body relative to placebo. Vitamin D_3 supplementation less than 20 μg (800 IU)/day without calcium [24, 25] did not reduce the risk of fractures.

Vitamin D Supplementation and Risk of Falling

Older nursing home residents have a high risk of falls and fracture. Fall-related fracture and injury is a serious problem affecting the quality of life and cost of care for these older nursing home residents. Approximately 50 % of nursing home residents fall at least once each year [23]. A serum 25(OH)D level below 50 nmol/L is associated with increased body sway and a level below 30 nmol/L with decreased muscle strength.

Several randomized clinical trials have shown that vitamin D supplementation reduces falls 23–53 % in residents of nursing homes or residential care and a meta-analysis of vitamin D supplementation and falls (including older adults nursing home participants) found a 19 % reduction [23]. Nevertheless, not all trials with older adults residential care populations have found an association between vitamin D supplements and falls [26] or fracture risk [27]. A recent meta-analysis looked at all of the high-quality, double-blinded trials came to the conclusion the supplemental vitamin D in a dose of 17.5–25 μg (700–1,000 IU) a day which bring serum 25(OH)D levels up to 75–100 nmol/L could reduced the risk of falling among older individuals [23].

Vitamin D Supplementation and Chronic Pain

Severe vitamin D deficiency, particularly with 25(OH)D levels less than 30 nmol/L, leads to osteomalacia characterized by severe muscle weakness and pain, with rapid resolution of symptoms after vitamin D replacement. The effect is that increased parathyroid hormone levels impair bone mineralization causing a spongy matrix to form under periosteal membranes covering the skeleton. This gelatin-like matrix can absorb fluid, expand, and cause outward pressure on periosteal tissues, which generates pain since these tissues are highly innervated with sensory pain fibers [28]. In a large study of 360 female patients with chronic back pain, vitamin D therapy produced symptomatic improvement in 96 % of all patients and in 100 % of those with severe 25(OH)D deficiencies [29].

Vitamin D Supplementation Strategies

In older nursing home residents, the UVB exposure on a large area, once a week for 8 weeks, leads to an important improvement of the vitamin D status but the vitamin D sufficiency, however, was not reached in most subjects [11]. Though brief UVB at 1-week intervals is an efficient and safe method for prevention of vitamin D deficiency in the older adults, it is time-consuming for the ward staff and thus less convenient than oral vitamin D supplementation.

It was suggested that a 25(OH)D concentration of 75–100 nmol/L was required for fall and fracture prevention [30]. Studies suggest that 17.5–25 μg (700–1,000 IU) of vitamin D per day may bring 50 % older adults up to 75–100 nmol/L [23]. The low baseline levels of plasma 25(OH)D between 20 and 40 nmol/L may require a daily dose of 55 μg (2,200 IU) vitamin D to reach and maintain 80 nmol/L [31]. These results indicate that individuals with a lower starting level may need a higher dose of vitamin D to achieve desirable levels, while relatively lower doses may be sufficient in individuals who start at higher baseline levels. It has been reported that the rate of increase of 25(OH)D levels was between 0.6 and 1.2 nmol/L per 1 μg (40 IU) daily of vitamin D_3 and that the response may well be an inverse function of the starting 25(OH)D concentration [31].

Food Fortification

The vitamin D is difficult to obtain from the diet because it is not naturally present in many foods. Fortification of foods with vitamin D provides an alternative approach. Supplementation of food with vitamin D is common in the USA, where milk is fortified with vitamin D, but is not common in Europe with the exception of some Northern countries (Belgium, Netherlands, and United Kingdom) where fortification is compulsory only to margarines [20]. Other countries have a voluntary fortification program (e.g., Finland allows addition of vitamin D to milk and margarine) [32].

Fortification of bread, other cereals, and margarine is focused on the older adults. There were insufficient data for definitive evaluation of the effect of vitamin D food fortification on serum 25(OH) D. Increases in serum 25(OH)D from vitamin D fortified foods may be influenced by a number of factors, including total vitamin D intake, bioavailability, and the actual vitamin D content within the fortified food source [33]. The individual treatment effects ranged from 14.5 to 34.5 nmol/L [33]. Studies of older adults conducted in the UK have found small increases in 25(OH)D concentrations in those consuming fortified margarine and milk [33]. Significant increases in 25(OH)D were observed in frail older adults Dutch subjects who every day consumed fortified dairy products that brought their intake of vitamin D up to recommended levels (from 3.2 to 11.6 μg/day) [34]. In contrast, no increases

in 25(OH)D were found in a Scottish study of long-stay residents of geriatric wards who received fortified foods (margarine, butter, or milk) as part of their daily diet for a period of 0.5–1 year [35].

Cost-Effectiveness

Cost-effectiveness analyses show that 20 μg (800 IU) of supplementary vitamin D daily in older people is suitably low-cost, low-risk, and effective strategy for reducing fractures. Beyond reducing the incidence of hip fractures, vitamin D supplementation in nursing home residents also plays a role in maintaining independence, reducing pain, and improving cognition. These benefits increase quality of life and further reduce health care costs.

An adequate fortification program should secure a supply of about 20 μg (800 IU) vitamin D per day to the older adults. Bread and edible fats (butter, oil, and margarine) would be obvious food items to enrich to reach this population group. This is an effective, safe, and cheap means of preventing osteoporotic fractures.

Impact of Fortified Bread on Health in Institutionalized Older Adults

Fortification of foods with vitamin D is an inexpensive approach to ensuring adequate vitamin D nutrition in older adults. Actually, the fortification of foods with vitamin D is not common in Europe and fluid milk and ready-to-eat cereals are the only routinely fortified with vitamin D in the United States and Canada. The usual fortification level for most ready-to-eat cereals is 1–3.5 μg [36]. However, the older adults, a group at greatest risk of vitamin D insufficiency, consume less milk and ready-to-eat cereal than do other groups [36]. Fortifying less expensive staple foods, such as cereals and grains, results in broader dissemination of vitamin D throughout the population. Vitamin D fortification of bread and cereals (flour) would help prevent osteomalacia and osteoporosis in adults and might provide additional potential health benefits, such as reduced risk of some common cancers.

Vitamin D Fortification of the Bread in Clinical Studies

Few studies have investigated the effects of vitamin D supplementation using bread as a vehicle. To address the problem of vitamin D deficiency in Europe, the OPTIFORD (Optimizing the Fortification of vitamin D in the European Union) project was conducted in four European countries. One of the aims of this project was to examine the potential of fortifying bread with vitamin D (10 μg vitamin D_3 in three slices of bread per day), which is expected to raise the mean 25(OH)D concentration by 10 nmol/L [37]. The vitamin D_3 (dry vitamin D_3 type 100, CWS, Roche) was incorporated with edible fats finely dispersed in a starch-coated matrix of gelatin and sucrose. Recoveries of cholecalciferol in bread samples varied between 79 and 109 %, indicating that cholecalciferol was stable during baking and analysis [38].

In the study conducted by Mocanu et al. [2], the safety and efficacy of food fortification of bread to provide high amounts of vitamin D and calcium to nursing home residents deficient in these compounds were examined. Older nursing home residents from Iasi, Romania (latitude 47°N) received daily one bread bun (100 g) fortified with 800 mg $CaCO_3$/day (320 mg elemental calcium) and 125 μg (5,000 IU) vitamin D_3/day. The oil containing the vitamin D_3 (Vigantol; Merck KGaA, Darmstadt, Germany) was added into the dough. The dough was baked at 260–270 °C for 15 min. Estimated loss during processing was 40–50 % [2].

Compliance to Vitamin D Fortified Bread

The bread is one of the most consumed food by older nursing homes residents and vitamin D fortified bread could be an efficient way to replace the pills. In the study conducted in Romania, the older nursing residents received vitamin D fortified bread (100 g) daily and the adherence to fortified bread intake was monitored by questionnaire. The rate of compliance was as follows: 91 % of patients reported that they consumed the fortified bread every day and 76 % of patients consumed the whole bun. Seventy-nine percent of patients were satisfied with the organoleptic qualities of bread and 21 % have reported some deficiencies. Sixty percent of patients said their health improved at the end of the study [39].

Bioavailability of Vitamin D from Fortified Bread

Little is known about the bioavailability of vitamin D from fortified bread. Nutri et al. [38] have assessed the bioavailability of cholecalciferol in two types of bread. In a 3-week study, 44 subjects aged between 25 and 45 years with low serum 25-OHD concentrations (<58.1 nmol/L) daily received vitamin D bread. The individuals were given fortified wheat bread, fortified rye bread, regular wheat bread (control), and a daily cholecalciferol supplement of 10 μg (vitamin D control). The average daily portion of bread was 85 g (four thin slices), with a mean daily cholecalciferol intake goal of 10 μg from fortified breads, the same amount as from the supplement. In the beginning of the study, 73 % of the women had a serum 25(OH)D concentration <38 nmol/L. The changes in serum 25(OH)D concentrations were 16, 15, 0, and 19 nmol/L in the fortified wheat, fortified rye, control, and vitamin D supplement groups, respectively. The results of this study showed that cholecalciferol was absorbed equally from both wheat and rye bread. The serum PTH concentration did not differ among the groups at the beginning or end of the study. Serum calcium concentrations and urinary calcium excretion did not change in any of the groups.

Efficiency and Safety of Vitamin D Fortified Bread

In older adults, the use of large dose of vitamin D is justified by very low levels of vitamin D (older nursing homes residents usually have 25(OH)D levels < 50 nmol/L). To achieve optimal levels between 75 and 100 nmol/L in all individuals, doses of vitamin D >50 μg/day have been suggested to be required [40].

Fortification of bread with high doses of vitamin D_3 (125 μg vitamin D_3/bun) was used to increase the serum levels of 25(OH)D from below 50 nmol/L to optimal range in older residents of a nursing home [2]. The older adults declared they eaten about 80 % of bun fortified with vitamin D, so the daily intake of vitamin D was 100 μg. After 12 months of intervention, serum 25(OH)D increased from its initial mean of 28.8 to 126.4 nmol/L. The median increase in 25(OH)D during the year with bread fortified with 125 μg/day was 98.0 nmol/L. There was no evidence of a sex difference in the 125 μg response to vitamin D. At every follow-up time point, serum 25(OH)D was significantly higher than that at baseline. However, there was no significant difference between 6 and 12 months suggesting that a final plateau had been approached by 6 months (Fig. 15.1). Despite the substantial fortification of a common food, 8 % of study participants did not reach the goal of >75 nmol/L after 12 months; this finding resulted because of either biologic variation or poor compliance. Subjects who were provided 125 μg vitamin D_3/day maintained 25(OH)D concentrations within the physiologic range (<220 nmol/L), as has been reported to be achieved through sun exposure.

The ratio of urinary calcium to creatinine was significantly higher at the 6-months follow-up visit than at baseline. Mean serum PTH was initially near the top of the reference range for PTH, but it was significantly lower at 6, 9, and 12 months.

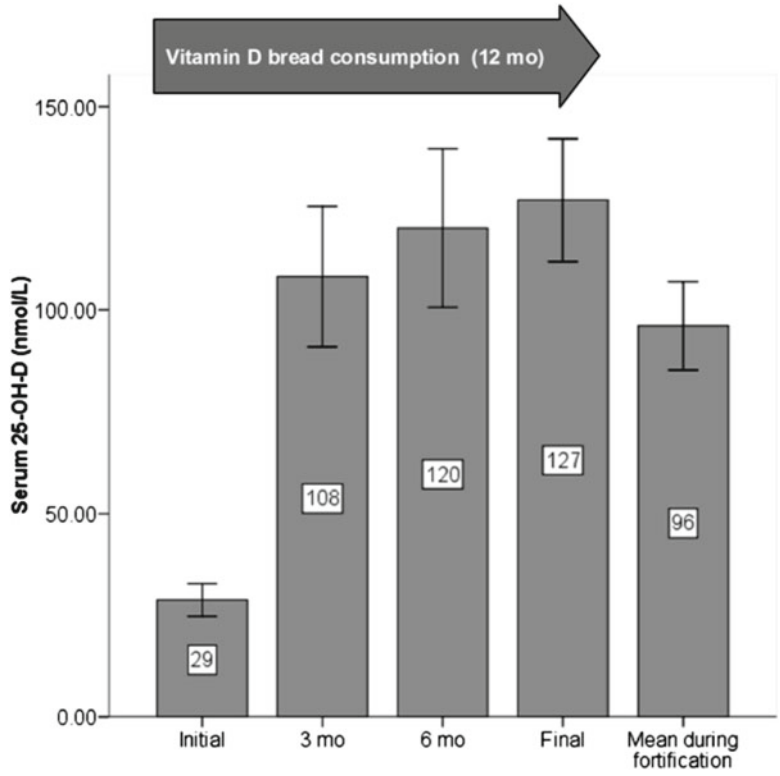

Fig. 15.1 Effects on vitamin D status of vitamin D bread in older adults ($N=45$) living in a nursing home from Iasi, Romania (latitude 47°N). Mean values of serum 25(OH)D concentrations during the consumption of bread fortified with 125 μg vitamin D_3. The Serum 25(OH)D concentrations were significantly higher than baseline at 3 months ($p<0.001$), 6 months ($p<0.001$), and 12 months ($p<0.001$) (adapted from [2])

In conclusion, fortification of bread with 125 μg vitamin D_3 was effective to maintain a serum 25(OH)D concentration >75 nmol/L and to suppress PTH with no detected evidence of adverse effects on serum and urinary calcium in older nursing home residents.

Health Effects of Vitamin D Fortified Bread

It should be emphasized that there are very few intervention studies demonstrating the effect of vitamin D bread fortification on health in general population and in older adults, in particularly. Optiford, a coalition of scientists representing various countries in Europe, investigated if food fortification with vitamin D is a feasible strategy to remedy insufficient vitamin D status [37]. In another study, the effects of vitamin D fortified bread on bone and general health were investigated in older nursing residents from Eastern Europe by Mocanu et al. [2].

Bone Health

The effects on bone mineral density of bread fortified with 800 mg $CaCO_3$/day (320 mg elemental calcium) and 125 μg (5,000 IU) vitamin D_3/day were investigated in vitamin D deficient older adults

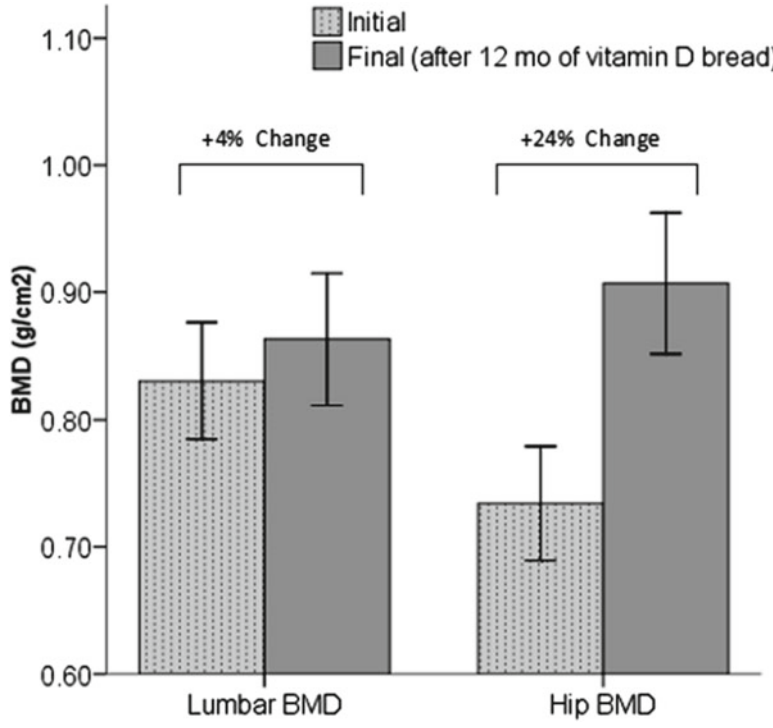

Fig. 15.2 Effects on bone health of vitamin D bread in vitamin D deficient older adults ($N=45$) living in a nursing home from Iasi, Romania (latitude 47°N). Bone mineral density data, before and after 12 months of eating bread fortified with calcium (320 mg) and vitamin D_3 (125 μg). Hip BMD increased substantially (+24 % from baseline; $p<0.001$). The rise in Z scores for hip BMD was highly statistically significant; $p<0.001$) (adapted from [2])

living in a nursing home from Iasi, Romania [2]. Substantial increases in BMD measures were observed after consumption of the fortified bread for 12 months (Fig. 15.2). Lumbar spine BMD increased by 4 % over the initial measure. Hip BMD increased by a surprising 23.4 % over the initial reading. These findings support the desirability of maintaining a serum 25(OH)D concentration >75 nmol/L. The increases in BMD were probably due to mineralization of osteoid in patients with vitamin D deficiency and osteomalacia. These findings represent the best data available concerning the response of vitamin D-deprived adults to calcium plus sufficient vitamin D to achieve the desirable serum 25(OH)D concentration of >75 nmol/L (Fig. 15.1).

In Optiford study, the bread was fortified with 10 μg (400 IU) vitamin D/100 g. This failed to ensure the target level for serum 25(OH)D (>50 nmol/L) after 3 weeks of supplementation [38].

Pain

The effects of 1 year vitamin D bread on Quality of Life (QoL) in institutionalized older adults were assessed in the study conducted by Mocanu et al. [2]. Patients were divided into two groups: with (F) and without (NF) vertebral fractures and they completed the Romanian version of QUALEFFO-41 questionnaire at baseline and after 3, 6, and 12 months of intervention. After 1 year supplementation (125 μg vitamin D_3/bun), the most important improvement was recording for pain domain (unpublished data).

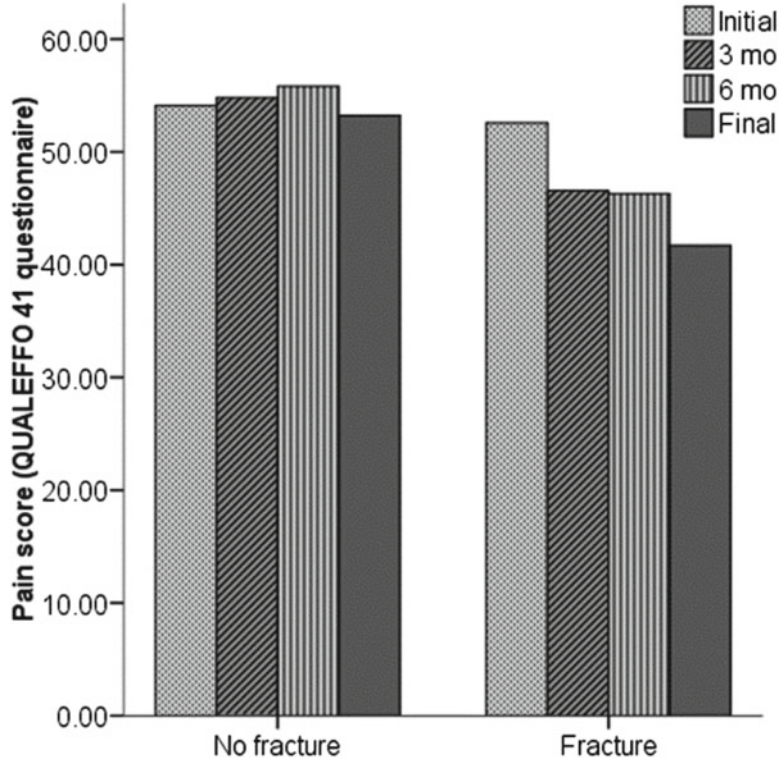

Fig. 15.3 Effect on pain dimension of QUALEFFO 41 questionnaire of vitamin D bread in vitamin D deficient older adults living in a nursing home from Iasi, Romania (latitude 47°N). In patients with fracture ($N=17$), the mean scores for pain domain were improved than baseline at 3 months ($p=0.422$), at 6 months ($p=0.209$), and at 12 months ($p=0.107$)

The fortification of bread with 125 μg vitamin D3 and 320 mg elemental calcium had the greatest impact on pain in patients with osteoporotic fractures (Fig. 15.3) (unpublished data). The mean score for pain domain improved with 19 % (vs. 6 % in NF group) and pain amelioration was noticed in 50 % of older adults with fracture as compared to 28 % of patients without fracture. The older nursing home residents showed a significant increase in hip BMD, *T*-score, and fracture reduction after 12 months of supplementation with high dose of vitamin D and calcium, explained mainly by the correction of osteomalacia [2]. Not surprisingly, the amelioration in osteomalacia symptoms was translated into a reduction of pain symptoms in a large percentage of F group patients, as recorded by the pain domain of the QUALEFO-41 questionnaire [41].

Well-Being

In the study carried out in an Eastern European nursing home [2], the effects of 1 year consumption of bread fortified with vitamin D and calcium on the quality of life (QoL) were also measured in older adults. The changes in QoL were evaluated using QUALEFFO-41questionnaire. After supplementation period, among domains of the QUALEFFO-41 questionnaire, the most important improvement was recording for pain (37 % of patients), daily activities (35 % of patients), locomotion functions (26 % of patients) and social activities (30 % of patients) (unpublished data) (Fig. 15.4).

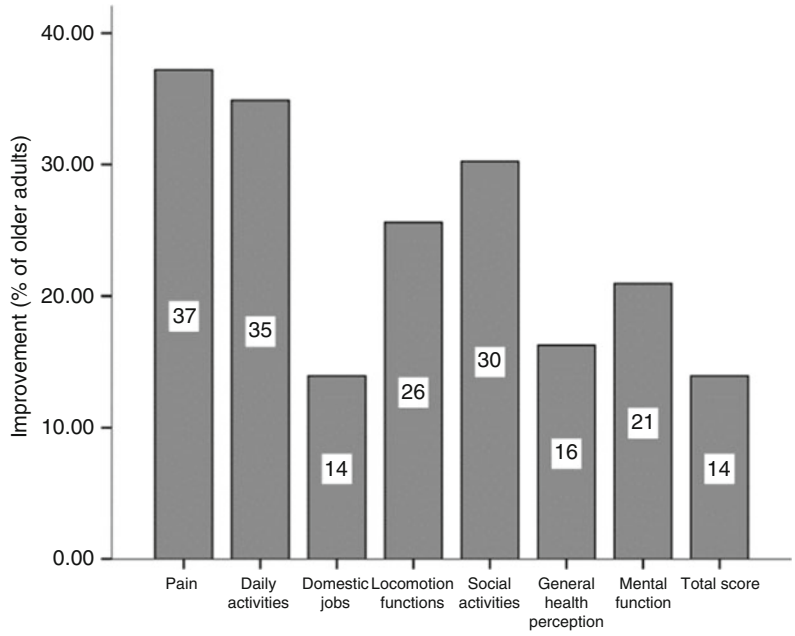

Fig. 15.4 Effect of vitamin D bread on quality of life measured by QUALEFFO 41 questionnaire in vitamin D deficient older adults ($N=42$) living in a nursing home from Iasi, Romania (latitude 47°N). The quality of life in older patients improved after 1 year consumption of vitamin D bread especially for the pain, daily activities, domestic jobs, locomotion functions, and social activities domains

In conclusion, the daily consumption of bread fortified with 125 μg (5,000 IU) vitamin D_3 and 320 mg elemental calcium during 1 year resulted in optimal status of vitamin D and osteoid mineralization. The reduction of fractures and pain contributed to greater improvement in QoL mainly related to daily activities, domestic jobs, locomotion functions, and social activities domains observed in older patients [23, 42].

The Benefits of Using Vitamin D Fortified Bread

The fortified wheat or maize products are food staples, consumed in significant quantities by all age groups and economic classes at nearly every meal. This makes them ideal vehicles for getting deficient nutrients to the general population. Together with vitamin D, calcium could be also added to milled cereals. Since vitamin D and calcium work as partners in bone health, fortifying with appropriate levels of calcium should be considered when fortifying with vitamin D. Cereal fortification is safe because a person cannot eat enough exceed the upper safety levels of vitamin D intakes. Fortification at the mill is relatively inexpensive and affordable [43].

Newly available yeast products are exposed to ultraviolet light to generate vitamin D_2 in the yeast which can be used for vitamin D_2-fortified bread; however, since vitamin D_3 is more potent than vitamin D_2, allowance needs to be made to provide higher amounts of vitamin D_2 to make up for its lesser potency.

Guidance on Levels to Be Added

In the long-term, an incremental intake of 1 μg (40 IU) vitamin D/day increased the mean serum 25(OH)D concentration by 0.5–1.5 nmol/L [32]. The average adult with an initial 25(OH)D concentration between 20 and 40 nmol/L requires 55 μg (2,200 IU) vitamin D_3/day to reach a 25(OH)D target concentration of 80 nmol/L [44]. This is not enough to ensure a concentration >80 nmol/L for all adults. In a recent study [45], nursing home residents received a minimum of 5 months of daily 50 μg (2,000 IU) vitamin D_3 supplementation. 94.1 % of nursing home residents had a 25(OH)D level in excess of 80 nmol/L after a minimum of 5 months of daily 50 μg (2,000 IU) vitamin D_3 supplementation. No residents had 25(OH)D levels in a toxic range. Aloia et al. [46] demonstrated that the mean daily dose predicted to raise levels to >75 nmol/L in all patients was 95 μg (3,800 IU) for those with a starting level of >55 nmol/L, and was as high as 125 μg (5,000 IU) for those with starting levels of <55 nmol/L. Some data suggest that for most older people (who are likely to have baseline 25(OH)D levels of <50 nmol/L), a dose of 20 μg (800 IU) per day or equivalent is sufficient to raise 25(OH)D levels to >50 nmol/L, but to raise levels to >75 nmol/L requires between 50 and 125 μg (2,000 and 5,000 IU) per day of oral vitamin D3 [46]. Efforts to correct vitamin D deficiency and secondary hyperthyroidism with doses of vitamin D >50 μg (2,000 IU) per day have been severely constrained in adults by the tolerable upper intake level (UL) for vitamin D. The UL is 100 μg (4,000 IU)/day in North America and 50 μg (2,000 IU)/day in Europe, whereas in the United Kingdom a similar safe intake limit—the guidance level—is 25 μg (1,000 IU)/day [47].

Recommendations

In older nursing home residents, the fortification of bread with a dose of 10 μg (400 IU)/100 g would cover 50 % of the RDA recommendations (800 IU) [21] and 20 % of the dose recommended by medical groups for bone health (2,000 IU/day) [46]. This dose is safe and represents only 10 % of the tolerable upper intake level (4,000 IU/day). The use of rye flour might decrease the vitamin D absorption by 30 % as compared to wheat flour [38]. In this case fortification could increase to 12.5 μg (500 IU)/100 g of rye bread. The vitamin D_3 is the most potent vitamin D and it should be used for food fortification [32].

Conclusion

Several factors contribute to the increased requirements for dietary calcium and vitamin D in older adults, including the age-related reduction in both calcium absorption and renal calcium conservation, been attributed in part, to inadequate vitamin D status from reduced dietary intake, less efficient absorption, reduced dermal capacity to synthesize vitamin D and/or inadequate exposure to sunlight. Based on evidence from literature, in order to improve health and well-being and to preclude preventable morbidity and mortality associated with 25(OH)D deficiency, all nursing home patients without contraindication could be routinely supplemented with (at minimum) 50 μg (2,000 IU) of vitamin D_3 on a daily basis, with a supplementation dose that leads to a maximum total daily calcium intake of 1,000–1,200 mg. Fortification of bread and cereals is a feasible way to improve vitamin D nutrition and a dose of 10 μg (400 IU) vitamin D_3 per 100 g serving from any sources is safe.

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Chapter 16
Learnings from the Postmenopausal Health Study for the Effect of Dairy Products Fortified with Calcium and Vitamin D on Bone Metabolism

George Moschonis, Angeliki Giannopoulou, and Yannis Manios

Key Points

- The main source of vitamin D in the human body is its endogenous production via skin exposure to sunlight (i.e. ultraviolet B irradiation).
- Studies in sunny Mediterranean countries have shown that the prevalence of vitamin D deficiency is high, despite the sunny climate.
- Supplementation with calcium (1,200 mg/day) and vitamin D (reaching up to 22.5 μg/day) via fortified dairy products was found to produce more favourable changes in dietary, hormonal, bone metabolism and bone mass indices in osteopenic postmenopausal women in Greece that participated in the "Postmenopausal Health Study".

Keywords Calcium and vitamin D • Bone metabolism • Fortified dairy products • Postmenopausal women • Intervention • Growth and calciotropic hormones • Osteoclastic differentiation molecules • Bone-remodelling indices • Bone mineral density

Abbreviations

DRIs	Dietary Recommended Intakes
IOM	Institute of Medicine
PTH	Parathyroid hormone
IGF-I	Insulin-like growth factor I
RANKL	Receptor activator of nuclear factor-kappaB ligand
OPG	Osteoprotegerin
CTx-I	Type I collagen cross-linked C-telopeptide
OC	Osteocalcin
BMD	Bone mineral density

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V.R. Preedy et al. (eds.), *Handbook of Food Fortification and Health: From Concepts to Public Health Applications Volume 2*, Nutrition and Health, DOI 10.1007/978-1-4614-7110-3_16,

Introduction

Osteoporosis and the subsequent risk of bone fracture occur most commonly among postmenopausal women who are middle-aged and older. These disorders account for a significant burden of morbidity and mortality worldwide and have become a major public health problem [1]. Epidemiological data reveal an increased incidence of hip fractures more likely of osteoporotic etiology in women >50 years old from several European countries (Table 16.1).

Whereas research so far has focused mainly on women, the disease has also taken great dimensions in men although this cannot be detected based on the current data due to low percentage of men who get a physician' s referral for bone mass examination.

The adequate intake of certain nutrients that are essential for bone metabolism, such as calcium and vitamin D, plays an important role in maintaining bone mass. With increasing age, however, both dietary calcium intake and intestinal calcium absorption decrease [2]. Furthermore, in the elderly, serum concentrations of 25-hydroxyvitamin D_3 [$25(OH)D_3$] decline, mostly because of decreased sunlight (ultraviolet B irradiation) exposure, which leads to a limited capacity for cutaneous vitamin D synthesis [3]. Combined with low dietary intake of vitamin D from staple foods, especially in countries without mandatory fortification policy, these factors contribute to lower blood concentrations of $25(OH)D_3$ and consequently to accelerated bone loss and greater risk of bone fracture [4]. It has been reported that meeting daily dietary requirements of calcium and vitamin D produces a significant reduction in the incidence of bone fracture [5].

According to recent evidence, supplementation with calcium or vitamin D or both has been reported to exert a significant favourable effect on bone mineral density (BMD) and on several bone-remodelling biomarkers and has been associated with lower risk of fracture [6, 7]. An abundance of clinical trials has indicated the beneficial effects of supplementation with calcium or vitamin D or their combination on the prevention of bone loss and on bone-remodelling indices [7, 8], but very few studies have examined the effect of increases in the intake of these micronutrients through fortified foods and especially through dairy products [4, 9, 10]. Even scarcer are the data coming from dietary intervention trials with fortified foods in Mediterranean populations. The common belief in Mediterranean populations is that because of the sunny climate, there is no need for additional vitamin D intake and

Table 16.1 Age-specific incidence figures for hip fracture in women in several EU member states (per 10,000 population)

	Age group							
Country	50–54	55–59	60–64	65–69	70–74	75–79	80–84	+85
Austria	3.4	7.1	14.1	26.5	47.7	82.4	138.0	351.0
Belgium	2.7	5.9	11.8	22.6	41.1	72.0	122.0	317.0
Denmark	4.1	8.6	17.0	31.9	57.2	98.4	164.0	416.0
Finland	2.7	5.9	12.1	23.4	43.1	76.2	130.0	346.0
Germany	3.4	7.1	14.1	26.5	47.7	82.4	138.0	351.0
Greece	2.5	5.4	10.8	20.4	36.9	64.2	108.0	232.0
Hungary	2.4	6.0	11.2	18.6	35.1	69.5	184.5	324.4
Ireland	1.8	4.3	9.3	19.1	37.3	69.5	125.0	362.0
Italy	1.6	3.5	7.2	13.9	25.6	45.4	77.6	172.0
Luxemburg	2.7	5.9	11.8	22.6	41.1	72.0	122.0	317.0
Netherlands	2.7	5.9	11.8	22.6	41.1	72.0	122.0	317.0
Portugal	2.6	5.2	9.6	17.1	29.0	47.7	75.8	151.0
Spain	0.6	1.7	4.4	10.5	23.7	50.3	102.0	290.0
Sweden	4.1	9.1	18.1	38.7	81.7	168.9	336.4	518.3
United Kingdom	1.8	4.3	9.3	19.1	37.3	69.5	125.0	362.0

Table 16.1 presents the incidence of hip fractures in women in several EU member states

Source: Report on osteoporosis in the European Community. Action on prevention. Luxembourg Office for Official Publications of the European Communities, p. 112, 1998

that calcium supplementation alone may be adequate to prevent bone loss. However, studies conducted in this region have shown a high prevalence of vitamin D deficiency [11–13]. The substantial risk of vitamin D deficiency in southern European countries, particularly during winter months, insufficient intestinal calcium absorption in the elderly and the recently published new Dietary Recommended Intakes (DRIs) for calcium and vitamin D by the Institute of Medicine (IOM) [14] all point to the need for additional calcium and vitamin D intakes.

The "Postmenopausal Health Study"

One of the very few studies that have thoroughly examined the effect of dairy products enriched with calcium and vitamin D on bone health is the "Postmenopausal Health Study". More specifically, the "Postmenopausal Health Study" examined the changes in bone metabolism indices at different skeletal sites in apparently healthy, self-dependent postmenopausal women throughout an intervention period of 30 months.

Methodology of the "Postmenopausal Health Study"

The methodology of the "Postmenopausal Health Study" has been described in detail elsewhere [15, 16]. In brief, the recruitment of the study participants included two screening phases after approval from the Ethical Committee of Harokopio University. This procedure yielded 112 postmenopausal women (55–65 years old) that were randomly assigned into a dietary intervention group ($n=42$), a calcium-supplemented group ($n=30$) and a control group ($n=40$).

After the first 12 months of the programme, women in the calcium-supplemented group were excluded from the study, which carried on for 18 more months with the other two groups. During their 12-month participation in the study, women in the calcium-supplemented group received an amount of 600 mg of supplementary calcium (87 % of calcium lactate gluconate and 13 % of calcium carbonate) per day in the form of water-soluble tablets aiming to a total of 1,200 mg per day, in addition to their daily dietary calcium intake that was estimated to be approximately 600 mg.

Women in the dietary intervention group were advised to consume three portions of low-fat fortified dairy products (milk and yogurt) on a daily basis. The dairy products were providing 1,200 mg of calcium and 7.5 μg of vitamin D_3 for the first 12 months. Vitamin D_3 increased to 22.5 μg for the remaining 18 months of the intervention by increasing the vitamin content of fortified milk from 2.5 to 10 μg/portion. To ensure compliance with the intervention scheme, these women also attended "Health and Nutrition Counseling" sessions biweekly. These sessions were aiming to increase their awareness on health issues and to improve their self-efficacy in adopting a healthier lifestyle.

Finally, no intervention was delivered to subjects in the control group (i.e. neither fortified dairy products nor nutrition and lifestyle counselling sessions) as they continued with their usual diets.

Results of the "Postmenopausal Health Study"

Effect of the Intervention on Dietary Intake Indices

The "Postmenopausal Health Study" showed certain favourable dietary changes in the dietary intervention group and to a lesser extent in the calcium-supplemented group. More specifically, both the dietary intervention group and the calcium-supplemented group increased calcium intake to >1,000 mg/day, reaching the recommended adequate intake of 1,200 mg/day. Furthermore, greater intakes of protein,

magnesium, phosphorus and vitamin D were observed for the dietary intervention group compared to the other two groups. The changes in the abovementioned micronutrients were mainly delivered by the increased consumption of fortified dairy products provided to women in the dietary intervention group. These favourable dietary changes were also indicative of the effectiveness of the Health and Nutrition Education component of the programme in increasing the self-efficacy of women in the dietary intervention group to comply with the given dietary instructions. Similar to the present study, other intervention studies conducted with middle-aged women and including nutrition counseling as an integral part of their intervention scheme also reported high adherence to the dietary guidelines provided [17], whereas compliance was considerably lower in intervention programmes not followed by regular counseling sessions [4].

Effect of the Intervention on Growth and Calciotropic Hormones

The favourable dietary changes observed in the "Postmenopausal Health Study" were more likely responsible for the changes observed in the growth and calciotropic hormones examined. More specifically during the 12-month intervention period, the results of the "Postmenopausal Health Study" regarding serum IGF-I levels are concordant with those in other studies showing significant positive associations between increases in dietary protein intake and serum concentrations of IGF-I [18, 19]. This anabolic hormone-like peptide has been reported by recent studies to stimulate bone formation activity in postmenopausal women, basically via the differentiation of osteoblasts [20].

Regarding $25(OH)D_3$ serum levels, significant increases were observed in the prevalence of vitamin D insufficiency in dietary intervention and control groups during winter months, while significant decreases were observed in both groups during summer months as presented in Fig. 16.1.

These changes, especially the increase in the prevalence of vitamin D insufficiency during winter months—i.e. the months when exposure to sunlight is limited—was similar to the one reported in other recent studies [4, 21, 22]. Although the seasonal effect on vitamin D status was obvious in both the dietary intervention and control groups, still changes in serum levels of $25(OH)D_3$ were more favourable for women in the dietary intervention group, who had a milder decrease during winter and a higher increase during summer compared to the relative changes in the control group.

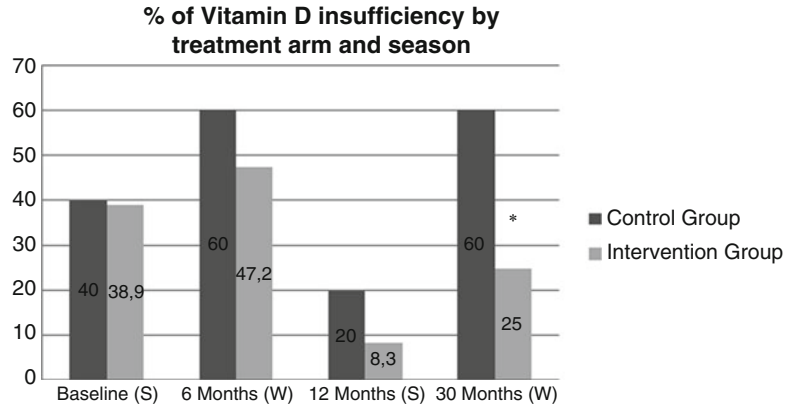

Fig. 16.1 Prevalence of vitamin D insufficiency (<20 ng/mL) for women in the dietary intervention ($n=30$) and the control group ($n=36$) at baseline, 6, 12 and 30 months of follow-up. Figure 16.1 presents the results of the Postmenopausal Healthy Study regarding the vitamin D insufficiency during summer and winter months. *$P=0.006$ for differences between control and intervention groups based on Fisher's exact test. *S* summer months; *W* winter months (*Source* [28])

Considering that the major source of vitamin D in the body is its endogenous synthesis via the photoconversion of 7-dehydrocholesterol to previtamin D_3 in the skin, measurements of sunlight exposure by personal ultraviolet dosimetry in the "Postmenopausal Health Study" showed no significant differences among groups. These data indicate that seasonal variations in serum 25(OH)D_3 concentrations were greater for women in the control group and calcium-supplemented group than for women in the dietary intervention group, who received supplementation with an additional amount of vitamin D_3. Nonetheless, the amount of vitamin D_3 supplemented to the dietary intervention group was obviously not enough to entirely prevent reduction of vitamin D serum levels during winter months. Contrary to these findings from the "Postmenopausal Health Study", other recent vitamin D supplementation studies have reported dose-dependent increases in serum 25(OH)D_3 levels during winter months with limited sun exposure ranging from 38.4 to 88 ng/mL after daily oral supplementation of 100 to 250 μg of vitamin D_3 [23].

Seasonal variations were also observed for serum parathyroid hormone (PTH) levels, but only for women in the control group, who maintained low dietary calcium intake (i.e. <700 mg/day) throughout the study. In contrast, the changes observed in the other two groups did not differ statistically significantly from baseline. This should be attributed to the additional calcium supplementation provided to women in the dietary intervention and the calcium-supplemented groups, which improved their calcium status and suppressed PTH secretion. Another regulating mechanism for serum PTH concentrations in the dietary intervention group could also be the maintenance of serum 25(OH)D_3 in levels above 25 ng/mL. Evidence suggests that serum concentration of 25(OH)D_3, which is considered adequate to prevent compensatory hypersecretion of PTH, ranges from 25 to 40 ng/mL [24]. The findings of the "Postmenopausal Health Study" are in agreement with similar dietary intervention studies providing either calcium supplements (with or without vitamin D) or fortified dairy products to postmenopausal women. These studies showed that suppression of serum PTH was positively associated with the dose of supplemented calcium or vitamin D (or both), as well as with the basal serum 25(OH)D_3 concentrations [4, 9, 25].

Effect of the Intervention on Osteoclastic Differentiation Molecules

The effect of the intervention in the "Postmenopausal Health Study" on *growth and calciotropic* hormones probably triggered the favourable changes also observed for serum levels of osteoclastic differentiation molecules. More specifically, the "Postmenopausal Health Study" showed a decrease in the serum levels of the receptor activator of nuclear factor-kappaB ligand (RANKL) (i.e. a marker linked to bone resorption) for women in the dietary intervention group that was found to differentiate significantly compared to the increase observed for women in the control group. In contrast to RANKL levels, serum osteoprotegerin (OPG) levels (i.e. a marker linked to bone formation) increased for women in the dietary intervention group, while serum OPG levels decreased in the control group.

The discovery of the unique role of the RANK/RANKL/OPG signalling has led to the targeting of this pathway as a novel therapeutic approach in the management of osteoporosis [26]. Although very little is known so far about the effect of dietary interventions on bone markers, there have been studies in postmenopausal women where 24 months of genistein (i.e. a phytoestrogen) administration has resulted in higher serum OPG and lower serum RANKL levels [27]. The "Postmenopausal Health Study" is, to our knowledge, the first study to examine the effect of a dietary intervention with fortified dairy products on these two osteoclasts' differentiation molecules.

Effect of the Intervention on Bone-Remodelling Indices

The effect of the intervention conducted in the "Postmenopausal Health Study" on the molecular circuit of OPG/RANKL could have resulted in the changes observed in certain bone-remodelling indices, i.e. in serum type I collagen cross-linked C-telopeptide (CTx-I) and osteocalcin (OC) levels.

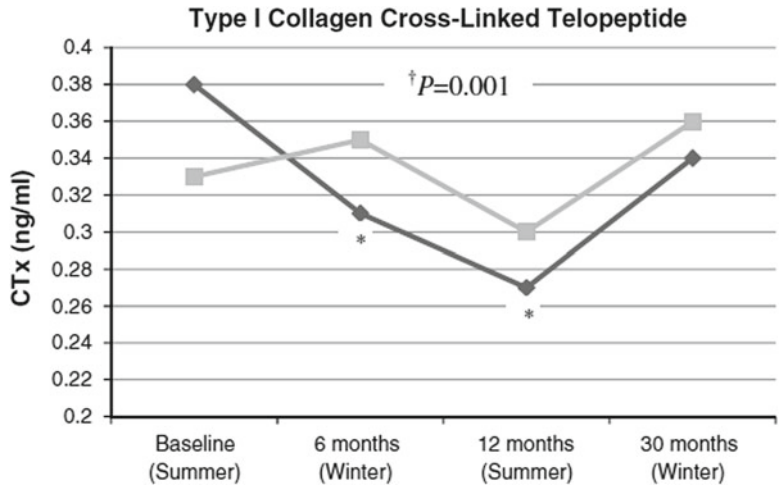

Fig. 16.2 Changes in serum levels of CTx-I for women in the dietary intervention ($n=20$) and control groups ($n=20$) after 6, 12 and 30 months of intervention. Intervention group — Control group - - -. †Treatment × time interaction effect; *$P<0.05$ vs. baseline within the same group (time effect). Figure 16.2 shows the changes of CTx-I (ng/mL) levels during the study period separately in the two study groups (*Source* [16])

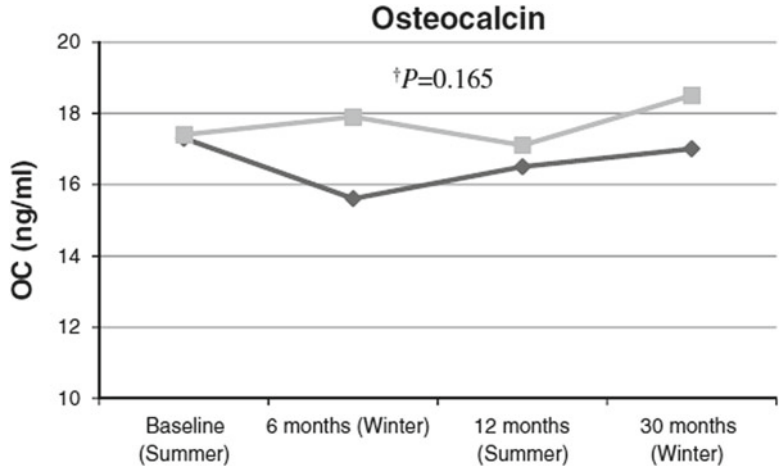

Fig. 16.3 Changes in serum levels of osteocalcin for women in the dietary intervention ($n=20$) and control groups ($n=20$) after 6, 12 and 30 months of intervention. Figure 16.3 presents the changes on OC (ng/mL) levels at baseline and three follow-up measurements in the two study groups. Intervention group — Control group - - -. †Treatment × time interaction effect; *$P<0.05$ vs. baseline within the same group (time effect) (*Source* [16])

Specifically, serum CTx-I significantly decreased only for women in the dietary intervention group, after 6 and 12 months of intervention, while it did not change in the control group during the 30-month intervention period. Nonetheless, a seasonal effect was evident in the control group, as mean serum CTx levels remained higher during the winter (6- and 30-month follow-up) and lower during the summer period (baseline and 12-month follow-up), as presented in Fig. 16.2.

Regarding serum OC levels, there were no significant differences between the changes observed in the two study groups. However, similarly to the changes observed for CTx, serum OC levels in the control group followed a seasonal pattern with higher levels during winter months and lower levels in summer months compared to baseline. On the other hand, as presented in Fig. 16.3, serum OC in the

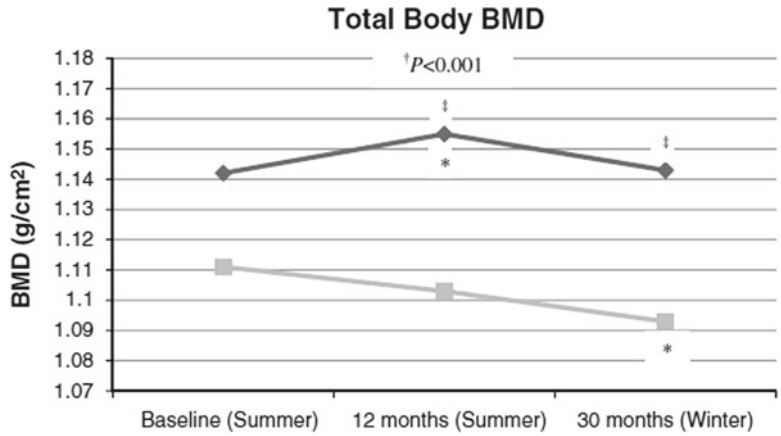

Fig. 16.4 Changes in total BMD for women in the dietary ($n=20$) and control groups ($n=20$) after 6, 12 and 30 months of intervention. Figure 16.4 shows how total body BMD (g/cm^2) changed during the study period. Intervention group Control group . †Treatment × time interaction effect; ‡$P<0.05$ intervention vs. control group (treatment effect); *$P<0.05$ vs. baseline within the same group (time effect) (*Source* [16])

dietary intervention group remained at much lower levels at all follow-up examinations compared to baseline, throughout the intervention period.

Overall, the changes observed in the control group were indicative of a clear seasonal effect on the rate of bone remodelling, showing increases during winter months and decreases during summer months. Still, the implemented intervention was proved to be effective enough in reducing the rate of bone remodelling for women in the dietary intervention group. Suppression of this process has been associated with prevention of bone loss from skeletal sites that are susceptible to fracture as well as from the total body [7].

Effect of the Intervention on Bone Mineral Density

The effect of the intervention in the "Postmenopausal Health Study" on bone remodelling indices coincided with the more favourable changes of total body BMD observed for women in the dietary intervention group. Specifically, at the first 12 months of the programme, women in the dietary intervention group were found to have higher levels of total body BMD than women in the other two study groups. Similarly, following the increase in the vitamin D content of the supplemented fortified dairy products (i.e. from 7.5 to 22.5 μg) after the first 12 months of the program, women in the dietary intervention group were found to have more favourable changes in total body BMD compared to women in the control group until the end of the 30-month intervention period, as presented in Fig. 16.4.

In agreement with the findings of the "Postmenopausal Health Study", Riggs et al. [25] reported that 12-month supplementation of 1,600 mg of calcium per day in postmenopausal women could preserve total body BMD. Similar findings are also derived from other dietary intervention studies on postmenopausal women after providing 1,000–1,200 mg of calcium and 6–10 μg of vitamin D_3 per day through the consumption of fortified milk or milk powder for 12 months [9]. In the same context, supplementation studies conducted with postmenopausal women that ensured daily intakes of either 1,000–1,500 mg of calcium and 12.5–14 μg of vitamin D_3 [6, 22], or 1,200 mg of calcium and 17.5 μg of vitamin D_3 [22] also showed more favourable changes in lumbar (L2–L4) and total spine BMD for those women receiving the supplements.

Guidance on Safe Levels and/or Guidance on Levels to Be Added

- According to recent guidelines released by the Institute of Medicine 2010, the tolerable upper levels (UL) for calcium and vitamin D are 2,000 mg and 4,000 IU (i.e. 100 μg), respectively. Although UL for calcium did not change, the UL for vitamin D has been doubled from 2001 (the 2001 UL was 50 μg) to 2010.
- The recommended intakes of calcium and vitamin D includes the cumulative intake from both supplements and dietary sources.
- According to the Institute of Medicine when intakes of vitamin D surpass 4,000 IU (i.e. 100 μg) per day, health risks begin to increase. Furthermore, very high intake levels of vitamin D (above 10,000 IU or 250 μg/day) are known to cause kidney and tissue damage. Strong evidence about possible risks of daily vitamin D intake at lower levels is limited, but some preliminary studies offer tentative signals about adverse health effects.
- Recent data that show multiple benefits of vitamin D in different aspects of health justify the doubling of the upper level threshold from 50 to 100 μg/day.
- According to the Institute of Medicine when intakes of calcium surpass 2,000 mg/day, health risks also increase.

Recommendations

- Based on recent recommendations provided by the Institute of Medicine (2010):
  - The RDA for calcium intake is 1,000 and 1,200 mg for women aged 31–50 years and >51 years, respectively.
  - The RDA for vitamin D intake is 600 IU (15 μg) and 800 IU (20 μg) for women aged 31–70 years and >70 years, respectively.
- Based on the 2001 recommendations from the Institute of Medicine, the RDA for magnesium intake is 320 mg for women aged 31–50 years, 51 years and older.

Conclusion

Osteoporosis is a very common disease that affects not only older women but also older men and has been related with increased fracture risk. Due to the fact that calcium and vitamin D are very important in reducing the risk of fracture, several supplementation studies have examined their effect on bone mass and bone metabolism indices. Still there are only a few clinical trials examining the effect of these nutrients when supplemented to susceptible population groups via fortified dairy products. The “Postmenopausal Health Study” is one such clinical trial with a duration of 30 months showing favourable changes in dietary, hormonal, bone metabolism and bone mass indices in postmenopausal women were provided with dairy products enriched with calcium (1,200 mg) and vitamin D_3 (ranging from 7.5 to 22.5 μg) for 30 months.

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Chapter 17
Cognition and Multiple-Micronutrient Fortification of Salt

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Key Points

1. Multiple-micronutrient deficiencies are one of the important causes of lowered concentration abilities and impaired memory skills. Two studies are discussed where we tested the change in memory and cognition when a multiple-micronutrient-fortified salt is the intervention.
2. Study on nutrition and cognition in older children:
   (a) A randomized controlled trial with a pre- and posttest design was used to study children 5–18 years of age, with an experimental ($n = 213$) and control group ($n = 189$) for 9 months where the experimental group received a multiple-micronutrient-fortified salt and the control group received iodized salt, and children between 11 and 18 years of age were given cognitive tests to assess memory and attention. Biochemical and cognitive tests were administered at baseline and post-intervention.
   (b) There was a significant improvement ($p < 0.05$) in all the biochemical measurements and memory tests in the experimental group when compared with the control group.
3. Study on nutrition and cognition in younger children:
   (a) The Research Design was a pre- and posttest design with experimental and control groups in the age group 7–11 years. In the experimental group ($n = 63$), the food in the school kitchen was cooked with the multiple-micronutrient-fortified salt for a period of 1 year. The control group ($n = 66$) consisted of day scholars who did not eat at the school. Biochemical tests and a battery of seven memory tests were administered to all the children at baseline and post-intervention.
   (b) There was a significant ($p < 0.05$) improvement in most of the biochemical tests and in four of the seven memory tests in the experimental group when compared with the control.
   (c) These studies show that the multiple-micronutrient-fortified salt is effective in improving multiple-micronutrient status and cognition in children.

Keywords Cognition • Multiple micronutrients • Fortification of salt • Nutrition • Multiple-micronutrient deficiencies

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V.R. Preedy et al. (eds.), *Handbook of Food Fortification and Health: From Concepts to Public Health Applications Volume 2*, Nutrition and Health, DOI 10.1007/978-1-4614-7110-3_17,

Abbreviations

AGP	Alpha 1 acid glycoprotein
CRP	C-reactive protein
ELISA	Enzyme-linked immunosorbent assay
IU	International units
IQ	Intelligence quotient
NIST	National Institute of Standards and Technology
RDA	Required daily allowance
RBC	Red blood cells
SF	Serum ferritin
sTfR	Soluble transferrin receptor

Introduction

Micronutrient deficiencies in developing countries are a consequence of the plant-based cereal diets typically consumed in these areas [1, 2]. Dietary phytate inhibits the absorption of many micronutrients, notably iron and zinc. Micronutrient deficiencies can cause impairments in physical development and cognition. Iron and iodine deficiencies affect more than 30 % of the global population [3]. Multiple-micronutrient deficiencies can be effectively tackled with multiple-micronutrient fortification.

Salt is an excellent vehicle of fortification because it is used universally by everyone everyday in about the same quantities. For salt fortification to be successful, the micronutrients should not change the color, odor, or taste of the food when the salt is used in cooking, should be stable at cooking temperatures, and should be bioavailable. With these concepts kept in mind, a multiple-micronutrient-fortified salt was developed that contained vitamin A, vitamin B1, vitamin B2, vitamin B6, vitamin B12, folic acid, niacin, iron, iodine, and zinc.

The iron in hemoglobin carries oxygen to all parts of the body including the brain. Iron deficiency results in decreased oxygen-carrying capacity of the blood. This affects cognition and memory. Many B-complex vitamins are also involved in cognition. We therefore tested the change in memory and cognition when the multiple-micronutrient-fortified salt is the intervention for the delivery of multiple micronutrients in children. The relationship between nutrition and cognition can be thus assessed. Two studies are discussed: one study which tests cognition on children 7–11 years of age and another study which tests cognition on children 11–18 years of age, when multiple-micronutrient-fortified salt is the intervention.

Study 1: Study on Nutrition and Cognition in Children 11–18 Years of Age

The study was a randomized controlled trial which had a pre- and posttest design with experimental and control groups. This study tested the stability of the fortified salt during storage and the absorption of iron, vitamin A, vitamin B12, folic acid, zinc, and iodine in the target group of children in residential schools aged 5–18 years. Children between 11 and 18 years of age were given cognitive tests to assess memory and attention and not younger children 5–10 years of age.

Three residential schools were randomly selected as the experimental schools and three other residential schools as the controls in the city of Chennai, Tamil Nadu, South India. The experimental schools were supplied with the multiple-micronutrient-fortified salt every month. The control schools

Table 17.1 Stability of the multiple-micronutrient-fortified salt during storage at 30 °C and relative humidity 45 % for 10 months in study 1

Micronutrient in 10 g salt	Initial concentration	Concentration after 10 months of storage	% loss
Vitamin A (3,000 IU)	2,915 ± 52.3	2,298 ± 90.68	21.18
Vitamin B1 (1 mg)	1.013 ± 0.009	0.966 ± 0.013	4.64
Vitamin B2 (1 mg)	1.015 ± 0.013	0.941 ± 0.042	7.28
Vitamin B6 (1 mg)	1.02 ± 0.017	0.946 ± 0.015	7.32
Niacin (5 mg)	5.058 ± 0.079	4.78 ± 0.054	5.48
Iron (1,000 ppm)	1012.7 ± 15.3	991.9 ± 9.91	2.05
Iodine (40 ppm)	41.57 ± 1.96	35.62 ± 1.97	14.31
Folic acid (100 μg)	107.38 ± 2.28	98.66 ± 2.42	8.12
Vitamin B12 (4 μg)	4.077 ± 0.023	3.939 ± 0.08	3.38
Zinc (10 mg)	10.121 ± 0.078	9.7 ± 0.089	4.12

Data given as mean ± SD of ten batches prepared for the study

were provided with iodized salt every month. Verification by weighing the salt leftover from the previous month confirmed the daily usage of the salt in all the schools. The main outcome indicators were the biochemical tests and the memory tests. The study began when the schools reopened after the summer vacation and continued for 9 months until the schools closed again for the next summer vacation. The experimental and control groups of children were homogenous in terms of age and socioeconomic status and diets. We have considered a *p*-level of 0.05 and power of 80 % with a two-tailed test for all sample size calculations based on our earlier experiences with the use of fortified salt in children [4]. All the children were given a tablet of albendazole (400 mg) at baseline, at 4 months, and post-intervention after 9 months. Clinical assessment of angular stomatitis, a condition caused by deficiencies of B-complex vitamins and clinical vitamin A deficiencies such as xerosis and the presence of Bitot spots, was conducted by a physician before the start of the study and after 9 months of intervention.

Dosage of Micronutrients

The salt was used in all the meals prepared for the children. It was found out that the average consumption of salt was 10 g per child per day. Therefore the fortified salt was prepared such that 10 g of the fortified salt contained about one RDA [5] of the micronutrients; the exception was iron, which was given at a dosage of 10 mg per day (30 % RDA) as the iron was chelated, instead of 28 mg iron, which was the RDA (Table 17.1). The cooking staff of all the six schools certified that the fortified salt did not change the color or taste of any food.

Blood Collection and Storage

Venous blood samples (5 mL) were drawn from each child, and 500 μL was transferred into vials with ethylenediaminetetraacetate as an anticoagulant. The hemoglobin measurements were performed on these samples. The remaining 4.5 mL of blood was transferred into vials covered with black paper to prevent exposure to light, and the blood was allowed to clot. Serum separation was performed in the laboratory and the samples were frozen at −20 °C. During vitamin A estimations, the samples were processed in a dark room with yellow lighting to prevent retinol isomerization.

Laboratory Analyses

The biochemical estimations done were for hemoglobin, serum ferritin, sTfR, CRP, AGP, serum vitamin B12, serum folic acid, serum zinc, serum retinol, and urinary iodine. Hemoglobin was measured in all the children in both groups ($n=213$ in experimental group and $n=189$ in control group) 3 times during the study, at baseline, after 4 months, and post-intervention after 9 months. All other biochemical measurements were done at baseline and post-intervention. Serum vitamin A was measured by high-performance liquid chromatography (HPLC) only in those children who were identified as having vitamin A deficiency by a physician who checked the eyes of the children for clinical signs of vitamin A deficiency, such as Bitot spots or xerosis. One hundred and nineteen children in the experimental group and 87 children in the control group had clinical signs of vitamin A deficiency. Serum folic acid, vitamin B12, serum zinc, sTfR, serum ferritin, CRP, and AGP were measured in the 50 children who had the lowest hemoglobin levels in the experimental group and 50 children with lowest hemoglobin in the control group. Urinary iodine was measured in a random subsample of 73 children in the experimental group and 70 children in the control group. Hemoglobin was estimated by the cyanmethemoglobin method with a spectrophotometer (UV double-beam model, Shimadzu, Japan) [21]. Serum vitamin A was measured by a rapid, reverse-phase HPLC method (HPLC-Shimadzu, Japan). NIST serum sample standards (SRM968c-lyophilized frozen serum sample with certified retinol values) were used to calculate the retinol values in the children. Vitamin B12 and folic acid assays were performed with a paramagnetic particle, chemiluminescent immunoassay for the quantitative determination of levels in human serum using the ADVIA Centaur Immunoassay System (Siemens, Germany). Serum zinc was determined by atomic absorption spectroscopy. Serum ferritin, sTfR, AGP, and CRP were determined by sandwich ELISA method [6]. Urinary iodine was measured by using the Sandell–Kolthoff reaction as modified by Pino et al. [7]. Iron deficiency (ID) was defined as SF <15 mg/L or sTfR concentration >7.6 mg/L [8]. Anemia was defined as a hemoglobin concentration <13 g/dL in boys aged ≥15 years, a hemoglobin concentration <12 g/dL in children aged ≥12 years and in girls aged ≥15 years, and a hemoglobin concentration <11.5 g/dL in children aged 5–11 years [8]. Iron deficiency anemia (IDA) was defined as simultaneous presence of ID and anemia. Body iron stores were estimated by the method of Cook et al. [9]. Infection was defined as CRP greater than 10 mg/L, and such data was removed before statistical analysis. Serum vitamin A deficiency was defined when serum vitamin A was less than 20 μg/dL.

Validation of Biochemical Measurements

For hemoglobin, serum vitamin A, urinary iodine, serum zinc, serum vitamin B12, and serum folic acid, estimations were measured in duplicate in 10 % of the samples. The coefficient of variation (CV) for estimation of serum vitamin A was 4.1 %. The ELISA tests for ferritin, sTfR, AGP, and CRP were done in duplicate for every sample; the CV for ferritin was 7.5 %; sTfR, 6.3 %; CRP, 12.4 %; and AGP, 7.8 %.

Cognitive Test

The investigators who carried out the memory tests were blinded to the group assignment of the schools (experimental and control schools). The children were divided into two groups based on their ages. Sixth- through eighth-grade children (11–13-year-olds) were in one group and 9th- to 12th-grade children (14- to 18-year-olds) were in another group. The younger children were given a test wherein an audio tape with 15 words was played to them. The 15-word sequence was repeated

10 times. The children had to memorize the words in the same sequence and write them down at the end of the test. The older children were given a test with a 20-word sequence. The test analyzed the ability of the children to memorize the words in the same sequence. One mark was allotted to each word written in the correct sequence. The words were chosen from Shellenberg's list and there was no connection or correlation between the words. The memory tests were given at baseline and post-intervention to 92 children in the experimental group and 70 children in the control group, in the age group 11–18 years.

Measurement of Stability of the Multiple-Micronutrient-Fortified Salt

To determine the stability of the multiple-micronutrient-fortified salt, its composition was analyzed initially and every month for a storage period of 10 months at 30 °C, humidity 45 % (Table 17.1).

Statistical Analysis

Statistical analysis was performed with SPSS 11.0 software (SPSS Inc., Chicago, IL, USA) and Microsoft Excel 2000 (Microsoft Corp., Seattle, WA, USA). Repeat measures of analysis of variance were used to compare the effects of group × time for memory tests, hemoglobin, serum ferritin, sTfR, body iron stores, CRP, AGP, serum vitamin B12, serum folic acid, serum zinc, and serum retinol. If the interaction effect of group × time was significant ($p < 0.05$), *t*-tests between groups and paired *t*-tests within groups were done. Proportions were compared by using chi-square tests. If data was not normally distributed, statistical analysis was done after log transformation. Binary logistic regression was done to compare the effects of group × time for the binary variables of anemia, iron deficiency (ID), IDA, vitamin A deficiency, and angular stomatitis. Significance was set at $p < 0.05$. The Mann–Whitney test and Wilcoxon signed-rank test were used to analyze urinary iodine (UI).

Ethical Issues

The study was approved by the institutional review board of the Sundar Serendipity Foundation. Informed written consent was obtained from the school directors, and informed oral consent was obtained from the parents or legal guardians of all of the children. The children with severe anemia (hemoglobin <8 g/dL) were treated with ferrous sulfate tablets and excluded from the study. Children found to be anemic at the end of the study in both the experimental and control groups were treated with ferrous sulfate tablets (60 mg elemental iron) for a period of 3 months.

The Impact of the Multiple-Micronutrient-Fortified Salt on Nutrition and Cognition in Older Children

Stability of the Multiple-Micronutrient-Fortified Salt

The percentage loss of the micronutrients over a period of 10 months is given in Table 17.1. It can be seen that the micronutrients are extremely stable on storage. In this study the salt batches were prepared every month and delivered to the schools on a monthly basis. The data given in Table 17.1 represents the mean ± SD of the ten batches of salt prepared for the study.

Efficacy Trial

There were 213 children in the experimental group (96 in school A, 50 in school B, and 67 in school C) and 189 children in the control group (21 in school D, 110 in school E, and 58 in school F) who completed the study (see Table 17.2). The mean baseline age of the experimental group was 12.31 years and that of the control group was 12.27 years; there was no significant difference in the age between the two groups. Changes in biochemical parameters over the study period in the experimental and control groups are shown in Table 17.2. There was a significant increase in the hemoglobin ($p=0.0001$) of the experimental group over the study period, whereas there was no statistically significant change in the control group hemoglobin. All indices of iron status (serum ferritin, sTfR, and body iron stores) improved significantly in the experimental group when compared with the control group at the end of the study ($p<0.05$) (Table 17.2). Serum retinol, serum vitamin B12, serum folic acid, and serum zinc improved significantly over the study period ($p<0.05$) in the experimental group when compared with the control group (Table 17.2). At baseline 12.8 % of the children in the experimental group and 14.8 % of the children in the control group had angular stomatitis, a condition attributable to B-complex deficiencies. At the end of the study, the prevalence of angular stomatitis in the experimental group had reduced significantly from 12.8 to 4.2 %, whereas in the control group the prevalence of angular stomatitis increased marginally from 14.8 to 16.4 % (Table 17.2). In the subsample of the children where we tested for anemia, iron deficiency, and IDA, there was a significant ($p<0.005$) reduction in anemia and IDA, but not iron deficiency, in the experimental group compared to the control (Table 17.3). In the subsample of children on whom serum retinol measurements were performed, there was a significant reduction in the prevalence of retinol deficiency in the experimental group ($p<0.0001$) when compared to the control group (Table 17.3). When median urinary iodine is considered, there is a significant improvement from baseline to post-intervention levels in both experimental and control groups, showing that the absorption of iodine from the multiple-micronutrient-fortified salt is similar to the absorption of iodine from iodized salt (Table 17.4).

Memory Tests

At baseline, there was no significant difference in the memory scores of the experimental and control groups of children, and hence they are comparable for memory outcomes. At the end of the study, the memory scores of the experimental group were significantly higher than those of the control group. ANOVA repeat measures showed significant ($p=0.045$) group × time interaction. If changes in the memory scores are considered (post-intervention scores minus baseline scores), the change in the experimental group is significantly higher than the change in the control group, showing that the use of the multiple-micronutrient-fortified salt helped in increasing memory scores in children (Table 17.5).

Study 2: Study on Nutrition and Cognition in Children 7–11 Years of Age

Subjects: The Research Design was a pre- and posttest design with experimental ($n=63$) and control ($n=66$) groups. The children residing in the residential school constituted the experimental group. The children who lived in communities nearby and attended the day school constituted the control group. The experimental and control groups of children were similar in age, intelligence, nutrient intake, and socioeconomic status. Baseline results showed that there was no statistical difference between the groups in Raven's progressive matrices showing that the groups were comparable in intelligence and hence can be compared for cognitive outcomes. The study was approved by the institutional review board of Sundar Serendipity Foundation, and all ethical procedures were similar to the previous study.

Table 17.2 Biochemical parameters in the experimental and control groups over 9 months in study 1

	Experimental group				Control group				
				p value				*p* value	*p* value
	N	Baseline	Post-intervention	Paired *t*-test	*N*	Baseline	Post-intervention	Paired *t*-test	Group × time ANOVA
Hemoglobin (g/dL)[a]	213	11.94 ± 1.4	12.61 ± 1.3	0.0001	189	12.22 ± 0.99	12.12 ± 0.96	0.099	0.0001
Ferritin (μg/L)[a,b]	45	8.43 ± 18.06	8.31 ± 42.12	0.889	43	12.17 ± 19.3	8.13 ± 15.75	0.001	0.013
sTfR (mg/L)[a]	45	12.86 ± 5.9	11.74 ± 5.25	0.005	43	9.77 ± 2.48	10.31 ± 3.27	0.226	0.005
Body iron stores (mg/kg)[a]	45	−2.68 ± 5	−2.4 ± 5.23	0.489	43	−0.55 ± 4.7	−2.14 ± 4.5	0.002	0.004
CRP (mg/L)	45	0.47 ± 0.92	0.41 ± 0.72	0.620	43	0.66 ± 1.24	0.57 ± 1.1	0.707	0.913
AGP (g/L)	45	0.84 ± 0.18	0.84 ± 0.23	0.987	43	0.82 ± 0.23	0.77 ± 0.22	0.285	0.447
Serum vitamin A (μg/dL)[a]	119	20.68 ± 8.23	25.34 ± 5.74	0.0001	87	19.08 ± 6.6	19.21 ± 5.24	0.872	0.0001
Serum vitamin B12 (pg/mL)[a]	45	5,612 ± 7,624	15,741 ± 10,979	0.0001	50	4,707 ± 5,950	557 ± 366	0.0001	0.0001
Serum folic acid (ng/mL)[a]	45	16.4 ± 11.6	10.12 ± 7.02	0.004	50	17.88 ± 11.98	5.08 ± 2.46	0.0001	0.018
Serum zinc (μg/dL)[a]	45	92.5 ± 39.6	142.5 ± 132	0.010	50	101.22 ± 48.3	102.73 ± 88.78	0.916	0.042
Angular stomatitis,[c] prevalence (%)	213	12.8	4.2		189	14.8	16.4		0.0228

Data given as mean ± SD

Hemoglobin was done 3 times, at baseline, midpoint, and post-intervention. The midpoint value of hemoglobin in the experimental group was 12.27 ± 1.42 g/dL, and in the control group it was 12.04 ± 1.06.

[a]Significant group × time interaction ANOVA repeat measures

[b]Geometric mean ± SD

[c]By binary logistic regression, there was a significant time × group interaction for angular stomatitis.

Table 17.3 Prevalence percentage of anemia, iron deficiency, iron deficiency anemia, and vitamin A deficiency in the two groups at baseline and post-intervention in study 1

	Experimental group—multiple-micronutrient-fortified salt			Control group—iodized salt			
	N	Baseline	Post-intervention	*N*	Baseline	Post-intervention	*p* values
Anemia	45	100	60	43	86	69.8	0.0039
Iron deficiency	45	91.1	84.4	43	83.7	86	0.6264
Iron deficiency anemia	45	91.1	51.1	43	74.4	58.1	0.0048
Serum vitamin A deficiency	119	57.1	16	87	62.1	59.8	0.0001

By binary logistic regression, there was a significant time × group interaction for anemia, iron deficiency anemia, and serum vitamin A deficiency but not for iron deficiency.

The fortified salt (Table 17.6) was used in all the meals cooked for the children in the experimental group for a period of 1 year. There was no intervention in the control group except for deworming. Deworming was done in all the children by giving a tablet of albendazole 400 mg at baseline, after 6 months of intervention, and at end line. The fortified salt was supplied to the school every month, and the continuous use of the fortified salt in all the meals prepared everyday was monitored. The period of the study was 1 year. The fortified salt did not change the color or taste of any food preparation. The average consumption of salt was 10 g per child per day, and the children of the experimental group consumed three meals per day and an afternoon snack, and all the food was prepared with fortified salt. The biochemical parameters that were estimated for all the children were hemoglobin, hematocrit, red blood cell count, urinary iodine, and serum vitamin A. Hemoglobin estimation was carried out before the start of the study, 6 months after the commencement, and 1 year after commencement. All other biochemical tests were done at baseline and end line only.

Blood Collection, Storage, and Laboratory Analysis

Three milliliter of venous blood was drawn from each child. Five hundred microliters of blood was transferred into vials which had EDTA as anticoagulant. Hemoglobin, hematocrit, and red cell counts were estimated in this sample, within a few hours of blood collection. The test for serum vitamin A was carried out in the remaining blood sample, transferred into vials wrapped in black paper to prevent sun exposure. When the blood was clotted, serum was separated from this sample and frozen at −20 °C. Hemoglobin was estimated by cyanmethemoglobin method [10]. Hematocrit was estimated by centrifuging blood in Wintrobe tubes [10]. Red blood cell count was done by counting the cells using the Neubauer counting chamber [10]. Serum vitamin A was estimated by the Carrprice method [11] using a spectrophotometer (UV double-beam Shimadzu spectrophotometer). Urinary iodine was measured by using the Sandell–Kolthoff reaction as modified by Pino et al. [7]. Hemoglobin was done in duplicate for all the samples. In hematocrit, RBC count, serum vitamin A, and urinary iodine, in 10 % of the samples, the test was done twice for validation.

Statistics

Statistical analysis was done using SPSS 11.0 (SPSS Inc., Chicago, IL, USA) and Microsoft Excel 2000(Microsoft Corp., Seattle, WA, USA). ANOVA was done to compare the effects of the nutritional intervention and cognition between groups and over time. Mann–Whitney test and Wilcoxon signed-rank test was used to test the significance of median urinary iodine analysis.

Table 17.4 Median urinary iodine excretion values μg/L (range) in the experimental and control groups over 9 months in study 1

	Experimental group—multiple-micronutrient-fortified salt			Control group—iodized salt			
	N	Baseline	Post-intervention	*N*	Baseline	Post-intervention	*p* values
Whole group, median urinary iodine (μg/L)	73	250 (20–500)[a,c]	365 (220–550)[b,c]	70	110 (20–500)[a,c]	317.5 (50–570)[b,c]	0.0001
Subjects with baseline median urinary iodine less than 100 μg/L—iodine deficient	13	75 (20–95)[d,c]	315 (275–450)[e,c]	30	50 (20–90)[d,c]	260 (50–570)[e,c]	0.001
Subjects with baseline median urinary iodine more than 100 μg/L—iodine sufficient	60	312.5 (105–500)[a,c]	370 (220–550)[b,c]	40	167.5 (100–500)[a,c]	337.5 (80–570)[b,c]	0.002

[a]Mann–Whitney test. Significant difference between the experimental and control groups at baseline, the control group significantly lesser than the experimental group
[b]Mann–Whitney test. Significant difference between the experimental and control groups post-intervention, the control group significantly lesser than the experimental group
[c]Wilcoxon signed-rank test. Significant improvement from baseline to post-intervention in both experimental and control groups, showing that the absorption of iodine from the multiple-micronutrient-fortified salt is similar to the absorption of iodine from iodized salt
[d]Mann–Whitney test. In subjects who are iodine deficient at baseline, there is no significant difference between experimental and control groups at baseline.
[e]Mann–Whitney test. In subjects who are iodine deficient at baseline, there is no significant difference between experimental and control groups post-intervention, so both groups have improved equally showing that the absorption of iodine from the multiple-micronutrient-fortified salt is similar to the absorption of iodine from iodized salt

Table 17.5 Memory test scores in study 1

	Experimental group					Control group					
					p value					*p* value	*p* value
	N	Baseline	Post-intervention	Change (post-intervention scores minus baseline scores)	Paired *t*-test	*N*	Baseline	Post-intervention	Change (post-intervention scores minus baseline scores)	Paired *t*-test	Group × time ANOVA
Mean memory test scores[a]	92	13.7 ± 0.588	16.22 ± 0.414	2.52 ± 0.4	0.0001	70	12.94 ± 0.564	14.16 ± 0.518	1.20 ± 0.521	0.025	0.045

Data given as mean ± standard error of mean

[a]Significant group × time interaction ANOVA showing improvement of memory scores in the experimental group

Table 17.6 Composition of the multiple-micronutrient-fortified salt in study 2

Ingredient	Nutrient status
Vitamin A (IU/g)	300
Vitamin B2 (mg/kg)	200
Calcium pantothenate (mg/kg)	200
Niacin (g/kg)	3
Vitamin B1 (mg/kg)	200
Vitamin B6 (mg/kg)	200
Folic acid (mg/kg)	5
Vitamin B12 (μg/kg)	400
Iron (ppm)	1,000
Iodine (ppm)	40

Tests for Memory, Concentration, and IQ

Iron Deficiency Anaemia is one of the important causes of lowered concentration abilities and impaired memory skills. The present study involves giving a battery of memory tests and concentration tests to see if there is an improvement in these memory and concentration abilities when anemia is reduced through the nutritive intervention. To test the memory in children, we used the children's memory test developed by NIMHANS (National Institute of Mental Health and Neurological Sciences, Bangalore, India). We gave the children only the nonverbal component of the test. This was possible because each test was individually scored. The individual scoring also helps us find out in which aspects of memory does improvement take place when iron and other micronutrients are given. The children in both the experimental and control groups were also administered the Raven's Children's Progressive Matrices, a test to assess the IQ of these children and a letter cancellation test to assess concentration. These tests were administered at baseline and post-intervention.

Description of the Memory Tests

Personal information: This test is a measure of remote memory which constitutes recall of past events of personal life. This is adopted from Wechsler memory scale and PGI memory scale.

Digit span: This subtest is taken from Wechsler memory scale. This comprises of span for digits forward and backward. The maximum number of digits used in the series is limited to 9. This test is a measure of attention and concentration.

Delayed response learning: This essentially requires the ability to delay the previous response in order to arrive at a final solution. This measures delayed memory span. There are four sets of fairly simple arithmetical problems. Each problem consists of two parts presented one after the other. In the first part a simple arithmetical problem is given, and the child solves it and keeps the result in mind and then solves the second part of the problem 10 s later incorporating the result from the previous part.

Mann–Suiter Visual Memory Screen for Objects: This is designed to assess the ability to revisualize pictures of common objects presented in groups. There are four cards: On the first card there are two pictures and it was exposed for 2 s. The second card has three pictures and it was exposed for 3 s; the third card had four pictures and the fourth card had five pictures, and it was exposed for 4 s and 5 s, respectively. The child was expected to recall the pictures in the same sequence. This test measures short-term visual memory.

Benton Visual Retention test: This test is designed to assess visual perception, visual memory and visual-constructive abilities. There are ten cards. Each card is exposed for 10 s and the child is asked to reproduce the design from memory. This test measures the visual spatial perception, visual and verbal conceptualization and immediate memory span.

Cattell's retentivity test: It consists of complex and unfamiliar designs of irregular geometric figures which cannot elicit any verbal associations. On a card ten geometrical figures are presented for 30 s; after a 2-min pause and from the second card, the child has to recognize the geometrical figures which he has already seen in the first card. This measures the visual recall for irregular geometrical designs and delayed memory span.

Letter Cancellation Test

This test is a measure of concentration. The children are given the test which has many alphabets typed out in rows, and the children are instructed to score out the A's and E's within a period of 2 min. If the child has omitted to score a letter or if he/she has scored a letter which is not A or E, it is considered as a wrong. If the child has correctly struck out an A or E, it is considered as a right. The final score is obtained by subtracting the total of wrongs from the totals of rights.

Raven's Colored Progressive Matrices

This is an IQ test to measure intelligence in children. The same tests were administered before the intervention program and repeated after 1 year of nutrition intervention.

The Impact of the Multiple-Micronutrient-Fortified Salt on Nutrition and Cognition in Younger Children

Efficacy Study

It can be seen that there was a significant ($p<0.05$) improvement in the experimental group in hemoglobin, red cell count, serum vitamin A, and urinary iodine, whereas in the control group there was a statistically significant decline ($p<0.05$) in hemoglobin, hematocrit, red cell count, and urinary iodine (Table 17.7).

Angular Stomatitis

In the experimental group the prevalence of angular stomatitis was 30.4 % at the start of the study, was totally eliminated within 2 months of the start of the study, and did not reappear throughout the whole study period. In the control group the prevalence of angular stomatitis was 3.25 % at the start of the study and increased to 25.5 % at the end of the study (Table 17.7).

Cognitive Study

We find that out of the seven memory tests administered, in four of the tests, namely, the Benton Visual Retention test, the Cattell's retentivity test, Mann–Suiter Visual Memory Screen for Objects

Table 17.7 Biochemical and clinical parameters at baseline and at the end of the study in study 2

	Experimental group, $n=63$		Control group, $n=66$	
	Baseline	End point	Baseline	End point
Hemoglobin (gm/dL)[e]	9.55 ± 1.21[a,c]	10.2 ± 0.77[a]	10.4 ± 0.59[b,c]	10.1 ± 0.75[b]
Hematocrit (L/L)[e]	0.32 ± 0.03	0.32 ± 0.03[d]	0.31 ± 0.02[b]	0.29 ± 0.02[b,d]
Red blood cells[e] (million/cmm)	3.47 ± 0.24[a,c]	3.97 ± 0.39[a,d]	3.8 ± 0.48[b,c]	3.48 ± 0.26[b,d]
Serum vitamin A (μg/dL)[e]	36.5 ± 12.5[a,c]	41.4 ± 14.8[a]	42.7 ± 12.8[c]	46.2 ± 18.6
Urinary iodine (μg/L)[f]	245 (100–600)[a]	510 (125–600)[a,d]	230 (55–600)[b]	70 (70–635)[b,d]
Angular stomatitis (%)	30.4	0	3.25	25.5

[a]Significant increase($p<0.05$) from baseline to endpoint in the experimental group
[b]Significant decrease ($p<0.05$) from baseline to endpoint in the control group
[c]Values differ significantly ($p<0.05$) at baseline between experimental and control groups
[d]Experimental group significantly higher ($p<0.05$) than control group at endpoint
[e]Data given as mean ± SD
[f]Data given as median (range) Mann–Whitney test and Wilcoxon signed-rank test used to test the significance

Table 17.8 Cognitive tests: increment in scores in study 2

Name of the test	Test measures	Experiment, $n=63$	Control, $n=66$
Benton Visual Retention test	Memory	15.5 ± 20.9[a]	8.48 ± 19.4[a]
Cattell's retentivity test	Memory	8.73 ± 19.2[a]	2.57 ± 16.1[a]
Mann–Suiter Visual Memory Screen for Objects	Memory	21.2 ± 29.6[a]	7.27 ± 16.1[a]
Delayed response learning test	Memory	22.2 ± 33.7[a]	5.90 ± 20.4[a]
Personal information test	Memory	31.6 ± 42.5	24.7 ± 35.8
Digit forward test	Memory	0.95 ± 11.0	1.6 ± 12.5
Digit backward test	Memory	2.38 ± 21.8	4.24 ± 19.1
Letter cancellation test	Attention and concentration	4.70 ± 8.43[a]	1.53 ± 8.83[a]
Raven's colored progressive matrices	Intelligence	5 ± 21.7	4.6 ± 18.8

[a]Significant improvement of the experimental group ($p<0.05$) over the control
Data given as mean ± SD

and delayed response learning test, the mean increment in scores in the experimental group is significantly more ($p<0.05$) than the control group. Only in the personal information test and the digit forward and backward test, the increment in scores in the experimental group is not statistically significant. In the letter cancellation test which is a measure of attention and concentration too, the mean increment in score in the experimental group is significantly more than ($p<0.05$) the control. There are no significant differences with respect to the intelligence test—Raven's colored progressive matrices in the experimental and control groups. This is understandable as there usually will not be an improvement in the overall intelligence but only in certain specific areas of memory which is measured by the memory tests (Table 17.8).

How Nutrition Impacts Cognition

There is strong evidence that among school-aged children, initially low scores on tests of cognition or school achievement due to Iron Deficiency Anaemia can be improved and in some cases reversed after iron treatment [12–15]. The adverse effects on cognitive and educational test performance due to Iron Deficiency Anaemia in preschool and school-aged children appear more transitory in nature than the effects on development in infants and imply that treatment of Iron Deficiency Anaemia in preschool and school-aged children through iron supplementation programs may have beneficial and immediate effects.

In these two studies, an improvement in memory scores was also seen in the control group. It can be reasoned out that there is always a familiarity element when a retest is given and this familiarity leads to improvement in scores in the control also. To offset this improvement in scores due to familiarity, the post-intervention score was subtracted from baseline score, and the change in scores was taken to consider whether there was an improvement of the experimental group over the control. We found a significant improvement in the change in memory scores in the experimental group compared to the control in both studies.

A recent study in India [16] showed that even twice weekly supplementation with iron and folic acid tablets improved cognition in adolescent girls. Thus it may be theorized that the chelated iron in these studies contributed substantially to the improvement in hemoglobin, decrease in anemia, and improvement in cognitive scores in the children. Other studies [17–19] which have given multiple micronutrients to children have shown improvements in cognition scores.

It is rather difficult to pinpoint which of the micronutrients, other than the iron in the multiple micronutrients, were responsible for the improvement of cognitive scores in children. There is a lot of interaction among micronutrients and several micronutrients work synergistically. It is well known that vitamin A improves iron status. Also, several B-complex vitamins are required during erythropoiesis. Riboflavin supplementation has been shown to improve hemoglobin in earlier studies [20]. It has beent shown that riboflavin has a direct role in the release of iron from ferritin [21]. Animal studies have shown that riboflavin deficiency impairs iron absorption. Moreover, other studies have shown [22] a better hematological response when riboflavin was given along with iron than when iron was given alone. Though we did not carry out biochemical analyses of B-complex deficiency in this study, we observed clinical signs of angular stomatitis, which may indicate B-complex deficiency. Angular stomatitis may also be due to infection, when it responded to topical applications of antibiotics or gentian violet as in earlier studies [23], or due to micronutrient deficiencies [24]. Because angular stomatitis was reduced significantly in our study when fortified salt was used, it may be assumed that the cause of angular stomatitis in our studies might have been micronutrient deficiencies and not infection. The improved B-complex status in the children could have led to the improvement in cognition scores as well. We feel that it is the synergistic interaction of the multiple micronutrients that led to improved micronutrient status in the children, which in turn led to improvement in cognition scores.

The bioavailability of all the vitamins and minerals has been studied extensively in the past when they have been delivered as supplements in the form of tablets or syrups, but what is different in these studies is that the these fortificants must be stable at the high temperatures of cooking and during storage in the harsh environment of salt. We found that all the fortificants except vitamin A are very stable during storage and cooking. Even with respect to vitamin A, its stability is considerably enhanced by microencapsulating it, as was done in these studies. With appropriate overages as was added in this study, we find that the vitamin A is highly stable and bioavailable and was able to decrease the prevalence of retinol deficiency from 57.1 to 16 % in the experimental group. The B-complex vitamins and iodine were also very stable during cooking and storage.

Several factors contributed to the absorption of all the micronutrients in this study. The salt was used in cooking in all the meals. The children consumed three meals and an evening snack each day; therefore the micronutrients were delivered in small doses throughout the day. Malaria is not a problem in this area and hookworms too are normally not present in this region. Ascaris infection may be commonly present and we tackled that through periodic deworming.

Guidance on Levels of Fortificants to Be Added

The level of the fortificants to be added depends on the severity of the problem of micronutrient deficiency. In the study on nutrition and cognition in children 7–11 years of age described above, we found a high prevalence of angular stomatitis—30.4 % at baseline. Therefore we added 2 RDA of B

group vitamins as fortificants, whereas it was only 1 RDA in the other study described. Though the RDA of vitamin A is 2,000 IU, we have added 3,000 IU in both these studies. This is because there is a loss of vitamin A during cooking and storage. Taking these losses into consideration, the addition of 3,000 IU vitamin A in 10-g salt seems necessary.

Conclusion

Global control of multiple-micronutrient deficiencies requires an integrated approach of food fortification, targeted supplementation, and dietary diversification. A stable and efficacious multiple-micronutrient-fortified salt could be useful in combating multiple-micronutrient deficiencies in many developing countries.

Acknowledgement Tables 17.1–17.5 were published in the International Journal for Vitamins and Nutrition Research. (Vinodkumar M, Erhardt JG, Rajagopalan S. Impact of a multiple-micronutrient-fortified salt on the nutritional status and memory of schoolchildren. Int J Vitam Nutr Res. 2009; 79(5):348–61)

Tables 17.6–17.8 were published in the Asia Pacific Journal of Clinical Nutrition. (Kumar MV, Rajagopalan S. (2007) Multiple-micronutrient fortification of salt and its effect on cognition in Chennai school children. Asia Pac J Clin Nutr. 16(3):505–11)

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Part III
Public Health, Concepts and Issues

Chapter 18
Food Fortification as a Global Public Health Intervention: Strategies to Deal with Barriers to Adoption, Application and Impact Assessment

Lada Timotijevic, Arnold Timmer, and Adebayo Ogunlade

Key Points

- This chapter focuses on food fortification policy formulation and implementation with a public health purpose in mind: the barriers and the strategies to overcome them.
- A simple representation of food fortification policy development is a stepwise model consisting of scientific evidence production, adoption of policy option, policy application and assessments of impact.
- Key barriers within scientific evidence gathering stage include varied terminologies used, heterogeneity of recommended micronutrient values, the lack of reliable indicators of micronutrient status, lack of strong evidence of intake–status–health association and the lack of evidence of effectiveness of food fortification as a policy.
- Barriers to adoption of food fortification go beyond the scientific evidence to include assessments of technical feasibility, existence of the infrastructure to achieve the policy implementation, the regulatory and institutional framework, vested interests and stakeholder interactions, consumer base and ultimately economic consequences of fortification.
- Barriers to implementation of food fortification include technical constraints; socio-economic, infrastructural, political, and ethical considerations; and consumer acceptance.
- Barriers to monitoring and impact assessment are fragmentation of development of the monitoring framework and evaluation, budgetary constraints, lack of sensitive methods to enable attribution of the observed changes in micronutrient status to food fortification, inability to draw conclusions and corrective action from the evaluation and lack of leadership.
- Continuous investment into research, stakeholder interactions and clear commitment to public health nutrition goals are vital for the food fortification approach to emerge as an option for policy, globally.

Keywords Food fortification • Policy development • Public health • Barriers to implementation • Application of policy • Consumer acceptance • Stakeholder involvement • Monitoring and evaluation

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V.R. Preedy et al. (eds.), *Handbook of Food Fortification and Health: From Concepts to Public Health Applications Volume 2*, Nutrition and Health, DOI 10.1007/978-1-4614-7110-3_18,

Abbreviations

EU European Union
EC European Commission
SME Small and medium enterprise

Introduction

Food fortification with micronutrients continues to be one of the possible policy options to tackle micronutrient malnutrition as it is considered a more sustainable and cost-effective method available to improve public health, especially when it is mandated [1, 2]. Food fortification can provide relatively rapid solutions to address low micronutrient intakes at a population level, as it does not require complex behaviour change and enables maintenance of traditional dietary patterns [3–5]. Whilst it is adopted in a number of developing countries where it remains one of the key strategies to tackle these serious issues, a relatively small proportion of foods are currently fortified on a mandatory basis in the Western Europe. Given the persistence of pockets of micronutrient malnutrition within Europe and a patchy approach to food fortification globally, questions can be raised about public health policy realities that act to constrain wider use of food fortification.

There are many guidelines developed about implementing a successful food fortification programme [5–7]; however relatively little is known about the reasons for a great deal of variation in the extent to which such programmes are recommended, adopted and applied in policy [8] and ultimately about its impact upon health [9]. Understanding the complex context of food fortification policy development is central to explaining these differences and illuminating the barriers to adoption, application and impact of food fortification.

The purpose of the current chapter is to provide an overview of processes of public health policy development relevant to food fortification adoption, application and impact. The chapter will start by proposing a policy development framework to aid structuring of the key public health considerations entering decisions about food fortification. Barriers relevant to each of the stages of the food fortification policy development process will then be reviewed, and finally some reflections on public health strategies to achieve food fortification will be offered. We will be focusing on food fortification with a public health purpose in mind, and where relevant the distinction will be made between mandatory and voluntary food fortification. Although the two are similar as they both involve addition of the existing nutrients to foods to the levels typical or above those usually found in these foods and both are government-led efforts to address a certain public health outcome, voluntary fortification is primarily practised by food producers, whilst mandatory fortification is legislated by governments.

Food Fortification Policy Development Framework

A simplified depiction of the food fortification policy development is presented in Fig. 18.1 as consisting of several steps: scientific evidence production, adoption of policy option, policy application (implementation) and assessments of impact (monitoring and evaluation). In reality the steps are not strictly chronological and mutually exclusive, but often occur at the same time and in different order.

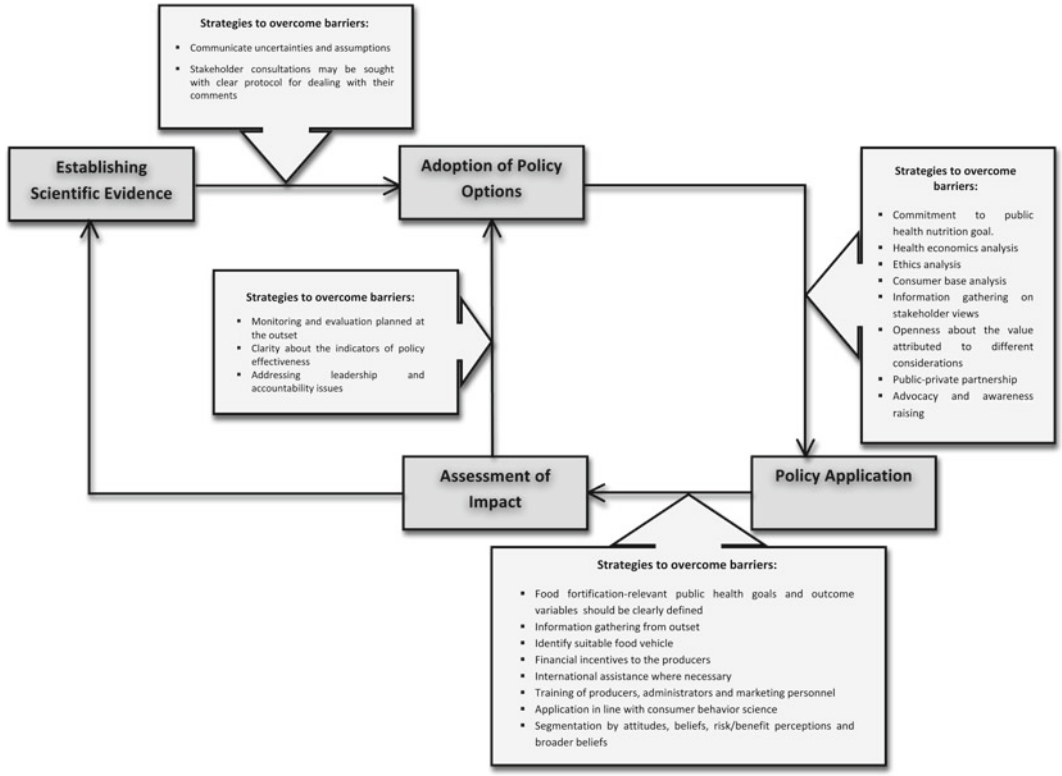

Fig. 18.1 A simplified framework for food fortification policy development and strategies to overcome barriers to implementation

Ideally, and in many cases, establishing the scientific evidence that there is a need to deal with micronutrient malnutrition in the whole population or a particular population group is the starting point of consideration of food fortification. Decision-making to adopt food fortification as a policy option is the next step, predicated not only on the scientific evidence about the link between nutrient intake and health status/outcome and the nutritional status of the population (and its subgroups) but also on a detailed and systematic information and data collection to establish the exact parameters for application, monitoring and evaluation of food fortification policy option. The evidence collation represents one step in the process that is managed through stakeholder interactions and negotiations [10]. The decision to adopt food fortification is made in the context of the existing policy and the policy options already selected to achieve public health outcome. Once food fortification is adopted as a policy option, application of the policy will depend on whether it is voluntary or mandatory. For mandatory fortification legislative framework must be developed to regulate it, whilst for both voluntary and mandatory, systems of regulation need to be put in place to ensure quality and safety control at all stages of food production, distribution and retail. In addition, application of food fortification must include considerations of monitoring and evaluation, as a feedback loop to policy to enable accurate assessments of the viability, cost-effectiveness and health impact of food fortification. Along each stage of this chain, different types of evidence are routinely drawn upon and considered in order to ensure that optimal strategies are in place to deal with possible barriers to achieving the ultimate aim of food fortification policy option—improving health.

Establishing Scientific Evidence for Adoption of Food Fortification

For food fortification policy to be recommended, evidence base needs to be established of the benefits as well as risks of fortifying food with a particular micronutrient, specifically that intake of a micronutrient (as the fortificant) by at-risk population will result in the desirable health outcome, and that there is low risk of overconsumption of the micronutrient in individuals outside the at-risk groups and those who choose to eat high amounts of fortified foods [1, 4, 5]. Furthermore, the inadequacy of intake, the prevalence of deficiency and the risk factors of deficiency need also be established [5, 11].

However, establishing such evidence base is complicated by several factors, including great variation in the terminology used for micronutrient requirements and heterogeneity of recommended micronutrient values, the variations in definitions of population groups and the various approaches to establishing micronutrient requirements (e.g. health outcomes and methods used when data are missing for subpopulations) [12]. In addition, often, the lack of reliable and widely accepted indicators of micronutrient status of adequate sensitivity (e.g. for zinc [5]) and the lack of gold standard evidence (randomised controlled trials) of micronutrient intake–status–health outcome associations constrain an ability to reach scientific consensus about the prevalence of micronutrient deficiency.

Due to the paucity of data, many assumptions are made by scientific advisors to policy makers, such as those about the attributes of the population group [12]. With each assumption, the associated uncertainty results in decreasing confidence in the reference standard. Selection of the criteria for the definition of population groups is driven by a range of criteria based on the evidence about physiology (such as life cycle, physical activity, energy needs, body weight and body composition) but also about social determinants (e.g. being an immigrant population, socially excluded or a member of a particular ethnic minority group) as well as consumer behaviour (e.g. routinely consuming or not particular types of food). In the context of the explicit requirement for science-based public health nutrition policies, the range and breadth of assumptions entering scientific decision-making is a challenge facing policy makers. Transparency about the areas of uncertainty and openness about the range of assumptions made in the process of scientific decision-making is an important aspect of communicating scientific conclusions to policy makers. Recommendations of food fortification should include an explicit reference to the uncertainties of the scientific evidence underpinning it.

In addition to providing scientific evidence (and rationale) of the micronutrient-relevant health for the whole population or the specific at-risk groups, governments need strong evidence that food fortification is an effective policy option for addressing micronutrient deficiencies. Several studies provide evidence that mandatory and voluntary fortification can help to address the problem of at-risk populations [3, 13]. Such evidence is not equivocal and is sometimes controversial, as is the case with iron [14]. However, the measures of effectiveness and cost-effectiveness will partly depend on the policy guidelines specifying when either voluntary or mandatory fortification can be adopted [11]. Different countries have different legislative requirements (in relation to fortification), and their nutrition policies vary. Alignment with the existing nutrition policy is a key to evaluations of effectiveness. Clarity and explicitness about the public health goals of food fortification (e.g. the expected health outcome and the degree of impact expected) is a prerequisite and when inappropriately defined can lead to erroneous expectations of higher impact and the resultant discontinuation of the programme.

Although scientific evidence is deemed to be a necessary consideration in the decision-making process about whether or not to adopt food fortification as a policy option for dealing with micronutrient malnutrition, indication from global practice is that empirical knowledge from other locations often plays a bigger role in moving policymaking agenda closer to the adoption of food fortification (Box 18.1).

Box 18.1: Strategies to Overcome Barriers Relevant to Establishing Scientific Evidence Base to Consider in Decisions About Food Fortification

Scientists ought to communicate uncertainties, assumptions and realistic objectives to policy makers.

Stakeholder consultations may be sought, whereby scientific advisors will identify the way in which they will deal with stakeholder comments in advance of consultation and ensure this is reflected in any report following it.

Adoption of Food Fortification as a Policy Option

In making a decision whether or not to adopt food fortification as a policy option, it is not sufficient to provide strong scientific evidence and clear rationale of the benefits and risks of food fortification. The parameters of the decision to adopt fortification policy are wide ranging and include the assessments of technical feasibility, existence of the infrastructure to achieve the policy implementation, the regulatory and institutional framework, vested interests and stakeholder interactions, consumer base and, ultimately, economic consequences of fortification. Whilst some of these considerations will be explicit (e.g. technical feasibility—whether food fortification is possible without changing organoleptic properties of the food), others will form the backdrop against which the decisions will be made (e.g. vested interests).

The decision about food fortification will be made in the context of a wide array of policy options available to the policy maker as well as the existing institutional (governing) context and regulatory framework within which this will be effected. Mandatory food fortification may be the last resort and considerations of other options are often recommended before legislating for food fortification [11]. The extent to which the existing legal/regulatory and institutional context determines suitability of food fortification as a policy option is clearly illustrated with reference to the European Union (EU) legal context: national regulations governing the fortification of foods intended for general consumption still vary widely throughout the EU. The effect is a possible ban on fortified foods thus potentially contravening the EU regulation on liberal trade. The European Commission (EC) regulations require member states to justify prohibitions through a provision of clear risk assessment of fortified foods, resulting in safety, rather than nutritional need acting as a key consideration in the adjudications about the proposed prohibitions [2]. The effect of this is that ultimately, financial and economic considerations (e.g. competitiveness on the European market) can override public health needs and be an abiding influence on whether food fortification policy is adopted.

The choice of a food vehicle for fortification is also routinely based on economic analysis as well as technical considerations and consumption patterns. The cost implications of food fortification are considered especially in the case of staples and condiments as a food vehicle, as they are used in large amounts and frequently, whereby small variations in price can have profound consequence [5]. This is particularly problematic in the context of globalised economy in which even the slighted increases in the cost can result in a disadvantage of the product on the global market. Similarly, the cost of establishing the regulatory framework (e.g. legislation of mandatory fortification, quality control and assurance throughout a product development cycle, the cost of legislation of health claims and labelling

of the fortified product) and an appropriate infrastructure (e.g. developing the technical know-how or an ability to deliver fortified food products from plants to consumers) is balanced against the public health burden of micronutrient malnutrition.

Thus, economic and financial considerations, and the need for short-term impact, regularly underwrite any decision to adopt (or not) food fortification policies. Horton [15] suggests that fortification works well if there are widespread deficiencies (e.g. iron), if the cost of the fortificant is not too high and if the target group is difficult to reach (e.g. women in preconception period in the case of folic acid) but also if legal context is amenable to adopting it. However, even when the economic analysis, legal realities and scientific considerations of benefits of fortification are favourable to the choice of this option, there are additional challenges that shape the decision-making at this stage, including political and ethical.

Vested interests represent an important backdrop against which the deliberation about food fortification takes place. Industry is often quick to respond to the calls for food fortification as it presents new marketing opportunities. This is increasingly the case in the context of greater emphasis upon optimal health rather than micronutrient deficiencies as a principal focus of nutrition policies. The move towards optimising health through the use of micronutrients such as calcium in prevention of osteoporosis, or antioxidant vitamins in the prevention of heart disease or cancer, has blurred the boundaries between fortified and functional foods [16], the latter forming an important strategy for manufacturers' product differentiation. Changes in eating patterns over the recent years (due to more women participating in the labour market, greater reliance upon convenience foods and urbanisation) are often invoked as an important social trend that calls for manufacturers to respond with innovation in food. Furthermore, prohibitions of fortification are represented by industry as curtailment of "consumer choice". Against these pro-fortification forces, there are consumer groups which frequently argue against excessive manipulation of basic food products and call for natural foods in consumer diets. Concerns about manipulating and changing the properties of basic food categories such as flour and about diets being increasingly based on "artificial", nutrient-poor, modified foods have prompted consumer groups to argue that education about healthy diet rather than fortification should be the preferred option for changing the nutrient status of populations. Blurring the boundary between healthy and unhealthy food through voluntary fortification of unhealthy food is raised as a concern as this is thought to obfuscate healthy eating messages to consumers [16]. Preference for regulated, mandatory fortification is then expressed in order to deal with the uncontrolled nature of voluntary fortification [17].

Ethical considerations enter decision-making explicitly in some cases (e.g. the UK Food Standards Agency has commissioned a report on ethical considerations of food fortification—both voluntary and mandatory; see Fuller-Deets and Dingwall [18]) and are often invoked in deliberations of stakeholders about adopting food fortification. The key consideration is balancing the right to the autonomy of the individual (e.g. to choose the type of food they consume and not be forced through mandated fortification) with the principles of equity and social justice (micronutrient malnutrition has a clear socio-economic gradient).

In short, a range of considerations are put forward in the decisions about the value, cost-effectiveness and acceptability of food fortification as a strategy for public health nutrition. However, once the decision to adopt mandatory or voluntary fortification is made (usually based on a combination of the above considerations), application stage requires systematic decision-making, planning and a review of additional evidence and information (Box 18.2).

Box 18.2 Strategies to Overcome Barriers to Adoption of Food Fortification

Commitment to public health nutrition goals is essential.

Health economics analysis (cost–benefit analysis) is a key argument in communicating with policymakers; however it should be transparent how values are assigned to different variables in the analysis.

Ethical analysis is essential when dealing with issues of food fortification both instrumentally (as a means to assuage the concerns of some stakeholders and the public) and substantially (to inform policy makers about the ethical aspects of food fortification).

Consumer base analysis is a prerequisite, but must go beyond a simple market research analysis and be informed by consumer science.

Information gathering on stakeholder views should include information about motivations, concerns and vested interests.

Openness about the weight attributed to different considerations is essential to achieve consensus and enable public–private partnership.

Public–private partnership is a prerequisite for success and its feasibility should be assessed early in the deliberation about whether or not to adopt food fortification.

Advocacy and awareness raising is important where there are no commitments to public health nutrition goals within the broader context of health policy.

Application of Food Fortification Policy

Policy application of food fortification requires a great deal of planning. Most guidelines specify the following steps in implementing:

1. Defining and setting programme goals should include collation of information (e.g. data on nutritional status, composition of the usual diet and dietary intakes of micronutrients).
2. Developing appropriate programme (with the requirement to consider not only the fortificant, food vehicle and safety levels, but the broader context in which the programme will be delivered as well as the consumer acceptance of fortified food).
3. Programme monitoring for course correction and evaluation of impact.
4. Feedback.

In this overview we will not be addressing the information needs phase that is necessary to inform the programme goals (this has partly been addressed in the section on Science in this chapter). From the public health strategies' point of view, understanding the broader context of food fortification programme application—both the constraints/barriers and the possible strategies to overcome these—will be the focus of this section.

Darnton-Hill [10] identified a number of constraints to successful application, which they categorise as technical constraints, socio-economic, infrastructural, political and other. Lack of consumer acceptance is certainly one of the key barriers to effective fortification of food [19], and we will deal with this at length in this section.

Technical constraints will range from the installation and maintenance of new machinery, the stability and bioavailability of added micronutrient fortificants in food and sometimes new technologies and quality assurance, to the choice food vehicles [10, 20]. The long-term periods and high costs required for the development of new combinations of fortificants must be taken into account when planning a food fortification programme. Who the key implementers of the programme are also determines its success: an evaluation should be made about the small producers' ability to make food fortification technically work as they typically serve those that are the target population.

Socio-economic constraints include the issue of reaching the target audience, whereby social exclusion and food insecurity (i.e. those in need of fortified foods being the least able to access these foods) represent a particular problem. A US study showed that fortification tends to increase micronutrient intakes of high consumers of any given fortified product (regardless of whether they are the targeted group) whilst failing to reach low consumers in the target population [21]. Thus, those who most need the exposure to fortified food are the least likely to consume it due to socio-economic constraints such as living in remote locations difficult to reach commercially and greater price sensitivity. Identifying a suitable food vehicle—consumed by those in need—that is financially accessible is a prerequisite to successful fortification [15]. Voluntary fortification can show price increments imposed by the traders due to the perceived added health benefit. Such costs of food fortification may then be transferred to the consumer, which might in turn deter purchase especially among those who are most in need.

The infrastructural constraints include an ability to engage industry in production, ensure adequate quality control systems and enable distribution of food products across the country, all of these particularly are relevant in the developing countries. Political support is crucial, though may be lacking for a number of reasons. Public health nutrition policy is often seen as of lesser importance than those of mainstream healthcare, thus lacking adequate budgets to develop an effective programme. The high cost associated with implementation as well as the need to achieve a buy-in of producers (many of which, as in the case of flour fortification, are small to medium enterprises (SMEs)) who are often constrained by financial implications of fortifying processes require concerted effort to ensure the cost-effectiveness of the process of fortification for manufacturers. Finally, a simple lack of awareness of micronutrient malnutrition, especially in the developing world where there are no regular nutrition status population surveys, could be an important political factor influencing the likelihood of achieving a buy-in of many stakeholders into the food fortification programmes. Raising awareness through political advocacy is important especially in developing countries.

Lack of broader stakeholder consensus will be a deterrent to the subsequent successful implementation. A review of the food fortification strategies across the developed world [22] showed that the key to successful implementation of food fortification programme is in forming viable private–public partnerships. Bringing on board principal private sector players (such as millers, breakfast foods, infant food, pharmaceutical industry) with public sector including public health agencies, research communities (traditionally well connected to the health agencies) and legislators provides for a solid base of a successful and impactful food fortification programme. Such infrastructures exist in industrialised countries such as Canada, the United Kingdom and parts of Europe but are less prominent in developing countries due to limited resources and structural inadequacies. It is important therefore to ensure that as broad a range of sectors and stakeholders as possible is involved in the process and that their motivations and concerns are addressed [19].

Lack of consumer acceptance whether or not a fortification programme will be successful will ultimately depend on consumer acceptance of fortified foods. Many food fortification implementation guidelines identify strategic and creative use of communication as essential for the success of the policy option both in terms of advocacy of food fortification (telling the success stories) and as a vehicle to consumer acceptance and behaviour change (leading to purchase and consumption of

fortified foods) [5, 19]. For instance, one of the frequent causes of programme failures is identified as a lack of consumer demand and take-up of fortified foods [23]. Factors affecting food choice are complex [24, 25] and any effect of interventions designed to change dietary patterns may not be in evidence for a long time. Even though food fortification is considered a policy option that requires minimal or no behaviour change on behalf of the consumer, there are still many considerations to be taken into account with respect to consumer behaviour in order to ensure effectiveness of the programme.

Relatively little research has been conducted in relation to consumer responses and attitudes to fortified foods, and when it has been done, this has been mainly within the framework of social marketing. A greater body of consumer research in the context of functional foods may provide leverage and useful insights into the consumer issues relevant to fortified food. Drawing from the consumer science relevant to functional foods as well as broader food choice literature, the key issues underpinning consumer acceptance of fortified food, apart from cost and taste implications, emerge as awareness/knowledge of food fortification, understanding (and the attendant beliefs and values) and use.

Consumer acceptance is often thought to be premised on the general nutritional knowledge (i.e. the extent to which certain vitamins and minerals are widely considered as beneficial to human health) but also upon the knowledge of specific illnesses. However, general consumer knowledge of nutrition beyond the basic concepts of healthy diet is poor [26]. Knowledge pertaining to specific nutrients and the more abstract nutritional concepts (such as percentages of recommended daily allowance) is even more limited [27]. A study on consumer awareness of food fortification demonstrated that consumers have little awareness of the current practices related to fortification in the UK [17]. When awareness of voluntarily fortified foods is present, this knowledge primarily comes from advertisements and is not supplemented with knowledge of the functional role of micronutrients and their association with a health outcome [17, 28]. Presentation of basic concepts relevant to micronutrient-health outcome association (e.g. folic acid—Neural Tube Defects Association) can lead to acceptance of mandatory fortification (e.g. of flour), though concerns are expressed about safety as well as choice (lack of control) [17]. A simple solution to this would be to develop nutritional educational campaigns; however, beyond the most basic five-a-day campaigns, these have been shown to have little impact on consumer diets [29]. A more nuanced explanation of the role of consumer knowledge in acceptance of fortified food is necessary for any strategy to induce consumer acceptance to be effective.

Evidence suggests that consumers tend to be happy for products already perceived as nutritious to be fortified, but they do not accept fortification of less healthy foods such as snack foods or soft drinks [28]. The functional food literature also informs us that the acceptance of food fortification will depend upon the type of food category and the food compound; however, the findings here are contrary to those just reviewed. Siró et al. [30] have demonstrated in relation to functional foods that consumers are more willing to accept enhanced foods which are processed rather than those which are perceived to be intrinsically healthy (e.g. yogurt). Fortifying foods with compounds that consumers are familiar with is also more likely to lead to acceptance of enrichment with a substance already present in the food than with the addition of a compound novel to the product or those that the consumers are less familiar with [24].

Within social marketing consumer research, there is a general assumption that people will be more accepting foods if the benefits to consumers are clear and concrete [19], and these have been thought to offset the perceived risks. However, people's risk/benefit perceptions associated with foods extend beyond their own personal health and encompass wider set of beliefs about the merits or disadvantages of technological processes used to produce them [31]. Indeed, recent literature is increasingly critical of the information-processing model of humans based on the assumption that people conduct simple cost–benefit analysis which leads to their choice of optimal behaviour patterns. Consumer judgments of benefit are not made independently of their overall belief system. The beliefs which are closely aligned, e.g. those about technological modification of a natural product (e.g. genetically

modified foods), may influence the perceptions of benefits incurred in the specific case of fortified foods. Cultural norms around nature and technology and in particular the technology used to produce fortified foods [32] are important determinants of acceptance as such. Broader values linked with the notions of "naturalness" and human interference with nature can also affect acceptance of fortified foods. Acceptance of fortified foods will be informed by consumer acceptance of the food technology used, and even though food fortification technology occupies the "low-risk" position on the scale among the range of food technologies [32], the consumer perceptions of technological risks (not just risks to health associated with consumption of the specific nutrients) should be measured. Thus, it is important to emphasise that when considering consumer understanding of food fortification, we need to take into account not only the consumer reactions to the product itself and the associated technology but more broadly to other developments in science, for example, in the field of genetically modified organisms (GMOs). Behavioural literature has demonstrated that consumer perceptions in one area of technological development will have spillover effects on perceptions of other technologies, especially when the awareness is low and knowledge/understanding is poor [33].

Broader values and belief systems held by consumers should be gauged not only in relation to the technology itself but the context in which the technology is offered. Thus, acceptance of fortified food is premised upon consumer trust in the regulatory body charged with managing development and distribution of such foods. Credibility of the information sources (e.g. of health claims) is an important factor though this is further complicated by increased cynicism about corporate interests pushing fortified products [24].

Even with an appropriate knowledge and understanding in place, there is still no guarantee of the ultimate use of the fortified food product. Our understanding of consumer use of the product can be usefully informed with reference to the dietary behaviour literature, which examines barriers to healthy eating in general. The take-home message from this large body of research is that most people are not preoccupied with long-term health, particularly when time periods greater than 20 years in the future are being considered. People are also resistant to changes in food purchase and preparation behaviours as these are highly habituated and embedded within a web of everyday practices. Low perceived threat (susceptibility to the illness) and low motivation to change can curtail acting upon the information even if the information is digested and adopted. Social norms of the referent group can often be a stronger basis for behaviour than any information about the benefits of a micronutrient-fortified food product. Lack of opportunities (e.g. cost implications) and taste is also a possible barrier to the use of fortified foods even if the benefits of it are not questioned.

In summary, there are many potential barriers and considerations that need to be addressed once food fortification has been adopted. However, all of these must be done in conjunction with an ongoing monitoring and planning of evaluation, which will inform impact assessment (Box 18.3).

Box 18.3 Strategies to Overcome Barriers to Policy Application

It is important to be explicit about public health goals specific to food fortification and to disentangle; the relative influence (upon the outcome variables) of a plethora of policy options implemented.

Information gathering is essential for planning.

Identifying suitable food vehicle: It is essential that the food vehicle is accessible especially to the hard to reach, its cost at the levels acceptable to the at-risk groups. The decision on food fortification should include a feasibility study that shows that, if this vehicle is selected, it is technically feasible and consumed by the target population and is accepted by industry and consumers.

(continued)

Box 18.3 (continued)

Financial incentives to the producers: Develop plans for offsetting any additional costs of fortification of foods, especially for the SME, whose endorsement is required for the application to be successful.

Ensure international assistance where necessary.

Train producers, administrators and marketing personnel.

Application of policy should be in line with consumer science.

It is necessary to address consumer concern associated with processing technologies, innovation in food and own health status in communicating about food products that have been fortified.

Segmentation should be by attitudes, values, risk/benefit perceptions and broader beliefs: identifying and understanding risks perceived by certain "at-risk" groups or populations may facilitate the development of a targeted information strategy which is better able to deliver relevant information to potential users.

Monitoring and Impact Assessment

In order for information from impact assessment to be used effectively, responsibilities for data collection at different levels of analysis must be clearly established, and the system must include feedback loops, which allow the information to flow in a timely manner to the entities responsible for taking action at different levels [5]. For optimal performance of a fortification programme, the activities (production, delivery, quality and behaviour change communication) and outputs (access and coverage, knowledge and appropriate use) require periodic monitoring. Once these indicators show satisfactory results, the impact on the determined public health outcomes can be measured.

Often, monitoring of the programme and/or evaluation of the impact is not included into programme planning, and budget plans are not carried out. Ironically, given that evaluation of the impact of food fortification policies is the key predictor of adoption of food fortification policies in the future, such an omission is puzzling. One of the main reasons for omitting monitoring and evaluation is the fragmentation of development of the monitoring framework and its implementation across the food industry, various government departments and nutrition institutes involved in the monitoring. Furthermore, monitoring is often omitted from the budget plans.

Another important reason is the difficulty in developing a valid, reliable and sensitive method which will allow attribution of the observed changes in micronutrient status to food fortification. The constraints which include time needed for food fortification to be fully implemented and a change in micronutrient intake observed, the difficulty in establishing consumption patterns especially in developing countries, and the multiple confounding variables affecting micronutrient status all play a role in developing appropriate monitoring instruments.

Even if evaluation is applied and achieves feedback, formulation of conclusions and corrective action are often hampered by operational challenges (e.g. SMEs facing difficulty in fortifying at the required standards or required resources, or when the know-how is inadequate), political sensitivities (especially to do with possible penalties for non-adherence) and ambiguity about what action to take. The latter can often be due to incomplete data to formulate conclusions, a weak capacity to analyse and interpret information, and communication of results that is not understood by the involved stakeholders with a wide technical background.

These challenges can be mitigated by investing in good technical leadership for developing the monitoring and evaluation plan, from the outset, with realistic and evidence-based targets and indicators,

Box 18.4 Strategies to Overcome Barriers

Monitoring and evaluation should be planned at the outset.
Ensure clarity about the indicators of policy effectiveness.
Address leadership and accountability issues.

taking into account lessons learned and good practices from other fortification experiences. A well-functioning national stakeholder committee or public health advisory board should have an oversight function including monitoring. Capacity development in all of these is central (Box 18.4).

Conclusions

This current chapter has offered some insights into the barriers encountered at different stages of food fortification formulation and implementation and some suggestions about the possible strategies to overcome these. Of course, the long-term aim is to ensure that most of people's nutritional needs can be met through diverse diets. Fortification cannot solve all micronutrient problems [15], but it provides a valuable contribution to addressing these in an effective, safe and sustainable way. One of the most successful public health programmes has been salt iodisation which saw a rapid scaleup in developing countries in 10 years from 20 % of households using iodised salt in 1990 to 70 % in 2000 (UNICEF, State of the World's Children) [34]. Food fortification is a complex process of decision-making and planning, involving many parties, commitment to long-term objectives and dedicated budgets, and requiring a long time between decision to adopt and any measurements of impact. Continuous investment into research, stakeholder interactions and clear commitment to public health nutrition goals are vital for a food fortification approach to emerge as an option for policy, globally.

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Chapter 19
Why Food Fortification with Vitamin B12 Is Needed?

Ilia Volkov and Victor R. Preedy

Key Points

- Vitamin B12 plays a functional role in a variety of organs and body systems. The list of conditions that are affected by vitamin B12 deficiency either directly or indirectly is growing.
- In some countries two factors can contribute to vitamin B12 deficiency, namely, changes in dietary pattern among segments of the population within the higher socioeconomic strata and the existence of poverty which leads to a low consumption of animal products (particularly red meat).
- There is an increasing prevalence of low vitamin B12 level in different segments of the general population.
- Vitamin B12 deficiency has various and serious health effects.
- The early detection of vitamin B12 deficiency is essential in order to prescribe opportune treatments.
- In order to prevent serious health problems, routine fortification with vitamin B12 should be seriously considered.

Keywords Vitamin B12 • Cobalamin • Vitamin B12 deficiency • Vitamin B12 routine fortification • Malignancy and vitamin B12 • Quality of life

Abbreviations

MRI Magnetic resonance imaging
RAS Recurrent aphthous stomatitis

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V.R. Preedy et al. (eds.), *Handbook of Food Fortification and Health: From Concepts to Public Health Applications Volume 2*, Nutrition and Health, DOI 10.1007/978-1-4614-7110-3_19,

Introduction

There are many articles indicating the increasing prevalence of low vitamin B12 (also called cobalamin) status in different segments of the general population [1–7]. The early detection of vitamin B12 deficiency is essential in order to prescribe opportune treatments, and there is evidence that such deficiencies are more common than would be expected. Vitamin B12 deficiency frequently occurs in individuals with dietary patterns that exclude animal food products and patients who are unable to absorb vitamin B12. Vitamin B12 deficiency has many causes, and pernicious anemia has been described as a widespread cause of this. Recent studies on vitamin B12, including the description of novel etiologies of vitamin B12 deficiency, have added to our understanding of this essential dietary component. For example, vitamin B12 deficiency can arise not only from insufficient dietary intake [8] but also from food-cobalamin malabsorption syndrome [9]. The latter is characterized by the failure to release vitamin B12 from food or a deficiency of intestinal vitamin B12 transport proteins or both, due to chronic overgrowth of *Helicobacter pylori* [10] and intestinal microbial proliferation. Changes in gut flora can arise from antibiotic treatment, long-term ingestion of biguanides (metformin) [11, 12], and antacids, including H2-receptor antagonists and proton-pump inhibitors [13] (mainly among patients with Zollinger–Ellison syndrome [14]). There are also genetic vitamin B12 metabolism diseases as Imerslund–Grasbeck syndrome which is a selective vitamin B12 malabsorption with proteinuria [15]. Chronic alcoholism, surgery (e.g., bypass surgery for obesity), and partial pancreatic exocrine failure can also contribute to vitamin B12 deficiency. Overall, this demonstrates that new approaches to the identification and treatment of subjects with vitamin B12 deficiency may be needed.

Persons with vitamin B12 deficiency may be asymptomatic. However, vitamin B12 deficiency should be suspected in patients presenting with myelopathy, cognitive decline, neuropathy, psychiatric disturbances, or specific hematological signs and symptoms.

In the following an attempt is made to demonstrate the critical role of vitamin B12 by surveying and analyzing available reports, as well as reporting the clinical experiences of the main author (IV).

Vitamin B12 Responsiveness in the Clinical Setting

Vitamin B12 influences the bone marrow, skin and the peripheral and central nervous systems, mucous membranes, bones, and blood vessels, as well as the normal development of children. Numerous studies emphasize the health problems of nutritional vitamin B12 deficiency and an obligation for the clinical, biochemical, and metabolic monitoring of infants born to mothers suffering from vitamin B12 deficiency. Dietary deficiencies of vitamin B12 for the period of pregnancy and lactation may result in health problems in exclusively breastfed children. Physical examination of these children has revealed failure to thrive, muscular hypotonia, irritability, anorexia, unusual movements, and psychomotor retardation. Laboratory analysis shows hematological pathology, such as a macrocytic anemia, a low level of vitamin B12, a high level of homocysteine and methylmalonic acid (MMA). Magnetic resonance imaging (MRI) of the brain reveals spread fronto-temporoparietal atrophy and problems of myelination [16]. Several researches have shown a relationship between maternal vitamin B12 status and birth weight, and one study extended those findings directly in terms of neonatal vitamin B12 status and birth weight. Vitamin B12 status in the mother was correlated to neonatal vitamin B12 status as determined by cord serum vitamin B12 level. In addition, low neonatal vitamin B12 levels are adversely associated with low birth weight [17].

Children have particular nutritional requirements in comparison with adults. Growth, tissue differentiation and maturation, whole body accretion of macro- and microcomponents, enhanced energy expenditure, and higher rates of protein turnover explain these differences. Dietary deviations and/or an imbalance between demand and supply will thus raise the risk of nutritional deficiencies along with

Table 19.1 Demographic, clinical, and nutritional characteristics of the patients by vitamin B12 levels

Characteristic	Low B12 levels (≤160 pg/mL) (*n* = 03)	Low-normal B12 Levels (161–300 pg/mL) (*n* = 64)	Normal B12 levels (>300 pg/mL) (*n* = 42)
	Number (%)		
Female sex	65 (63)	44 (69)	29 (69)
Age group (*years*)			
18–40	52 (50)	34 (53)	22 (52)
41–60	38 (37)	26 (40)	12 (29)
>60	13 (13)	4 (6)	8 (19)
Symptoms and signs			
Impaired vibration sense	37 (36)*	10 (16)	2 (5)
Impaired position sense	18 (17)**	0	0
Hyperactive tendon reflexes	25 (24)**	9 (14)	2 (5)
Extensor plantar response	14 (14)***	3 (5)	0
Impaired sensation	28 (27)****	8 (12.5)	4 (10)
Hypoactive tendon reflexes	12 (12)	5 (8)	3 (7)
Optic atrophy	5 (5)	0	0
Fatigue	14 (14)	9 (14)	4 (10)
Recurrent strokes	2 (2)	0	0
Mental disturbances[a]	27 (26)	12 (19)	5 (12)
Meat consumption (*per week*)			
<50 g	42 (41)*	9 (14)***	0
51–100 g	39 (38)****	23 (36)	8 (19)

*$P < 0.001$ compared with normal B12 level group
**$P < 0.005$ compared with normal B12 level group
***$P < 0.02$ compared with normal B12 level group
****$P < 0.05$ compared with normal B12 level group
[a]Obtundation, impaired concentration, memory loss, disorientation, psychosis

Table 19.2 Laboratory findings in patients with low or low-normal vitamin B12 levels

Laboratory findings	Low B12 level (≤160 pg/mL) (*n* = 103)	Low-normal B12 level 161–300 pg/m (*n* = 64)
	Number (%)	
Anemia[a]	58 (56)	35 (54)
Iron deficiency[b]	20 (19)	14 (22)
Macrocytosis[c]	23 (22)	11 (17)
Low serum folate levels[d]	19 (18)	6 (9)

[a]Hemoglobin <14 g/dL in men or <12 g/dL in women
[b]Iron level <59 μg/dL in men or <37 μg/dL in women
[c]Mean corpuscular volume >94 fL
[d]Serum folate level <3.5 ng/mL

consequent health problems. Thus, if dietary restrictions are needed for children with medical conditions (e.g., procedures on the intestine, food allergies, or intolerances), particular attention must be paid to avoiding micronutrient deficiencies such as vitamin B12 [18]. This is because children are very vulnerable to the deficiency of this vitamin (e.g., see (INCECIK2010, ABDELGAWADA2002)) with long-term consequences.

Vitamin B12 deficiency can cause peripheral neuropathy (Table 19.1) and combined system diseases including demyelination of the dorsal columns and the corticospinal tract. An extensive assortment of neuropsychological symptoms and signs has been found, such as spasticity, muscle weakness, reduced or hyperactive reflexes, urinary or fecal incontinence, ataxia, orthostatic hypotension, loss of vision, dementia, psychoses, and disturbances of mood. Several neurological syndromes or symptoms

are often seen in a single patient. The severity of pathology before treatment is undoubtedly related to the duration of symptoms prior to diagnosis [19].

Multiple sclerosis (MS) and vitamin B12 deficiency have common inflammatory and neurodegenerative pathophysiological characteristics. As a result of these similarities in the clinical appearance and MRI findings, the differential diagnosis between vitamin B12 deficiency and MS may be not easy. Moreover, low or decreased levels of vitamin B12 have been demonstrated in MS patients. In addition, current studies suggest that vitamin B12, in addition to its known role as a cofactor in myelin formation, has significant immunomodulatory and neurotrophic effects. These findings raise the questions of the potential causal association between the two disorders and merit further studies into the need to routinely determine vitamin B12 levels in MS patients [20] or more routinely in the hospital or clinical setting.

The role of vitamin B12 deficiency in psychiatric illness has been studied and discussed since the 1940s. Vitamin B12 has essential roles in brain functioning as well as cognitive processing. The broad psychiatric appearances of vitamin B12 deficiency are mood disorders, depression [21], psychotic status [22], mania, and obsessive compulsive disorders. The severity of disease and the therapeutic efficacy of treatment depend on the duration of the pathology. Consequently, the testing for serum vitamin B12 levels and consideration of vitamin B12 deficiency are recommended in patients with atypical psychiatric symptoms and the spectrum of organic brain syndromes.

There is a well-characterized association between levels of vitamin B12 and homocysteine which has been implicated as a risk factor for cardiovascular diseases, as well as brain atrophy. Some data supports the finding that increased circulating homocysteine is a risk factor for cognitive impairment in dementia (e.g., Alzheimer's disease) through vascular involvement as well as direct neurotoxic influence [23–25]. This may be due to the reason that in some research, increased plasma total homocysteine levels have been associated with ischemic stroke risk [26–28]. Several retrospective and prospective studies have revealed a consistent, independent relationship between hyperhomocysteinemia and cardiovascular disease, as well as all-cause mortality.

According to some guidelines the treatment of hyperhomocysteinemia is recommended for the apparently healthy general population [29]. Some large studies confirm that a supplementation with group B vitamins do not reduce the risk of major cardiovascular events or all-cause mortality in patients with vascular disease [30, 31]. It is possible that the outcomes of these and similar trials could be different if the studies had addressed the following points:

1. Using vitamin B12 or B complex as secondary prevention (i.e., curative aspects) of cardiovascular events for patients with irreversible changes of blood vessels has limited efficacy. Rather, vitamin B12 or B complex should be used within the context of primary prevention (i.e., preventative aspects).
2. Using high doses of vitamin B12 to reduce cardiovascular risk factors will probably be more effective than using cocktails of group B vitamins. Furthermore, using folic acid alone for prevention of cardiovascular diseases has been proven to be ineffective [32], while very high doses of vitamin B12 (60 mg every day for 6 months) has been used effectively, without any toxic side effects, for the treatment of other diseases [33].

The vitamin B12 carrier proteins, the transcobalamins (TC), are elevated during trauma, chronic infections, and inflammatory diseases. The scientific basis of this is not fully known, but it is possible that such elevations in transcobalamins could signal a compensatory reaction that primes the potential delivery of vitamin B12 to counteract inflammation via a multitude of processes such as the regulation of NFkappaB [34] There is clinical data to suggest that high doses of vitamin B12 ameliorate sepsis, traumatic shock, and systemic inflammatory response syndrome [34]. For example, septic shock has a high mortality rate. In the United States approximately 200,000 people die annually from this disease. The high mortality results in part from severe hypotension secondary to high serum nitric oxide (NO) concentrations [34]. In relation to current clinical data, cobinamide, a precursor of vitamin B12, has been proposed as a scavenger and cytoprotective agent to bind and inactivate NO [35].

Vitamin B12 has a fundamental role in hematopoiesis, i.e., red cell production (Table 19.2). Usually, low vitamin B12 produces the classic picture of macrocytic anemia, with a mean corpuscular volume (MCV) greater than 100 fL. The MCV correlates extremely well with vitamin B12 deficiency: an MCV of 80–100 fL indicates less than 25 % probability of vitamin B12 deficiency, MCV of 115–129 fL indicates a 50 % probability, and MCV greater 130 indicates 100 % probability [36]. This is a classic *textbook* picture of vitamin B12 deficiency. However, hematological abnormalities, such as anemia or macrocytosis, may be absent at the time of neurological presentation [37]. It is well known now that vitamin B12 deficiency may be accompanied by iron deficiency, and this association could mask macrocytosis [38, 39].

There are no generally accepted guidelines for the definition, diagnosis, treatment, and follow-up of vitamin B12 deficiency. Total serum vitamin B12 may not reliably indicate vitamin B12 status. The probability of "functional" vitamin B12 deficiency decreases upon increasing the blood level of vitamin B12. To increase the specificity and sensitivity of diagnosing vitamin B12 deficiency, it has been proposed that homocysteine, MMA, and holotranscobalamin II (holoTC), a subfraction of biologically active vitamin B12, should be measured [40].

Observations on Recurrent Aphthous Stomatitis

Recurrent aphthous stomatitis (RAS), the most common oral mucosa lesions seen in primary care, is seen in up to 25 % of the general population. Most treatments given to patients suffering from RAS achieve short-term therapeutic goals, such as alleviation of pain, reduction of ulcer duration, and recovery of normal oral function. A few treatment regimens have achieved long-term therapeutic goals, such as a reduction in the frequency and severity of RAS and maintenance of remission. With 10-year clinical experience of this disease, the lead author of this Chapter IV has shown that vitamin B12 treatment achieves long-term therapeutic goals and can be effective for patients suffering from RAS, regardless of their serum vitamin B12 level (Fig. 19.1).

A randomized, double-blind placebo-controlled trial has been carried out to confirm this observation [41]. The results of this study, conducted in the primary care setting, indicated that vitamin B12 treatment

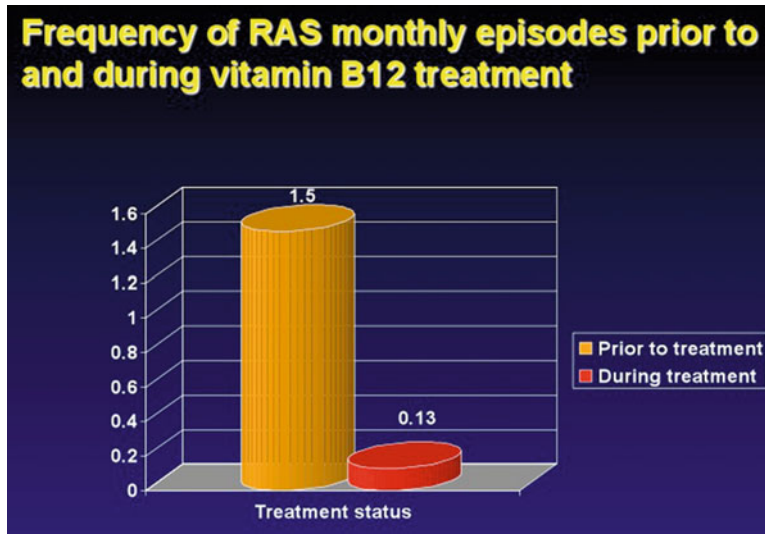

Fig. 19.1 Frequency of RAS episodes prior to and during vitamin B12 treatment (episodes per month)

can achieve long-term therapeutic goals in the management of RAS, in terms of various disease parameters such as pain, the number of ulcers, and duration of outbreak. This outcome did not depend on initial level of vitamin B12. Although a statistical significance was found between the interventional and control groups, the results need to be carefully interpreted due to the small sample size (58 people in the study) even though a prospective double-blind study design was used. The results nonetheless confirm previous clinical observations from our group. More than 74 % of patients in the interventional group were free from aphthous ulcers at the end of the treatment period, in comparison to 32 % in the control group. No adverse events were associated with vitamin B12 treatment in our study, and it is worth pointing out that this is the most comprehensive study on this disease to date.

Fertility

A potential association between vitamin B12 and problems of fertility and early recurrent abortions has been under discussion for long time [42–44]. In a meta-analysis of five studies, a significant correlation between serum vitamin B12 and early recurrent abortions was found [45]. No difference was noticed between early recurrent abortions and controls for folate [45].

Observations on Bone Disease

Osteoporosis is a common problem, which frequently has destructive health outcomes because of its association with fragility fractures and functional disabilities. The total number of fractures, and hence the cost to society, will increase dramatically over the next 30–50 years as a result of demographic changes in the amount of elderly people. Thus, prevention of osteoporosis by identifying risk factors or risk indicators, as well as the development of new treatment strategies, is a major health issue. Some data suggest that vitamin B12 status affects bone metabolism, bone quality, and fracture risk in humans [46]. A preventive regimen of vitamin B12 supplementation for healthy people with risk factors for osteoporosis or treatment of patients suffering from osteoporosis with vitamin B12 is advocated. Controlled clinical trials should thus be conducted to confirm the safety and effectiveness of vitamin B12 therapy and prophylaxis for osteoporosis.

Observations on the Aged and Aging

As mentioned above there is a changing demographic profile in most countries in which the proportion and number of elderly will rise in the foreseeable future. For example, recent data shows a world population of 7.0 billion [47]. This will increase to 9.3 billion by 2050. In Europe one third of the population will be over 60 years of age by 2050, and worldwide the proportion of people over 60 years of age will increase from 11 % in 2011 to 22 % in 2050. The relevance of this pertains to the incidence of vitamin B12 deficiency in the elderly. The prevalence varies but it is not uncommon to see figures of over 50 % being reported in some vulnerable groups though the general figure is probably about 10–20 % of elderly patients [9]. Vitamin B12 deficiency may arise as a consequence of poor intakes, food-cobalamin malabsorption syndrome, or pernicious anemia [48]. In pernicious anemia there is damage in the stomach with a consequential loss of intrinsic factor [49]. Intrinsic factor (also called gastric intrinsic factor) is needed for the binding of vitamin B12 and its subsequent absorption across the mucosa of the small intestinal ileum. The relevance of vitamin B12 deficiency

relates to the fact that cognitive decline as well as neuroelectricphysiological disturbances are associated with reduced plasma vitamin B12 which may occur independently of homocysteinemia [50]. Furthermore vitamin B12 supplementation in the elderly improves cognitive measures [51]. It is not unreasonable then to suggest more routine vitamin B12 supplementation in the elderly, preferably within the food matrices. However, the efficiency at which vitamin B12 is bioavailable varies depending on the food sources, i.e., less than 10 % from eggs and approx. 40–90 % from meats [52]. Obviously, the mode at which vitamin B12 is supplemented needs to be explored further [53], but the outcome of some studies has been negative [54].

Necessity of a New Approach to the Problem of Vitamin B12

Folic acid fortification of some foods has prevented the occurrence of neural tube defects in many countries. However, excessive folic acid fortification may be harmful to those with vitamin B12 deficiency. For example, among participants with vitamin B12 deficiency in the National Health and Nutrition Examination Survey, high serum folate (>59 nmol/L) was linked with a higher prevalence of anemia and cognitive impairment when compared with normal serum folate. Researchers also found a rise in serum levels of homocysteine and MMA, two useful indicators of low vitamin B12 status [55].

According common guideline (Recommended Dietary Allowances), the vitamin B12 intake is about 2.4 μg/day [56]. This contrasts with the some novel recommended daily intakes of between 2 and 6 μg [57]. Today there is a tendency in modern society to change habits, for example, cessation of smoking, "fighting" with overweight, accentuating physical exercise, and adopting correct eating habits. We have come to the conclusion that as a result of media information disseminating the relationship between meat, cholesterol, and cardiovascular diseases, consumption of meat, particularly beef, has decreased. We suppose that the decrease of level of vitamin B12 in the population with higher educational level is caused by a premeditated decrease in consumption of animal products. Also in modern society there is a tendency for ideological motives, particularly among the younger generation, to be vegans. To address the above, the issue of food fortification needs to be addressed. The advantage of vitamin B12 supplementation or fortification is that toxicity is rare, and at the same time it will address the needs of vulnerable groups.

Conclusion

Changes in lifestyle among segments of the population with a high socioeconomic level, on one hand, and the existence of poverty, on the other, are two main factors in the decreasing consumption of animal products (particularly red meat). This causes a decrease in the level of vitamin B12 in the general population, and as a consequence, this will increase pathology due to vitamin B12 deficiency (such as neurological and hematological disorders). As mentioned, vitamin B12 deficiency has various and serious health effects. In lieu of these possible developments and in order to prevent serious health problems, routine vitamin B12 fortification should be seriously considered and discussed.

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Chapter 20
Folic Acid to Prevent Neural Tube Defects: Success and Controversies

Philippe De Wals

Key Points

- The impact of folic acid food fortification in Canada is described and current controversies are discussed.
- Mandatory fortification of many cereal products was implemented in 1998.
- Level of fortification is set at 0.15 mg/100 g for flour and cornmeal.
- The estimated average additional intake is approximately 150 μg/day.
- Folate deficiency in the population was virtually eliminated.
- The overall frequency of neural tube defects was reduced by a factor of 2 and geographical variation almost disappeared.
- A decrease in the birth frequency of congenital cardiovascular malformations, of Wilms' tumor and neuroblastoma in children, and of stroke mortality in adults was also observed.
- Currently, there is no consensus regarding the folic acid supplement dose to recommend for women of childbearing age, the safety and potential effectiveness of increasing folic acid fortification level, and the potential impact and feasibility of fortifying food with vitamin B12.

Keywords Fortification • Folic acid • Congenital anomalies • Cardiovascular diseases • Cancers • Canada

Abbreviations

FA Folic acid
NTD Neural tube defect

Introduction

This chapter contains the story of folic acid (FA) food fortification in Canada. The first section describes the context in which the decision was made to pass a regulation requiring flour producers to fortify a wide range of products with the aim of preventing neural tube defects. In Part II, the results

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V.R. Preedy et al. (eds.), *Handbook of Food Fortification and Health: From Concepts to Public Health Applications Volume 2*, Nutrition and Health, DOI 10.1007/978-1-4614-7110-3_20,

of impact studies are described: increase in dietary intake, improvement in folate status, and reduction in the frequency of neural tube defects and possibly other congenital anomalies, cardiovascular disease, and some pediatric cancers. The difficulties involved in studying the adverse effects of fortification are discussed. Finally, three current controversies are summarized: the folic acid recommendation for women of childbearing age, increasing FA fortification levels, and fortifying food with vitamin B12.

Implementation of Folic Acid Fortification in Canada

How the Decision Was Made in Canada

Irrefutable evidence of the protective effect of folic acid was provided when the results of a randomized trial piloted by the Medical Research Council at 33 centers in 7 countries were published in 1991 [1]. This sparked an immediate debate in the United States regarding the merits of different preventive strategies such as improved nutrition, taking vitamin supplements, and fortifying the food chain [2, 3]. The debate received a lot of publicity and involved a large number of public organizations (Centers for Disease Control and Prevention, Food and Drug Administration, Institute of Medicine), professional bodies (American Medical Association, American College of Preventive Medicine, American College of Medical Genetics, American Academy of Paediatrics, American College of Obstetrics and Gynaecology, American Academy of Family Physicians), the industry (flour producers, vitamin manufacturers), and academia. With some difficulty, a consensus was reached to recommend a policy of fortifying a wide range of food products [4, 5]. Impact analyses were done to determine which food products should be fortified in order to increase the dietary intake of the largest possible number of women of childbearing age while ensuring that average daily intakes remained within safe limits. In the end, a regulation was passed making the fortification of certain foods mandatory starting January 1, 1998 [6].

In Canada, regulations involving food are the responsibility of Health Canada. At that time there was no public health agency operating with a certain degree of independence of the government as was the case with the Centers for Disease Control in the United States. The debate received little attention and took place behind the scenes at Health Canada. In March 1996, a workshop was organized to examine the issue of the prevention of neural tube defects [7]. There was not enough support for a regulation making fortification mandatory, and the only recommendation was to conduct a fortification pilot project (which was never done). In the next few years, pressure was exerted by the agrifood industry in general and flour producers in particular, who were unhappy about having to meet different standards for products sold in the USA and Canada. This pressure, combined with pressure from the public health network in the United States, finally persuaded Health Canada to support a common policy. The Food and Drug Regulations were amended in December 1996 to allow the fortification of certain products with folic acid [8] and then again in November 1998 making fortification mandatory in order to bring the fortification levels of flour in Canada in line with those in the United States [9].

How the Program Was Implemented in Canada

The level of fortification set in Canada is 0.15 mg/100 g for flour and cornmeal and 0.20–0.27 mg/100 g for pasta, as in the United States. In the USA, but not Canada, rice may be enriched at 0.154 mg/100 g up to a maximum of 0.308 mg/100 g and corn grits and farina at 0.15 mg/100 g. Breakfast cereals may be enriched up to 400 μg per serving in the USA and up to 60 μg per serving in Canada. In order to meet US requirements for imported flour on January 1, 1998, the Canadian industry started fortification early in 1997 [10]. Biochemical analysis of food products in Canada suggested that actual FA levels

in food could be somewhat higher than recommended as manufacturers were extra careful about meeting required levels [11].

There are no specific data concerning the extra cost of this fortification in Canada. Synthetic folic acid is not expensive to make, and flour producers should already have been doing some fortification to offset the losses in the cooking process. In the United States, the total cost of the operation was estimated to be three million dollars per year, or less than 1 cent per person [12]. For the industry, this cost is minimal and did not result in any price increase for the fortified products.

Although the avowed aim of the policy was to prevent neural tube defects, virtually no consideration had been given to measuring the impact of the policy. Canadian researchers had to step in to study this aspect.

Impact of Folic Acid Food Fortification in Canada

Intake of Folic Acid

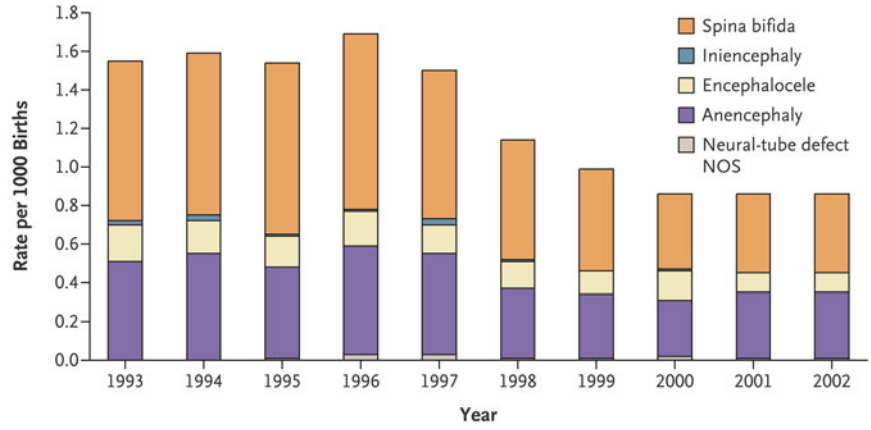
The goal of fortification was to provide an additional 100 μg/day of FA to women of childbearing age [9]. A study of pre-fortification vs. post-fortification folate levels in Ontario women of childbearing age found geometric mean red cell folate levels of 527 nmol/L vs. 741 nmol/L, with serum B12 levels remaining unchanged [13]. On the basis of the increase in serum folate concentration reported in Ontario, it was estimated that the fortification program increased folic acid consumption by 150 μg/day [14]. Based on four provincial dietary surveys conducted in Canada since fortification and assuming addition at the levels required by regulation, the average estimated additional intake was 131 μg/day among women 19–30 years of age (Lee NS, Food Directorate, Health Canada, written communication, August 25, 2006).

Folate Status

A cross-sectional study was conducted between August 1996 and July 1997 among pregnant women in Newfoundland during the first prenatal visit to obtain pre-fortification baseline data [15]. On the basis of the interpretive criteria used for red blood cell folate status, 11 % of the women were deficient (<340 nmol/L) and a further 13 % were classified as indeterminate (340–420 nmol/L). In 2007–2009, the folate status of a nationally representative sample of Canadians, including a subset of women of childbearing age, was assessed [16]. Less than 1 % of Canadians showed folate deficiency (red blood cell folate <305 nmol/L) and 40 % showed high folate concentrations (>1,360 nmol/L). Among women of childbearing age, 22 % showed concentrations below those considered optimal for maximum neural tube defect risk reduction (<906 nmol/L). No differences by age or income were found among women of childbearing age. Today, folate deficiency is virtually nonexistent in the Canadian population. As a result, ordering folate assays is no longer recommended for the investigation of anemia [17].

Neural Tube Defects

Changes in the frequency of neural tube defects before and after food fortification with folic acid were assessed in a multicentric study in seven Canadian provinces from 1993 to 2002 [10]. The study population included 1.9 million live births, stillbirths, and terminations of pregnancies because of fetal



All neural-tube defects	1.55	1.59	1.55	1.69	1.50	1.14	0.99	0.86	0.86	0.86
Spina bifida	0.83	0.84	0.89	0.91	0.77	0.62	0.53	0.39	0.41	0.41
Iniencephaly	0.02	0.03	0.01	0.01	0.03	0.01	0.00	0.01	0.00	0.00
Encephalocele	0.19	0.17	0.16	0.18	0.15	0.14	0.12	0.15	0.10	0.10
Anencephaly	0.51	0.55	0.47	0.56	0.52	0.36	0.33	0.29	0.34	0.34
Neural-tube defect NOS	0.00	0.00	0.01	0.03	0.03	0.01	0.01	0.02	0.01	0.01

Fig. 20.1 Prevalence of neural-tube defects, according to diagnostic category, in seven Canadian provinces from 1993 through 2002 (Reprinted with permission from the New England Journal of Medicine [10])

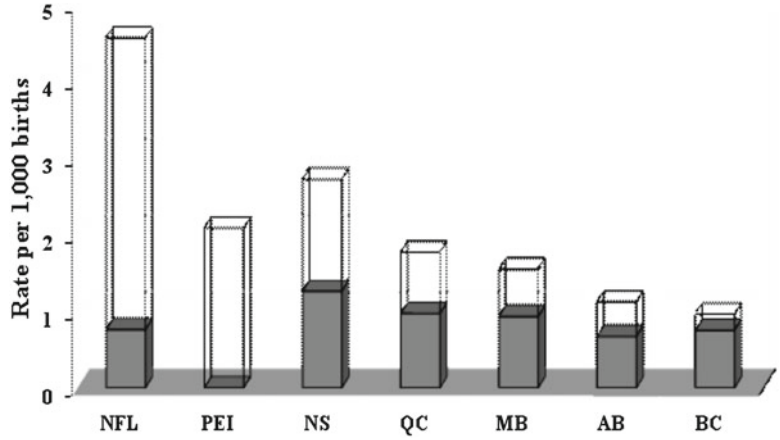


Fig. 20.2 Prevalence of neural tube defects in seven Canadian provinces before (*upperline*) and after (*lower line*) folic acid food fortification. *NFL* Newfoundland and Labrador; *PEI* Prince Edward Island; *NS* Nova Scotia; *QC* Quebec; *AB* Alberta; *BC* British Columbia (Adapted from the New England Journal of Medicine [10])

anomalies. The prevalence of neural tube defects decreased from 1.58 per 1,000 births before fortification to 0.86 per 1,000 births during the full-fortification period, a statistically significant reduction of 46 % (Fig. 20.1). The observed reduction in rate was greater for spina bifida (a decrease of 53 %) than for anencephaly and encephalocele (decreases of 38 % and 31 %, respectively).

In Canada the prevalence of neural tube defects was historically higher in the eastern than the western provinces. Interestingly, a greater reduction in rates was observed in regions with a higher baseline prevalence of neural tube defects than in regions with a lower prevalence (Fig. 20.2). The greatest reduction in birth prevalence was seen in Newfoundland and Labrador, which had a difference in rate of 3.80 per 1,000 births, as compared with British Columbia, which had a difference in

rate of 0.21 per 1,000 births. During the post-fortification period, geographical differences almost disappeared although a small east–west gradient persisted with rates of 1.26/1,000 in Nova Scotia, 0.97/1,000 in Quebec, 0.93/1,000 in Manitoba, 0.67/1,000 in Alberta, and 0.75/1,000 in British Columbia.

For spina bifida, frequency reduction following FA fortification was higher for the more severe upper cranial, cervical, and thoracic lesions than for the less severe lumbar and sacral defects [18]. No significant decrease was seen for the pathogenically distinct lipomyelomeningocele [19]. The effect of supplementation was also evaluated among women who underwent maternal serum screening in Ontario, and the prevalence of open neural tube defects declined from 1.13 per 1,000 pregnancies before fortification to 0.58 per 1,000 pregnancies thereafter [20].

Other Congenital Anomalies

The use of multivitamin supplements during the periconceptional period has been associated with a decreased risk of several categories of congenital anomalies. Results of a meta-analysis of controlled trials and observational studies suggested consistent protection against cardiovascular and limb defects but a lower degree of consistency for cleft palate and/or oral cleft, urinary tract anomalies, and hydrocephalus [21]. No statistically significant protection could be demonstrated in another meta-analysis including randomized and quasi-randomized trials only, but the small number of subjects limited the power to detect a beneficial effect [22]. Results of studies on multivitamin supplementation cannot be readily extrapolated to fortification using a single vitamin with a much lower dose.

In the province of Quebec, live and stillborn infants with severe congenital heart defects were identified in three administrative databases and time-series analyses were performed [23]. There was no change in the prevalence of heart defects in the 9 years before fortification (1990–1998), while in the 7 years after fortification (1999–2005), there was a statistically significant steady decrease (Fig. 20.3). In the study, medical records were not reviewed and congenital anomaly cases in pregnancy terminations were not included. Results should thus be interpreted with caution. The frequency rate of heart defects started to decrease in 2001, as compared with 1998 for neural tube defects, and the shapes of the two curves were also very different [10]. In Ontario, a retrospective study of all women who underwent maternal serum screening at 15–20 weeks gestation was performed and there was no evidence of a decline in the prevalence of orofacial clefts and trisomy 21 in the offspring following fortification [24, 25]. More studies are needed to assess the effect of folic acid food fortification on the risk of congenital anomalies other than neural tube defects.

Cardiovascular Disease

Epidemiological studies have shown a positive correlation between homocysteine plasma concentration and the risk of cerebral, coronary, and peripheral vascular disease [26]. Increasing folic acid intake results in the lowering of homocysteine plasma concentration, but the results of clinical trials to reduce the incidence of stroke and myocardial infarction in high-risk individuals have been disappointing [27–29]. The effect of folic acid at low doses in mostly low-risk individuals may be different. A time-series analysis of stroke mortality rate was conducted in the United States and Canada where folic acid fortification was implemented in 2008 and in England and Wales where no fortification is required [30]. An acceleration in the decline of stroke mortality following fortification was observed in the USA and Canada but not in the UK. It would be interesting to repeat this type of analysis in other countries.

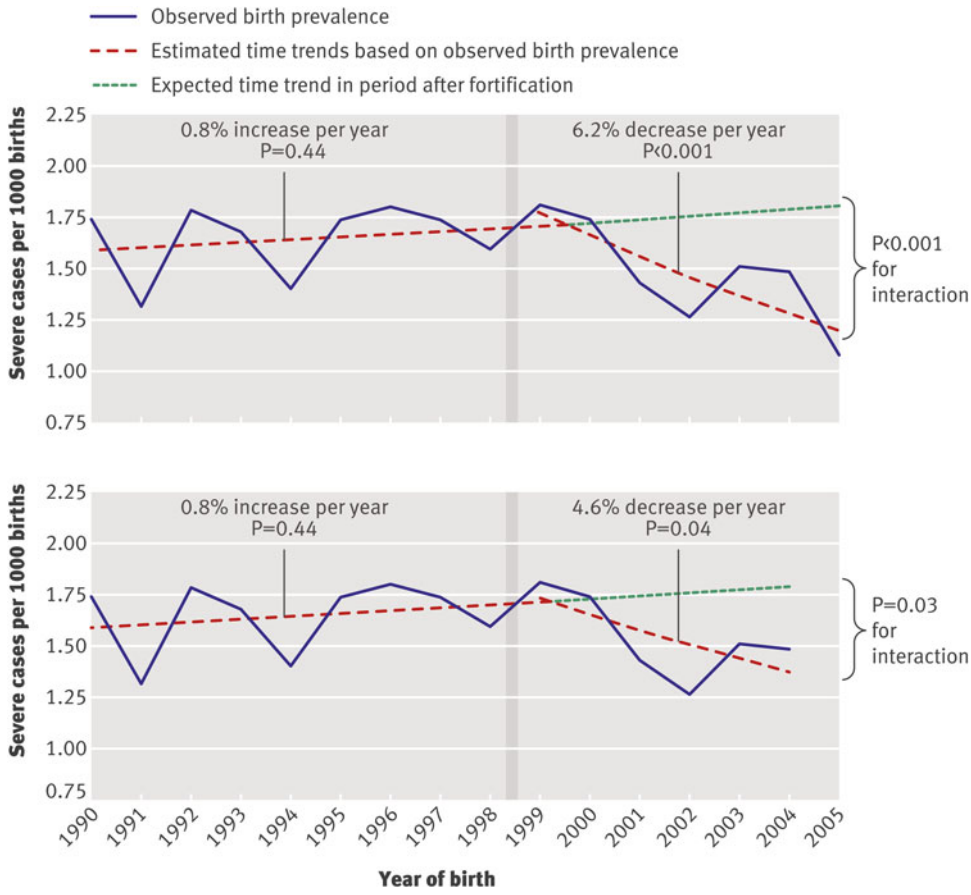


Fig. 20.3 Time trends in birth prevalence of severe congenital heart disease before and after January 1, 1999, cutoff representing introduction of mandatory fortification of flour and pasta products with folic acid in Canada (Reprinted with permission from the British Medical Journal [23])

Pediatric Cancer

The role of natural folates and folic acid in DNA biosynthesis, damage, and repair and in cell differentiation and multiplication is complex and not completely understood [31]. Observational studies were conducted in Ontario comparing the incidence of several types of pediatric cancers before and after the introduction of folic acid supplementation. A significant decrease in rate was observed for Wilms' tumor and neuroblastoma but not for acute lymphoblastic leukemia, brain cancers, and other embryonal cancers [32, 33]. Although there is a biological plausibility in these observations, more evidence is needed to establish causation.

Adverse Effects

High doses of folic acid (≥5 mg/day) given to individuals with severe anemia caused by B12 deficiency have been associated with an improvement in the anemia, but they also could mask and even accelerate

the degeneration of the nervous system [34]. The additional intake of folic acid resulting from supplementation at the current levels is far beyond the therapeutic FA doses used at a time when it was difficult to differentiate folate from B12 deficiency [35]. As yet, there has been no good study on the incidence and severity of neurological disorders before and after FA supplementation. There are many reasons for this: the clinical symptoms of B12 deficiency are not very specific and are relatively mild at an early stage, patients usually have comorbidities and are not necessarily referred to neurologists or hospitalized, and there have been major changes in diagnostic tests in recent years, both for B12 status and neurological disorders.

An association between folic acid supplement use and twin pregnancies has been reported in some studies [36]. A systematic review of epidemiological studies comparing twinning rates before and after FA fortification in the USA and Chile provided conflicting results [37]. In recent years, there has been a steep rise in the frequency of multiple births in Canada, resulting mainly from increasing use of assisted reproduction technologies [38]. This factor is related at the individual level to nutrition and FA supplement use and at the ecological level to fortification. Thus, it is almost impossible to identify the potential role of FA fortification in the increase.

Epidemiological studies have shown an inverse relationship between dietary folate intake and the risk of colorectal cancer in the population [39–42]. Conversely, clinical trials using high FA doses in patients with precancerous colorectal lesions did not show a protective effect, and an increased risk of progression of advanced adenomas was observed in some studies [43–45]. A "dual-modulator" role of folate in colorectal carcinogenesis has been proposed, in which moderate dietary increases initiated before the establishment of neoplastic foci have a protective influence, whereas high intake once early lesions are established increases tumorigenesis [46]. In Canada, the incidence of colorectal cancer rose between 1980 and 1985 in both sexes and then declined until the mid-1990s, more markedly in females than males. Rates then rose through 2000, only to decline significantly thereafter, this time more markedly in males than females [47]. Whether FA fortification played a role in these trends is difficult to assess as there have been changes in nutrition patterns in the last decades as well as changes in screening and diagnostic practices.

Current Controversies

Folic Acid Recommendation for Women of Childbearing Age

In Canada, the first recommendations regarding folic acid supplementation for the prevention of neural tube defects were published in the early 1990s [48–50]. For low-risk women, a daily dose of 0.4–0.8 mg was recommended. In 2003, the Society of Obstetricians and Gynaecologists of Canada updated its recommendations, and 0.4–1.0 mg of folic acid was recommended for women who could become pregnant [51]. In 2007, when the impact of fortification was demonstrated, guidelines were changed: 0.4–1.0 mg was recommended for women with no personal health risks, planned pregnancy, good diet, and good compliance with supplement use on a daily basis, while 5 mg/day was recommended for all other women (the majority) [52]. Reasons for increasing the dose were as follows: FA intakes in the population were suboptimal at current fortification levels, 5 mg per day in young women is safe, and there may be a subset of women with certain genetic and/or physiological characteristics requiring high doses. As yet, this recommendation has not been endorsed by public health authorities.

Interestingly, two studies conducted in the fortification era in the USA did not show a clear benefit of multivitamin supplement use for the prevention of neural tube defects [53, 54].

Increasing Fortification Levels

After FA fortification was implemented in Canada, NTD rates decreased and then stabilized with a reduced but still discernible east to west gradient [10]. This is a powerful argument supporting an increase in FA food fortification levels to eliminate geographical variation that is most probably caused by nutritional and not genetic factors. In the USA, this debate is going on for years and no consensus has been achieved so far [55–57]. In the MRC trial, 72 % protection against NTD was recorded using a daily FA dose of 4 mg [1]. However, the confidence interval around this estimate was large. In a community intervention in China using pills containing 400 μg FA, the lowest NTD rates (0.6–0.7/1,000) were recorded among women who were highly compliant with supplementation during the periconceptional period [58]. These rates were also observed in the western part of Canada following fortification at low levels and may well represent the lowest achievable level with FA supplementation or fortification [10]. In Chile, fortification of wheat flour at 220 μg/100 g was associated with a 43 % reduction in NTD rate and an absolute rate of 1/1,000 births in exposed women [59]. These results are not very different than those reported in Canada using lower FA fortification doses [10]. Controlled trials testing the relative effects of different FA fortification levels would be almost impossible to organize, and indirect evidence could only be provided by high-quality studies on the effect of FA supplementation alone in a context of fortification.

Fortifying Food with Vitamin B12

Vitamin B12 deficiency is frequent in the Canadian population, especially in the elderly [60]. Results of a case–control study in Ontario showed a tripling risk of NTD in the presence of low maternal B12 status as compared to women in the highest quartile of serum holotranscobalamin levels, a marker of B12 status [61]. The authors of the study estimated that up to 34 % of NTD cases occurring in the context of folic acid fortification could be due to maternal B12 deficiency and could thus be prevented through additional B12 fortification [61]. Adding B12 in cereal products is technically feasible, of reasonable cost, and there is evidence that B12-fortified flour, consumed as bread, can improve B12 status among persons with no impairment of gastrointestinal absorption [62]. The implementation of this public health measure has been endorsed by the Society of Obstetricians and Gynaecologists of Canada [52]. In the USA, B12 fortification is a subject of a controversy as there are many scientific uncertainties regarding the potential effectiveness of the measure [62–64]. Up to now, there has been no controlled trial demonstrating the effectiveness of B12 supplementation or fortification to prevent the occurrence of neurological disorders in patients with mild subclinical deficiency (individuals with severe B12 deficiency caused by specific malabsorption will not respond to increased oral intake). The same can be said for neural tube defects and the only evidence is from epidemiological studies comparing NTD risk in women who had or not taken multivitamin containing B12 during the periconceptional period [21]. Randomized clinical trials will have to be conducted to justify a mandatory B12 food fortification policy as it was done for folic acid.

Conclusion

In Canada, a mandatory FA food fortification policy was implemented to reduce the occurrence of neural tube defects in the population and the effectiveness of the measure was higher than anticipated although the mean additional FA intake among women of childbearing age was quite low. There is certainly room for further NTD risk reduction if fortification levels are increased. However, because

of the uncertainty regarding the impact of this measure and the possibility of cancer promotion, public health authorities will request more evidence before considering such move. As history has told, decisions in Canada will most probably follow and be in line with those adopted in the USA. So, let us follow the debate south of the border.

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Chapter 21
Vitamin D Fortification in North America: Current Status and Future Considerations

Mona S. Calvo and Susan J. Whiting

Key Points

- In Canada and the United States the adequacy of foods fortified with vitamin D to meet the needs of all race, gender, and age groups has been called into question.
- Much of the intake of vitamin D from foods in the United States and Canada is from fortified foods.
- Fortification practices are different between United States and Canada, the former having a voluntary approach and the latter stressing mandatory fortification.
- Novel approaches to vitamin D enrichment of foods include the use of "bio-addition" which is the enrichment of a food staple with another food rich in a specific nutrient and to the postharvest or preprocessing manipulation of foods that result in high vitamin D content.
- Current efforts to seek new food sources of vitamin D have focused on the postharvest exposure of edible mushrooms to ultraviolet light.

Keywords Vitamin D fortification • Vitamin D3 (cholecalciferol) • Vitamin D2 (ergocalciferol) • Future considerations • North America

Abbreviations

1,25(OH)2D	1,25-Dihydroxyvitamin D
25(OH)D	25-Hydroxyvitamin D
AI	Adequate intake
CHMS	Canadian health measures survey
DRI	Dietary Recommended Intake
EAR	Estimated average requirement
IOM	Institute of Medicine of the National Academy of Sciences

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V.R. Preedy et al. (eds.), *Handbook of Food Fortification and Health: From Concepts to Public Health Applications Volume 2*, Nutrition and Health, DOI 10.1007/978-1-4614-7110-3_21,

NHANES	National Health and Nutrition Education Survey
RDA	Recommended Daily Allowance
UL	Upper level of safe intake
USDA	US Department of Agriculture
Vitamin D2	Ergocalciferol
Vitamin D3	Cholecalciferol

Introduction

Until the last decade, adequacy of vitamin D status was not a public health concern, because vitamin D synthesis from sun exposure was thought to meet most of the North American populations' needs and the ubiquitous fortification of milk was thought to provide sufficient intake when sun exposure was limited. More recently, a growing incidence of rickets in infants and evidence from national surveys showing high prevalence of poor vitamin D status in children and adults is slowly eroding our confidence in the vitamin D adequacy of many Americans and Canadians, as well as in other developed countries [1–3]. Concern about the high prevalence of poor vitamin D status stems from the significant association of low plasma 25-hydroxyvitamin D {25(OH)D} levels with the increased risk of both chronic and infectious disease, but most strongly with the chronic bone diseases, osteoporosis, and rickets in children [1–4].

In North America, realization of the inadequacy of sun exposure to meet our vitamin D needs prompted the joint efforts of the US Department of Health and Human Services and the US Department of Agriculture (USDA) to emphasize the need for more vitamin D rich foods in the 2010 Dietary Guidelines for Americans [5]. A similar message to consume more vitamin D from food sources rather than dietary supplements was issued by the Institute of Medicine of the National Academy of Sciences in their 2011 Dietary Recommended Intakes (DRI) [6]. In contrast, the 2007 Canada Food Guide indicated that adults over age 50 years should take a vitamin D supplement of 400 IU to meet the previous recommendation of 400 IU for adults 50–70 years and 600 IU for adults over 70 years [7]. The new DRI values, the Recommended Daily Allowances (RDA), serve as the official nutrient intake guidelines for both Americans and Canadians and are now threefold higher than the previous 1997 intake.

With respect to vitamin D intake, Canada and the United States differ from the European countries in their significant reliance on fortified foods rather than dietary supplements, but recently the adequacy of these fortified foods to meet the needs of all race, gender, and age groups has been called into question [8, 9]. Poor vitamin D status from reduced sun exposure that occurs in winter is made worse by limited access to vitamin D-containing foods. The key problem is that there are very few foods that are naturally rich in or fortified with vitamin D and most of these are expensive or not accessible to the entire population, such as fresh or canned fatty fish like salmon or mackerel. Most of the vitamin D consumed in North America comes from the fortification of specific food staples such as milk, some fruit juices, and ready-to-eat cereals in the United States and milk and margarine in Canada [10]. The fortification of food staples is a tried and true public health strategy to reduce poor vitamin D status and the risk of associated chronic diseases in a large population, in contrast to supplementation, which only addresses the needs of the individual [11–13]. In this review, we focus on the use of food fortification as a mechanism to correct this vitamin D problem in North America, present the dietary guidelines governing intake, the regulatory aspects governing the addition of this potentially toxic fat soluble vitamin to the food supply, and demonstrate the impact and efficacy of current fortification practices and the potential of future strategies to assure adequate intake or beneficial changes in disease biomarkers.

Prevalence of Poor Vitamin D Status, Dietary Guidelines, and Intake Goals for Vitamin D

Nutritional status of vitamin D is evaluated by measuring the intermediate or transport form of vitamin D, 25(OH)D, which is produced when cholecalciferol (vitamin D_3) that is produced in skin when exposed to sunlight or ergocalciferol (vitamin D_2) and vitamin D_3 contained in foods is hydroxylated in the liver and subsequently released to the circulation [1]. This intermediate or transport form of vitamin D serves to supply tissues with the necessary precursor to the active form of vitamin D, 1,25-dihydroxyvitamin D {1,25$(OH)_2$D}, which is critical to the regulation of many cell processes including the synthesis of proteins essential to cell differentiation, proliferation, apoptosis, and immune response [2]. Controversy surrounds just what the optimal level of 25(OH)D should be [3]. Threshold levels for vitamin D adequacy have been officially established for the North American population by the Institute of Medicine of the National Academy of Sciences only as vitamin D status relates to maintenance of bone health [1, 3, 6, 14–17]. Determining the need for vitamin D is difficult as factors such as sunlight exposure with appropriate strength, age-related changes in cutaneous vitamin D formation in skin, and availability of foods containing vitamin D all contribute to our difficulty to provide a single amount as a recommendation for intake [1]. Figure 21.1a, b show the prevalence of vitamin D deficiency and insufficiency in adult men and women of different race ethnicities in the United States and Canada, respectively, using the recently established threshold values for maintenance of healthy bone [6, 15–17]. The high prevalence of poor vitamin D status shown for both countries in individuals with darker skin underscores the difficulty in determining appropriate vitamin D dietary guidelines for the entire population using a "one- size- fits- all" approach.

Table 21.1 shows a selection of recommendations for dietary vitamin D that have been set for individuals, in most cases to provide enough dietary vitamin D to maintain a level of 25(OH)D of approximately 50 nmol/L or higher. Most of these recommendations relate to bone health, but the relation of poor vitamin D status to other chronic diseases, despite their well known associations [3, 14], are thought to have too little direct evidence to support officially establishing specific optimal goals for 25(OH)D levels to reduce disease risk [3, 6]. In establishing the dietary intake recommendations shown in Table 21.1, the specific conditions of year-round sun exposure, latitude, and skin pigmentation that blocks sunlight were taken into account in a few, but not all of these recommended intake levels for North America. Of these guidelines, the Estimated Average Requirement or EAR is deemed the most appropriate for evaluating the nutritional intake adequacy of both the American and Canadian populations (Table 21.2) [18].

Vitamin D intake from foods recently estimated from nationally representative surveys conducted in the United States varies with gender and age (Fig. 21.2a). There are higher intakes in men than women when food sources alone are considered and a decrease in vitamin D intake with increasing age with food intakes only. Much of the intake of vitamin D from foods in the United States is from fortified foods [19]. This explains why intake from foods is higher in the United States, as much as twice the intake in the United Kingdom where few fortified foods are in the marketplace. Data from the NHANES surveys show the significant racial and ethnic differences in vitamin D nutritional status and intake that occur in the United States, Fig. 21.2a. Black men and women in the United States have significantly lower serum 25(OH)D than Whites and consume significantly lower vitamin D from milk, ready-to-eat cereals, and dietary supplements (Figs. 21.1a and 21.2a). In general, with one exception, those in greatest need of dietary sources of vitamin D due to aging and/or darker skin have the lowest intakes of vitamin D, thus further contributing to their low circulating 25(OH)D levels. Black and Hispanic adults and other darker skinned individuals also have reduced potential for adequate sun exposure to synthesize vitamin D and would need more dietary intake than White Americans.

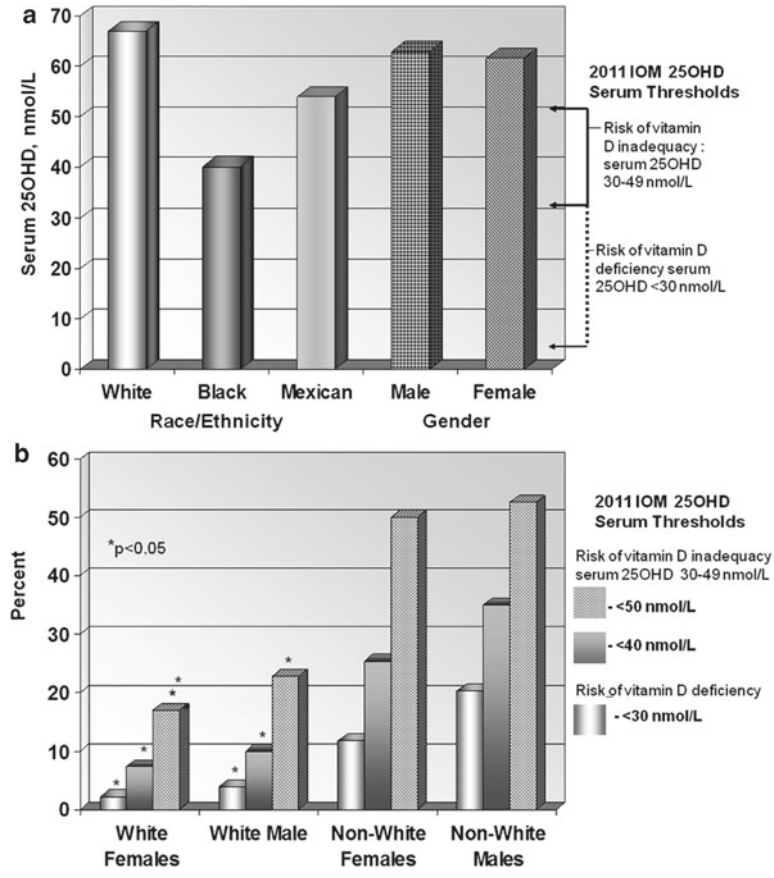

Fig. 21.1 (**a**) Age- and season-adjusted mean serum 25(OH)D among persons aged 1 year and over: United States NHANES, 2000–2004 [17]. Mean serum 25(OH)D levels grouped according to race/ethnicity and gender. Unpublished figure plotted from data presented in Looker et al. 2008 AJCN 88(6):1519–27 and NCHS Data Brief No. 56. March 2011. The 2011 serum 25(OH)D threshold values for risk of vitamin D inadequacy and deficiency are defined as 30–49 nmol/L and less than 30 nmol/L, respectively [6]. (**b**) Year-round prevalence of vitamin D deficiency and insufficiency in a representative survey of Canadian White and non-White males and females 6–79 years old: 2007–2009 [16]. Percent of Canadian White male and female adults relative to their non-White counterparts at risk for vitamin D inadequacy or deficiency. Symbol (*) indicates significant difference between race/ethnicities of the same gender ($p < 0.05$). Unpublished figure plotted from data taken from Whiting et al. 2011 AJCN 94:128–35. The 2011 serum 25(OH)D threshold values for risk of vitamin D inadequacy and deficiency are defined as 30–49 nmol/L and less than 30 nmol/L, respectively [6]

Similar low intakes and poor vitamin D status are observed in Canadian Aboriginal populations [20], especially among urban dwellers who no longer consume traditional diets, and among darker skinned Canadians of Asian ancestry who tend to consume traditional vegetarian or vegan diets [20–22]. Figures 21.1b and 21.2b are nationally representative data from the Canadian Community Health Survey (CHMS), and in contrast to the race/ethnicity differences observed in the US NHANES data, show less difference in dietary intake between those with European ancestry and those with non-European ancestry and darker skin [16, 22]. This observation may reflect a greater need for higher vitamin D intakes in Canada where the opportunities for sun exposure are more limited year-round than in the lower 48 states. Figure 21.2a, b clearly demonstrate that the intake of vitamin D from food sources alone rarely meets the EAR in either country, stressing the need for more vitamin D rich food sources in North America.

Table 21.1 A selection of recent dietary recommendations and expert guidelines for health professionals for vitamin D intake by adults in Canada and the United States

Organization and date	Age group	Recommendation	Comment from organization
Endocrine society, 2011, www.endo-society.org	1–18 y 19+	600–1,000 IU 1,500–2,000 IU	A serum level of 25(OH)D above 75 nmol/L for patients at risk for vitamin D deficiency
Institute of Medicine (IOM), 2011, www.iom.edu	1–70 y 71+ y	600 IU 800 IU	A serum level of 25(OH)D above 50 nmol/L to ensure adequate bone health
Osteoporosis Canada, 2010, www.osteoporosis.ca	19–50 y 51+ y	400–1,000 IU 800–2,000 IU	A serum level of 25(OH)D above 75 nmol/L consistently improves clinical outcomes such as fracture risk
Dietary Guidelines for Americans, 2010, www.health.gov/dietaryguidelines	Men and women at risk for low sun exposure or >50 y	N/A	In 2010 "When necessary, individuals may consider vitamin D supplementation if they are having difficulty meeting the AI through vitamin-D rich foods."
National Osteoporosis Foundation, 2008, www.nof.org	<50 y 50+ y	400–800 IU 800–1,000 IU	"Advice on adequate amounts … of vitamin D for individuals at risk of insufficiency"
Health Canada, Canada Food Guide, 2007, www.hc-sc.gc.ca/fn-an/food-guide-aliment/context/index-eng.php	>50 y	+ 400 IU	"Have 500 mL (2 cups) of [fortified] milk every day for adequate vitamin D." and, for "Men and women over 50 the need for vitamin D increases after the age of 50". In addition to following *Canada's Food Guide*, everyone over the age of 50 should take a daily vitamin D supplement of 10 μg (400 IU)
Canadian Cancer Society, 2007, www.cancer.ca	19+ y	1,000 IU	"Due to our northern latitude … we recommend that Canadian adults consider taking … 1000 international units (IU) a day during fall and winter months."

Table 21.2 The 2011 dietary reference intake values for vitamin D [6]

Age/sex group	EAR IU/d	RDA (AI*) IU/d	UL IU/d
0–6 mo M&F	NA	(400*)	1,000
6–12 mo M&F	NA	(400*)	1,500
1–3 y M&F	400	600	2,500
4–8 y M&F	400	600	3,000
9–18 y M&F[a]	400	600	4,000
19–50 y M&F[a]	400	600	4,000
51–70 y M	400	600	4,000
51 y and over F	400	600	4,000
Over 70 y M	400	800	4,000

To convert to micrograms, divide IU values by 40
NA not applicable (infant values are AIs which are based on the composition of breast milk)
[a]Includes pregnancy and lactation values

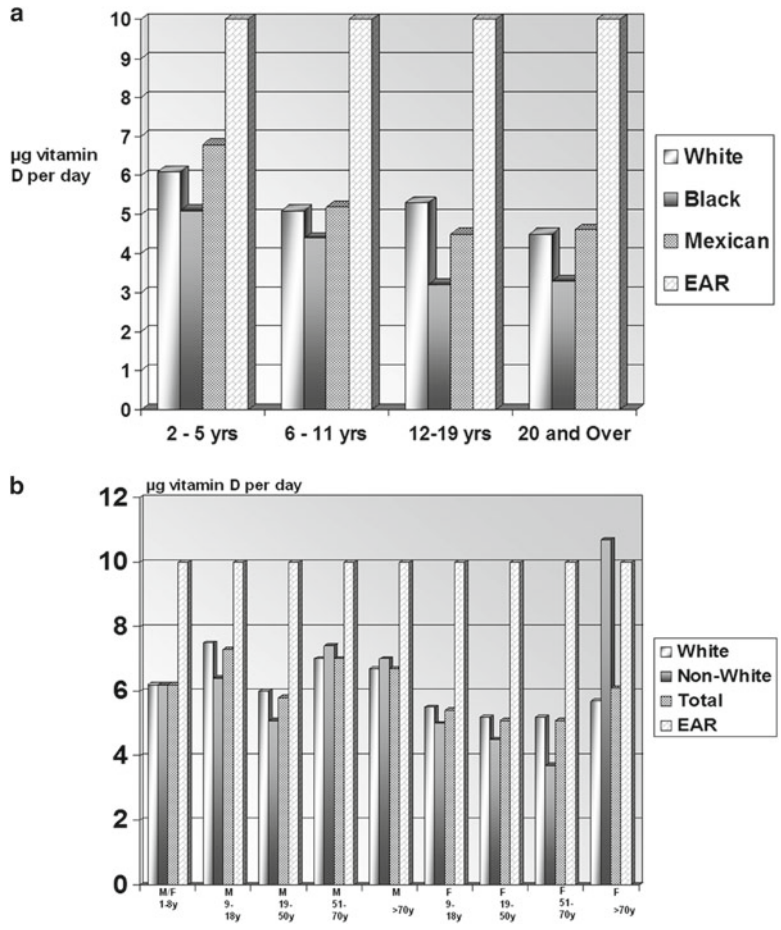

Fig. 21.2 (**a**) Vitamin D intakes from food: mean amounts consumed per individual, by race/ethnicity and age, in the United States, NHANES 2007–2008. Mean daily vitamin D intake from food (micrograms of vitamin D/day) among White, Black, and Mexican Americans in various age groups plotted against the Estimated Average Requirement for vitamin D. Unpublished figure plotted from data taken from What We Eat in America, NHANES 2007–2008, individuals 2 years and over (excluding breast-fed children) day 1 dietary intake data, weighted (revised August 2010). (**b**) Vitamin D intakes from food: mean amounts consumed per individual, by race/ethnicity and age, in Canada: 2004 Canadian Community Health Survey [9]. Mean daily vitamin D intake from food (micrograms of vitamin D/day) among White and non-White Canadians in various age groups plotted against the Estimated Average Requirement for vitamin D. Unpublished figure plotted from data taken from Vantanparast et al. 2010 J Steroid Biochemistry and Molecular Biology.121:301–3

Regulation of Vitamin D Addition to Food in Canada and North America

Canadians and Americans are largely dependent on fortified foods and dietary supplements to meet their vitamin D needs because foods that are naturally rich in or fortified with vitamin D are less frequently consumed. While Canadians and Americans use the same dietary guidelines (DRI) for vitamin D and calcium and the same upper levels (UL) of safe intake for these nutrients, they have very different regulatory approaches to the allowed addition of vitamin D and calcium to foods [10]. Both countries recognize the potential for toxicity if vitamin D is consumed at very high doses and

Table 21.3 Regulation of the addition of Vitamin D to foods in the United States and Canada

Food category	USA (μg)[a]	Canada (% DV = 5 μg)[b]
Enriched Farina	8.75/100 g (350 IU)	NA[c]
Ready-to-eat breakfast cereal	8.75/100 g (350 IU)	NA
Enriched rice	2.25/100 g (90 IU)	NA
Enriched cornmeal products	2.25/100 g (90 IU)	NA
Enriched noodle products	2.25/100 g (90 IU)	NA
Enriched macaroni products	2.25/100 g (90 IU)	NA
Infant formula[d]	Minimum 1 μg/100 kcal (40 IU) Maximum 25 μg/100 kcal (100 IU)	Mandatory level 400 IU/L
Fluid milk	1.05/100 g (42 IU)	44 % of DV/250 mL
Acidified milk	1.05/100 g (42 IU)	44 % of DV/250 mL
Cultured milk	1.05/100 g (42 IU)	44 % of DV/250 mL
Non-fat dry milk, A & D fortified[d]	1.05/100 g (42 IU)	44 % of DV/250 mL[h]
Evaporated milk, fortified[d]	1.05/100 g (42 IU)	6 % of DV per 15 mL (100 IU/250 mL diluted)
Dry whole milk	1.05/100 g (42 IU)	44 % of DV/250 mL[h]
Yogurt	2.22/100 g (89 IU)	May use fortified milk providing 15 % DV/100 g
Low-fat yogurt	2.22/100 g (89 IU)	May use fortified milk
Non-fat yogurt	2.22/100 g (89 IU)	May use fortified milk
Cheese	2.22/100 g (89 IU)	May use fortified milk
Margarine[e]	8.3/100 g (331 IU)	Mandatory level 13.25/100 g (530 IU)
Calcium-fortified 100 % fruit juice	2.5/240 mL (100 IU)	44 % of DV/250 mL
Calcium-fortified fruit juice drink	2.5/240 mL (100 IU)	NA
Soy-protein meal replacement drink[f]	3.5/240 mL (140 IU)	5%DV (10 IU)/55 g
Meal replacement and other bars[f]	2.5/40 g (100 IU)	5 % DV (10 IU)/55 g
Cheese and cheese products[f]	2.02/30 g (81 IU)	NA
Soy beverages[g]	1.25/100 g (50 IU)	44 % of DV/250 mL as D2
Soy beverage products[g]	2.23/100 g (89 IU)	45 % of DV/250 mL
Soy-based butter substitute spreads[g]	8.25/100 g (330 IU)	NA
Soy-based cheese substitute products[g]	6.25/100 g (270 IU)	NA

[a]Maximal level of vitamin D that can be added in accordance with 21 CFR 184.1(b)(2) for the category of food in the United States
[b]Maximal level of vitamin D that can be added in accordance with Canadian Food and Drugs Act and Food and Drug regulations
[c]Not a lawful addition in Canada
[d]Mandatory fortification with vitamin D in the United States and Canada
[e]Mandatory fortification with vitamin D in Canada, optional in the United States
[f]Maximum level allowed as vitamin D_3 only in the United States. Canada allows 5 % of DV per 55 g of a meal replacement cereal or liquid formulated diet with a required range of not less than 100 IU and not more than 400 IU and does not specify form
[g]Maximum level allowed as vitamin D_2 only in the United States
[h]Reconstituted

therefore both countries carefully regulate vitamin D addition to food. The regulatory limitations to the addition of vitamin D to specific food categories for the United States and for Canada are summarized in Table 21.3. While both countries use the same dietary guidelines for optimal and safe levels of vitamin D intake, they have very different approaches to labeling the vitamin D content of foods. Figure 21.3 presents the American and the Canadian version of a label that would appear on a box of cereal such as corn flakes. Neither Canada nor the United States require vitamin D content on the Nutrition Facts Panel of the food label. Because Canada does not currently allow the addition of

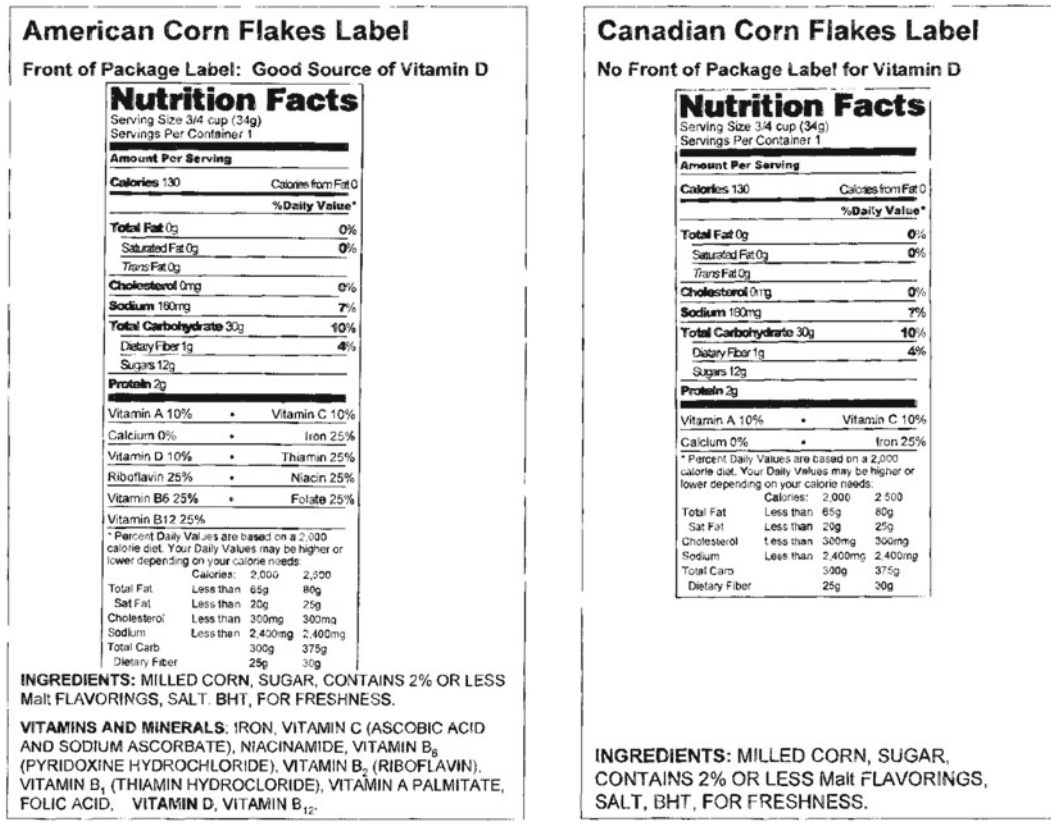

Fig. 21.3 American corn flakes label and Canadian corn flakes label. Comparison of vitamin D labeling in the United States and Canada. The Daily Value used in Canada is currently lower than the value used on American food labels, 200 vs. 400 IU

vitamin D to corn flakes, vitamin D does not appear in the ingredients list, but is included in the American label and can be shown in the Nutrition Facts panel if the manufacturer chooses to do so. A key fundamental difference in labeling of vitamin D content of foods between Canada and the United States is the Daily Value which is an amount used to convey information about the adequacy of the level of nutrient in the food to meet consumer needs. The Canadian Daily Value for vitamin D is 200 IU/serving, while that used on American food labels is 400 IU/serving. This is confusing since Canadians are considered to have a greater need for dietary vitamin D given their high latitude location and longer winters.

Vitamin D Fortification in the United States of America

The approach to vitamin D fortification of foods in the United States is very carefully regulated, but is largely optional for a number of food categories and is required only for fortified fluid milk and fortified evaporated milk (Table 21.3). The addition of vitamin D to foods as a nutrient supplement is in accordance with the US Code of Federal Regulations (CFR): 21 CFR 184.1 (b) (2) which imposes strict limitations with respect to the categories of foods, functional use, and level of use [10]. Such regulatory limitations provide a control mechanism that limits over-fortification with vitamin D.

In accordance with 21 CFR 184.1 (b) (2), any addition of vitamin D to foods not in compliance with each of these established limitations requires a food additive regulation. More recently, a number of new food categories shown in Table 21.3 have been approved for the addition of either vitamin D_3 or vitamin D_2 as a nutrient supplement in accordance with 21 CFR 172.380. In contrast to Canada, where fortification with vitamin D is mandatory for designated food staples, the lawful addition of vitamin D to eligible foods in the United States is voluntary in most cases, with the exception of fluid milk. Vitamin D fortification is required when the label declares that the milk is fortified.

Although many food categories are eligible for controlled levels of vitamin D fortification in the United States, there is a large discrepancy between the number of eligible foods and the number and variety of vitamin D fortified foods currently in the US market place [1, 10]. Fluid milk and ready-to-eat cereals are the major contributors to vitamin D intake in the United States, while milk and margarine are the major fortified foods contributing to Canadian vitamin D intake [10, 11]. Selective fortification of only a few staple foods that are not commonly consumed by individuals at greatest risk of poor vitamin D status is a major barrier to optimizing vitamin D intake [14]. By encouraging US manufacturers to utilize these fortification options, the vitamin D intake of groups at risk could be significantly improved. Consideration could also be given to shifting optional fortification to mandatory for those eligible food staples commonly consumed by the entire American population. Cereal grain products (pasta, bread, and other baked goods) are frequently consumed by the general population and would serve as a good candidate for mandatory fortification with vitamin D [11].

Vitamin D Fortification in Canada

Current Situation

The Canadian approach to vitamin D addition to food is that of mandatory fortification of food staples through the Canadian Food and Drug Regulations [10]. Milk and milk alternatives and margarine are required to be fortified in Canada. Fluid milk in Canada is labeled as providing 44 % of the recommended daily intake which is currently set at 5 μg (200 IU) per 250 mL serving (Table 21.3). Other milk products that require vitamin D fortification include: evaporated milk, powdered milk, goat's milk, and milks of plant origin (soy, rice, and other grains), which also must be fortified with calcium [10]. All margarines in Canada are fortified with vitamin D at the level of 13.25 μg (530 IU) per 100 g. Other foods for which vitamin D addition is permitted are meal replacements, nutritional supplements, and formulated liquid diets. Addition of vitamin D to such foods is optional but may be no less than 2.5 μg (100 IU) and no more than 10 μg (400 IU) per 1,000 kcal, as long as the intended total energy intake is <2,500 kcal. Industrial milk used in baked goods, yogurts and cheeses does not need to be fortified; however, some yogurts are currently in the market place having been made with fortified milk; they tend to have 15 % DV in 100 g servings. Examination of vitamin D status of Canadians from CHMS shown in Fig. 21.4 indicates that milk ingestion is associated with better status, i.e., higher 25(OH)D levels compared to those not ingesting milk [22], indicating that fortification is having a positive effect on the vitamin D status of Canadians. Other foods that are fortified include calcium-fortified juices and calcium-fortified soy beverage.

History of Vitamin D Fortification in Canada

The history of vitamin D fortification in Canada differs from the experience in the United States which has seen little change since its inception in the 1930s. The Canadian experience follows the

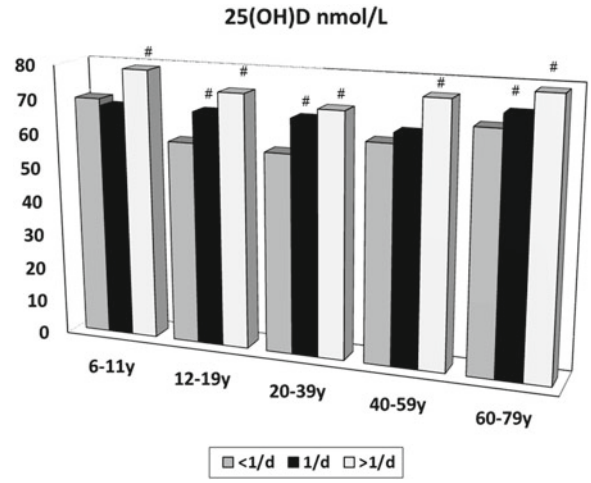

Fig. 21.4 Mean serum levels in Canadians consuming varying levels of milk daily [22]. The effect of fortified milk intake (per day) on plasma 25-hydroxyvitamin D levels in the Canadian Health Measures Survey (2007–2009). Symbol (#) indicates significantly different from group having <1 serving of milk per day. Unpublished figure plotted from data taken from Langlois et al. 2010 Canadian Health Measures Survey. Health reports 2010;21(1)47–55

cautious approach of limiting food fortification based on concerns about potential for adverse effects due to over-fortification. As a result of rickets being a major cause of death due to malnutrition in Canada in the 1930s, measures were taken at this time to fortify foods with vitamin D [23]. By 1942, foods could be fortified with vitamin D at levels of 400 IU; then in 1949, a maximum of 800 IU was set. Foods that contained vitamin D at this time included fruit drinks, biscuits, and ready-to-eat cereals in addition to milk and margarine. By the mid-1960s, it was found that some children were getting too little vitamin D, while at the other extreme, some children were getting doses that were too high (~1,800 IU). This led to a situation where both deficiency and excess existed at the same time. Although no events of excess were reported, in 1964–1965, the Canadian government set limits to fortification, allowing only standardized staple foods (milk and margarine) to be voluntarily fortified with vitamin D. However, cases of rickets continued, and health professionals mounted lobbying activities to encourage dairies to fortify milk. In 1975, addition of vitamin D to milk was made mandatory.

Future of Fortification

More recently, in response to growing reports of vitamin D deficiency and inadequate intakes of other nutrients, new regulations for allowing addition of vitamins and minerals to Canadian foods were announced in 2005. However, these proposed new regulations have not been implemented at the time of writing this chapter. The proposed policy is outlined in the document, *Addition of Vitamins and Minerals to Food*, 2005: *Health Canada's Proposed Policy and Implementation Plans* (http://www.hc-sc.gc.ca/fn-an/nutrition/vitamin/fortification_factsheet2-fiche2-eng.php). As noted in this document, the proposed policy would create a new provision for food fortification done at the "discretion" or "choice" of the manufacturer (within defined limits set by Health Canada) to meet a market demand, a process known as discretionary fortification. Even so, in Canada, voluntary (discretionary) fortification requires the following of specific rules, for example, to fortify a soy beverage with calcium, all the nutrients present in cow milk must be added, including vitamin D, vitamin B_{12}, vitamin A, and so forth.

In the proposed policy to permit addition of vitamins/minerals, this can be done for restoration of losses during processing, nutritional equivalence of substitute foods, and fortification to correct or

prevent problems of public health significance. Voluntary fortification does have some of the same risks as mandatory fortification, i.e., modest increase in average intake with concurrent risk of exceeding the Upper Level of safe intake (UL shown in Table 21.2). Allowable food vehicles, and the option not to fortify, mitigate some of these risks. Under the new proposed policy, those who do not consume any milk can choose fortified orange juice, while those who have adequate milk intake can choose unfortified juice and would be able to increase their vitamin D intake. In the new proposed policy, nutrients are assigned a risk level. Vitamin D is deemed a Risk Category B nutrient, a nutrient with serious adverse effects, but with low risk of excessive intake at the proposed level of addition for discretionary fortification. If a nutrient is added, the minimum level of total nutrient (naturally occurring and added) in the food must be 5 % of the Daily Value per reference amount of the food (i.e., the food will qualify for a "source" claim). For Risk Category B nutrients such as vitamin D, the total amount of the nutrient (naturally occurring and added) permitted in the food after addition is up to 10 % of the Daily Value per reference amount of the food. If the food contains 10 % of the Daily Value the food will qualify for a "good source" claim. In Canada, the current Daily Value (DV) is 200 IU yet the RDA for most age groups is 600 IU [6]. Thus, an update in the Canadian DV is anticipated prior to implementing this new policy.

Sacco and Tarasuk have provided some possible scenarios for this new fortification policy, although not specifically relating to vitamin D. They contend that Health Canada's proposed discretionary fortification policy is misaligned with the nutritional needs of Canadians because it is not rooted in an assessment of current nutrient intake patterns [24]. In a more recent analysis [25], they concluded that consumption of foods slated for discretionary fortification is associated with lower nutrient intakes and suboptimal food intake patterns.

Novel Approaches to Vitamin D Enrichment of Food

The effectiveness of fortifying foods other than milk and milk products with vitamin D needs to be assessed since a significant number of North Americans do not drink milk [14]. The addition of vitamin D to food is an effective approach to correcting vitamin D deficiency or insufficiency. This was recently established in a systematic evidence-based review that specifically focused on the relation of vitamin D to bone and muscle health [26] and in another study that examined trends in vitamin D intake from food sources over the past 2 decades [27]. In the meta analyses [26], the authors examined the effect of vitamin D supplementation and food fortification on 25(OH)D levels, reporting a significant association between intake, either as vitamin D_2 or as vitamin D_3, and 25(OH)D concentrations. They determined a 1–2 nmol/L increase in 25(OH)D for each additional 100 IU of vitamin D which provides important evidence of the efficacy and safety of using food fortification to increase 25(OH)D [26]. Harnack et al. [27] more recently reported the alarming trend for decreased vitamin D intake from food sources in a population study over the last 2 decades, particularly in men, and this trend was consistent with the reported decline in serum 25(OH)D discussed earlier [17]. These findings draw attention to the need to evaluate the adequacy of the current food fortification practices in the United States and Canada and to explore new approaches to enriching the vitamin D content of food.

Novel approaches to vitamin D enrichment of foods include the use of "bio-additions." We use the term "bio-addition" to describe the enrichment of a food staple with another food rich in a specific nutrient and to the postharvest or preprocessing manipulation of foods that result in high vitamin D content. This form of "biofortification" has been proposed for bread and other important food staples, especially where individuals prefer a nonanimal source of vitamin D for religious, cultural, or dietary reasons [11]. Biofortification is a term that normally refers to producing crops with higher levels of

Fig. 21.5 Soup label showing use of vitamin D enriched mushrooms in Canada. Soup label taken from product currently available in the Canadian market place and used with permission from the manufacturers

nutrients in foods through changes in plant breeding or genetic alteration, thus modifying nutrient content preharvest rather than postharvest (the latter being consistent with traditional food fortification). In terms of vitamin D, however, one may expand this concept to consider those foods, whether plant, animal, or fungal/yeast, in which vitamin D levels are increased during growth or harvest of the food source.

Current efforts to seek new food sources of vitamin D have focused on the postharvest exposure of edible mushrooms to ultraviolet light [28, 29]. Exposing white button mushrooms to ultraviolet light markedly increases their vitamin D_2 (ergocalciferol) content much the same way that exposing human skin to sunlight (ultraviolet light) increases the synthesis of vitamin D_3 (cholecalciferol). The safety, bioavailability, and efficacy in support of bone growth of vitamin D_2 from ultraviolet light-treated edible mushrooms have recently been established using a regulatory approach in chronically fed growing rats [28]. Light-treated mushrooms show great potential in correcting vitamin D deficiency during the winter or in individuals consuming vegan diets and living in countries with limited sunlight [30, 31]. Light-exposed edible mushrooms are the only natural plant food source commonly consumed in the United States and Canada and are now available in the market place and in Canada serve as the source of vitamin D enrichment in specific brands of mushroom soup as shown in Fig. 21.5. High levels of vitamin D_2 from irradiated white button mushrooms made into a soup [30] was effective in significantly raising serum 25(OH)D levels in young vitamin D-deficient Germans during the winter as shown in Fig. 21.6 and in Table 21.4. It is also possible to irradiate yeast with UV light, as was done originally to provide vitamin D_2 for fortification of milk in the early twentieth century. The company Lallemande (www.lallemande.com) has a (patent pending) process to convert ergosterol in baker's yeast to ergocalciferol (vitamin D_2) while allowing the baker's yeast to maintain leavening and flavor properties. Many different kinds of baked goods leavened with yeasts irradiated in this process could be produced with higher vitamin D content, but as yet are not available in the North American market.

Fortification of animal feeds with higher levels of vitamin D during rapid growth or egg production is another novel approach to bio-addition of vitamin D to foods. In Canada meat contribution to vitamin D intake was second only to the contributions from traditionally fortified milk products [9]. Strategies are being developed for the natural enhancement of vitamin D content of animal foods through changes in diet composition of poultry, livestock, and farmed fish [1, 32, 33].

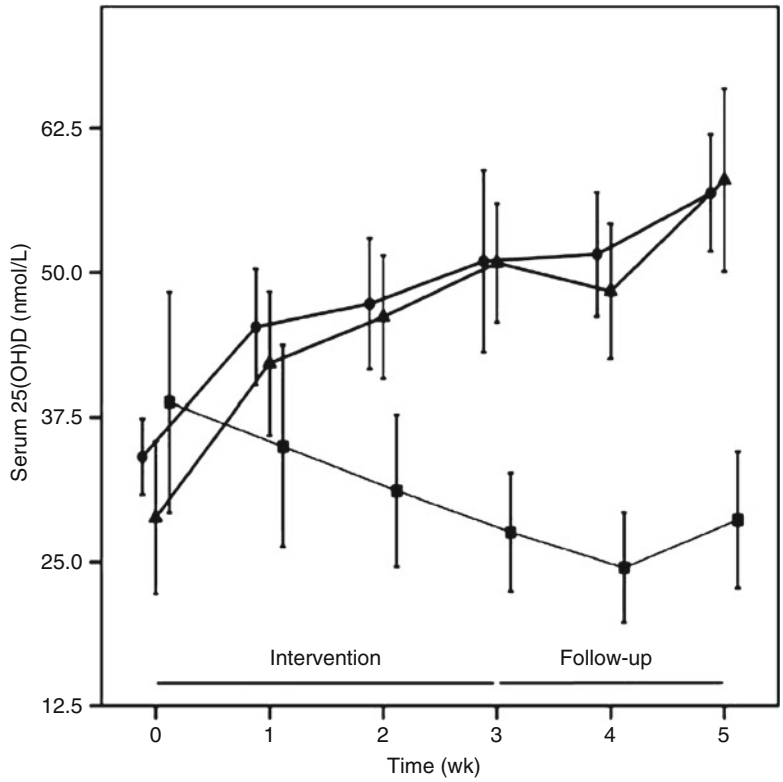

Fig. 21.6 Group mean serum 25(OH)D response to consuming soup made with or without vitamin D_2 enhanced mushrooms [30]. Time course of the mean changes in serum 25(OH)D over the 5-weeks study period in subjects who consumed four times (weeks 0, 1, 2, 3) mushrooms enhanced with vitamin D_2 via UV-B irradiations (mushroom group, $n=8$, *filled circle*) or vitamin D_2-containing supplement (supplement group, $n=9$, *filled triangle*) or placebo (placebo group, $n=9$, *filled square*) at the end of winter. Error bars are 2 S.E. Time courses for serum 25(OH)D over the study period in the mushroom and supplement groups did not differ significantly. Published with permission of the publishers and authors [30]

Conclusion: Efficacy of Current Fortification Practices and Proposed Bio-Addition Approaches for Vitamin D Enrichment of Food sources

In conclusion, we present selected examples of clinical trials demonstrating the efficacy of fortification of different food vehicles with vitamin D in improving vitamin D status and other end-measures in different age and gender groups in Table 21.4 [34]. In all of these studies, despite a wide range of fortification levels, there were no reports of adverse effects even in those using very high levels of vitamin D [30, 35–48]. Table 21.4 presents a sampling of effective food vehicles for vitamin D fortification that could be used in future strategies to increase the variety of vitamin D rich foods in the North American food supply. Fortification of these different foods with vitamin D is effective in improving vitamin D status and reducing the risk of bone disease and possibly other chronic diseases. We have presented evidence suggesting that current levels of fortification may need to be reevaluated to meet the needs of specific target populations in greater need such as the very elderly and certain racial and ethnic groups. However, we have also shown that even in otherwise normal healthy individuals, a greater variety of fortification options is necessary in order to achieve required intakes.

Table 21.4 Selected examples of clinical trials examining efficacy of food vehicles for vitamin D fortification shown by food category tested in different age and gender target populations

Study	Study population	Food vehicle and level of vitamin D fortification	Outcome establishing efficacy of vitamin D fortification [34]
Infant formula/fluid milk			
Carpenter et al. [35]	Infants, 6–36 mo ($n=781$) 64 % Hispanic, 23 % African American, 2 % White	Commercial infant formula Minimum vitamin D content 10 μg/L (400 IU/L)	Significantly greater 25(OH)D concentrations observed with formula feeding
Houghton et al. [36]	Toddlers, 12–20 mo ($n=225$)	Fortified milk 3.7 μg/d (148 IU/d)	17.9 nmol/L higher 25(OH)D levels than the control group
Chan et al. [37]	Pre-pubertal girls, Caucasian, age 11 y, Tanner stage 2 ($n=48$)	Variety of dairy products Daily vitamin D intake: 288 vs. 128 IU control group	Significantly greater increase in lumbar spine density relative to control (22.8 ± 6.9 % vs. 12.9 ± 8.3 %)
Rich-Edwards et al. [38]	Mongolian school children, 9–11 y Baseline 25(OH)D levels: 20 ± 10 nmol/L ($n=579$)	Fortified UHT milk from Mongolia and the United States fortified to provide 300 IU vitamin D/d	Significantly raised 25(OH)D levels : Mongolian milk: 50 ± 15 nmol/L US milk: 72.6 ± 25 nmol/L
Green et al. [39]	No-pregnant women, 18–47 y ($n=73$)	Milk powder providing 5 μg/d (200 IU/d)	19 % higher 25(OH)D levels (10 nmol higher) than control group
Daly et al. [40]	Older men, 61.9 ± 7.7 y) ($n=167$)	400 mL/d UHT milk fortified with 1,000 g Calcium + 800 IU vitamin D3 for 2 y	Percent change in BMD (bone loss), 0.9–1.6 % less in fortified milk group at femur neck and ultradistal radius ($p<0.008$ to $p<0.001$)
Yogurt			
Nikooyeh et al. [41]	Type 2 diabetic adults, baseline 25(OH) D levels: 35 % deficient ($n=90$)	Vitamin D—fortified yogurt drink—12 wks intervention 250 mLs, 2× per day of: 500 IU D3 + 150 mg Ca or 500 IU D3 + 250 mg Ca	Both vitamin D fortified yogurt drinks improved glycemic status and increased serum 25(OH)D: 32.8 ± 28.4 nmol/L 28.8 ± 16.1 nmol/L
Cheese			
Johnson et al. [42]	Older men and women, >60 y ($n=110$)	Vitamin D_3-fortified process cheese containing 600 IU/daily dose, 2 mo winter intervention	Vitamin D fortified process cheese was bioavailable, but did not increase 25(OH)D during limited sun exposure
Wagner et al. [43]	Adults ($n=80$)	Vitamin D_3-fortified hard cheese (34 g with 28,000 IUD3/wk) equivalent to daily dose of 4,000 IU D3	Changes in 25(OH)D from baseline: Cheddar cheese + vitamin D: 65.3 ± 24.1 nmol/L increase Low-fat Cheddar + vitamin D: 69.4 ± 24.7 nmol/L

Bread			
Natri et al. [44]	Women, 25–45 y Baseline 25(OH)D range 12–45 nmol/L ($n=41$)	Fortified rye or wheat bread 10 μg D_3/d, for 3 wks	Significant ($p<0.001$) rise in serum 25(OH)D levels: Rye: 14.9 ± 6.2 nmol/L Wheat: 16.3 ± 6.6 nmol/L
Mocanu et al. [45]	Nursing home elderly men and women ($n=45$)	Bread bun fortified with 5,000 IU D_3/d for 12 mo	After 12 mo, mean 25(OH)D level was 125.6 ± 38.8 nmol/L with 92 % exceeding 74 nmol/L and Lumbar spine and hip bone density z scores increased significantly ($p<0.001$)
Mushrooms			
Urbain et al. [30]	Vitamin D-deficient Young German adults ($n=30$)	UVB-irradiated button mushrooms, 28,000 IU D_2/serving/wk for 5 wks during German winter	3.9 nmol/L/wk rise in serum 25(OH)D in those consuming vitamin D rich mushrooms
Orange juice			
Biancuzzo et al. [46]	Adults, 18–84 y At baseline 64 % of subjects serum 25(OH)D levels were <50 nmol/L ($n=60$)	Compared bioavailability of 1,000 IU oral supplements with orange juice fortified with either 1,000 IU of D_3 or D_2 for 11 wks at the end of winter	Bioavailability of both vitamin D_2 and D_3 from orange juice was not significantly different from their respective dietary supplements taken in capsules, further demonstrating efficacy of an aqueous, fat-free vehicle
Tangpricha et al. [47]	Adults, 22–60 y ($n=26$)	Compared Ca-fortified orange juice with Ca and vitamin D fortified orange juice consumption for 12 wks: 240 mL/d OJ with 350 mg Ca 240 mL/d OJ with 1,000 IU D_3 and 350 mg Ca	From baseline to 12 wks, vitamin D + Ca-fortified orange juice increased serum 25(OH)D by 150 % without change in serum calcium A change from 50.0 ± 10 to 73.0 ± 8.0 nmol/L, $p<0.01$
Foods for special medical use			
Adolphe et al. [48]	Long-term care residents, men and women, >50 y ($n=12$)	Four puréed foods were fortified with vitamin D_3 and fed for 8 wks as: Vegetables and meats served at lunch and dinner Fortification level was determined based on estimated intake and AI for each individual	Mean serum 25(OH)D levels rose from 41 ± 21 to 66 ± 11 nmol/L after 8 wks, $p<0.003$

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Chapter 22
Profiling National Mandatory Folic Acid Fortification Policy Around the World

Mark A. Lawrence and Karishma Kripalani

Key Points

- There exists compelling evidence that exposure to sufficiently high levels of folic acid during the periconceptional period can help reduce the risk of a neural tube defect-affected pregnancy in certain individuals.
- There are a number of potential policy responses to this epidemiological evidence.
- Mandatory folic acid fortification in different countries has been shown to help prevent 20–50 % of neural tube defects; however, concerns persist about potential risks associated with raised levels of exposure to folic acid for the target individuals and population in general.
- Mandatory folic acid fortification is a controversial policy intervention associated with a number of scientific uncertainties and ethical dilemmas because it is non-discriminatory and exposes everyone in the population, including infants, children, older adults and men, all for whom there often is no apparent benefit, to unprecedented levels of synthetic folic acid.
- Mandatory folic acid fortification has appealed as a policy intervention because it introduces folic acid into the food supply environment and therefore can passively expose at-risk individuals to folic acid.
- Sixty-six countries have recommended mandatory folic acid fortification—mostly in wheat flour and within the range of 100–300 μg/100 g.
- Three countries (Ireland, New Zealand, United Kingdom) initially recommended mandatory folic acid fortification and subsequently have chosen to delay putting this recommendation into practice.
- One country (United States) is reviewing its mandatory folic acid fortification policy to consider increasing the number of food products mandated to be fortified with folic acid.
- Nutrition education activities and monitoring and evaluation programmes towards potential risks and benefits are essential complementary policy interventions in countries where mandatory folic acid fortification is being implemented.

Keywords Folic acid • Folate • Mandatory fortification • Neural tube defects • Evidence • Ethics

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V.R. Preedy et al. (eds.), *Handbook of Food Fortification and Health: From Concepts to Public Health Applications Volume 2*, Nutrition and Health, DOI 10.1007/978-1-4614-7110-3_22,

Abbreviations

A2Z	The USAID Micronutrient and Child Blindness Project
CDC	Centers for Disease Control and Prevention
Codex	Codex Alimentarius Commission
FAO	Food and Agriculture Organization
FFI	Flour Fortification Initiative
GAIN	Global Alliance for Improved Nutrition
MI	Micronutrient Initiative
NTD	Neural tube defect
RiFoRG	Rice Fortification Resource Group
UNICEF	United Nations Children's Fund
USAID	United States Agency for International Development
WFP	World Food Programme
WHO	World Health Organization

Introduction

Mandatory food fortification "occurs when governments legally oblige food producers to fortify particular foods or categories of foods with specified micronutrients" [1]. Because mandatory food fortification exposes everyone in the population who consumes the fortified food(s) to raised levels of the specified micronutrient, it is one of the most powerful policy instruments available to influence dietary intake and population health outcomes. Many national food regulatory authorities have established policy guidance on mandatory food fortification. From an international perspective, the Codex Alimentarius Commission (Codex) provides principles for the addition of essential nutrients to foods [2]. According to these Codex principles, mandatory food fortification with additional vitamins and minerals is recommended where there is proven public health and nutrition need (Note: these principles are under review at the time of writing).

Mandatory folic acid fortification has been developed and implemented as a food policy intervention for two purposes:

1. *Prevention and control of folate-related micronutrient malnutrition.*
   Mandatory folic acid fortification has been recommended as an intervention to help prevent and control micronutrient malnutrition such as child and maternal anaemia associated with folate deficiency [3]. For example, in 1996, Ecuador issued a decree which specified rules of fortification and enrichment of wheat flour for the prevention of nutritional anaemia [4].
2. *Reduction in risk of neural tube defects.*
   Twenty years ago, compelling epidemiological evidence emerged that sufficient folic acid consumed during the periconceptional period helps to reduce the risk of neural tube defects (NTDs) [5, 6]. Since then a number of other studies have added to the evidence base for this protective effect of folic acid. Neural tube defects are congenital malformations that result from the failure of the neural tube to close during embryogenesis and most commonly include spina bifida and anencephaly. Neural tube defects place an exceptionally high social, financial and emotional burden on affected individuals and their families. National NTD birth prevalence varies widely depending on genetic and environmental conditions and has been recorded as up to 6 per 1,000 live births in China [7]. In response to the epidemiological evidence, authorities in several countries have recommended that all women of childbearing age consume 400 μg of folic acid daily to help prevent NTDs [8–10]. There are a number of potential interventions available to help at-risk individuals

achieve this recommended folic acid intake level, including supplementation, voluntary folic acid fortification and mandatory folic acid fortification. Mandatory folic acid fortification in particular has received a substantial amount of attention.

This chapter profiles national recommended or mandatory folic acid fortification policies around the world. Since 1992, folic acid also has been added to certain food products for the purpose of "restoration". Guatemala was the first country to specify that folic acid, at the level of 0.35–0.45 mg/kg, be added to wheat flour to restore its folate to preprocessing levels ("la Ley General de Enriquecimiento de Alimentos, Decreto número 44-92") [11]. Codex defines fortification levels beyond those used for restoration and as such restoration policy interventions will not be considered further.

Background

Mandatory folic acid fortification is an especially complex and vexed policy response to the epidemiological evidence of the folic acid—NTD relationship. On the one hand, it is an immediately attractive policy intervention to achieve the recommended dietary folic acid intake. It introduces folic acid into the food supply environment and therefore can passively increase the folic acid exposure of women at risk of having an NTD-affected pregnancy. This passive exposure means that at-risk women do not need to consciously change their dietary behaviour to increase their folic acid exposure; a particular advantage given that it has been estimated that almost half of all pregnancies are unplanned and by the time a woman might realize that she is pregnant, it may be too late to prevent a neural tube defect. On the other hand, it is a policy intervention that is associated with a number of scientific uncertainties and ethical dilemmas.

Scientific uncertainties associated with mandatory folic acid fortification include the unknown mechanism by which folic acid helps reduce the risk of NTDs and the unknown optimum dose of folic acid required to exert this protective effect. In addition there are significant uncertainties related to potential health risks associated with raised exposure to folic acid. High levels of circulating folic acid in blood serum may delay a diagnosis of vitamin B12 deficiency, thereby exacerbating subsequent neurological damage [12]. Overriding this health risk is the concern that raised folic acid exposure may promote the progression of colorectal cancer in certain individuals [13].

Ethical dilemmas associated with mandatory folic acid fortification to help reduce the risk of NTDs relate to its non-discriminatory nature. This policy intervention exposes everyone in the population, including infants, children, older adults and men, all for whom there is no apparent benefit, to unprecedented levels of synthetic folic acid. This ethical dilemma is compounded given that the mechanism by which folic acid might exert its protective effect is unknown (see Table 22.1).

Table 22.1 Key benefits and risks of mandatory folic acid fortification

Benefits	Risks
Does not require behaviour change on the part of at-risk individuals	Ethical concerns associated with raising the population's exposure to folic acid for no apparent benefit to the majority
Fortification of staple foods promotes equitable exposure to folic acid	Health concerns associated with masking the symptoms of vitamin B12 deficiency and the possible relationship with the progression of colorectal cancer
A simple and rapid policy intervention relative to several policy alternatives	Many unknowns with the folic acid—NTD relationship including the mechanism, the role (if any) of other nutrients and the optimum folic acid dose for protection

Summary of key benefits and risks associated with mandatory fortification of food staples with folic acid as a policy intervention to reduce the risk of NTDs

These scientific uncertainties and ethical dilemmas have stimulated intense policy debates about whether or not to pursue mandatory folic acid fortification as a policy intervention to help reduce the risk of NTDs. Typically, the policy-making process is based on a risk-analysis approach rather than an application of the precautionary principle. Critically, USAID and important nongovernment organizations (NGOs) have supported mandatory folic acid fortification and include the Flour Fortification Initiative (FFI), United Nations Children's Fund (UNICEF) and Centers for Disease Control and Prevention (CDC), World Health Organization (WHO), Food and Agriculture Organization of the United Nations (FAO), World Bank, World Food Programme and The USAID Micronutrient and Child Blindness Project (A2Z).

Methods

Data Collection

A systematic and comprehensive method was used to review national policies towards mandatory folic acid food fortification. The starting point for the present review was the 2006 Report of the National Committee on Folic Acid Food Fortification published by the Food Safety Authority of Ireland [14], complemented with data from the *Micronutrient Initiative Handbook* [15]. Further data were obtained from the FFI website [16]. Country reports, expert reports, levels of fortification and fortification vehicles were identified from this source. Where possible, data was independently cross-checked with Ministry of Health or other national or regional reports. Country data on food vehicles and levels of fortification were not always complete nor were they always available from official government websites. Occasionally these data were reported inconsistently among secondary sources. Where data gaps and discrepancies existed, judgments were made by deferring to available data published in the FFI database. The FFI focus is on wheat and maize flour. To address gaps in information on fortification through other food vehicles, data was supplemented by other NGOs involved in folic acid or micronutrient fortification, including Global Alliance for Improved Nutrition (GAIN) and its affiliate, Rice Fortification Resource Group (RiFoRG); WHO; Micronutrient Initiative (MI); and Helen Keller International. Resources on the websites of these groups included press releases, recent news and updates on folic acid fortification. This allowed supplementation and updating of the FFI listing of countries reporting folic acid fortification initiatives that, at the time of writing, was based on 2009 data.

In addition relevant academic and medical resources were accessed from the publication databases ProQuest, GALE Health Reference Centre, EBSCO (Health Policy Reference Center, Medline (full text) Academic Search Complete and Global Health) and PubMed. A Boolean search was entered into each database: "folic acid" AND ("national policy" OR "mandatory" OR "voluntary"). Limits were English language and articles published between 1985 and (24 July) 2011. An initial screen resulted in ProQuest = 404, GALE = 53, EBSCO = 348 and PubMed = 223. From these, titles and abstracts were reviewed and relevant articles accessed. These were cross-referenced to identify further articles, including government reports and publications, which were sourced online where available.

Data Interpretation

The circumstances and technical details associated with recommendations to mandate the addition of folic acid to food were not always clear from the data available. Two particular inclusion/exclusion criteria were applied to standardize the interpretation of data available in relation to mandatory folic

acid fortification policy. First, fortification was defined in accordance with the Codex Alimentarius Commission's Principles for the addition of Essential Nutrients to Foods [2]. The application of this definition meant that a policy that related to the addition of folic acid for the purpose of restoring folate levels to those that existed preprocessing (restoration) was not considered consistent with the definition of food fortification. This purpose was not always specified in the literature. To ensure consistency in data interpretation, countries that had recommended policies based on fortification levels up to and including 70 μg folic acid/100 g flour were not included in the results as this is the restoration level of cereal grain products, as specified by the US Food and Drug Administration (FDA) [17]. For example, Nigeria is currently considering a revision to their flour fortification premix, to include folic acid at 006.0 mg/kg [16]. As this level is sufficient to replace the folate lost during processing of cereal grain, it was classified as restoration rather than fortification and therefore is not included in the data.

Second, mandatory folic acid fortification was recognized as a dynamic policy area. The decision to fortify or not, at what level and for which food vehicle(s) is under ongoing review in many countries. The data presented in this chapter relates to the findings from an audit of national mandatory or recommended folic acid fortification policies as of November 2011.

Results

The results of the systematic review of national mandatory folic acid fortification policies are presented in Table 22.2. This table lists those countries organized alphabetically by geographical region where mandatory folic acid fortification policy is officially recommended or mandated, the food vehicle and fortification level. Sixty-six countries recommend mandatory folic acid fortification as a policy intervention. Wheat or maize flour, and/or flour products such as noodles or pasta, are the food vehicles of choice used by all countries. Additional vehicles include rice and milk. Fortification levels range between 100 and 300 μg/100 g. Primary sources such as a government decree or statement on an NGO website are indicated in the table.

In addition to the results presented in Table 22.2, authorities in three other countries initially recommended mandatory folic acid fortification but subsequently have delayed its implementation for varying reasons.

United Kingdom: In 2006, the Scientific Advisory Committee on Nutrition, which advises the UK Food Standards Agency and the UK Department of Health, recommended that mandatory folic acid fortification should proceed together with controls on the intake of folic acid from voluntarily fortified foods [18]. However, before mandatory folic acid fortification policy was regulated in the UK, concerns were raised about the possible relationship between folic acid and the promotion of colorectal cancer. In November 2011, there had been no action taken in response to the original advice.

Ireland: In 2006, the Minister for Health and Children in Ireland accepted the recommendation of the National Committee on Folic Acid Food Fortification to introduce a programme of mandatory folic acid fortification of bread [14]. However, in 2009, the Food Safety Authority of Ireland announced that because of the increased availability of folic acid in the food supply since the original mandatory folic acid fortification recommendation and the reduced incidence of NTDs to 9.3 per 10,000 live births, there would be limited public health benefit from introducing mandatory folic acid fortification at that time [19].

New Zealand: In September 2007, following 14 years of at times intense lobbying, Food Standards Australia New Zealand recommended that Australia and New Zealand implement a programme of mandatory folic acid fortification of bread-making flour. Both governments agreed to phase in the

Table 22.2 Countries that have a recommended/mandatory folic acid fortification policy: the food vehicle and fortification level

Country	Food vehicle[a]	Fortification level
Africa		
Cote d'Ivoire[a] [40]	Wheat flour	260 μg/100 g [16]
Mauritania [16]		
Senegal[a]		
Benin[a]	Wheat flour (at least)	260 μg/100 g [16]
Burkina Faso[a]		260 μg/100 g [16]
Cabo Verde[a]		N/A
Gambia[a]		N/A
Guinea-Bissau[a]		260 μg/100 g [16]
Liberia[a]		N/A
Mali[a]		260 μg/100 g [16]
Niger[a]		260 μg/100 g [16]
Sierra Leone[a]		N/A
Togo[a]		260 μg/100 g [16]
Zambia	Maize flour	206 μg/100 g
	Maize meal	189–240 μg/100 g
	Wheat flour	124–136 μg/100 g
Ghana[a]	Wheat flour	200 μg/100 g [16]
Malawi	Wheat flour[a]	210 μg/100 g
Morocco	N/A	150 μg/100 g
South Africa [41]	Wheat and maize	
Guinea[a]	Wheat flour	135 μg/100 g [42]
Tanzania [43]	Wheat and maize flour	N/A
Cameroon		N/A
Nigeria[a]		N/A
Americas		
Latin America and Caribbean		
Paraguay	Wheat flour[a]	300 μg/100 g
Uruguay [44]	Wheat flour	240 μg/100 g
Chile [45, 46]	Wheat flour	220–240 μg/100 g
Cuba	Wheat flour[a]	250 μg/100 g
Argentina	Wheat flour[a]	220 μg/100 g
Mexico	Wheat flour[a]	200 μg/100 g
Costa Rica	Wheat and maize flour	180 μg/100 g [11]
	Milk and rice [47]	
Guatemala [48]	Wheat and maize flour	180 μg/100 g
El Salvador	Wheat (and maize) flour [49]	
Belize	Wheat flour	150 μg/100 g
Bolivia	Some maize flour	
Colombia		
Brazil [50]		
Jamaica		
Haiti		
Barbados	Wheat flour	150 μg/100 g
Belize	Maize flour, rice and milk in some countries	Up to 300 μg/100 g
Grenada		
Guadeloupe		N/A
Guyana		N/A

(continued)

Table 22.2 (continued)

Country	Food vehicle[a]	Fortification level
Puerto Rico		N/A
St. Vincent		N/A
Peru	Wheat flour[a]	120 μg/100 g
North America		
Canada	White flour in foods as consumed	150 μg/100 g
	Enriched uncooked pasta	200 μg/100 g
USA	Food as consumed (flour, rice, breads, rolls and buns, pasta, corn grits, corn meal, farina, macaroni, noodles [27])	140 μg/100 g
Asia		
Indonesia	Wheat flour	200 μg/100 g
Kazakhstan	Wheat flour	N/A
Kyrgyzstan		
Pakistan		
Turkmenistan		
Nepal	Wheat flour	150 μg/100 g [51]
Uzbekistan	Wheat flour	100 μg/100 g
Western Asia		
Yemen	Wheat flour	160 μg/100 g
Bahrain	Wheat flour [16]	150 μg/100 g
Egypt		
Jordan		
Kuwait		
Oman [34]		
Iran		
Qatar		
Saudi Arabia		
Iraq	Wheat flour [52]	N/A
Oceania		
Australia [53]	Wheat flour for bread-making [54]	200–300 μg/100 g
Fiji	Wheat flour [55]	150 μg/100 g [16]
Total = 66		

Source: National food vehicle and fortification level for folic acid by world region; unpublished table adapted from multiple sources

Notes

[a]*Africa*: In West Africa, the Helen Keller Foundation is working with 13 millers to implement the equivalent of mandatory folic acid fortification [56]. This program has the endorsement of the 15-nation Economic Community of West African States (ECOWAS). Therefore, it was judged that the governments of these countries recommend folic acid fortification (Additionally, in Ghana, public sector—NGO partnerships have enabled a 5-year folic acid fortification program [57])

Pacific Island countries: Many Pacific island countries do not have milling facilities and rely to a large extent on flour imported from Fiji. The mandatory folic acid fortified flour prepared by the Fiji Flour Mills is exported to Wallis and Futuna, Vanuatu, Tuvalu, Tonga, the Solomon Islands, French Polynesia, Micronesia, American Samoa and Samoa [16]. However, because there is no explicit national policy of mandatory folic acid fortification, and it was not possible to quantify the precise proportion of folic acid fortified flour available in these Pacific Island countries, they were not included in the list of countries with mandatory folic acid fortification

Palestine: At the time of writing, the occupied Palestinian territories were not recognized as a state by the UN; therefore, it is not included in the table. However, mandatory folic acid fortification is being implemented at 100 μg/100 g wheat flour [16]

Where there were gaps in information, the default position was to assume that the food vehicle was wheat flour if the country was listed on the FFI database

implementation over the following 2 years to allow millers and manufacturers time to adapt their processing mechanisms to the new food standard requirements [20]. Australia proceeded to implement mandatory folic acid fortification as scheduled in September 2009. However, during the phase-in period, further lobbying was undertaken in New Zealand raising concerns about the safety of mandatory folic acid fortification and in August 2009, the New Zealand government decided to defer mandatory folic acid fortification until at least 2012 [21].

Meanwhile, in 2011 in North America there were calls for the scope of mandatory folic acid fortification to be extended. Commentaries in Canadian [22] and US medical and health journals [23] were complemented by efforts to implement higher levels of fortification and to extend fortification to corn masa flour in response to the higher rates of NTDs observed in Hispanic women. On 5 January 2011, Mr. Sessions submitted "that the Commissioner of Food and Drugs should evaluate the scientific evidence on the question of whether to add more folic acid to enriched grain products and expand folic acid fortification into cornmeal and corn-based food products to help prevent further serious birth defects". The resolution was referred to the Committee on Energy and Commerce [24]. Subsequently, the CDC has initiated research into Hispanic women's reactions to and beliefs about folic acid fortification of corn masa flour. It is also investigating childbearing age women's folic acid awareness and knowledge and their reactions to existing folic acid educational materials [25].

Discussion

In 1996, Oman was the first country to successfully implement nationwide mandatory folic acid fortification with 5 mg/kg folic acid to white flour to reduce the risk of NTDs [26]. The USA followed in 1998 (based on 1996 legislation), with all enriched cereal grain product, including flour, rice, breads, rolls and buns, pasta, corn grits, corn meal, farina, macaroni and noodles fortified at 140 μg/100 g cereal grain [27]. To date, a further 64 countries have recommended mandatory folic acid fortification as national policy, and the US policy recommendations in particular may be viewed as providing a particular impetus for the adoption of this policy internationally. Although these countries are spread across the world, it is apparent that there is some degree of clustering. A substantial proportion of Africa and the Americas have recommended mandatory folic acid fortification. However, the vexed nature of this policy recommendation is illustrated by the significant number of countries that are yet to adopt this recommendation, especially in Western Europe, and that three countries (Ireland, New Zealand, UK) that initially made this recommendation subsequently have deferred the implementation of the recommendation. Conversely, the USA is now considering extending the scope of its mandatory folic acid fortification policy recommendation to include additional food vehicles.

For those countries that have recommended mandatory folic acid fortification, it is not possible to be certain of the primary purpose for this policy. In particular, it may not be possible always to distinguish whether mandatory folic acid fortification has been recommended as a public health intervention to prevent the occurrence of conditions such as anaemia associated with evidence of folate deficiency among the population, or as a response to reduce the risk of NTDs through increased exposure to folic acid for certain individuals. In some countries such as Nepal [28] and Kyrgyzstan [29] mandatory folic acid fortification is clearly recommended to address both purposes. However, given the temporal association between the awareness of the folic acid—NTD relationship and the endorsement of the mandatory folic acid fortification recommendation—it is presumed that the majority of those countries who have made this recommendation since 1996 have done so as a primary intervention to reduce the risk of NTD*s*.

For certain countries, it is apparent that it is the local recommendations of national food regulatory agencies that have driven the mandatory folic acid fortification policy [30]. In other countries, it is apparent that it is the effective advocacy of external agencies towards national authorities that have

been influential in driving the policy recommendation and then supporting joint implementation. Formed in 2004, the work of the FFI focuses on wheat flour, with increasing attention to other flours (e.g. maize). Complementing their work is the Global Alliance for Improved Nutrition (GAIN) and their affiliate, Rice Fortification Resource Group (RiFoRG), in recognition that rice is a staple for over half of the world's population [31]. These groups work with governments and industry, providing advocacy, technical and regulatory expertise. Their rationale for folic acid fortification is to address NTDs and also general malnutrition. In addition to work at a national level, local and regional initiatives are supported through financial backing of pilot projects, technical support and building capacity in developing economies [31]. In other countries, the final mandatory folic acid fortification recommendation has emerged from a truncated process of shifting policy positions shaped by advocacy from pro-and anti-mandatory folic acid fortification interest groups. For example, in 1998, the Indonesian Ministry of Health mandated the addition of folic acid to the existing fortification of wheat flour produced in the country. A national standard (SNI) was issued by the Ministry of Health in 2001 for all flour traded, and by 2003 these were streamlined for fortification to the SNI for imported and domestically produced flour. The industry association APTINDO, responsible for 85 % of the market, complied with this legislation. The remaining market is made up of flour imports, and lobbying from importers led to the revocation of the SNI in 2008. The rationale used was that the fortification was a barrier to imports and created higher prices. International, industry and pro-fortification pressure led to the reinstation of the SNI within the same year, with simplified procedures for imports [16].

For those countries where mandatory folic acid fortification had been recommended, it was observed that wheat flour was always at least one of the food vehicles. Flour fortification is prioritized by lobby groups, presumably because this is the vehicle with which they can offer technical support. However, local staples are not always the food vehicle that is fortified, as observed in certain NGO-led interventions. Therefore, it is not always the case that the food vehicle is necessarily the most culturally and socially appropriate for the country [32]. Nevertheless, national adaptations of the food vehicle for fortification have been undertaken and include corn and rice as the food vehicle of choice.

The level of folic acid fortification varies significantly among countries, ranging from 100 μg/100 g in Uzbekistan—slightly higher than restoration levels—to 300 μg/100 g in Paraguay, Belize and Australia. This range was spread equally around the world with some clustering patterns observed among certain countries located in geographically similar parts of the world and where agreements to streamline folic acid levels have been implemented, e.g. countries in Central and South America. Typically folic acid was added to a food vehicle at a level of 150–200 μg/100 g. This is approximately similar to the level originally implemented by the USA in 1998 and suggests that the USA's policy has been a template for the recommended policy in many other countries. The variation in fortification levels to a large extent reflects the interplay of consumption patterns and target intakes of the fortified food vehicle. In countries such as Indonesia [16] relatively high levels of folic acid fortification were mandated because the consumption of wheat flour was considered to be low [33]. In addition, other dietary sources of folic acid such as supplements and voluntarily fortified food products need to be monitored to determine an appropriate level of fortification of a food staple.

Among countries that have implemented mandatory folic acid fortification there has been a reported reduction in NTD prevalence of between 20 and 57 % [33]. It is not possible to accurately determine what proportion of the reduction in NTD prevalence can be attributed to mandatory folic acid fortification, the increased availability of voluntarily folic acid fortified food products or the promotion of folic acid supplements undertaken during the same time period. For example, when voluntary folic acid fortification of food products was first permitted alongside supplementation in Australia, it was observed that there was a reduction in NTD incidence (32 % for anencephaly, 23 % for spina bifida, 34 % for encephaloceles) [34]. Evaluation studies indicate that the relative effectiveness of mandatory folic acid fortification varies with the background prevalence of NTDs and the folate status of the population prior to the implementation of mandatory folic acid fortification. It may be that for

those countries that have relatively high NTD prevalence and low folate status, the evidence for recommending mandatory folic acid fortification is more persuasive than for those countries which have a relatively low background NTD prevalence and are folate replete, as they would be operating on diminishing returns. Of particular concern is whether adequate monitoring and evaluation is in place to detect potential adverse health outcomes arising from mandatory folic acid fortification [35].

In 2006, guidelines for Target Fortification Level, Minimum and Maximum Fortification Level and Legal Minimum Level of folic acid to facilitate flour fortification were disseminated by the WHO and the FAO of the United Nations. These guidelines recommend 1.0–5.0 ppm, depending on wheat flour consumption per capita [36].

Limitations

A thorough effort has been made to systematically search, collect and collate the data presented in this chapter. Nevertheless there are some potential limitations. In setting the parameters for what was included in the table, there are a number of time-limited NGO-initiated interventions (such as in Afghanistan and Bangladesh where flour is fortified through the United Nations World Food Programme) [37] that were not classified as national mandatory folic acid fortification, but nonetheless may have otherwise similar effect in practice for the populations targeted. In particular, voluntary folic acid fortification can have the same effect as mandatory folic acid fortification. However, because responsibility for such implementation rests outside national authorities, assumptions cannot be made about the ongoing implementation of folic acid fortified foods, which foods are being fortified and at what level of fortification. Therefore, countries that do not recommend mandatory folic acid fortification may have available voluntarily folic acid fortified foods, including many European countries, China, India, Malaysia, Sri Lanka and Vietnam. Voluntary fortification policies were not included in the present analysis.

Moreover, although certain countries were not identified as recommending a policy of mandatory folic acid fortification, this policy may be being implemented in regions and states within those countries, but not always at a national level. For example, in India there is no national mandatory folic acid fortification policy, yet mandatory fortification is already being implemented in Gujarat, West Bengal and Tamil Nadu [38].

Establishment of regulations does not always indicate implementation [39]. This may be due to manufacturing challenges, where mechanisms are not in place and substantial investment may be necessary. However, for countries already setup to fortify with iron, for example, the addition of another micronutrient is easier than for countries not yet equipped for micronutrient fortification. This is illustrated by the case of Turkmenistan where mandatory iron fortification was implemented in 1996; this was supplemented with folic acid fortification in 2000 [16]. For those countries that do report that they have adopted mandatory folic acid fortification recommendations, we could not make any assumptions about what stage of implementation a country is at or if it is implementing as outlined in legislation.

Mandatory folic acid fortification is a dynamic food fortification policy intervention. We were only able to collect data that was publically available. In so doing we could not be certain that all mandatory folic acid fortification policies are being reported. Many national food regulatory authorities are maintaining a close watch on the evidence available for this topic. Specific countries are continually assessing whether or not they will recommend mandatory folic acid fortification, and if they have recommended this policy intervention, the level of fortification and the food vehicle is subject to ongoing review. Policies may change. However, to the best of our knowledge, the presented data provide as comprehensive and up-to-date profile of mandatory folic acid fortification policy and practice around the world as reasonable on November 2011.

Conclusions

Mandatory folic acid fortification is a rich case study of food fortification policy and practice. It provides a powerful example of the complex considerations that can be involved in translating scientific evidence into food fortification policy.

The findings of this systematic review highlight that approximately one third of countries have adopted mandatory folic acid fortification. Within these findings there are interesting dynamics. At least one country is considering increasing its level of fortification; three countries that had recommended mandatory folic acid fortification subsequently have put the final decision on hold. Moreover, within certain countries, some local or regional mandatory fortification is being implemented.

These findings illustrate that, however compelling epidemiological evidence for a nutrient and health relationship might appear, assumptions cannot be made about the process of translating this scientific evidence into food fortification policy and practice. The analysis presented in this chapter suggests that at least in part the explanation for this variation among countries might be explained by different interpretations of the precautionary approach to policy-making and different framings of ethical discourses.

In addition to mandatory folic acid fortification policy recommendations, in many countries there are diverse policies permitting certain food products to be voluntarily fortified with folic acid as well as campaigns to promote the consumption of folic acid supplementation by women of childbearing age. This means that a proportion of the world's population now is being exposed to a potentially significant background level of folic acid in the food supply. Ongoing nutrition education and monitoring and evaluation to complement these various policy activities will be a priority public health objective into the future.

Recommendations

It will be valuable to continue and extend this form of systematic review for mandatory folic acid food fortification. There are five particular priorities:

1. Continue the auditing and data verification on a regular basis to address gaps and inconsistencies and to keep the data as up to date and valid as possible.
2. Extend the scope of the database by collecting detailed technical information on the food vehicles, the level of food fortification, and when fortification occurred.
3. Investigate why various countries do or don't adopt mandatory folic acid fortification—this critical policy analysis will help inform food fortification policy-making more broadly.
4. Undertake comprehensive and timely monitoring and evaluation of the intervention's implementation process and resulting health outcomes, including folic acid intake, folic acid status, NTD prevalence and other potential benefits and risks associated with mandatory folic acid fortification.
5. Build a comprehensive database that incorporates supplementation, voluntary fortification and education initiatives.

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Chapter 23
In-Home Fortification of Complementary Feedings: Chinese Perspectives

Chunming Chen, Yuying Wang, Wu He, Suying Chang, and Victor R. Preedy

Key Points

- Young children are particularly vulnerable to the long-lasting effects of micro- and macro-nutrient deficiencies.
- In China there is evidence that particular groups of children under the age of 5 suffer from stunting and iron deficiency anemia. This may be due to limitations in the traditional complementary feeding practices combined with economic and education deficits of affected households.
- We describe the development of *Ying Yang Bao*, which is a low-cost soybean-based product used for the in-home fortification of complementary feedings. This supplement is culturally acceptable and amenable for mass production, distribution, and marketing.
- In the initial studies each sachet contained iron, zinc, calcium and vitamins B_2, and D_3 with a small amount of protein, lipid. In subsequent projects vitamins B_1, B_{12}, and folic acid were added to each sachet.
- Usage of Ying Yang Bao was shown to improve the nutritional profiles of children, reduce stunting and anemia. It also improved scores for the Development Quotient (indicators of gross and fine motor skills, adaptation, language skills, and social behavior), Full-Scale Intelligence Quotient (IQ), as well as verbal IQ, and performance IQ.

Keywords Child nutrition • Complementary feeding • Under-nutrition • Anemia • Prevention • Home fortification • Complementary food supplement • Intervention trial

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V.R. Preedy et al. (eds.), *Handbook of Food Fortification and Health: From Concepts to Public Health Applications Volume 2*, Nutrition and Health, DOI 10.1007/978-1-4614-7110-3_23,

Abbreviations

CDC China	Chinese Center Disease Control and Prevention
CDRF	Chinese Development Research Foundation
CFNSS	Chinese Food and Nutrition Surveillance System
DQ	Development quotient
HAZ	*Z* score for height-for-age
Hb	Hemoglobin
IQ	Intelligence quotient
UNICEF	United Children's Fund
WAZ	*Z* score for weight-for-age
WHO	World Health Organization
Ying Yang Bao	*Ying Yang Bao* (Nutrition Pack)

Introduction

The nutritional status of children under 5 years of age has a definite impact on subsequent health outcomes, particularly child mortality. Prevailing information on global health indicates that among the 556 million children under 5 in the low-income countries, 178 million (i.e., 32 %) are stunted, 121 million (20 %) are underweight, and 55 million (10 %) are wasted [1].

Altogether under-nutrition is responsible for 21 % of the overall child mortality rates and a similar 21 % of the total disease burden [1]. To prevent subsequent impacts on health it is imperative that monitoring and interventions take place within the early years. This is to circumvent any subsequent failures in growth, especially in children who are between 6 and 18 months of age [2].

In terms of China, data has been collected over 20 years as part of the Chinese Food and Nutrition Surveillance System (CFNSS). The CFNSS has access to information from 600 sites provided by the State Statistic Bureau which samples and collates household data. In one study on 40 randomly sampled surveillance sites all over China, 16,000 children under 5 years old were examined [3]. Eight rounds of surveillance data were collated in 1990, 1995, 1998, 2000, 2005, 2008, 2009, and 2010. Data from the CFNSS demonstrated that the prevalence of stunting among children increased until about age 24 months, after which there was no overt increase in the prevalence of stunting. In other words, the prevalence of stunting reached a "plateau" or "leveling" at 24 months no matter the prevalence under 24 months [3]. This trend was observed year-on-year despite the overall secular reduction in stunting (Fig. 23.1). In 2010, the prevalence of stunting in 6–12 months children was reduced to 6.8 %, and in children age 24–60 months, this was between 11% and 14 % (see Fig. 23.1).

Further analysis of the CFNSS data illustrated that the nutritional status of children under 2 years old has improved dramatically during the past 20 years. This reflects the rapid economic growth of China. The change in stunting in 2010, the age-transition from <24 months to >24 months, was moderate, i.e., a change of under 10 %. This markedly compares with the 1990 data which shows more dramatic changes in the prevalence of stunting in the same age-transition <24 months compared to >24 months, i.e., a change of about 20 % (Fig. 23.1).

The data on anemia in children under 2 years of age shows a high prevalence and very slow or no reduction during the period of economic growth (see Fig. 23.2) [4]. The avoidance of infantile and juvenile anemia is critical for intellectual development and maintaining immune function. It is well known that long-term anemia impacts in later adult years [5]. Thus, iron deficiency anemia prevention is an important public health issue for the whole Chinese population. As a consequence, the prevention of anemia during the early formative childhood years has to be treated with great urgency.

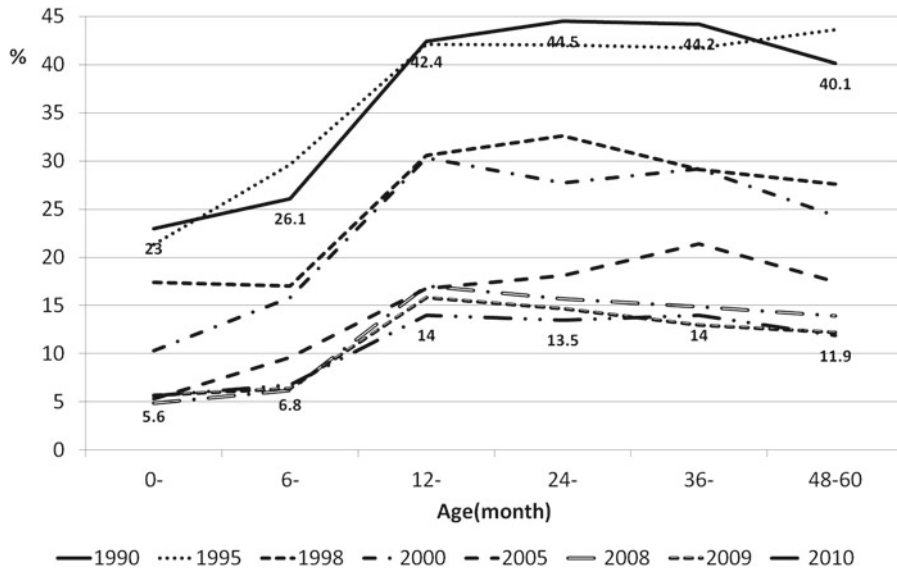

Fig. 23.1 Prevalence of stunting of children under 5 years old during 1990–2010 in the rural China (mostly cited from Chen CM et al. with permission [4])

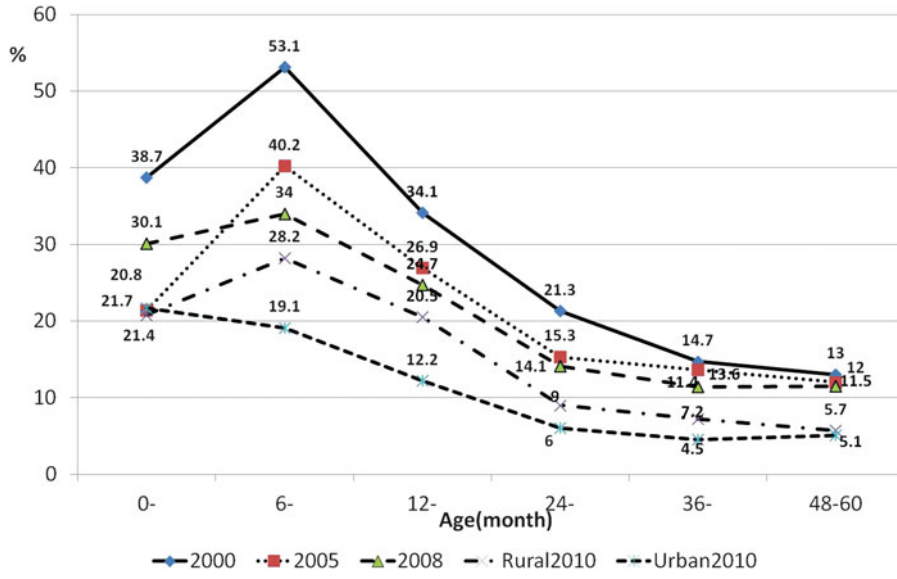

Fig. 23.2 Prevalence of anemia (%) in children under 5 in rural and urban China (by age groups) (unpublished)

The changing pattern of anemia in China can be explained by the deficiencies in complementary feeding. Complementary feeding arises when breast feeding no longer fulfils the need of the child and additional family foods are provided in the diet. Chang et al. [6] describe the status and problems of complementary feeding in China during 1990–2005 and its relationship with the prevalence of stunting in rural China. There was no correlation between the use of starchy foods in complementary feeding with the prevalence of stunting. However, the Xi'an survey in 2001 indicated that the iron and

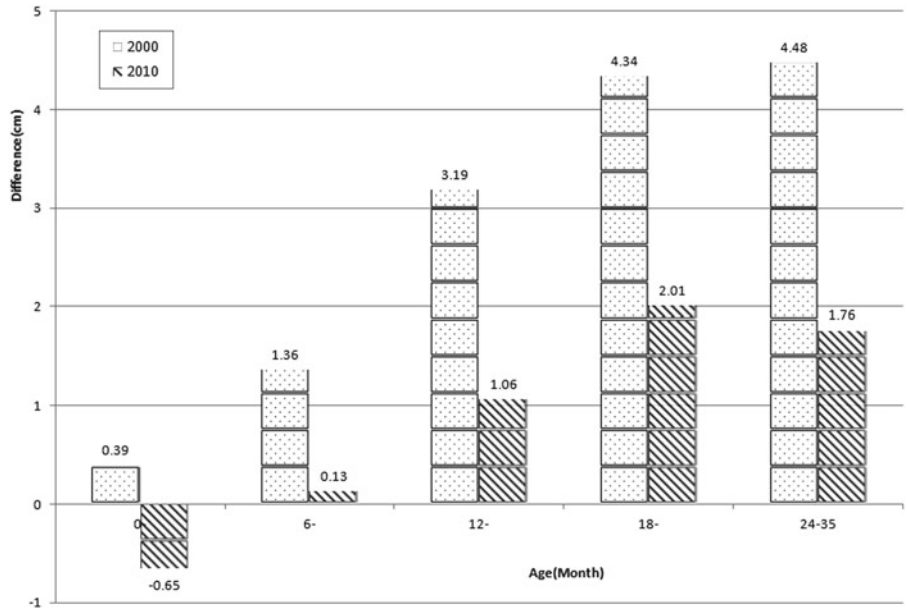

Fig. 23.3 Difference in stature between Chinese rural children and median of WHO Child Growth Standards in 2000, 2010 (unpublished)

zinc intakes of 7–18 months old children were 20–30 % and 27 % of the recommended level, respectively [7]. This suggests that stunting may arise as a consequence of a micro-nutrient deficiency rather than a macro-nutrient deficiency.

The common background of iron deficiency anemia in China pertains to the dietary pattern of the Chinese population, which is dominated with cereal-based foods (rice, wheat, maize). In cereal-based diet, the bioavailability of iron is low: that is below 4 % [8]. Meat contains heme iron which has a much greater bioavailability, at about double the value for non-heme iron in the diet. However, dietary modification alone is unlikely to provide a solution for the high prevalence of anemia, since culturally red meat in Chinese diet is only a moderate amount. Thus, overall, the quality of complementary feeding should focus on the combination of both protein and micronutrients deficiency to address the impaired nutritional status of children.

In the past decade there have been improvements in the linear growth of children. Figure 23.3 indicates the difference of height of children under 5 years old when compared with the WHO standard median [9]. However, nutritional deficits still remain in China and the pattern is somewhat different to other countries such as Malawi and Cambodia [10]. If compared with the median of the length in WHO Growth Standards, the deficit in height/length of Chinese children in the rural at the age of 3 was 77 % in 2000, but only 22 % happened before age of 12 months, and the length deficit reached 56.8 % at age of 12–18 months. The picture is very much different from that of Malawi and Cambodia [10]. Such difference illustrated the major cause of stunting of Chinese children under 3 years of age is the consequence of inadequate complementary feeding after 6 months of age rather than the feeding before age of 6 months.

We have been working on improving the quality of complementary feeding since 2000 and trying to formulate a supplementary food for Chinese infants and young children. The aim was to provide a food which could be acceptable and useful in rural households in terms of nutrition quality, texture, taste, and price [11].

The ensuing text relates to the development and application of home level fortification of complementary feeding in China.

Research on Supplement Food for Complementary Feeding

1. Formulation of the food
   The usual complementary feeding practice in China is making complementary foods at home. Thus, there was a prerequisite that improvements should be easily adapted for home use with minimal or no further preparation. The formula of the supplemental food was based on the following principles:

   (a) The improvement should be based on a food supplement for feeding that was *complementary*, in other words as an *addition* to the normal complementary food.
   (b) The daily supplement should be sufficient to meet the daily nutritional requirement of infants and young children and be compatible with the traditionally complementary feeding practice of Chinese mothers.
   (c) The daily supplement should increase protein and micro-nutrient intake by usage of a small sachet which is easy to use and low priced.
   (d) Soybean powder is a food base which not only increases protein intake but can be used as a carrier or vehicle for micronutrients. Iron, zinc, calcium, and vitamin B complex (riboflavin and thiamin) were used in the initial studies. The soybean powder has the flexibility of being amenable for usage with other micronutrients should the need arise.
   (e) Whole soybean is traditionally used by the Chinese and has an excellent amino acid profile. The long-chain polyunsaturated fatty acids comprise about 58 % of the total lipids and are beneficial to child health and development.

   The initial formula for the nutrient-dense complementary food was developed in 2001 as a "once-a-day" sachet. It was called *Ying Yang Bao* in Chinese (translated as "Nutrition Pack" in English). Each sachet comprised:

   - 10 g Whole soybean powder
   - 6 mg Iron (as sodium iron EDTA)
   - 4 mg Zinc (as zinc sulfate)
   - 385 mg Calcium (as calcium carbonate)
   - 0.2 mg Vitamin B_2
   - 7 μg Vitamin D_3
   - 44 kcal Energy
   - 3.8 g Protein

2. The field trial in Gansu Province during 2001–2003
   The field trial recruited 1,478 children aged between 4 and 12 months old. The population was derived from five counties in Gansu Province, which is located in the northwest part of China. All the children were given the traditional routine complementary food, as was the usual practice within the home.

   A total of 978 of children were provided with the *Ying Yang Bao* supplement (Formula 1 group). A total of 500 children were given rice formula with 10 g of rice powder with vegetable oil to meet the same energy intake as in Formula 1 (Formula 2 group). In addition, a massive dose of vitamin A was given to children in both groups (every 6 months, 1 dose of 100,000 IU vitamin A for children aged under 12 months and 200,000 IU for children over 12 months). During the baseline period and at 6, 12, 18, and 24 months follow-up, a food questionnaire was used to identify compliance rate, and measurements were made for weight, length, and blood hemoglobin. The trial ended when the child was 24 months old, at which point additional developmental tests were made. These developmental tests included, for example, the Development Diagnostic Scale of Children aged 0–6 years, motor and language skill, social behavior, and other indices.

Table 23.1 Prevalence of anemia in children in different age groups (%) (Cited from Wang YY et al. with permission [12])

	Formula 1[a]	Formula 2[b]	*p*
Baseline	34.8	34.9	
6-Months follow-up	19.1	28.0	0.008
12-Months follow-up	8.2	12.4	0.074

[a]Children in Formula 1 group were given *Ying Yang Bao* supplement
[b]Children in Formula 2 group were given rice formula with 10 g of rice powder with vegetable oil to meet the same energy intake as in Formula 1

Table 23.2 Differences in hemoglobin concentration during intervention follow-ups (g/L) (Cited from Wang YY et al. with permission [12])

	Formula 1	Formula 2	Effect size
6-Months intervention	+5.2	+2.6[a]	0.27
12-Months intervention	+9.7	+7.5[b]	0.21

[a]Formula 1 vs. Formula 2, $P=0.0003$
[b]Formula 1 vs. Formula 2, $P=0.005$

After 24 months of age, a follow-up study was carried out between 2004 and 2006. Children in both the Formula 1 and Formula 2 groups were assessed for weight, height, hemoglobin, and developmental parameters. In September 2004, about 40 age-matched children from the nearby villages with similar socioeconomic status in each county were recruited as new community controls. These children did not receive either Formula 1 or Formula 2 or vitamin A supplementation and were followed up in the subsequent periods (age 4–6 years). Identical developmental tests were carried out on this cohort for comparison with the Formula 1 and Formula 2 groups.

3. The effectiveness of Ying Yang Bao

   (a) Reduction of anemia prevalence [12].
   The prevalence of anemia at baseline in the Formula 1 and Formula 2 groups was 34.8 % and 34.9 %, respectively, showing no significant difference. After 6- and 12-months intervention, the prevalence of anemia dropped significantly to 19.1 % and 8.2 %, respectively, in the Formula 1 group, and to 28.0 % and 12.4 %, respectively in the Formula 2 group ($P<0.05$) (Table 23.1). The *effect size* of the change in hemoglobin in the Formula 1 group over the Formula 2 was at a middle level, 0.27 and 0.21, respectively after 6- and 12-months intervention (Table 23.2). The hemoglobin concentration distribution curves showed significant rightwards changes (see Fig. 23.4).

   (b) Significant higher increase in length during the intervention period [13].
   The lengths at the age of 24 months in the Formula 1 group was 1.3 cm, significantly higher than the Formula 2 group. The *Z* score for the height-for-age (HAZ) of those in the Formula 1 group was –0.29 which is higher than –0.45 seen in the Formula 2 group.

   (c) Improved intelligence development and lasting IQ performance until 6 years of age [14].
   Intelligence development was assessed at the end of the intervention (i.e., at the age of 24 months) among 30 % of randomly selected children in both groups. Furthermore, subsequent follow-up assessments were made after completion of the intervention trial, at the ages of 3.5–4, 4.5–5, and 5.5–6 years. The Development Diagnostic Scale of Children aged 0–6 years was used for assessing the Development Quotient (DQ) of children under 5. The DQ includes indicators of gross and fine motor skills, adaptation, language skills, and social behavior [15, 16]. The revised Wechsler Preschool and Primary Scale of Intelligence (WPPSI) was used for children aged 5.5–6 years and the full-scale intelligence quotient (FIQ), verbal IQ, and performance IQ were calculated [17].

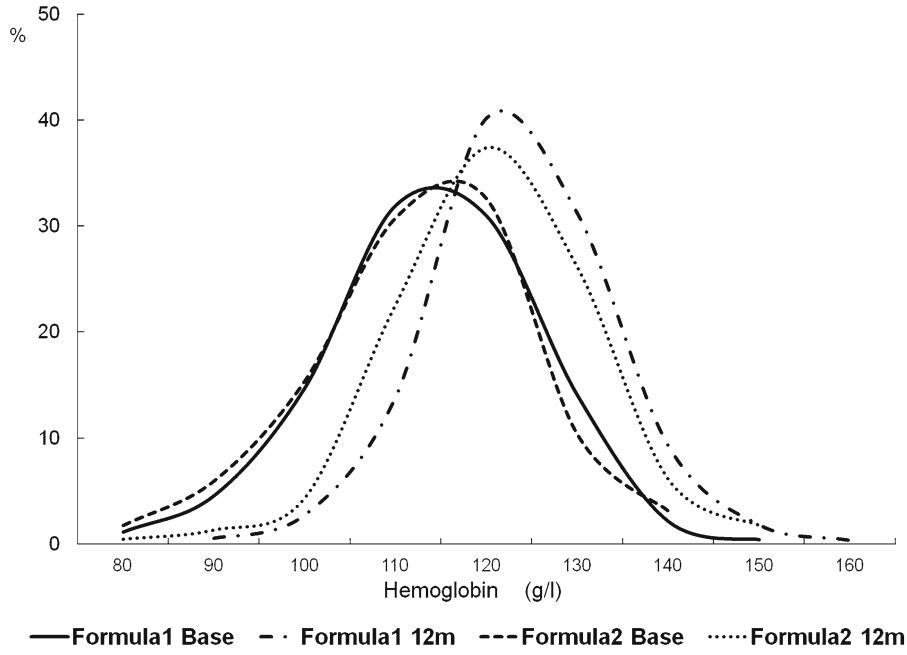

Fig. 23.4 Hemoglobin concentration distribution at baseline and 12-month follow-up survey. (Cited from Wang et al. with permission [12])

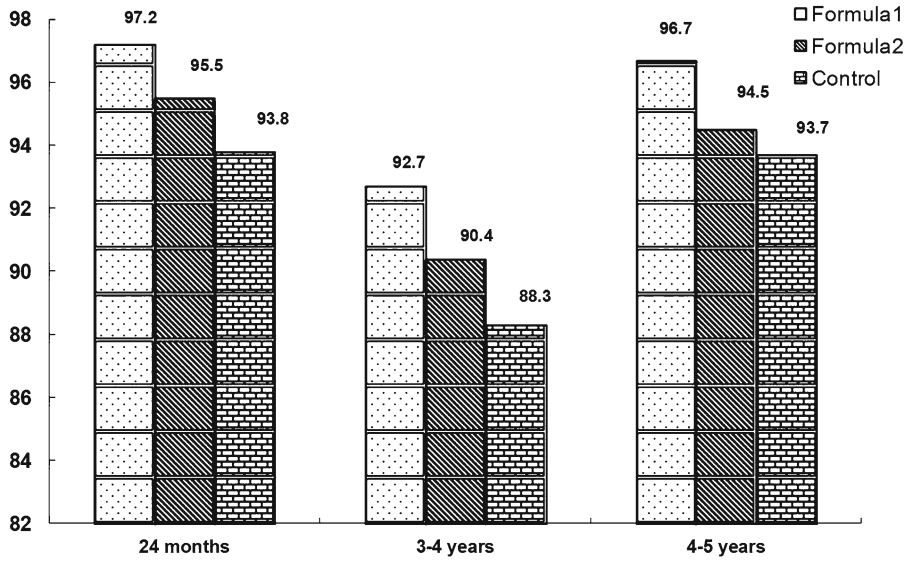

Fig. 23.5 The development quotient and component scores for children (Adapted from Chen CM et al. with permission [14])

The DQ was 97.2, 95.5, and 93.8 in the Formula 1 group, Formula 2 group, and control group for children aged at 24 months, respectively. It was 92.7, 90.4, and 88.3 at 3.5–4 years, and 96.7, 94.5, and 93.7 at ages of 4.5–5 years, respectively (see Fig. 23.5). At the age of 5.5–6 years, the Full-IQ of children in the Formula 1 group was 3.1 and 4.5 points higher than those

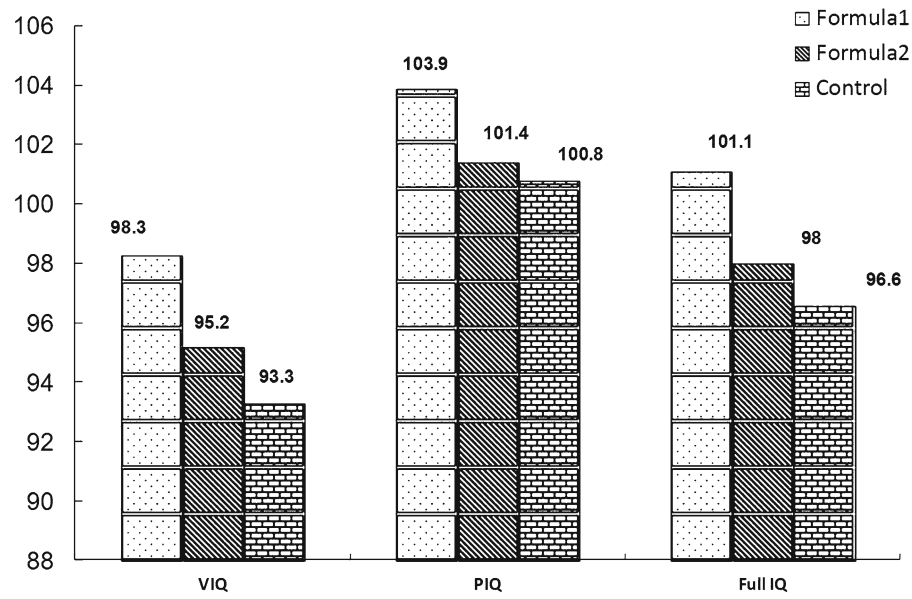

Fig. 23.6 The intelligence quotient and component scores for children (Adapted from Chen CM et al., cited with permission [14])

in the Formula 2 group or the control group. The Verbal IQ was 2.1 and 5 points higher and the Performance IQ was 2.5 and 3.1 points higher, respectively, in the Formula 1 group vs. the Formula 2 group or the control group (see Fig. 23.6). All the differences were statistically significant ($P < 0.05$).

The findings proved that the nutrient-dense fortified supplement for complementary feeding improved the intellectual development of children at the age of 24 months and 6 years onward. It demonstrates the extreme importance of nutritional status in early life and the potential for improving social and economic development in whole communities.

Establishment of a National Standard of Supplement Foods for Complementary Feeding

Ying Yang Bao is a novel supplement just for complementary feeding. It has a micro-nutrient density much higher than the level used in standard formulations used in China or the FAO/WHO Codex Alimentarius. Just one sachet a day achieves more than 65 % of the RNI for infants and young children. However, for wide usage and applicability, *Ying Yang Bao* must be manufactured commercially, distributed, and be readily available. Certainly, to maintain the quality, safety, and nutritional value of such a food product, it has to be guaranteed and approved by the statuary regulatory bodies.

The first step in establishing a food standard for *Ying Yang Bao* was a field intervention trial in two earthquake-affected counties, namely Lixian and Beichuan. This was initiated in September 2008, 4 months after the disaster struck. Based on the above research data, 12 g sachets of *Ying Yang Bao* of soy flour with eight nutrients were manufactured in the Shandong province. Each sachet contained the following:

Vitamin A 250 μg
Vitamin D_3 5 μg

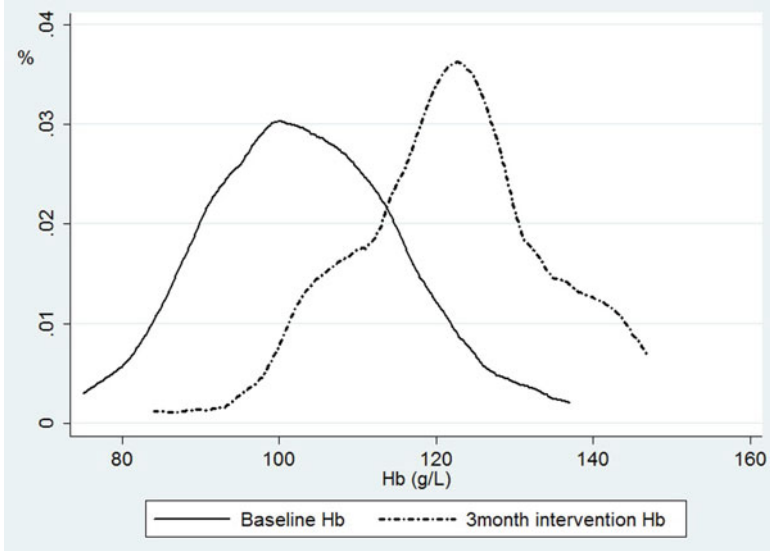

Fig. 23.7 Hemoglobin concentration distribution curve of children consumed >4 times/week (baseline and 3-month intervention) in Lixian (unpublished)

Vitamin B_1 0.3 mg
Vitamin B_2 0.3 mg
Folic acid 50 μg
Vitamin B_{12} 0.3 μg
Iron 5 mg
Zinc 5 mg
Calcium 250 mg

All the children aged 6–24 months were provided with one sachet of *Ying Yang Bao* a day for free and they were distributed through the Maternal and Child Health Care channel in the counties. The hemoglobin concentration of children who consumed *Ying Yang Bao* more than 4 times per week was significantly increased after 3 months of project implementation (see Fig. 23.7, Table 23.3) ([18] unpublished data).

As a consequence of the field trials, a new food category called "Complementary Food Supplements" was added to the National Food Standard list. In this list, the food-based nutrient supplements were defined as "a food base fortified with multiple high-density vitamin and minerals, which could be powder, particle, or semi-solid and provide some high quality protein and a small amount of energy" [19]. The Standard was issued in December 2008 and was implemented on March 1, 2009.

The National Food Standard defines the complementary food supplements as being applicable to infants and young children aged 6–36 months. Based on the RNI recommended by Chinese Nutrition Society, the National Food Standard provides detailed guidelines for adding nutrients to complementary food supplements (Table 23.4).

The National Food Standard defines that this product *Ying Yang Bao* requires at least vitamins A, D, B_1, B_2, iron, and zinc as mandatory fortified nutrients. The other nutrients listed in Table 23.4 are optional. For food-based nutrient supplements, protein (>2.5 g/sachet) and calcium (120–360 mg/sachet) are also required and protein could come from dairy, beans, or others sources.

The promulgation of the standard for complementary food supplements greatly promoted the production of *Ying Yang Bao* in China. With the standard-compliant *Ying Yang Bao* interventions for infants and young children in disaster-relief areas, social equity initiatives and other schemes became possible.

Table 23.3 The effect of 3-months intervention at the earthquake-affected counties (unpublished)

	Total anemia %			Medium degree anemia %		
Age at the beginning	Baseline	3-Months intervention	Nearby "no intervention" county	Baseline	3-Months intervention	Nearby "no intervention" county
County A						
6–12	85.6	44.4	53.8	25.6	1.4	11.8
12–18	79.4	25.0	25.9	16.2	7.5	3.7
18–24	70.1	10.0	37.5	16.4	3.3	4.2
County B						
6–12	59.2	30.3	48.4	7.2	2.2	12.9
12–18	48.7	34.4	23.8	8.3	0	0
18–24	37.8	18.5	30.8	5.1	3.7	3.8

Table 23.4 Nutrients in the daily ration which can be used in complementary food supplements (cited with permission)

	Daily ration	
Nutrients	6–12 months	13–36 months
Protein (g)	>2.5	2.5
Calcium (mg)	120–240	180–360
Magnesium (mg)	21–42	30–60
Iron (mg)	3–9	3.6–10.8
Zinc (mg)	2.4–7.2	2.7–8.1
Selenium (μg)	6–12	6–12
Copper (mg)	0.18–0.36	0.24–0.48
Vitamin A (μg)	120–360	150–450
Vitamin D (μg)	3–9	3–9
Vitamin E (mg)	0.9–2.7	1.2–3.6
Vitamin K (μg)	3–9	4.5–13.5
Vitamin B1 (mg)	>0.12	0.24
Vitamin B2 (mg)	>0.2	0.24
Niacin (mg)	1.2–10	2.4–10
Vitamin B6 (mg)	>0.12	0.2
Folic acid (μg)	32–150	60–300
Vitamin B12 (μg)	>0.2	0.36
Pantothenic acid (mg)	>0.72	0.8
Choline (mg)	>60	80
Biotin (μg)	>2.4	3.2
Vitamin C (mg)	>20	24

Complementary food supplements require at least vitamins A, D, B_1, B_2, iron, and zinc as mandatorily fortified nutrients. The other nutrients listed in this are optional. The Daily Ration is the reference requirements for populations within China. It is equivalent to the RNI (Reference Nutrient Intake in the UK)

From Science to Action

It is important to recognize the importance of adequate food and nutrition security for children aged 6–24 months. However, the process of translating nutritional knowledge into improved complementary feeding practices takes time. It is even more challenging in the rural areas. There is a need to coordinate (1) the child health care services, (2) nutrition education of the mother, (3) distribution, and (4) marketing.

There is a fundamental need to scale up the availability and usage of *Ying Yang Bao*. However, the networks for distributing *Ying Yang Bao* in the rural areas are still rudimentary. There is a need to make *Ying Yang Bao* more readily available in village shops. Stimulating the demand for *Ying Yang Bao* is one way of enhancing its supply; i.e., the relationship between supply and demand should be driven by local communities. This involves partnerships between governmental leaders, food entrepreneurs, and local officials and representatives.

Since 2009 several projects on the use of *Ying Yang Bao* have been initiated. Large scale examples are: (1) a project organized by the Society Experiment on Social Equity, which was established in 2009. This is being conducted in Ledu, a poor county in Qinghai Province, which covers a population of 100,000. It was initiated by the Chinese Development Research Foundation (CDRF) with collaboration with the Chinese Center for Disease Control and Prevention (CDC China). The project was designed with the leadership of the County government and *Ying Yang Bao* was distributed through the local maternal and health care systems. (2) A UNICEF China supported project on Nutrition Improvement of Infants and Young Children in earthquake-affect provinces. This project covered eight counties in three provinces. It is led by the National Institute of Nutrition and Food Safety of CDC China. The project covers 1,648 villages and 30,000 children aged 6–24 months. The model aims to investigate the efficiency of the overall production and distribution framework of *Ying Yang Bao* with the support of local government leaders with distribution through established CDC channels. (3) A new model of social business development has also been initiated as a NutriGo Project by a commercial food company, with intention of implementing corporate social responsibility to explore the ways of making the *Ying Yang Bao* available, accessible, affordable, and acceptable to the village shops and households. This project aims to run a factory solely with this public health purpose.

The improvements resulting in the ongoing community-based projects are striking. In the Ledu trial, after 20 months of project implementation (from September 2009 to May 2011), the coverage of a minimal consumption rate of three sachets per week was achieved by 70 % of the children 6–24 months of age. The prevalence of stunting in those aged 18 months and upwards was as high as 20.2–26.4 % in the control counties. In the intervention group it was 7.7–9.7 %. The average height of 2–3 years old in the intervention group was 1.8 cm higher than the controls which did not receive supplementation with *Ying Yang Bao*. The prevalence of anemia among children aged 6–24 months was reduced from 66% to 38 %. Importantly, the reduction of the 2-week incidence of diarrhea and fever (referred to as respiratory infection) was reduced by 50–80 %. Concomitantly, the medical expenses for the 2-week incidence of the two diseases were reduced by 70–80 % [20].

The UNICEF/CDC China project in eight counties demonstrated a 50 % reduction of anemia prevalence in aged-matched children. The prevalence of moderate anemia was reduced by 73 % after 6 months of intervention [21].

To facilitate the process of developing a sustainable action on home level fortification of complementary feeding, the following issues need to be addressed:

1. Demand
   Intensive public nutrition education as well as peer education on the importance of complementary feeding and the benefits of applying home level fortification should be an essential component in child health services, especially in the rural areas. The information must be simple, focused, culturally specific, and be routine, i.e., in a reinforcing manner. The educational material imparted to mothers in the rural setting must match their educational attainments. In other words, there is no point in imparting degree-level information to mothers with basic literacy skills. In the current trials, with an intensive education within a 15-month time frame, the usage of *Ying Yang Bao* at a rate of more than three sachets a week was only 70–80 %. Only 50–60 % of the caregivers were willing to feed their children with *Ying Yang Bao*. Thus, a substantial proportion of the population does not have their requirements met; this cohort needs to be targeted in a more structured way to achieve efficient usage of *Ying Yang Bao*.

2. Supply
   The development of *Ying Yang Bao* requires attention to production costs, retail prices, acceptance, and appearance. Improvements in manufacturing technology are key determinants to achieve these features. Marketing and distribution mechanisms must be in place so that village shops and local retailers are able to meet the demands of targeted households. The food industry and the national/local government need to coordinate their activities to help achieve these requirements.
3. Leadership and public–private partnerships
   The balance between demand and supply is subjected to a variety of factors such as production, distribution, quality control, wholesaling, retailing, public health principles, education channels, media involvement, scientific and social evaluation, etc. As a consequence of this complexity, the success in promoting the nutritional well-being of infants and young children is focused around leadership. Under the leadership of the local or national government, the production and distribution mechanism can be implemented in partnership with the commercial sector. The scientific community can play a more positive role only within such a partnership-leadership environment. Leadership is the heart of the action and for scaling up the programs for home level fortification with *Ying Yang Bao*.

The Future

The human-centered policy in China has placed people's livelihoods as the highest priorities in the 12th Five-Year Plan. Considerable investments are now being made to improve the health and nutrition of infants and young children as part of the developmental agenda of the country. The *Ying Yang Bao* projects have proven to be biologically effective and very successful. There is now a need to scale up the usage of *Ying Yang Bao* to alleviate nutritional deficiencies and address economic and educational poverty.

Conclusion

This chapter describes the process of the development of *Ying Yang Bao*, which is a low-cost soybean-based nutrient-dense supplement, as an in-home fortification for complementary feeding. Research data collected during 2001–2003 in Gansu province, showed that *Ying Yang Bao* could improve the nutritional profiles of children, reduce stunting and anemia. It also improved scores for the Development Quotient, FIQ, as well as verbal IQ, and performance IQ until age of 6 years old. Additional observation in the application of *Ying Yang Bao* in the earthquake-affected area, a new food category called "Complementary Food Supplements," was added to the National Food Standard list and the standard GB/T 22570–2008 was issued in December 2008 and was implemented on March 1, 2009. From science to action, large scale examples have proven *Ying Yang Bao* projects to be biologically effective and very successful. And it is also implied that there is a need to coordinate (1) the child health care services, (2) nutrition education of the mother, (3) distribution, and (4) marketing. To facilitate the process of developing a sustainable action on home level fortification of complementary feeding, integrating demand, supply, leadership, and public–private partnership into nutrition policy should be focused in the future.

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Chapter 24
Food Fortification: A Regulator's Perspective

Dorothy Mackerras, Dennis Thomas, Jason March, and Jenny Hazelton

Key Points

- Food regulations vary among countries. Manufacturers and importers need to check the relevant national legislation.
- Countries have processes for changing food regulations, including regulations for mandatory and voluntary micronutrient fortification.
- Different countries place different emphases on factors such as economic analysis, deregulation and consumer choice. This may lead to different solutions being adopted in countries with apparently similar health problems.
- The decision about the food vehicle to fortify and the concentration of fortificant to use should be based on modelling using detailed representative food consumption surveys from the population. The technological feasibility of possible food-vehicle-fortificant concentration combinations needs to be considered.
- The impact of fortification needs to be estimated in both target and non-target sub-populations to avoid excess intake in some groups.
- Consideration of the technical ability of industry to comply and government capability to enforce is needed when designing a mandatory regulation.

Keywords Regulation • Risk analysis • Dietary modelling • Economic analysis • Food technology

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V.R. Preedy et al. (eds.), *Handbook of Food Fortification and Health: From Concepts to Public Health Applications Volume 2*, Nutrition and Health, DOI 10.1007/978-1-4614-7110-3_24,

Abbreviations

AR	Average requirement
CBA	Cost–benefit analysis
FeNaEDTA	Ferric sodium edetate
FSANZ	Food Standards Australia New Zealand
RIS	Regulation Impact Statement
MTHF	L-5-methyltetrahydrofolate
NTD	Neural tube defect
UL	Upper level of intake
WHO	World Health Organization

Introduction

Food fortification generally refers to the deliberate addition of essential micronutrient(s) during food processing to correct an inadequacy in the diet and/or improve the health in part or all of the population [1]. Fortification of food is regulated in most countries. The Codex Alimentarius Commission, an intergovernmental body of the World Health Organization and the Food and Agriculture Organization, has established a set of principles for fortification for use by governments [1, 2].

In addition to estimating the impact on the health problem that is being addressed by the legislation, the development of laws, including food regulations, often has specific processes and rules which must be followed by the regulatory agency. These requirements potentially apply to primary and subordinate legislation and other instruments of a legislative or regulatory nature where there is an expectation of compliance. Overarching government policies (such as ensuring the greatest net benefit or the minimum amount of regulation to achieve the desired outcome) and the interplay between food regulations and other laws, such as consumer protection laws, need to be considered, as well as the ability to enforce the regulation. Members of the World Trade Organisation also need to consider the requirements of the Agreement on Technical Barriers to Trade [1, 3].

In this chapter we outline many factors considered by a food regulation agency before adopting new regulation or changing an existing regulation. We focus on mandatory fortification, but comment on the differences that can arise when a regulator is considering permitting an addition which is not mandatory (called voluntary or discretionary fortification). Differences between countries in which factors predominate can lead to countries with apparently similar health situations deciding to adopt different regulations in response to the situation.

Fortification of Food with Essential Micronutrients During Food Processing

We assume that the policy analysis process has examined the extent of the problem, identified a range of possible options and that mandatory fortification of food has been selected as a strategy that should go forward, either alone or in combination with other strategies.

The Risk Analysis Framework

In the food regulation arena, a clear distinction is made between two activities referred to as Risk Assessment and Risk Management (Fig. 24.1). There is an iterative cycle between these two activities [4].

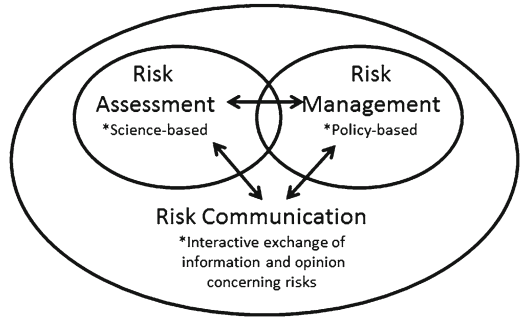

Fig. 24.1 The risk analysis framework, redrawn from [4]. (Reproduced with permission, WHO)

Risk, in this context, is a generic term that includes both favourable and adverse effects. Risk Assessment focuses on characterising the risks associated with nutrient inadequacy and excess in the population, but can include some aspects of food technology and social sciences. Sciences, such as food technology, the social sciences and health economics, as well as policy considerations and societal values, are used during Risk Management to identify one or more possible options for intervention. Risk Assessors forecast the change in risk of inadequate or excessive nutrient intake under the various identified options. Risk Managers combine this information with other factors that need to be considered to identify the option that should proceed. As well as linking Risk Assessors and Managers, Risk Communication includes stakeholders outside the regulatory agency—other government areas, health advocates, consumer advocates, the general public, industry, trade partners and legal advisors—to ensure that all relevant factors are considered during decision-making.

Depending on local structural arrangements, the Risk Assessors and Risk Managers might be different agencies (as is the case in the European Union) or in different branches of the same agency (as in Australia and New Zealand). In small agencies, both functions might be fulfilled by the same person. The essential feature is to separate the description of the risk from the decision about what action to take.

Risk Assessment

Figure 24.2 shows the four steps of Risk Assessment using both the generic risk terminology [5, 6] and nutrition-oriented terminology [5]. All four steps are followed to describe the current situation in the population. After identifying the nutrient/hazard (Step 1), the dose–response relationships for both benefits and adverse effects (if available) are described (Step 2). Population intakes, preferably using data from a representative survey, are calculated (Step 3) and then compared to health reference standards to determine the proportion with inadequate, adequate and excessive intakes (Step 4). The last two steps are repeated as many times as is necessary to forecast the change in situation under different scenarios, for example to explore the change in the intake of a nutrient under a range of possible concentrations in a single food vehicle or if different combinations of food vehicle were fortified [7].

Identifying the nutrient/hazard and the dose–response relationships for benefits and adverse effects may be more complex than simply naming the nutrient.

Vitamins can exist as different vitamers. When considering fortification to reduce neural tube defects (NTD), Food Standards Australia New Zealand (FSANZ) determined that the available evidence supported an effect of folic acid on NTDs, but that there was little evidence available examining whether naturally occurring folate affected NTDs (Steps 1 and 2) [8, 9]. Therefore, only folic acid (not total folate) was included in the estimates of pre-fortification intake and when projecting the effect of fortifying possible food vehicles at different concentrations (Step 3) [8]. This literature also influenced

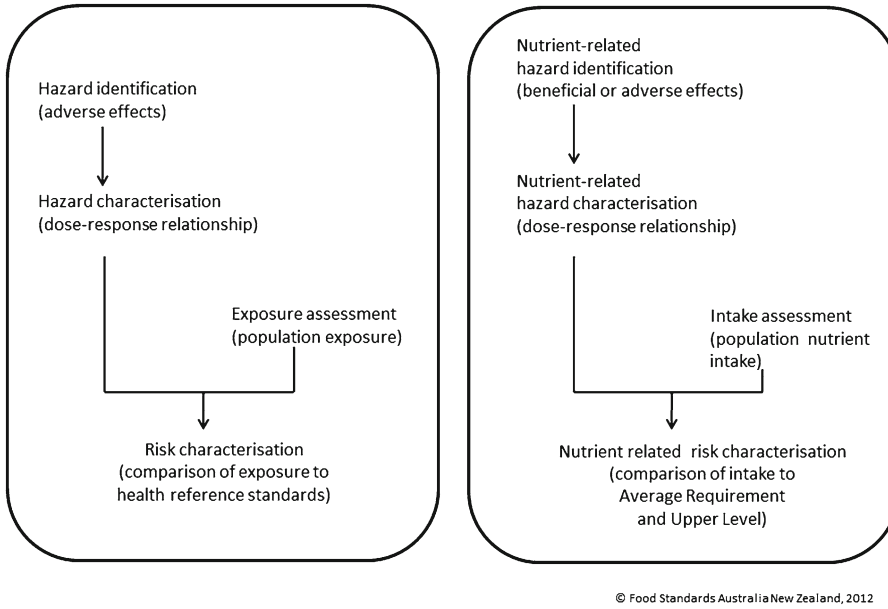

Fig. 24.2 Steps in risk assessment using standard terminology (*left-hand box*) [5, 6] and terminology adapted for nutritional risk assessment (*right-hand box*) [5]. (Reproduced with permission, FSANZ)

a subsequent request to permit L-5-methy-tetrahydrofolate (MTHF) to be added to food as a form of folate. As there was no human or animal data showing that MTHF would reduce NTDs (Step 2), MTHF was not permitted for mandatory fortification which has the purpose of reducing NTDs. However, it was permitted for voluntary fortification for the purpose of increasing folate intake [10].

Minerals are available in a range of inorganic salts and organic compounds. Hazard identification (Step 1) also involves identifying these other components. FSANZ considered a request to add ferric sodium edetate (FeNaEDTA) to a pre-existing list of permitted forms of iron that could be used as voluntary fortificants [11]. Permitting this form would not increase the amount of iron in the diet, because it would be used instead of another form of iron. However, using this form would increase the EDTA exposure in the population. FSANZ determined the population exposure to EDTA based on the use of previously permitted compounds containing EDTA (Step 3). Projections of the possible future use of FeNaEDTA across the food supply indicated that this might lead to exceedances of the Acceptable Daily Intake for EDTA (Step 4). Therefore, permission to use this form of iron was restricted to a limited range of foods to ensure that the population EDTA exposure would remain below the Acceptable Daily Intake for EDTA [11].

Determining population exposures (Step 3) requires information about the nutrient and chemical composition of foods and also the food intake in the population. The latter ideally comes from a probability sample of the population. Because health reference standards for nutrients and most food chemicals assume that long-term diet has been assessed, it is important to remove within-person day-to-day variability from the dietary information obtained via methods such as 24-h recalls. There are several possible methods for doing this [12, 13]. If the data cannot be corrected, then the user needs to be aware that the population distribution will over-disperse (the standard deviation will be too wide) and allow for this when interpreting the data to make decisions regarding fortification.

Finally, the calculated intake must be compared to health reference standards (risk characterisation, Step 4). Essential micronutrients, as a group, are unusual compared to other food chemicals in having both a lower bound (for dietary adequacy) and an upper bound (for dietary excess). The absence of an upper level of intake (UL) indicates that no apparent risk has been associated with very high intakes.

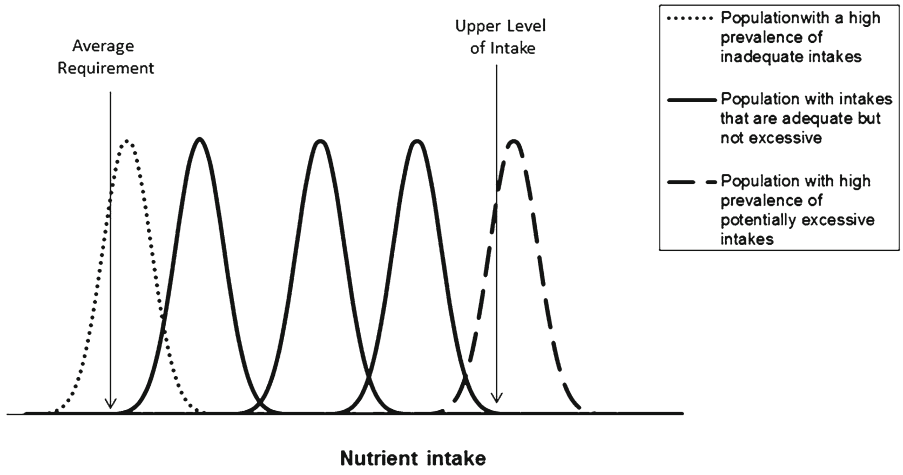

Fig. 24.3 Diagram showing the target zone for the population nutrient intake

It is important to match the identified nutrient/hazard to an appropriate health reference standard. In Australia and New Zealand, there is a UL for folic acid, but no UL for total folate intake [9]. Therefore, intakes of folic acid were compared to the UL during the work leading up to mandatory fortification [8]. Not all nutrients or forms of nutrients (such as MTHF) have an UL. Consequently, it was not relevant to consider possible excessive intakes when FSANZ examined the impact of permitting MTHF additions to food [11].

The aim of fortification is to shift the population intake so that it lies in the range between the cutpoint for adequacy and the cutpoint (if there is one) for excess intake (Fig. 24.3).

In past years, the proportion of the population with an intake below the Recommended Nutrient Intake (a value set to meet the nutritional requirements of approximately 97–98% of the healthy population) was often calculated to estimate the prevalence of inadequate intakes. This approach gives inaccurate results because most people require less than this level of intake. A better method is the Probability Approach which uses the full requirement distribution for a nutrient [14]. A short-cut method, calculating the proportion below the average requirement (AR), yields a good approximation to the result of the Probability Approach calculation provided certain assumptions are met [1, 12]. Except for energy and iron (for menstruating women), the proportions of the population with intakes below the AR and above the UL are used to describe the prevalence of inadequate and excess intakes, respectively [1, 14, 15]. In low and middle income countries, the focus of fortification is on preventing deficiencies. In high income countries, the range in intake can be so wide that the possibility of excessive intake must be examined even when mandating fortification to correct a population deficiency.

Guidance on Levels to be Added

Figure 24.3 shows that it is simple to calculate the average increase in nutrient intake that would move the distribution to the right so that a low proportion had intakes below the AR. This assumes that all members of the population eat the same amount of food, which is unlikely to be true. A more accurate method applies the proposed level of fortificant to the vehicles and recalculates the distribution so that the proportion below the AR and above the UL can be estimated. This may require several iterations for a particular vehicle and there may be several competing options that could to be examined. An allowance for losses of the fortificant during processing, storage and cooking should be included.

The situation is more complicated than suggested by Fig. 24.3 because different population subgroups have different ARs and ULs. For example the UL for folic acid in Australia and New Zealand for children aged 2–3 years is 300 μg/day, whereas the recommended intake of folic acid to reduce NTDs in adult women is 400 μg/day [9]. Children eat more food per kilogramme of body weight than do adults and, in Australia and New Zealand, eat a similar range of foods. We found that the food supply could not be sufficiently fortified to achieve the goal for adult women (for folic acid) or pregnant women (for iodine) unless a large proportion of children aged 2–3 years had intakes exceeding the UL for their age group. In both cases, FSANZ selected a fortification level that partially achieved the goal for adults but limited the proportion of young children that would exceed the UL [8, 16]. If the micronutrient under consideration has no UL, then it would be possible to define the amount that needs to be added by examining the additional amount needed in the most deficient population subgroup [1]. The same analyses are needed before allowing a new voluntary fortification permission to ensure that uptake of the permission by all foods in a category would not lead to excessive intake in the population.

These calculations require the ability to manipulate national survey data and compare it to reference health standards. Food regulation agencies in high income countries tend to have their own custom-designed software. WHO is funding software to do these calculations [17]. It will be able to analyse surveys with complex sampling designs (such as weighting), make adjustments to remove within-person variability (including doing necessary transformations if data are not normally distributed) and make comparisons to nutrient reference values [17]. If per capita availability of foodstuffs is the only available data, then it is not possible to do a full risk characterisation (Step 4) of the effects of fortification by comparing projected intakes in population subgroups to the relevant ARs and ULs. Tables of nutrient levels to add to various commodities depending on apparent per capita consumption exist and can be used when these data are all that are available [18].

Health reference values for micronutrients often include an allowance for variable bioavailability either by expressing different forms as an “equivalent” (in the case of vitamins) or by increasing the intake that is recommended (in the case of minerals). Depending on the form of fortificant to be used, the presence of inhibitors in the diet and the nutritional status of the population, a different assumption about bioavailability might be needed from that assumed when the reference values were set.

Fortification might also be used to replace micronutrients which are lost during processing (e.g. vitamin C in fruit juice) or to make selected alternate foods similar to a reference food (e.g. calcium in soy drink to match cow milk). The level in the reference food determines the amount. These purposes are referred to as restoration and nutritional equivalence respectively [5].

Risk Management

The Risk Management aspects for regulations controlling voluntary fortification are different from those for mandatory fortification. The decision to proceed with voluntary fortification is at the discretion of manufacturers and is often based on the ability of the manufacturer to create a niche market or a premium product that carries a higher price. Consequently, the dose, the food vehicle and possibly even labelling and quality assurance for voluntary fortification might be left to the manufacturer to be determined in some countries.

Selection of Vehicle/s for Fortification

For mandatory fortification, a primary consideration is that the food vehicle chosen is consumed regularly by the target population. The quantity of potential food vehicle consumed by the target

population may also be an important consideration depending on whether the concentration of the fortificant can be varied without changing organoleptic and technological properties of the vehicle. Information is also required for non-target groups so that their exposure can also be determined.

Countries with little domestic manufacturing capability are reliant on imports in the case of processed food. This may substantially limit the choice of food vehicles for mandatory fortification because the regulation must be implemented in another country. Enforcement of the mandatory fortification regulation at the border of the importing country might not always be a high priority with customs and quarantine officers who have many responsibilities outside the food fortification area. It may only be practical to consider fortification of basic commodities such as flour or salt. Even in a country such as Australia, processed foods are imported from many countries and it may be difficult to mandate fortification for a wide range of processed food products. Furthermore, although the World Trade Agreement allows member countries to impose mandatory fortification where a public health need can be shown, it also requires that the least trade restrictive option to achieve the goal be selected [3].

Nutritional Suitability of Potential Vehicles

The nutritional suitability of a food vehicle is an important consideration for both mandatory and voluntary fortification. Public health and consumer advocates are often concerned about the potential for fortified foods to distort consumption patterns in both the target and non-target population, especially where there is a risk of adverse health effects, e.g. from increasing intakes of saturated fat, sodium, sugars or alcohol. This is where consumer research can be an important source of information to guide food vehicle choice. Countries might limit the range of foods that can be voluntarily fortified using criteria defining some minimum level of "healthiness" for the vehicle [1]. In the case of mandatory fortification, there may be sound rationale to choose an otherwise less than desirable vehicle because it most effectively achieves the public health outcome (e.g. salt iodisation programs).

Technological Considerations

Food technological advice is essential to ensure that the method of fortificant addition is technically feasible for the vehicle selected, especially for mandatory fortification where the addition of the fortificant can impact on quality (e.g. colour, taste), manufacturing processes (e.g. equipment) and, as a result, impose undue regulatory burden. A range of factors must be examined in relation to both the food matrix of the vehicle (Table 24.1) and the fortificant (Table 24.2).

The low concentration of a fortificant may pose special problems. It may lead to inaccurate estimates of the cost of a program if only the costs of the fortificant are included and the costs of the dosing system are ignored. In Australia for example, previous mandatory fortification of wheat flour for bread-making with thiamin specified a minimum amount of thiamin and so the level of overage was unimportant. The new regulation for folic acid in the same vehicle [8] stipulated a minimum and maximum amount and millers incurred additional costs (e.g. purchase of new equipment) to meet this requirement. There are further costs if not all of a vehicle is fortified (e.g. only one type of flour) because the different streams must be kept separate.

There are a number of facets of industry quality assurance processes (Table 24.3) that might affect how a regulation needs to be worded. Low concentrations make it difficult to ensure that the fortificant is evenly mixed throughout the vehicle. This has consequences for both enforcement and labelling because it may not be possible to ensure that any single small sample of the food contains the required amount of fortificant. There needs to be clarity between manufacturers and the enforcement agency about how the sampling will be conducted to test for compliance with the fortification and labelling regulations. Analytical tests have lower variability when concentrations are higher. Therefore, laboratory performance checks or development of new analytical methods and reference materials might also be required as part of a fortification program involving very low concentrations [19].

Table 24.1 Technological considerations related to the food matrix

Area	Specific considerations
Level of addition	The quantity of fortificant preparation added should not impact on the food's general properties, for example colour or texture
	A very small addition rate is hard to control on a continuous basis
Method of addition	Typically, the mandatory requirement is for such a low level that the fortificant is added as a preparation, e.g. in an emulsion or diluted with a suitable carrier. It is important that the other components do not impact on the activity of the fortificant (e.g. potential for bread-making additives to react with folic acid)
	In a long-run continuous operation, such as for flour or salt, the fortificant (often on a carrier) can be added continuously
	If fortification occurs in a batch process, then the quantities are much smaller and will need to be diluted or used as an existing pre-mix (e.g. a bread pre-mix containing all the minor ingredients). Controlling levels and obtaining a good distribution may be much more difficult (e.g. using iodised salt in a brine process)
Maintenance of activity and distribution	Any expected changes to fortificant activity and distribution during manufacture and storage need to be understood
	Whether this is a critical issue depends on whether the fortification is based on the addition rate or the amount required to be present in a food sample at the end of the shelf-life

Table 24.2 Technological considerations related to the fortificant

Area	Specific considerations
Ability to add fortificant	If the fortificant is solid, is it appropriate to choose a liquid food vehicle?
	Is it more feasible to add the fortificant to an ingredient (e.g. flour, salt) or the final food (e.g. bread)
Concentration and distribution of the fortificant	Too low a concentration may result in manufacturing and analytical costs significantly higher than food industry norms; too high a concentration may lead to functional changes in the food or incur costs to prevent such functional changes
	Physical or chemical characteristics of the fortificant can limit even distribution in the vehicle (e.g. separation of potassium iodate in salt crystals)
Stability in the food before sale	A number of vitamins deteriorate during manufacturing (e.g. pasteurisation) and storage and may also be impacted by the food's characteristics (e.g. high acid). Rates of these changes are generally known, but can be high enough to impact substantially on the fortification assumptions, e.g. folic acid may be less stable in wholemeal flour than white flour
	Some of these changes may be managed through food technology processes, e.g. encapsulation, but this increases the manufacturing cost and possibly complexity
Stability in the food as prepared for eating	Storage and preparation in the home needs to be considered because a fortificant may be stable in a manufactured food until it is prepared for eating (e.g. mixing with other foods, or re-heating)
Relative cost of the fortificant	The relative cost of the fortificant should be considered but is often quite small for the common fortificants (e.g. 6–10 c per tonne of final food)
	For voluntary fortification, cost may be comparatively higher for less commonly used fortificants but in this can be recovered in the cost of the product
Cost of the dosing system	The capital and consequential operations costs of food manufacturers may be quite high, particularly where a food or ingredient manufacturer does not have an established blending system. (e.g. the addition of vitamins and minerals to flour requires a dosing system that is synchronised with the mill's production rate. It requires multi-dosing and control systems if the mill is manufacturing both unfortified and fortified flour. The systems need to be more complex if the mandatory concentration must be within a prescribed range rather above a minimum.)

Table 24.3 Quality assurance, control process and labelling considerations when selecting a fortificant

Area	Specific considerations
Long run versus single sample requirements	The level of process control and quality assurance will be materially impacted depending on whether each sample is expected to be within a specified range for the fortificant, or whether it is sufficient to show long-term compliance. (e.g. for iodine in salt significant "real time" variation might be acceptable provided that, in the long run, the level of fortification is in the correct range)
Whether to measure/ prescribe based on the ingoing fortificant or level in the final product	As a generalisation, it is inexpensive and simple to measure the ingoing rate of a fortificant, as crudely as comparing the weight of fortificant added to the weight of each batch of food (a common measure with fortification of flour)
	Prescribing the limit in the final food and measuring on that basis alone is expensive, typically occurs too late to be useable for quality control, and includes the sum of a number of expected variations including manufacturing rate, rate of addition, adequacy of mixing, blending in of other fractions
Sampling and analytical methodology	If the final food must be analysed, then an allowance for laboratory variability and accuracy is needed
	For continuous fortification there will be variation in both the manufacturing rate and the addition of the fortificant, although these will even be out in the long run. For flour fortification for typical mills, a minimum of five sub-samples has been shown to be sufficient to obtain a fair batch sample. Truth in labelling regulations in some countries requires each sample or retail pack to be in conformance and so manufacturing that in the long run is within guidelines may not be on a sample by sample basis
	For batch fortification there should be almost no variation in the addition rate. In this case, mixing is critical before sampling or use
	The analysis of fortificants ranges from simple colorimetric through to laboratory methods such as high performance liquid chromatography. It is critical to recognise the limits of accuracy of the method, even for very accurate methods. The coefficient of variation of most of tests is in the range of 10-15%. Folic acid testing in Australia revealed that accuracy is often plus or minus 10% [19]. Lack of standardised reference material for the food type being analysed increases these inaccuracies
Minimum/maximum issues	The quality assurance and process controls are quite different depending on whether the regulation prescribes a minimum or a range for the fortificant
	Where the manufacturing and quality assurance systems are focussed on ensuring a minimum level of addition, assumptions will typically include allowances for fluctuating manufacturing rates and known laboratory variations. A simple dose rate is established for the 'worst case'. This leads to an overage
	If the fortification must be within a prescribed range, then more sophisticated quality assurance systems are required. These include, for continuous production systems, being able to alter the addition rate in real time to match the production rate and other issues (e.g. blending of other streams) and the necessity for defined laboratory accuracy and variability to be able to use these results
	The quality assurance system may also need to take into account changes during a raw material's or product's shelf-life. This may be more difficult when staying within a range than achieving a minimum level
Possible conflict between label declaration/claims and actual levels of fortificants	For example, a manufacturer may fortify to ensure that the required minimum level is exceeded but the labelling may require statement of an average level. Often these will not be the same

Economic Analysis

Careful examination of the food vehicle, and fortificant and associated costs, including the cost of compliance, must be done prior to the introduction of mandatory fortification because manufacturers are compelled to add the fortificant even if it increases costs that they cannot recoup via increased

prices. A number of possible solutions might yield approximately the same increase in micronutrient intake in a population. For example a wide range of food could be fortified at a low concentration or a narrow range of food at a higher concentration. These would have differing costs, regulatory burdens and restrictions on consumer choice. In this situation, economic analysis provides a valuable decision-making framework not only assisting to decide which option should proceed, but also whether fortification should occur at all.

Many countries have formalised the requirement to conduct economic analysis, most commonly cost–benefit analysis (CBA), on significant new regulation. A requirement to fortify food would, in most circumstances, be sufficiently significant to require economic analysis. FSANZ, for example, needs to create a regulation impact statement (RIS) that complies with the Australian government requirements for significant regulation.

From a regulatory analysis perspective, RISs are an attempt to clearly define the problem being addressed and to set out the different options that could be adopted to address the problem. Generally there are three broad options that have to be considered: the status quo (i.e. no change), a non-regulatory option and the regulatory option. Non-regulatory options range from industry self-regulation to educational campaigns, although in some instances, these might have been considered and rejected during the policy process that leads to identifying food regulation as a strategy to manage the health problem. More than one regulatory option can also be considered.

The RIS needs to be comprehensive and identify the various groups in the community who are likely to be affected, favourably or adversely, by each of the options considered. Using the status quo as a base-line, the costs and benefits are aggregated to arrive at a net benefit or cost for each option. In Australia, a RIS needs to clearly demonstrate that a regulatory problem is being addressed, that decision makers clearly understand the implications of regulation and that the public can access the information on which regulations are based.

Such a process is an important discipline given the nature of regulation. A regulator could theoretically keep reducing risk by increasing regulation that passes costs onto others outside government. At some point the cost to the community as a whole would exceed the value gained through the regulatory intervention. The aim of the RIS is to help the regulator describe and identify where additional regulation does not deliver a net benefit to society. Alternatively, where several options are available, a RIS helps the regulator choose the one that creates the largest total benefit to the community as a whole.

Economic analyses, and CBA specifically, can be criticised as not sufficiently reflecting real world costs and benefits. However, a well-done analysis should provide all assumptions and limitations of the analysis clearly to decision makers to allow them to assess the value of the analysis in relation to the decision before them. Developments in the fields of health economics, behavioural economics, the economic assessment of regulatory interventions generally, and the use of input from the other social sciences are making it an increasingly valuable tool in the context of food regulation.

Risk Communication

Where mandatory fortification is applied within a population, there should be communication and education strategies developed to support and enhance the public health outcome. Public communication may be important at the commencement of mandatory fortification, particularly where a sub-population or non-target group is at risk of possible side effects. When Australia and New Zealand introduced mandatory iodine fortification of salt in bread, key messages of a general nature were disseminated to consumers and a targeted communication strategy was aimed at medical practitioners, health professionals and certain consumer groups to lessen the likelihood of adverse health impacts on individuals with pre-existing thyroid conditions and reduce perceived risks from concerned consumers.

Implementation Aspects

Labelling

A transition period between the date that a regulation is adopted and the date on which it becomes enforceable is usually necessary to allow time both to adjust manufacturing processes and also to update labelling on packaged foods. With voluntary fortification, opportunity exists for manufacturers to distinguish their products from others through label claims providing a competitive advantage. In the case of mandatory fortification this opportunity no longer exists and labelling changes are usually necessary to comply with mandated ingredient/nutrient declaration regulations. Labelling changes related to mandatory fortification impose costs on manufacturers. Allowing a longer transition period might lessen costs because required changes could then be accommodated as part of scheduled labelling updates by the manufacturer. The choice of vehicle can also impact on the extent of labelling changes. For example, if a staple food is fortified (e.g. iodised salt), then, in countries such as Australia and New Zealand where there is mandatory ingredient listing on food labels, this would generate concomitant label changes on all products that contain the fortified item.

Monitoring

Monitoring of a mandatory fortification program is required on several levels. In addition to the industry quality controls (Table 24.3), food composition and label surveys might be done by government. Monitoring of biomarkers and food intake in representative national surveys of the whole population and target groups is useful for assessing both benefits and adverse effects. Other methods might include assessing trends in birth defect registers and hospital admissions. It might be important to ensure that the fortification program has not had unintended effects in selected groups (e.g. women ceasing to take certain supplements).

Related Topics

Fortification is the addition of micronutrients during food processing for the purpose of changing population nutrient intake. There are several other related additions to food which do not meet this definition. They will be briefly mentioned here as some are becoming more common.

Addition of Essential Micronutrients to Food for Non-nutritional Purposes

Certain micronutrients are added to food because they perform technological functions. For example, beta-carotene is a pre-cursor of vitamin A and also a permitted colouring. Vitamin E is a permitted anti-oxidant in oil. Technological uses are not classified as fortification, but they increase the micronutrient intake in the population and so the impact of their use on intake needs to be included. Food composition analyses would identify these micronutrients in the food supply. If food composition tables have been compiled using foreign data, then any variations in national usage compared to the foreign composition data would need to be assessed.

Addition of Non-essential Food Components to Food for Stated Health Reasons

Compounds which are said to have health impacts are called functional foods and might be added to foods voluntarily by manufacturers. These range from some foods which have a long history of use but are being promoted for their health benefits, to others that are derived from non-food sources or which are not traditionally used in the country of interest to those which are derived from genetically modified crops. Many countries have requirements for regulators to assess this type of ingredient or food before manufacturers are permitted to add them to food. Addition of some of these ingredients could potentially increase population intakes to the point of exceeding health reference values such as Acceptable Daily Intakes [6]. This needs to be assessed by the regulator to ensure that the population is not over-exposed.

Phytosterols are an example of a functional food. Small amounts of phytosterols are found naturally in foods such as unrefined olive oil. More recently they have been extracted from "tall oils" which are by-products of wood pulp. Tall oils are not a traditional food in Australia and New Zealand and so phytosterols derived from them are classed novel food ingredients by FSANZ. Specific permissions following pre-market safety assessment are required before they can be added to foods.

Increasing Nutrient Concentration During Primary Production

It is well known that the addition of fertiliser at certain stages of cereal growth alters its protein content. Nutrient concentration in plants can also be increased by plant breeding (called biofortification [20]) either by conventional breeding or genetic engineering. Recent examples of biofortification are golden rice (vitamin A), sunflower with higher content of oleic acid and soybeans with stearidonic acid (a fatty acid on the pathway between alpha-linoleic acid and eicosapentaenoic acid) [21]. Different countries have different approaches to the safety assessment of genetically engineered crops. Crops that are used as human food, or animal feed that might become mixed with human food (e.g. maize), would be assessed by a range of regulators.

Changing animal feed is one way to alter the composition of the animal, and hence the food available to human populations. For example, iodine can be added to salt licks or other animal feed [22] to increase the iodine content of animal products used as human food.

Changing nutrient concentrations by breeding or husbandry raises the possibility of excessive intake of nutrients or other food components by the human population. Human food and agricultural regulators need to work together to monitor uptake of new technologies, analyse the national food supply and assess risk in the population.

Adventitious Contamination

The use of iodophors to clean vats in the dairy industry is thought to have controlled iodine deficiency in Australia for about 50 years. The resurgence of iodine deficiency is postulated to be due to a change to a different cleaning agent [23]. This illustrates the importance of local analyses of food and the role that contaminants can play in maintaining nutrition. Total diet studies [24] might monitor the levels of contaminants such as zinc in food to ensure that intake is not too high. Ignoring these data, or using food composition data from other countries because national data are not available, could lead to inappropriate fortification decisions.

Recommendations and Conclusion

Policy planners need to advocate for good food composition data, food intake surveys and, where relevant, biochemical measures surveys, to ensure that the nutritional status of the population is well understood. If nutritional status is less than desirable, then food fortification is one of several different strategies that might be useful. Monitoring the strategy/ies implemented in both the target and non-target populations is desirable so that the approach can be refined if necessary. Especially in high income countries where micronutrient intakes are generally higher than lower and middle income countries, an assessment of the possibility of excessive intakes in some population subgroups needs to be made, as well as the impact of potential fortification strategies in the target subgroups.

Advocates need to be aware that the overarching policy environment and legislative framework affect how legislation is developed and implemented. Some food technology options might not be as simple as they appear to those outside the field. A detailed assessment is needed to ensure drafting of a regulation that industry can comply with. It must be feasible to enforce and monitor a regulation if the regulation is to achieve its purpose. Given the differing degrees of inadequate intake in different countries, different food habits and different legislative frameworks, different regulations are to be expected.

Manufacturers and importers should remember that food regulations vary around the world. Fortificants that are permitted in certain foods in the country of manufacture may not be permitted in other countries. Even if fortification is allowed in the country of sale, the permitted concentration might differ from that allowed or required in the country of manufacture. The food regulations in the proposed country of sale need to be identified and adhered to.

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Part IV
International Perspectives

Chapter 25
Fortification of Flour and Outcomes: Oman's Perspective—Contextual Considerations and Outcome

Deena Alasfoor and Medhat K. ElSayed

Key Points

- Oman is a stable country transiting from being a developing to developed country; education, income, and health services are on the rise.
- Oman was a leader in making flour fortification a national legislation, and the coverage rate was more than 80 % consistently over the years.
- Political well: a well set up industry and open communication channels facilitated the initiation and sustainability of the fortification program.
- Process and coverage was established because of the small number of mills and adequate resources.
- The most important impact was on NTDs which declined significantly to 20 % of its original levels and saved about 1,350 births from 1996 to 2010.
- Anemia rates went down among women of childbearing age and adolescents, but had increased among preschool children and remained constant among men. It continues to be a public health problem of concern.
- Impact of iron and folate fortification is dependent on the bioavailability of each nutrient, and other confounding factors, such as the presence of comorbidities and genetic haemoglobinopathies.
- Anemia control is a complex issue that requires a comprehensive and holistic approach in policy and management.

Keywords Fortification • Oman • Iron • Folate • Flour fortification • Anemia • NTDs • Spina bifida • School children • Women • Men

Abbreviations

UAE	United Arab Emirates
KSA	Kingdom of Saudi Arabia
Km	Kilometers

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V.R. Preedy et al. (eds.), *Handbook of Food Fortification and Health: From Concepts to Public Health Applications Volume 2*, Nutrition and Health, DOI 10.1007/978-1-4614-7110-3_25,

Sq	Square
GDP	Gross Domestic Product
US	United States
$	Dollars
UNICEF	United Nations Children Fund
PEM	Protein energy malnutrition
Hb	Hemoglobin
g/dL	Grams per deciliter
mg	Milligram
μg	Microgram
IU	International units
μg/dL	Microgram per deciliter
WHO	World Health Organization
EMRO	Eastern Mediterranean Regional office of the World Health Organization
CDC	Centers for Disease Control
USA	United States of America
ppm	Parts per million
Mt/day	Metric tons per day
OR	Omani Rials
RNI	Recommended nutrients intake
UN	United Nations
EAR	Estimated average requirements
mg/d	Milligram per day
NTDs	Neural tube defects
QALYs	Quality adjusted life years
MoH	Ministry of Health

Introduction

Flour fortification was initiated in Oman in 1996, making this small Middle Eastern nation the first to mandate folic acid fortification globally. In that year, as the United States of America was fortifying on a subnational scale, Oman health authorities took the courageous first step to issue a national legislation that requires all white flour in the country to be fortified with iron and folic acid. Currently, 63 countries require iron fortification, and 57 of them require folic acid fortification of flour.

The impact of folate fortification is well documented in Oman and the world. However, the question that presents itself: Was the decision to fortify flour with iron and folate in 1996 a lucky draw or an educated guess by the Omani health authorities? Does the current outcome meet the expectations at that time? These and contextual factors will be discussed in order to gain a more in depth understanding of Oman's perspective on flour fortification.

Overview of Oman: Geography and Economy

The Sultanate of Oman is an Arab state in southwest Asia on the southeast coast of the Arabian Peninsula. It is bordered by the United Arab Emirates (UAE) to the northwest, Kingdom of Saudi Arabia to the west and Yemen to the southwest. The coast is formed by the Arabian Sea on the southeast and the Oman Sea on the northeast. Madha and Musandam enclaves are surrounded by the UAE on their land borders, with the Strait of Hormuz and Gulf of Oman forming Musandam's coastal boundaries [1].

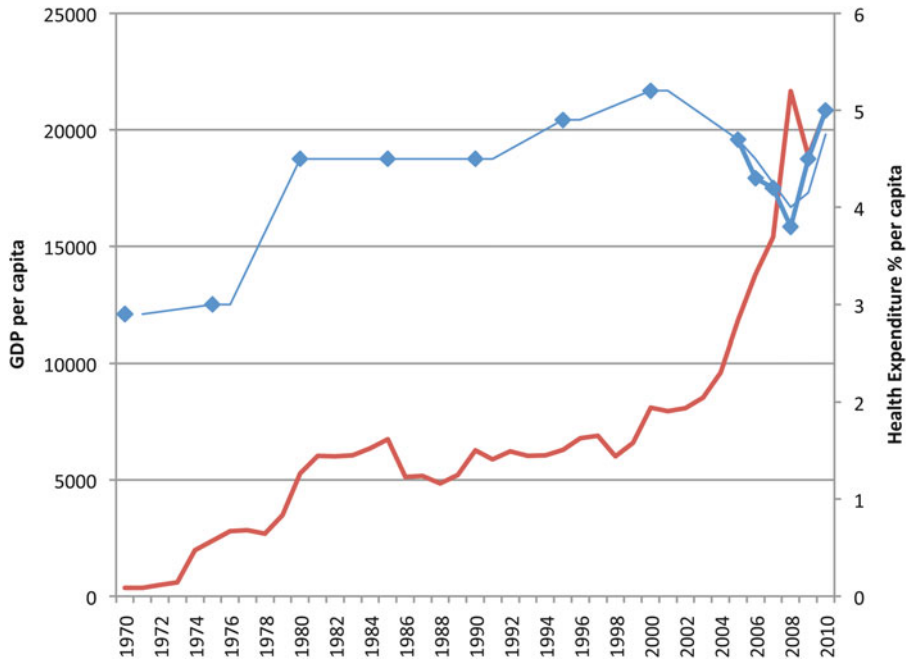

Fig. 25.1 Gross domestic product per capita and percentage expenditure on Health for Oman from 1970 to 2010 (Adapted from World Bank; Ministry of National Economy, Oman [43, 51])

The total area of Oman is estimated to be 309,500 km^2 and its coastline extends for 3,165 km [2, 3]. It has a variable landscape of desert, beach, and mountain terrains and has 9,000 ha of irrigated land and 39,000 ha of permanent crops [4].

Despite its dependence on oil, Oman's production is comparatively low compared to countries like KSA and Libya. Almost 67 % of the national revenue comes from oil, and an additional 11–12 % from gas [5].

GDP per capita reached a high of US$21,065.6 in 2010, showing a steady increase from US$8,283.9 in 2000 and US$12,334.4 in 2005 [6]. Health expenditure however did not increase significantly as it was only 2.1 % of the GDP in 2008, which may have been caused by high cost of the developmental projects in order diversify sources of income [7]. In the year 2000, a controversial report by the World Health Organization ranked Oman first on performance on level of health [8, 42, 43, 47, 51] (Fig. 25.1).

Demographics, Health and Nutrition

In 2010, Oman population size was estimated to be 2,750,000 of which 29.5 % were expatriates; males were 102.5 and 138 per 100 females for the Omani and total populations respectively. About 13.5 % of the population were under 5 years and another 35.3 % were under 15 years whereas only 3.6 % were 60 years and over. Women in reproductive age comprised about 27.2 % of the population. Crude birth rate in 2010 was estimated to be 31, crude death at 3.3 per 1,000 population and the total fertility rate was 3.7 and life expectancy was 76.1 years [2, 9].

The State of the children 2011 of UNICEF reported that 88 % of the population had access to improved drinking water sources, 97 % had improved sanitation facilities, both improving from 80 % in 1990. Immunization coverage went from less than 80 % in 1990 whereas under 5 mortality rate was 11.3/1,000 live births in 2010 from 35 in 1990 [10, 11].

The first documented investigation of protein energy malnutrition (PEM) and micronutrients deficiencies was in 1980 when a United Nations mission to the Gulf Region reported that 62.9 % of preschool children were underweight and 24.1 % were stunted [12]. In 1992, the rates had decreased to 32–46 % for stunting and 25–37 % for underweight [13]. These continued to decline as stunting was reported to be 10.6 % in 1999 and 9.8 % in 2009, whereas underweight was 17.9 % in 1999 and 8.6 % in 2010 [14, 15].

A national strategy to combat child malnutrition was introduced in 2004 and included early screening and management, social marketing campaign and a number of other multi-sector activities. Malnutrition was reduced probably because of the economic, social, educational, and health progress in addition to the interventions that took place during that period [12, 16].

Status of Micronutrients Malnutrition in 1996

Micronutrients Malnutrition was one of the most important issues tackled in health since 1980s when population surveys showed high prevalence estimates of anemia, vitamin A, and iodine deficiency disorders.

Anemia: A national study in 1986 reported that 54 % of pregnant women in Oman had hemoglobin (Hb) values less than 11 g/dL [17]. In the same year, an iron supplementation program for all pregnant women was implemented, which required all pregnant women to receive 200 mg of ferrous sulfate and 5 mg of folic acid daily [18]; however, 48.5 % of Omani pregnant women were still anemic in 1993 [19, 45].

Vitamin A: In 1981, the first study of vitamin A status in Oman found that 1.5 % of preschool children had Bitot's spots [20]. In 1994, a national study of vitamin A status in children 7 and 18 months, 3 and 6 years of age found that 18.7 % of the study population had serum retinol levels below 20 μg/L and 2.1 % had serum retinol levels below 10 μg/L indicating severe deficiency [21]. Following that (in 1995), universal supplementation was initiated for postpartum women at 200,000 IU, as well as 100,000, 200,000 IU for children at 9 and 15 months consecutively. In 1999 the prevalence of serum retinol levels less than 20 μg/L among children was reduced to 5.2 % and no children had serum retinol levels below 10 μg/L [22].

Iodine deficiency disorders: Universal salt iodization was legislated in Oman in 1996 after a household survey revealed that 22 % of households used iodized salt, 50.2 % of schoolchildren had urinary iodine levels below 100 μg/dL, and 1.2 % had goiter [23].

Initiation of the Flour Fortification Program in Oman

As flour fortification was being initiated in 1996, vitamin A and iodine deficiencies were on the decline among infants and children in Oman; at the same time child malnutrition and anemia among all age groups were known to be unacceptably high. Extrapolating from international data, iron deficiency was assumed to be the main cause of anemia in the region and thereby fortification was considered the intervention of choice [24].

A multi-organization consultation was held in Muscat, Oman and it concluded that fortification would potentially contribute to the reduction of anemia among women and children in Oman [25]. The consultation was a coordinated effort between the WHO offices in Oman and EMRO (personal communication, Dr. Anna Verster), and WHO, UNICEF, Micronutrients Initiative and Program on Micronutrients Malnutrition (CDC/USA). It was decided that "fortification of flour in most countries in the region will be simple and cheap and will be a major strategy to prevent anemia", and "ferrous

sulphate is the compound of choice, folic acid can be added at little additional cost". Oman consented to 30 iron and 1.5 ppm folic acid fortification level.

Flour is a stable commodity and does not change in color, taste, or consistency upon fortification which makes it an attractive option as a vehicle. Criteria for choosing a fortificant are explained in Chap. 7 with more detail. Population coverage was expected to be high as almost 80 % of the flour was supplied by one mill [26].

Oman Flour Mills (OFM) was the only mill in the country and supplied more than 80 % of the flour and bread demand with 800 Mt/day capacity. Main types of flour produced were the atta flour with high extraction rate of 87 % and accounted for 60 % of the production in 1996, and low extraction rate flour that accounted to 40 % of the production. Fiber content of high extraction flour inhibits iron absorption therefore it was decided to fortify the white flour only [27, 52].

Political Support

Early 1997, a legislation to mandate fortification of flour with 30 ppm iron and 1.5 ppm folic acid was issued by Ministry of Commerce and Industry and Oman Flour Mills complied with the legislation [27, 28]. Swift fortification of flour in Oman had elements of political support, dedicated management, and a convenient technical and logistic set up.

The Minister of Health at the time. was also the Chairman of Board for Oman Flour Mills. a rare incident that was detrimental in expediting the process. Concerned about the public health implications of anemia, he consulted with the World Health Organization in Muscat and EMRO and requested expert advice on the feasibility of flour fortification in Oman (personal communication).

Oman Flour Mill capacity was recognized by experts and it was stated that fortification could proceed without delay. There were no requirements for additional equipment, supplies, or manpower besides procurement of the fortificant. Fortification and the quality schemes it required could be incorporated easily because of the modern technology used in the mills. Shortly after this evaluation, a consultation gathered experts from Eastern Mediterranean Region (EMRO countries) and the rest of the world in the capital of Oman. At the time, the United States was in the process of mandating fortification which persuaded the crowd who knew that many commodities were already fortified for more than 50 years in that country.

The workshop consensus was the basis for the legislation issued by Ministry of Commerce and Industry shortly after. As for the operations management team, the expert visit and discussions of technical and finance queries paved the way to prompt implementation. In addition, low cost of fortification and high market share made it less difficult for them to take part.

In 2004, an evaluation of the flour fortification and micronutrients program took place. including qualitative and quantitative studies to assess anemia and flour fortification knowledge, attitude and practices among Omani families, and to determine the coverage and impact of fortified flour on anemia and iron status of women and children [29, 30].

Knowledge, Attitudes of Anemia and Flour Fortification

A number of studies demonstrated that knowledge of anemia and fortification was very low among Omanis. In 1993, about 51 % of pregnant women had some knowledge about anemia. but only 2.4 % related it to iron deficiency [31]. In 2003, a qualitative study indicated that there was a general lack of knowledge about micronutrients deficiencies and fortification, although many people were aware of additives and their hazards. There was no clear distinction between nutritional and genetic anemia in the population studied, moreover fortification was discussed with suspicion on the basis of possible

side effects. carcinogenesis and allergic reactions. Most respondents were not aware that flour is fortified. including a baker and health staff, although many were aware that salt was iodized possibly because there was a marketing campaign for the latter. When asked, most participants chose non-fortified flour [29].

A quantitative study a year later reported that 4.5 % of women and 7.9 % of the men knew that bread is fortified, and 4.5 % of the women and 4.7 % of the men knew that flour is fortified. Only 14.9 % of the women and 33.2 % of the men reported choosing fortified products in the stores. Fortification ranked higher on men's priority list when purchasing food as 10.8 % of them took it into considerations compared to only 4.2 % of women. Younger women chose fortified products more than older women ($p<0.05$) [30].

Flour was commonly purchased as almost 90 % of the households reported to have flour, and 79 % reported to consume Chappati and Omani bread which are home made. The coverage of flour fortification was 81 %.

In the same study, households with higher per capita income were more likely to have fortified flour. Only 51.6 % of lower income households had fortified flour compared to 84.2 % for the highest income quartiles. The percentage of fortified flour in low income households (per capita <R.O. 30[1]) was 64.9 %, compared to 83.4 %, in high income households (p-value = 0.005). Flour bought from local mills was the least fortified (44.4 %), though only 2.7 % of the houses bought from those mills.

Knowledge about flour fortification is low among this population; however, consumption of fortified flour seems to be high. None of the studies investigated folic acid fortification.

Bioavailability, Consumption and Nutrient Adequacy

Iron: Animal foods and ascorbates are important enhancers of non-heme iron absorption, while phytates, polyphenols, tannins, and fiber are considered as inhibitors. Recommended nutrition intake of cereal-based diet and low ascorbate content is set at <5 % (very low bioavailability) or 5–10 % or low bioavailability.

An intermediate diet with a bioavailability of 11–18 % is based on plant foods with some animal protein and ascorbic acid. High bioavailability diet (19 % absorption), is predominantly animal protein diet and with fruits rich in ascorbic acid. Omani diet was considered of intermediate bioavailability and was estimated to be 12 % [32, 33].

Consumption of flour was estimated to be 286 Mt per capita daily in 1995 [34]. Fig. 25.2 shows the percentage contribution of iron from fortification to the total iron intake among men and women. Iron intake of diet alone was lower than the estimated average requirements for children, women, and adolescents, whereas fortification at 30 ppm provided additional 8.0 mg per day for adolescents (50 %), 7.5 mg for women (46.6 %), and 8.6 mg for men (42 %). This addition exceeded the requirements for adolescents and men but achieved only 80 % of the requirements for women.[2]

Folate: Before fortification only 35 % of the folate requirements were satisfied by the diet, which went up to 93 % after fortification of folic acid. At least 59 % of the folate is supplied by wheat flour [33] (Table 25.1).

[1] Exchange rate: US$1 = 0.388 Omani Rial (OR); 30 OR = US$77.

[2] Calculated from the national dietary intake survey.

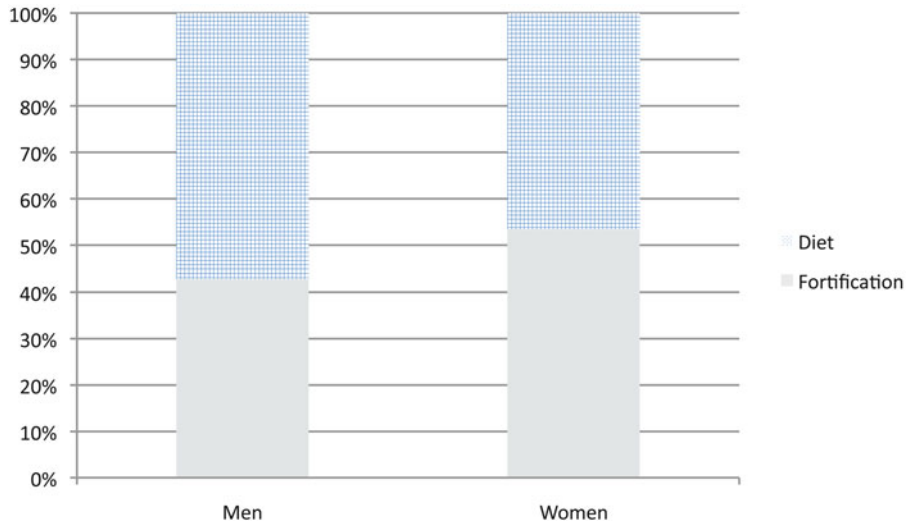

Fig. 25.2 Contribution of fortification to iron intake among Omani men and women (2004) (Adapted from National Nutrition Survey, Oman)

Table 25.1 Iron and folate requirements for Omani population, 2007 (MoH, Oman) [33]

Population category	Age group (years)	Energy (kcal)	Iron (mg)	Folate (μg)
Young children	1–3	1,000	5.5	150–200
Children	4–8	1,400	7.7	210–280
Adolescent males	9–13	2,000	11.0	300–400
	14–18	3,000	16.5	450–600
Adolescent females	9–13	1,900	10.5	285–380
	14–18	2,400	13.2	360–480
Adults males	19–30	2,100	11.6	315–420
	31–50	2,400	13.2	360–480
	51–70	2,200	12.1	330–440
	>70	1,800	9.9	270–360
Adults females	19–30	2,000	11	300–400
	31–50	2,000	11	300–400
	51–70	1,800	9.9	270–360
	>70	1,600	8.8	240–320
Pregnant women	All	2,100–2,700	11–15	315–540
Lactating women	All	2,400–2,900	13–16	360–580

Impact of Flour Fortification

As fortification of cereals with iron was practiced as early as 1952 in USA and Canada, it was considered an acceptable and safe strategy but the impact of fortification on anemia was more pronounced in developing countries where the reduction was 30–70 % compared to <20 % in developed countries. Moreover some subgroups were found to consume less fortified products or at higher risk of anemia (refer to Chap. 7). The human development index 2011 placed Oman higher than other eastern

Mediterranean countries based on this nation's health, education, and income. Because of that, and the ever rapid changing pattern of nutrition and morbidity, Oman cannot be compared to developed or developing countries nor can anemia improvement be predicted [35].

Anemia Among Preschool Children

Diet of children below the age of 5 years goes through several stages starting from breastfeeding up to the age of 6 months; complementary feeding to the age of 12 months and transition to family diet [36]. Iron status is determined by dietary practices as well as the health and nutritional status of the child. These are beyond our scope, although estimated iron intake and anemia status in this population are going to be investigated.

Flour intake of children below the age of 2 years is small or minimal, and may be confounded by the extent of breastfeeding therefore flour fortification is not expected to have a significant impact on anemia in this age group.

The national nutrition survey in 2004 indicated that iron intake for infants and young children was above the recommended intake, whereas the trend indicates an increase in anemia from 1995 to 2009 as shown in Fig. 25.3. At the age of 0–2 years however the rates were comparable, whereas a gradient increase is observed at the 3–5 age group, resulting in an overall increase of 20 % in 14 years [14, 37, 48, 50].

Anemia Among Adolescents

Mean intake of iron was 14.6 mg compared to the 18.6 RNI, indicating that dietary iron among Omani adolescents does not satisfy biological requirements. In 1980, a UN mission indicated that anemia rates among adolescents was 58.5 %, and in 1996 a school children survey found low Hb level of

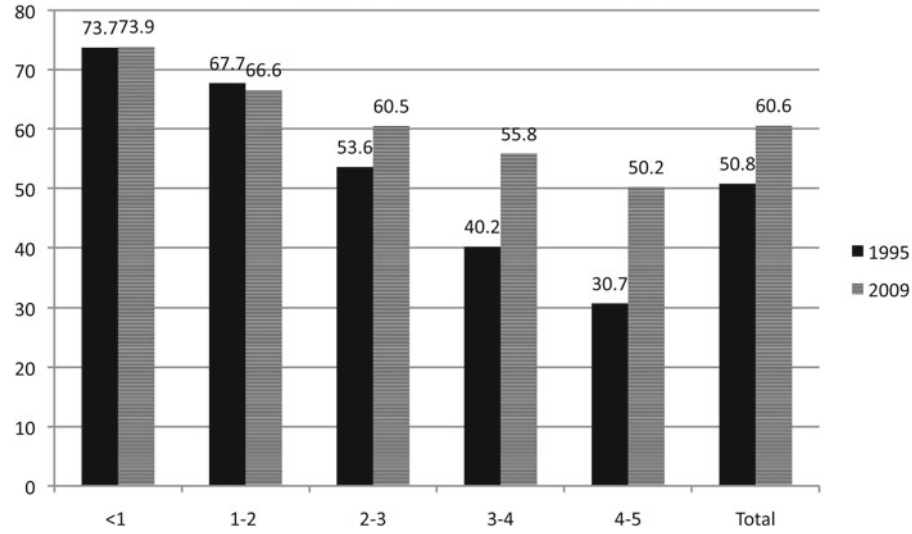

Fig. 25.3 Anemia rate among infants and young Omani children in 1995 and 2009 (Adapted from ElSayed et al. [15]; Al-Riyami et al. [19])

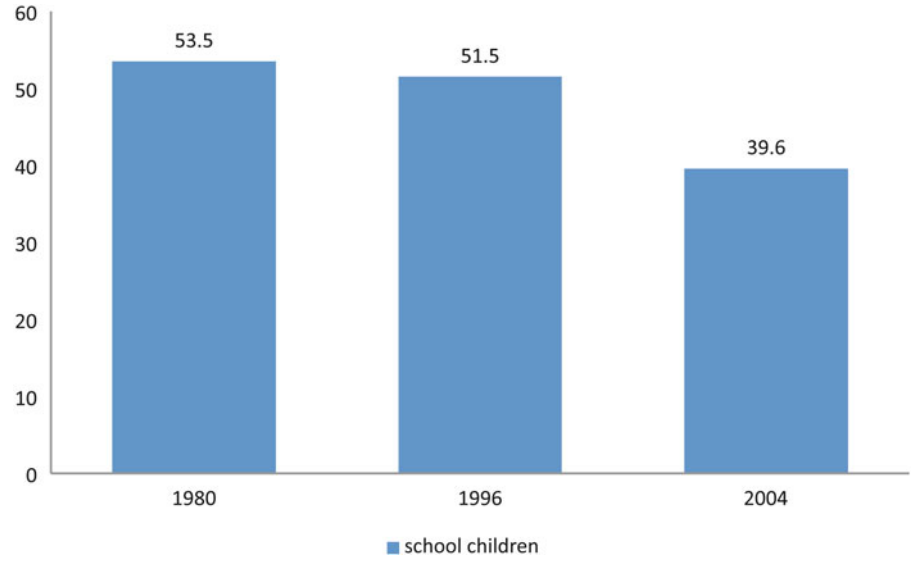

Fig. 25.4 Rate of anemia among Omani school children (Amine [12]; Alasfoor [36]; MoH, Oman, 2009)

51.5 % (95 % CI = 47.7–55.3), and in 2004 dropped by 23–39.6 % (95 % CI = 28.5–51.8) as shown in Fig. 25.4 [12, 38], The rate of decline in 1980–1996 before fortification was 0.31 per year, whereas it was 1.48 after fortification.

Anemia Among Women of Childbearing Age

Women in childbearing age had low iron intake similar to adolescents, as the mean intake was 16.1 ± 10.3, whereas the RNI was 24.5 mg/day and the EAR = 20.4 mg/day. The contribution of iron in fortified flour however was significant as indicated earlier, as more than 50 % of iron was provided through fortification (Fig. 25.2).

A surveillance system was established to monitor the hemoglobin levels as they enter into pregnancy in the early 1990s. As shown in Fig. 25.5, anemia levels witnessed a consistent decline of 0.8 % annually over the last 16 years, which indicates a positive response to fortification. It can be argued however that developmental progress, education, and wealth could have significantly impacted the nutritional status of women.

Neural Tube Defects

The most widely used outcome measure of folic acid fortification is reduction of neural tube defects, because of the severity of the problem and the outstanding reduction shown worldwide [39]. Similarly in Oman a reduction of spina bifida was observed consistently from 1996 to 2010, contrary to earlier dates where other NTDs were constant. Figure 25.6 shows the incidence of spina bifida as well as other congenital disorders reported from 1991 to 2010. Spina bifida went down by 86 %, from 3.48 to 0.68 per 1,000 births [40].

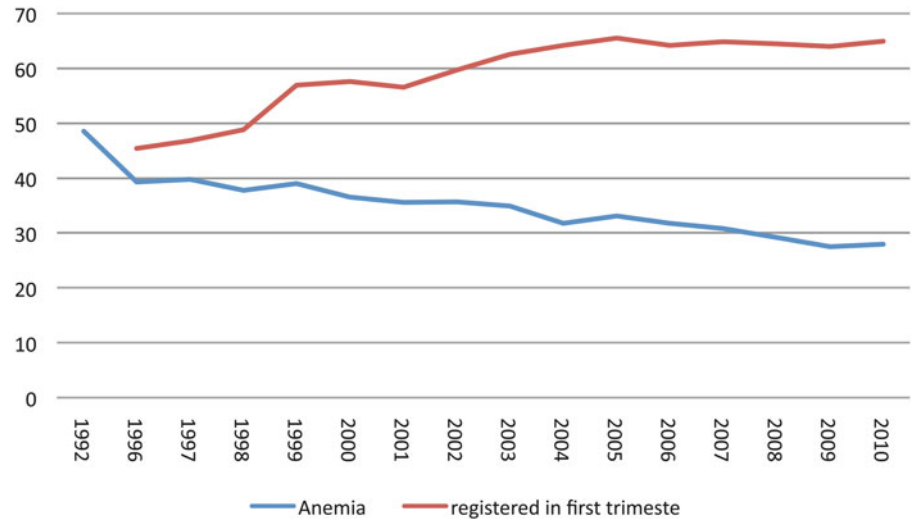

Fig. 25.5 Trend of anemia among pregnant women at first booking, and the percentage screened at the first trimester (unpublished, Department of Statistics and Information, MoH, Oman 2011)

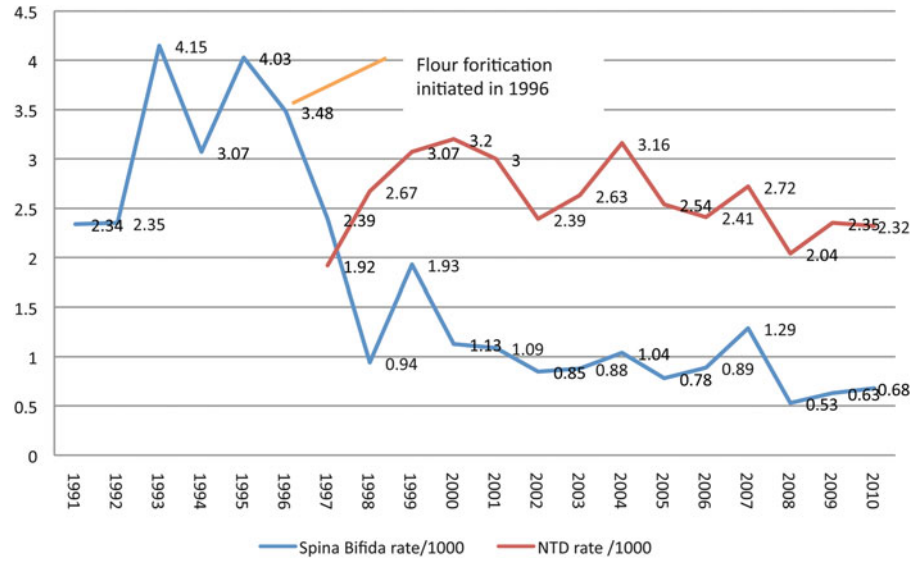

Fig. 25.6 Neural tube defects and spina bifida in Oman 1991–2010 (unpublished, Department of Statistics and Information, MoH, Oman 2011)

Conclusions

Political and Contextual Factors

Oman had been blessed with unique circumstances due to the fact that the Mills industry is contained within two large factories that are well equipped to cope technologically and politically with the introduction of flour fortification. The status Oman Flour Mills acquired among the millers society is a

driving factor to sustain the program (personal communication, Oman Flour Mills, Oman 2011). The low cost of fortification for the millers, estimated at less than US$1/t adds a convenience factor, especially with the large production capacity. However, this may not hold true in all countries where there are multiple mills and sources of wheat and flour.

Quality control and supervision was an achievable component of the program which facilitated issuing the Ministerial decree and was not challenged with hurdles such as limited manpower for supervision and control, and lack of financial resources.

Iron and flour supply from fortification are very different in bioavailability and requirements. Folate had been estimated to have a bioavailability of 60–80 % of the requirements consistently among age groups and it can be added safely to flour at levels that could provide almost 90 % of EAR. Iron on the other hand have low bioavailability, requirements vary greatly between different population groups and its consumption is affected by diet composition, nutritional and health status of the subject, and the form of iron introduced in the flour. Moreover addition of high amounts of Iron could alter the physical properties of bread and baked products making these less palatable.

Another factor of concern is that iron bioavailability is very low in high extraction rate (high fiber) flour and therefore its fortification may not be effective which introduces an additional issue which is striking a balance between fiber and iron recommendations of the diet. In Oman, almost 50 % of the flour produced in 1996 was whole wheat, which is reduced to less than 10 % in 2010. This had been evident in the National Nutrition Survey 2004 which showed that 1–2 % of the grain consumed was whole grain, and various population groups consumed about 40–50 % of the required whole grains [32].

Comorbidities and Genetic Factors

The health and nutritional status of a population are important factors that could promote or confound the effectiveness of flour fortification. The years from 1996 to 2010 in Oman have seen improvement of primary health care services, substantial health education activities, and improvements in wealth and education of the population. On the other hand it was observed that 9.5 % of the children suffered genetic haemoglobinopathies. One cannot ignore that these children could suffer nutritional anemia in addition to the genetically predisposed low hemoglobin levels. On the other hand, it should be noted that their hemoglobin levels will not improve through fortification. Realistically, the minimum prevalence of anemia this population could achieve is above 9 %. Anemia among men in Oman was 12.1 % in 2004 (95 % CI = 7.8–18.2), compared to 14 % in 1993[3] [30].

Outcome

The most pronounced outcome observed was that of folate fortification. Reduction of spina bifida to less than 20 % of its original rate is a significant achievement that has important implications on health cost savings, let alone the value of emotional lifelong strain that affects families and their communities. Number of deliveries in Oman went up from almost 45,000 in 1991 to about 60,000 in 2010. Consistent with Maize fortification (Chap. 7), flour fortification is estimated to avert NTDs of 1,350 births in Oman from spina bifida alone. In the United States, the annual QALYs gained were US$15,842 million from 182 cases gained because of fortification. It was also observed that folate fortification has an important impact on Myocardial infarction, colon cancer, and B12 deficiency masking.

[3] Analysis of data from the National Diabetes Survey 1993.

The QALYs cost of NTDs could be significantly different in Oman, but even with the most conserved approximation it can be concluded that fortification saves millions of dollars annually due to NTDs reduction alone. Colon cancer and Myocardial infarction had more QALY gains than NTDs, which leads to the conviction that fortification may have contributed significantly to national economy.

On the other hand, the impact of iron fortification is inconsistent among various population groups, despite findings of clinical trials (Chap. 7), which illustrates the importance of the context. Trials that are carried under controlled conditions may overestimate the true impact at a population level. In Oman, children had increased rates of anemia over the last few years, and this was more pronounced among the age of 3–5 years who consumed fortified flour. But the rates among women and adolescents are declining. In 2004 anemia among school children was 39 % and 31.8 % among women of childbearing age, both went down to 27.9 % in 2010. Yet these rates rank Oman among countries of severe public health problems for preschool children and pregnant women unlike neighboring gulf countries such as Kingdom of Saudi Arabia and United Arab Emirates, whereas it ranks in medium public health category for women of childbearing age [41].

Recommendations

High rates of anemia among preschool children indicate that concerted and evidence based efforts to control the problem are needed, especially in the view of the increasing trend and the overall rates that exceed 40 %, the threshold for a severe public health problem.

In 2010 a ministerial decree was issued to increase iron in flour and provide more options for fortificants. Surveillance of anemia among women, and newly introduced surveillance of anemia among children at the age of 9 and 18 months will show the impact of this amendment (personal communication, Department of Statistics and information, MoH, Oman).

Finally, the impact of fortification is contextual and is dependent on adequate process and coverage, population health, as well as the economic and social settings. Additional interventions should target high risk population groups and considerations of whole diet approach and prevention of chronic diseases through implementation of food-based dietary guidelines should be incorporated into the design, implementation, monitoring, and evaluation of fortification [44, 46, 49].

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Chapter 26
Strategies to Improve Micronutrient Status of Infants and Young Children with Special Attention to Complementary Foods Fortified with Micronutrients: Perspectives from Vietnam

Jacques Berger, Frank T. Wieringa, Arnaud Laillou, Phu Pham Van, and Marjoleine A. Dijkhuizen

Key Points

- Stunting and micronutrient deficiencies are still prevalent in Vietnam and other developing countries, and effective actions are needed.
- Integrated interventions should be directed to women in reproductive age before and during pregnancy and breastfeeding, and infants and young children.
- Appropriate complementary feeding practices are particularly important and should follow the WHO guidelines.
- Complementary foods of good quality can be manufactured locally and made available and accessible to all families especially the poorest, in accordance with the International Code of Marketing of Breast-milk Substitutes.
- The efficacy of appropriate complementary feeding on the nutritional status, growth, and development of infants and young children has been proved in Vietnam and in many other settings.
- The awareness, commitment, and support of communities and government are essential to ensure the effectiveness and sustainability of complementary feeding interventions.
- The fortification of staple foods and condiments with micronutrients may be an alternative strategy to improve the micronutrient status of populations including women of childbearing age, infants, and young children.

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V.R. Preedy et al. (eds.), *Handbook of Food Fortification and Health: From Concepts to Public Health Applications Volume 2*, Nutrition and Health, DOI 10.1007/978-1-4614-7110-3_26,

Keywords Micronutrient deficiencies • Stunting • Infants • Young children • Complementary foods • Developing countries • Vietnam

Introduction

Undernutrition is a major public health problem among infants and young children in many Asian countries, especially in South Asia. Malnutrition, including micronutrient deficiency, has a direct impact on morbidity and mortality of infants and children. Indeed, vitamin A deficiency alone has been associated with 0.6 million child deaths/year, and zinc deficiency is estimated to cause another 0.4 million child deaths/year [1]. Malnutrition during the first years of life, even before birth, also has long-term effects, and has been related to the development of non-communicable chronic diseases [2]. Moreover appropriate infant and young child feeding practices contribute to the prevention of overweight and obesity in early childhood, thus mitigating the dual burden of malnutrition experienced in many countries.

In Vietnam, the health and particularly the nutritional status of Vietnamese children have improved over the last 2 decades due to a period of rapid economic growth [3] and reduction in poverty [4, 5]. This is clearly demonstrated by the sharp decline in the prevalence of underweight among children under 5 years of age, from 51.0 % in 1990 to 26.6 % in 2004 [6] and then to 17.5 % in 2010 [7]. Similarly, the prevalence of stunting decreased from 60 % in 1985 to 30 % in 2005 [8] and to 29.3 % in 2010 [7]. But this prevalence of stunting close to 30 % also shows that there is still reason for concern, as the reduction of stunting has slowed down in the last years and remains of public health importance.

A recent survey on nutritional status of women and young children carried out in 2010 in a subsample of rural and urban households from 19 randomly selected provinces in Vietnam confirms these data. It showed that 30.1 % of children under 2 years of age were stunted, 10.4 % were underweight, 2.1 % were wasted, and 8.4 % were overweight (personal communication). In addition, 15.3 % of children under 2 years of age had anemia (Hb <110 g/L) and 7.1 % had a hemoglobin concentration between 110 and 115 g/L. Moreover, 41.1 % of the children <2 years of age had iron deficiency (plasma ferritin <12 μg/L), 53.2 % had zinc deficiency (plasma zinc <9.9 μmol/L), 11.8 % vitamin A deficiency (plasma retinol <0.70 μmol/L), 50.6 % marginal vitamin A status (plasma retinol between 0.70 and 1.05 μmol/L), 58.7 % vitamin D deficiency (plasma 25(OH)D <50 nmol/L), and 98.6 % mild hypocalcaemia (between 0.9 and 1.15 mmol/L).

Preventing malnutrition in infants and young children is thus an urgent current challenge. It is now widely accepted that preventing malnutrition of infants and young children requires an integrated approach that includes complementary actions to improve maternal, infant, and young child nutrition [9]. Different stages in the life cycle have to be considered: the period of preconception with the nutrition of adolescent girls and women of reproductive age [10], the pregnancy period [11], the perinatal period including delivery practices (e.g., delayed cord clamping) and the early neonatal period [12], the period of breastfeeding with a special attention to exclusive breastfeeding from 0 to 6 months of age, and the period for complementary feeding (6–24 months).

Infant and young child feeding interventions are critical to promote growth, development, and survival of children. Over a third of all deaths of children under 5 years of age are directly or indirectly caused by undernutrition. Inversely an estimated 13 % of child lives could be saved through improved breastfeeding practices alone [1] and an additional 6 % of child lives through improved complementary feeding practices [13]. In this chapter, we will therefore focus on the complementary feeding period from 6 to 24 months. This chapter will also discuss the importance of continued breastfeeding during the two first years as well as approaches to improve the quality and accessibility of foods available for complementary feeding from 6 months of age.

The Complementary Food Approach in Vietnam

Definition and Production of Complementary Foods

Adequate complementary feeding practices (Table 26.1) includes the timely use of appropriate complementary foods as a necessary condition to prevent malnutrition [14]. However, very often simply encouraging mothers to prepare appropriate food products at the appropriate time from local available foods has proven to be a challenge in developing countries, especially in populations with low economic resources. The cost and availability of high-quality foods, such as animal products, and the time and knowledge required to prepare balanced meals severely constrain the effectiveness, sustainability, and acceptability of such an approach. To address these issues, the *Fasevie* project had been developed in Vietnam, in partnership between the National Institute of Nutrition in Hanoi (subsidiary directly under the Vietnam Ministry of Health), the GRET, a French NGO (*Groupe de Recherche et d'Echanges Technologiques*), and the IRD (the Institute of Research for Development, France). The *Fasevie* project was conceived to develop easy-to-use micronutrient-fortified complementary foods targeted at low-income families. Different approaches and products, as well as production and potential marketing strategies, were investigated.

Under the *Fasevie* project, two types of products were developed. First, a rice-based instant flour since it was determined that caretakers were aware of microbiologic risks and safe water is easily available in Vietnam. The instant flour was made with rice (51.3 %), soybeans (20.8 %), sugar (15.0 %), sesame (5.0 %), dry milk (5.0 %), iodized salt (0.7 %), $Ca_3(PO_4)_2$ (1.2 %), vitamin–mineral premix (0.8 %), and aromas (0.2 %). The technology of extrusion cooking that was used to produce the instant flour enabled the inactivation of some of the antinutrient factors (such as phytates) and the preparation of gruels without involving other thermal treatment, making the product suitable for "instant" use without further cooking [18]. The second product was a soybean-based food complement with amylase. It was made with soybeans (87.7 %), iodized salt (3.1 %), $Ca_3(PO_4)_2$ (6.1 %), vitamin–mineral premix (3.1 %), and amylases (0.03 %). This product was intended to be added to the gruel that was cooked again for 10 min, to enrich the normal traditional gruel as prepared by the mother and to decrease the viscosity of the gruel. Both the extrusion cooking process for the fortified instant flour product and the addition of amylase in the food complement increased the digestibility and the energy density of complementary foods [15]. The nutrient composition of

Table 26.1 Guiding principles for complementary feeding (adapted from [27])

1. Practice exclusive breastfeeding from birth to 6 months of age, and introduce complementary foods at 6 months of age (180 days) while continuing to breastfeed
2. Continue frequent, on-demand breastfeeding until 2 years of age or beyond
3. Practice responsive feeding, applying the principles of psychosocial care
4. Practice good hygiene and proper food handling
5. Start at 6 months of age with small amounts of food and increase the quantity as the child gets older, while maintaining frequent breastfeeding
6. Gradually increase food consistency and variety as the infant gets older, adapting to the infant's requirements and abilities
7. Increase the number of times that the child is fed complementary foods as he/she gets older
8. Feed a variety of foods to ensure that nutrient needs are met
9. Use fortified complementary foods or vitamin-mineral supplements for the infant, as needed
10. During illness, increase fluid intake, including more frequent breastfeeding, and encourage the child to eat soft, varied, appetizing, favorite foods. After illness, give food more often than usual and encourage the child to eat more

Table 26.2 Amounts of nutrients to be supplied daily by complementary foods by age groups

Age group	Unit	6–8 months	9–11 months	12–23 months
Energy[a]	kcal	202	307	548
	kcal	356	479	772
Lipids (30/45 %)[b]	g	0/8	2/13	10/26
	g	0/13	3/20	15/36
Protein	g	3.9	4.5	7.2

[a]First line: mean energy requirements. Second line: energy requirements for 97.5 % of infants
[b]First line: to meet mean energy requirements. Second line: to meet energy requirements for 97.5 % of infants and children. On each line, first number: in order for 30 % of total energy to be from lipids, the percentage of energy in complementary foods as lipids is 0 %, 5 %, and 17 %, respectively, for 6–8 months, 9–11 months, and young children. Second number: in order for 45 % of total energy to be from lipids, percentage of energy in complementary foods is 34 %, 38 %, and 42 % of energy as lipids, respectively, for 6–8 months, 9–11 month,s and young children

Table 26.3 Amounts of vitamins to be supplied daily by complementary foods by age groups

Age group	Unit	6–8 months	9–11 months	12–23 months	UL[a](/day)
Vitamin A[b]	µg RE	163/63	192/92	25/125	600
Biotin[c]	µg	0.61	1.07	3.61	ND
Choline[c]	mg	42.2	51.4	112.2/103.3	1,000
Folic acid	µg	22.7	27.6	113.3	300
Niacin	mg	2.8	2.9	5.0	10
Pantothenic acid	mg	0.32	0.44	0.79	ND
Riboflavin	mg	0.16	0.18	0.31	ND
Thiamin	mg	0.16	0.17	0.38	ND
Vitamin B6	mg	0.21	0.22	0.43	30
Vitamin B12[b]	µg	0.25/0.45	0.24/0.44	0.67	ND
Vitamin C[b]	mg	23/3	25/5	0/8	400
Vitamin D	µg	1.2	1–2	1–2	25/50[d]
Vitamin E[c]	mg	2.8	3.0	4.2	200[e]
Vitamin K[b]	µg	1.4–8.9	1.5–9.0	10	ND

[a]*UL* upper limits
[b]According the reference values from IOM—FAO/WHO 2004
[c]Reference value only from IOM
[d]For infants/young children respectively
[e]Alpha-tocopherol equivalent

the products was based on the latest recommendations available at that time for a breastfed child 6–24 months of age [16]. Since then, we conducted a review of available literature and Tables 26.2, 26.3, and 26.4 present the amounts of nutrients that should be supplied daily by complementary foods (Prigge S and Berger J, personal communication) assuming an average intake of breast milk of 674 mL/day for 6–8 month old infants, 616 mL/day for 9–11 month old infants, and 549 mL/day for 12–23 month old children [17].

The nutrient compositions of both complementary foods produced in Vietnam is summarized in Table 26.5 (adapted from [18]). As a result, both products allowed the preparation of porridges of adequate viscosity, high-energy density, and adequate macronutrients and micronutrients. The nutritional composition of gruels prepared from both products was significantly better than traditional rice gruels as indicated in Table 26.6 (adapted from [15]). Both products were easy and convenient to use, with the instant product giving considerable savings in time and effort, and shelf-life was comparable between the two products.

Table 26.4 Amounts of minerals to be supplied daily by complementary foods by age groups

Age group	Unit	Bioav.	6–8 months	9–11 months	12–23 months	UL[a] (/day)
Calcium[b]	mg		0/28	0/70	115	–
Chloride	mg		287	321	1,269	2,300
Copper[c]	mg		0.2–0.4	0.2–0.4	0.2–0.4	1
Fluoride[d]	mg		–	–	–	
Iodine	µg		35–0	40–0	10–0	200
Iron	mg	5 %	18.6	18.6	11.6	40
		10 %	9.3	9.3	5.8	
Magnesium	mg		51–30	53–32	61–41	65
Manganese[e]	mg		0.6	0.6	1.2	2
Phosphorus[d]	mg		200	200	385	3,000
Potassium[d]	mg		346	377	2,712	ND
Selenium	µg		1.4–0.6	11.4–15.6	12.4–9.4	60/90[f]
Sodium[d]	mg		276	284	923	1,500
Zinc[g]	mg	30 %	2.0	2.1	1.1	6–8[h]
		23 %	2.6	2.7	1.5	
Zinc[i]	mg	30 %	2.0	2.1	2.1	23–28[j]
		15 %	4.0	4.1	4.2	

[a]*UL* upper limits
[b]According to accretion rate of 80/100 mg/day
[c]Recommended range (Rosado, 2003)
[d]Reference value from IOM
[e]Lutter and Dewey, 2003
[f]For infants/young children respectively
[g]IZiNCG 2004 with 30 or 23 % zinc absorption
[h]NOAEL IZiNCG 2004
[i]FAO/WHO 2004 with zinc absorption of 30 and 15 %
[j]FAO/WHO 2004

Efficacy of the Complementary Foods on Nutritional Status of Infants and Young Children

Once both products were available, an efficacy study was conducted in Tam Ky district, Quang Nam province, in central Vietnam. Twenty-nine villages were randomly divided into those receiving the energy-dense gruels prepared with the instant flour, the energy-dense gruels prepared with the micronutrient-fortified food complement, or nothing (control) [18]. The energy-dense fortified foods were distributed free of charge through canteens especially set up for the study, which were open daily from 0600 to 1800 hours. The goal of this intervention was to ensure that all infants received at least 2 meals/day of adequately and safely prepared experimental gruels. The duration of distribution of energy-dense fortified foods was 6 months. Nutritional status was assessed by measurement of anthropometry, hemoglobin and plasma ferritin, transferrin receptors, zinc, and retinol at baseline and the end of the 6-month intervention period. The products were designed to provide 100–120 kcal/100 mL of porridge.

Due to the incorporation of amylases, gruels made from the food supplement had a slightly higher energy density and a more appropriate consistency, which resulted in higher intakes per meal than gruels made from instant flour. In comparison with homemade complementary foods, both products resulted in significantly higher energy and nutrient intakes and increased sufficiently both energy and nutrient intakes of infants to complement their breastmilk intake [16].

Table 26.5 Composition of the raw instant flour and the food complement (adapted from [18])

Nutrients	Instant flour unit/419 kJ	Food complement unit/419 kJ
Raw protein, g	3.9	9.7
Digestible protein, g	3.6	8.8
Lipids, g	2.24	5.3
Vitamins[a]		
all-*trans* Retinol μg RE	38	154
Ascorbic acid, mg	20	87
Thiamin, μg	68	229
Riboflavin, μg	95	309
Nicotinamide, μg	368	535
Folic acid, μg	12	46
Pantothenic acid, μg	330	904
Cyanocobalamin, μg	0.06	0.16
Pyridoxine, μg	53	98
Phylloquinone, μg	8.4	38
Cholecalciferol, IU	117	516
Minerals		
Sodium, mg	73	343
Potassium, mg	143	512
Chlorine, mg	121	553
Calcium, mg	138	710
Phosphorus, mg	117	473
Magnesium, mg	21.5	61
Iron, mg	7.6	33
Zinc, mg	1.7	5.9
Iodine, μg	5.1	22
Copper, μg	92	284
Manganese, μg	319	942
Selenium, μg	2.1	4,5

[a]Chemical form of vitamins and minerals: dry retinol-acetate, thiamine monohydrate, riboflavin, niacinamide, calcium-D-pantothenate, vitamin B12 0.1 % WS, sodium ascorbate cryst., dry vitamin D3 100 CWS/A, potassium iodine, iron fumarate, zinc sulfate monohydrate, magnesium sulfate dried, and potassium dihydrogen phosphate

Table 26.6 Energy and nutrient contents and nutrient densities of daily average samples of gruels and other complementary foods

	Instant flour gruel	Food complement gruel	Homemade gruels
Energy and nutrient contents (per 100 g of ready-to-eat food)			
Energy (kcal)	99 (18)[a]	105 (12)[b]	71 (39)[c]
Protein (g)	3.61 (0.6)[a]	4.30 (0.6)[b]	2.62 (1.3)[c]
Lipid (g)	2.07 (0.04)[a]	1.63 (0.24)[c]	1.85 (1.82)[a, c]
Calcium (mg)	116 (22)[a]	175 (37)[b]	18 (39)[c]
Iron (mg)	5.37 (1.0)[a]	8.59 (2.1)[b]	0.43 (0.3)[d]
Zinc (mg)	1.51 (0.25)[a]	1.85 (0.29)[b]	0.39 (0.33)[c]
Energy and nutrient densities (per 100 kcal)			
Protein (g)	3.65 (0.1)[a]	4.13 (0.3)[b]	3.70 (1.2)[a]
Lipid (g)	2.03 (0.34)[a]	1.70 (0.38)[c]	1.35 (1.67)[d]
Calcium (mg)	113 (26)[a]	166 (20)[b]	28 (46)[c]
Iron (mg)	5.51 (0.4)[a]	8.17 (1.3)[b]	0.60 (0.5)[c]
Zinc (mg)	1.50 (0.14)[a]	1.77 (0.23)[b]	0.65 (0.61)[c]

Medians (interquartile ranges). Labeled medians without a common letter (a, b or c) differ ($p < 0.05$; Mann–Whitney's test)
Adapted from [16]

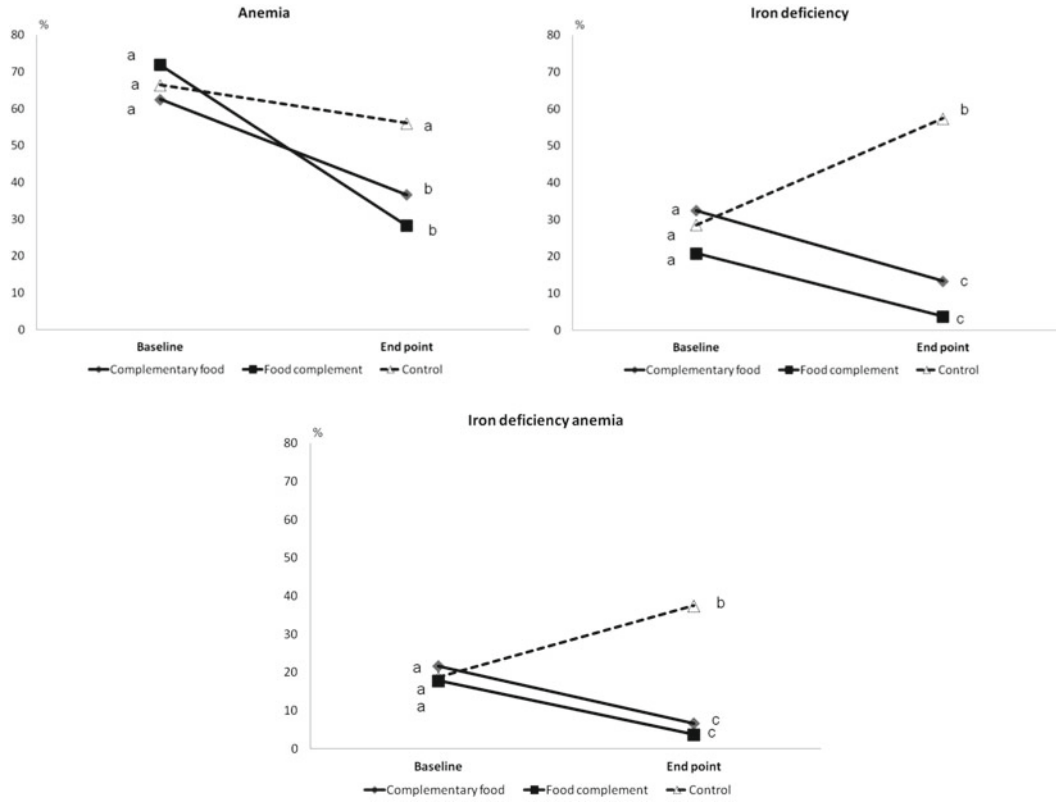

Fig. 26.1 Prevalence of anemia, iron deficiency, and iron deficiency anemia before and after the intervention (Labeled prevalence without a common letter (a, b or c) differ, $p < 0.05$)

This intervention resulted in a better iron status and lower prevalence of anemia, iron deficiency (ID), and iron deficiency anemia (IDA) in infants compared with those following usual feeding practices in the region [18]. Whereas prevalence of anemia, ID, and IDA decreased significantly at the end of intervention in both the experimental groups, prevalence of anemia did not change and prevalence of ID and IDA increased significantly in the control group (Fig. 26.1). At the end of the intervention, plasma zinc and retinol did not differ significantly between the three groups, but endpoint prevalence of zinc deficiency was lower in the group who received the instant flour compared with the control group.

Several elements of the intervention may explain the positive impact on the micronutrient status of infants including the hygienic preparation of meals in the intervention groups. However, the regular consumption of gruels fortified with ferrous fumarate and other micronutrients was the main cause of improvement of anemia and iron status in the infants. Indeed, the experimental gruels were consumed mainly in place of homemade gruels, did not affect the total duration and frequency of breastfeeding, and allowed higher micronutrient intakes of iron (>13 times) and zinc (>2.7 times) [15]. The daily micronutrient intakes in infants in the instant flour and food complement groups met about 80 % and 119 % of the iron requirements, respectively, assuming an iron bioavailability of 5 %, and 102 and 127 % of zinc requirements assuming a zinc bioavailability of 23 % for cereal-based foods, whereas the control group met only 8 % of iron and 27 % of zinc requirements.

Only a few studies have evaluated the impact of fortified complementary foods fortified on micronutrient status and growth. A recent review on fortified complementary food [19], mostly fortified

with iron and sometimes other micronutrients, estimates an impact on mean hemoglobin concentrations of 4–6 g/L and a reduction of 13–17 % in the prevalence of anemia. In some studies, ferritin was also measured, showing that in most cases, the impact was greater on the prevalence of iron deficiency (ID) than on IDA. A randomized control trial carried out in South Africa examined the effect of a micronutrient-fortified, maize-based porridge on anemia and micronutrient status of infants aged 6–12 months compared with a control group that received an unfortified maize porridge [20]. Plasma ferritin and hemoglobin concentration increased by 9.4 μg/L and 9 g/L, respectively, and the proportion of anemic infants significantly decreased. However, the consumption of fortified porridge for 6 months had no consistent effect on plasma retinol concentrations and did not improve plasma zinc concentrations. Our results are consistent with these findings, with an increase of ~10 μg/L in ferritin concentrations and increases of 3.3 and 6.7 g/L in hemoglobin concentrations for the instant flour and the food complement groups, respectively.

The interventions with the complementary food and the food complement also had an impact on the growth of the infants. After the 6-month intervention, weight, length, height-for-age (HAZ), and weight-for-age (WAZ) were significantly higher in the two experimental groups compared to the control group with an estimated effect size >0.20 HAZ scores for both the intervention groups. Children were again measured 6 and 18 months after the end of the intervention. Six months after the end of the intervention, the difference in HAZ scores between the control and the instant flour group was almost the same as directly at the end of the intervention. Eighteen months after the intervention, the difference between the same two groups was still 0.15 HAZ score, although, mainly due to a lack of power because of loss to follow-up, this difference was not statistically significant anymore. In contrast, ponderal growth (WHZ score and BMI z-score) was significantly higher in the instant flour group than in the food complement group with the control group positioned in between both intervention groups. This result suggests that the fortified instant complementary food provided a more complete array of nutrients, including proteins and lipids, enabling a more optimal growth process with perhaps more, denser and more diverse tissue formation, than the food complement which stimulated growth by providing a limited number of micronutrients without providing all nutrients needed for optimal growth. This could have led to growth that may be incomplete (e.g., no lean body mass increase) or not sustained (until the next limiting nutrient is depleted). Optimal growth would mean optimal new tissue formation, and this would in fact increase the growth potential of the subjects for a longer period after the intervention.

In conclusion, this efficacy study showed that the daily consumption of complementary foods made with local staple foods fortified with iron and other micronutrients and adequately prepared and used significantly decreased the prevalence of anemia, improved iron status, and prevented the decline of iron stores, which is often seen in infants in developing countries between 6 and 12 months of age. Moreover, this study shows that in Vietnam the general pattern of growth faltering during late infancy, which is observed in many developing countries, can be partly prevented by interventions including the provision of complementary foods with a higher energy, and a higher macro- and micronutrient density than traditional gruels.

Effectiveness of the Complementary Food Project on the Nutritional Status of Vietnamese Infants and Young Children

The production of the two complementary foods was firstly carried out at district level in two provinces by four median size companies (two public, one semiprivate, and one private). The instant infant flour was packed in plastic 400 g boxes containing 11 servings of 35 g each. In 2008, the consumer price was around 17,000 VND (US$1.06 per box, US$ 2.65/kg or US$0.10 per serving of infant flour). The complementary food supplement was packed in 150 g bags. Its consumer price was 6,000 VND (US$0.38) per bag containing 13 servings of 11 g each (US$ 0.03/serving) [21]. Promotion and

marketing of the two complementary foods were made in compliance with the "International Code of Marketing Breast-Milk Substitute" adopted by the World Health Assembly in 2001.

The distribution system at the village level was based on a network of volunteers from the Vietnamese Women's Union, in which the volunteers benefitted from a small margin between retail price and the price sold to the end consumers. A program of seven home visits was developed, including visits from the third trimester of pregnancy until the child reached the age of 24 months. During these visits, women were given adequate information on their status about pregnancy, hygiene, vaccination, breastfeeding, and infant and young child nutrition. The complementary foods were introduced and sold only when the infant reached 6 months of age, in line with the WHO guidelines. Most of the women preferred the instant flour. Attempts were also made to market the products through the existing distribution sales networks. Unfortunately, this tentative was unsuccessful, for a variety of reasons including lack of interest from wholesalers, insufficient sales volume to interest grocery shops over the long term, the costs of promoting a new product, and, probably most importantly, the unwillingness of the Women's Union to allow others to sell the product also.

The monitoring activities of the project showed that the proportion of target families that purchased the flour was only 13 % with an average of 1.2 box of 400 g per month corresponding to only three servings per week. Although many factors were probably involved in the low purchase rate, two main reasons have been identified. The first reason was the relative large container size of the instant flour and thus the high cost for a one-time purchase despite that the price was only half of that of the cheapest fortified infant flour available on the market and representing only 4 % of per capita monthly income. The second reason was the limited distribution of the instant flour due to gaps in the distribution network and to delays from the Women Union to collect the funds generated by the sales of the product to pay producers on time [21]. Consequently, the enterprises stopped their activities within a few years after the launch of the production due to insufficient support from the local authorities and insufficient sale volumes of the infant flour.

Upscaling of the Project

As planned at the start of the project, support from GRET and IRD ceased after some years. In 2006, the National Institute of Nutrition received a Grant from the Asian Development Bank (ADB) to reduce the incidence and severity of malnutrition among low-income, vulnerable, and primarily rural children by expanding access to improved feeding practices, including giving fortified complementary foods to children 6–24 months of age. The expansion of the project from the *Fasevie* model to a government-managed model encountered a number of unexpected difficulties, and a new program design, centered on the Protein Energy Malnutrition Control Program (PEMCP) of the MOH, was developed. The program had four main components: Expanding and building localized production of low-cost nutritious complementary food; implementing community-based complementary food sales and enhanced nutrition Education; developing innovative approaches for expanding distribution and access to reach the poorest and most vulnerable; and project management, monitoring, and evaluation. However currently available information let us think that no effective actions have been performed until now, 5 years after the start of this program.

Perspectives

The Complementary Food project implemented in Vietnam demonstrates that production of high-quality complementary foods and/or food complement made with local staple foods and local material is feasible. The regular consumption of nutrient balanced complementary foods to supplement

breast milk after 6 month of age improved the micronutrient status of infants, especially of iron status, and had a positive effect on stunting. However, physical and economical availability of these complementary foods as well as their adequate use by families, especially the poorest ones, are keys factors for their efficiency.

In the 2008 Lancet Series about Maternal and Child Nutrition, Bhutta et al. reviewed the potential interventions that can benefit maternal and child undernutrition and survival. The interventions included were very broad, ranging from promotion of breastfeeding, to strategies to promote complementary feeding (with or without provision of food supplements), to general supportive strategies to improve family and community nutrition to overall reduction of disease burden. They showed that although strategies for breastfeeding promotion have a large effect on survival, their effect on stunting is small. In contrast they concluded that appropriately designed interventions can have a positive effect on feeding practices. In populations that had sufficient means to procure appropriate food, education strategies alone to improve complementary feeding practices were of most benefit and increased HAZ score by 0.25 (95 % CI 0.01–0.49). In food-insecure populations provision of food supplements (with or without education) increased the HAZ score by 0.41 (0.05–0.76), a figure that is consistent with the results obtained with the Complementary Food project in Vietnam.

A review on infants and young child feeding practices in Vietnam was recently published [22]. The proportion of children aged 0–24 months ever breastfed is high and has remained consistent over time in Vietnam. In contrast, the median duration of breast feeding is less than recommended by WHO, even among the 2-month-old age group. Prevalence of exclusive breast feeding for the first 6 months is very low, however, with wide regional variability, and overall rate is apparently declining during the last years. As seen in most developing countries, exclusive breast feeding rates are lower in urban areas compared to rural areas. Early introduction and poor quality of complementary foods are also of substantial concern in Vietnam. This review shows that the feeding practices found at the beginning of the *Fasevie* project have not changed dramatically over the last decade, underlining the continuing need for the development of cheap, nutritious complementary foods for the poor. The review especially evaluated barriers to adequate infant and young child complementary feeding in Vietnam. Although many barriers were identified, including the lack of political will and coordination in enforcement and compliance with the WHO Code on marketing and usage of infant formula, three barriers are of importance within the context of this chapter: poor infant and young child complementary feeding practices among health providers; poor knowledge and practical skills related to infant and young child complementary feeding, and financial constraints. Clearly, there is a lot of scope to improve infant and young child feeding practices in Vietnam. The strong and active *Vietnam Women Union*'s and the consistent support from family members should provide a stable basis to build a comprehensive framework for this.

Surveys conducted at the beginning of the *Fasevie* project and the review by Phuong et al. indicate a low consumption of animal source foods such as meat or eggs and other food such as fruits by young children. Thus strategies to improve the intake of essential nutrients in this age group have to be developed. As outlined above, the micronutrient-fortified complementary foods could be one of such a strategy, although the effectiveness of the scale-up of this intervention was never evaluated properly. Other strategies could include micronutrient powders to be added to meals, improving the micronutrient content of traditional homemade complementary food or Energy-dense fat-based spreads [23]. However, if their efficacy has been demonstrated in controlled trials, their effectiveness on stunting prevalence has not been proved and their use in Vietnamese populations not assessed.

Fortification of staple food or/and condiments could be very cost-effective strategies to improve the overall micronutrient status of the Vietnamese population including young children. Vietnam is one of the largest rice producing countries in Asia. Moreover the per capita consumption of rice in Vietnam is one of the highest in the world with 165 kg milled rice/person/year (FAOSTAT Database, 2007. FAO, Rome), providing >60 % of daily energy requirements. However, rice is commonly milled to

yield white rice with >50 % of key vitamins and minerals being lost during milling. New technologies have resulted in the development of techniques to fortify rice without increasing costs substantially. Important, the taste, smell and appearance of this fortified rice is well accepted in South East Asia (FT Wieringa, personal data).

In Vietnam, in addition to rice, several staple foods and condiments can be fortified with micronutrients such as vegetable oil with retinyl palmitate, wheat flour with iron, zinc and folic acid, fish and soy sauces with iron. In the 2010 micronutrient survey in Vietnam, a subsample of children aged 6–75 months was surveyed and individual food consumption assessed using the 24-h recall method combined with controlled food weighing. Among the 6–11 months old children 81 % consumed rice daily, 56 % vegetable oil and 38 % sauces (mainly fish and soy sauces) and almost none wheat flour. The corresponding mean (±SD) daily consumption among 6–11 month old children was 105 g (±17 g) for rice, 8 g (±3 g) for vegetable oil, and 8 g (±6 g) for sauces. In the 12–23 month old children the daily consumption of rice was almost doubled whereas consumption of others foods did not change significantly. In this age category, 18 % consumed wheat flour with a mean daily consumption of 48 g (±10 g).

We thus calculated the potential contribution that these fortified products would bring to the Vietnamese recommended daily allowances [24] for children assuming that the selected foods would be fortified with levels of micronutrients set to the most current national or international recommendations [25].

In the 6–11 month old children fortified rice would bring on average 8 % of iron RDA (1.0 mg) assuming a 10.0 % iron bioavailability, 8.1 % of zinc RDA (0.3 mg) and 43.8 % of folic acid/Vitamin B9 RDA (36 μg). Fish and soy sauces would provide about 10.0 % of iron RDA (1.2 mg) and vegetable oils 15.8 % of vitamin A RDA (63 μg RE).

In the 12–23 month old children, fortified rice would cover on average 23.1 % of their iron RDA (1.8 mg), 14.5 % of zinc RDA (0.6 mg) and 39.1 % of folic acid/Vitamin B9 RDA (63 μg). Fish and soy sauces would provide about 16.1 % of iron RDA (1.24 mg) and vegetable oils 15.8 % of vitamin A RDA (63 μg RE). Wheat flour would provide higher micronutrient quantity respectively 19.7 % of iron RDA, 46.3 % of zinc RDA and 62.5 % of Vitamin B9 RDA but wheat flour was used by only 18 % of 12–23 month old children.

These results show that fortified foods that are often used daily in combination will provide a non-negligible part of required micronutrients, higher for the 12–23 month old children as they consumed higher quantity of staple foods, and consequently higher quantity of micronutrient regarding their RDA. The fortified products will also be consumed by women in reproductive age, before and during pregnancy and lactation that will be a very important contribution to improve their nutritional status and those of their babies. In Vietnam, the use of iron-fortified fish sauce in meals during 1 year prevented anemia and iron deficiency and increased iron stores of women of reproductive age [26]. After 1 year iron stores were in average of 350 mg (300–410 mg) and 369 mg (310–485) after 18 months, corresponding to the ideally quantity of 300 mg or more iron reserves prior to conception to meet iron needs during gestation [10].

Conclusion

To conclude, micronutrient deficiencies continue to affect a large portion of the infants and young children in Asia, thereby negatively affecting health, growth, and development. In this chapter we have outlined several feasible, cost-effective strategies which could substantially improve the micronutrient status of infants and young children. For all of these strategies, efficacy has been proven in Asia or other settings. Therefore, introduction and effectively scaling up of these interventions are needed now. But for this, the political will and commitment to invest in the new generation are needed.

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Chapter 27
Food Fortification Programmes in Pakistan

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Key Points

- People in Pakistan are confronting micronutrient deficiency especially vitamin A, iodine, and iron, identified as major preventable risk factors.
- Fortification of nutrients in staple diet can reduce deficiency diseases.
- Most Pakistanis in the Northern areas, Khyber Pakhtunkhwa, and some parts of Punjab suffer from iodine deficiency disorders.
- Iron deficiency anaemia is a major micronutrient problem especially among preschool children and pregnant and lactating women.
- Pakistan launched several programmes to combat nutritional disorders including Universal Salt Iodization Programme, Iodine Nutrition Programme, Global Alliance for Improved Nutrition, Assisted National Wheat Flour Fortification Project, and World Food Programme.

Keywords Micronutrients • Fortification • Vitamin A • Iodine • Iron • Pakistan

Abbreviations

AJK	Azad Jammu and Kashmir
CIDA	Canadian International Development Agency
GIS	Geographic information system
INACG	International Nutritional Anaemia Consultative Group
KPK	Khyber Pakhtunkhwa
MI	Micronutrient Initiative
NFA	National Fortification Alliance
NNSP	National Nutrition Strategic Plan
USI	Universal salt iodization

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V.R. Preedy et al. (eds.), *Handbook of Food Fortification and Health: From Concepts to Public Health Applications Volume 2*, Nutrition and Health, DOI 10.1007/978-1-4614-7110-3_27,

Introduction

Pakistan, with a population of over 180 million people, faces malnutrition as a major public health problem. Half of its children aged 5 years or less are stunted, over a third (38 %) are underweight, and a quarter of all births are low birth weight [1, 2]. This high level of malnutrition contributes to about half of the 7,40,000 child deaths occurring every year. People are confronting vitamin A, iodine, and iron malnutrition worldwide [3]. According to WHO, 2.0 billion people are suffering from iodine (35 %) and iron (37 %) deficiencies, worldwide. However, the quotient is more in South-East Asia. Approximately 0.8 million deaths/year (1.5 %) may be ascribed to iron deficiency [4]. The combined impact of such deficiencies hampers socio-economic progress in developing countries. These have adverse effects on physical and mental health, work capacity, education, and economic efficiency of the population. The upshots of IDD include goitre, growth retardation, mental retardation, and increased neonatal and postnatal mortality. Iron deficiency anaemia (IDA) impairs thyroid metabolism and reduces efficacy of iodine prophylaxis [5]. It may enhance maternal mortality, compromised development of motor skills and learning capacity, lethargy, and reduced immunity. Iodine and iron deficiencies that often coexist are established and globally identified as two of the four major preventable risk factors for compromised child development [6].

Food fortification provides extra nourishment to reduce the dietary deficiencies. Diets lacking variety can be deficient in certain nutrients; even staple diet may be deficient in particular nutrients. In such cases, fortification of staple diet can prevent large-scale deficiency diseases. Food fortification is a cost-effective intrusion that does not require any conscious action by the consumer and needs no changes in the dietary habits of the targeted populace [7].

Vitamin A Deficiency

Vitamin A deficiency (VAD) is a common cause of childhood blindness and also affects growth and development among children. Even moderate deficiency of vitamin A can lead to stunted growth, increased infection, and mortality. The extent of clinical VAD at the national level remains unclear. About 50 % child deaths are estimated to occur annually in Pakistan as a consequence of diarrhoea and respiratory tract infections that are associated with VAD. WHO have included Pakistan in the list of countries with severe sub-clinical VAD. VAD exists in 50 % of sampled children from a squatter settlement, where both morbidity and mortality are high. Fortification of Vanaspati ghee and oils to overcome VAD has been legislated since 1970s, but the law is not enforced; resultantly no oil/fat is fortified [8].

Micronutrient initiative (MI) is providing technical assistance for vitamin A supplementation in the country. Vitamin A capsules are distributed semi-annually through national polio immunization programme. While continuing to support current activity, MI is focusing on the children who are missing out on the immune-boosting benefits of vitamin A which is estimated at below 5 % children under the age of 5.

Iodine Deficiency Disorders

Iodine deficiency produces a spectrum of disorders such as impaired cognitive development and function, congenital abnormalities, cretinism, hypothyroidism, and endemic goitre—collectively known as IDD. Its deficiency affects one-third of the global population, including 260 million school-age children.

Table 27.1 Progress towards universal salt iodization in WHO regions [13]

WHO region	Coverage (% of household)	No. of countries with legislation on iodized salt
Africa	63	34
USA	90	17
South-East Asia	70	7
Europe	27	20
Eastern Mediterranean	66	14
Western Pacific	76	6
Total	68	98

Published data taken from [13]

Although pockets of severe iodine deficiency still exist, probably more than 80 % of the current iodine deficiency is of mild-to-moderate severity. More than half of the population in Western and Central Europe is at risk of iodine deficiency, and about one-fourth of the countries where iodine deficiency is public health problem still have weak or non-existent public health programmes to address this issue [9].

Natural foods and beverages contain little iodine in many parts of Pakistan; many inhabitants are affected by IDD. Approximately 50 % people nationwide, mostly in the Northern areas, the Khyber Pakhtunkhwa (KPK), and some parts of Punjab, are iodine deficient with one of the highest reported rates of iodine deficiency disorders (IDD) in the world. The incidence of IDD in Pakistan has been estimated at 30 % among the inhabitants aged 6–12 years. However, total population is likely to be much higher, considering that ten million people are identified at high risk.

Salt Iodization

Iodates and iodides are used for fortification as potassium calcium or sodium salts. Iodates are less soluble in water than iodides, and are more resistant to oxidation and evaporation. These are more stable under adverse climatic conditions and do not require co-addition of stabilizers. Potassium iodate is preferred especially in hot and humid climates, and is recommended as an additive for many foods, including salt. Countries in Europe and North America still use potassium iodide, while most others with tropical climates prefer potassium iodate [10].

Losses of iodine due to iodide oxidation increased by moisture, humidity, exposure to heat and sunlight, and impurities in the salt. To curb this problem, it is usually added to salt after its refinement and drying by any of the two techniques. In the wet method, a solution of potassium iodate (KIO_3) is either dripped or sprayed at a uniform rate onto salt passing on a conveyor belt. This technique is cost-effective. The dry method involves sprinkling potassium iodide (KI) powder or potassium iodate (KIO_3) over the dry salt. This technique is more challenging because it requires salt of small homogenous crystals or granules and thorough mixing after addition of the compound to ensure an even distribution of iodine. Poor mixing results in inappropriate salt iodization [11].

During the past few years the minimum dose of iodine fortification has been set at 150 μg/day per person. Many countries aim at much higher levels [12]. The actual iodate addition levels to salt are based on the average per capita salt intake and predictable losses of iodine during distribution. Fortification level is based on the assumption of 50 % iodine loss between iodization and consumption [3]. Progress towards universal salt iodization in WHO regions is presented in Table 27.1, which shows that the highest coverage in salt iodization is in American households followed by Western Pacific and South-East Asia regions.

Micronutrient Initiative has assisted in rejuvenating Pakistan's salt iodization programme by transferring expertise and providing equipment and supplies. At the moment, more than 70 % table salt is iodized. The programme has been stretched to far-flung districts through designing of a digital map of salt sources and producers, which are making iodized salt available to more than 157 million people. Some areas have reported a reduction after an intensive programme of consumption of iodized salt over the last few years.

At the start of salt iodization programme, only 2 % iodized salt was available. By August 1995, the number increased to 17 %. According to Public Sector Information in Pakistan, approximately 30 % of all edible salt has been iodized with the introduction of 35 million new users between 1993 and 1995. The number of salt processors producing iodized salt is now over 400. Pakistan has about 600 salt processors and most of them use simple technology on a small scale. Sindh provincial government is set to make iodization of salt compulsory since over 50 % of its population is at threat. Moreover, Pakistan has Universal Salt Iodization Programme (USIP), which is said to cover 102 districts and benefit 154 million people. The aim is to cover the entire country by 2013 [14].

Monitoring Iodized Salt Through Geographic Information System

Pakistan, in collaboration with MI, launched the Geographic Information System (GIS) in March 2011 to monitor and improve salt iodization across the country. This is expected to reduce preventable brain damage caused by lack of iodine in the diet by monitoring the quality of iodized salt produced in the country. In Pakistan with a high incidence of iodine deficiency, such technology is likely to be very useful to improve the quality of life of the millions.

To prevent iodine-related diseases in Pakistan, baseline analytical data for nutritionally and radiologically important elements in typical Pakistani diets have been generated. Iodine concentrations of representative Pakistani diets have been determined by using epithermal and radiochemical neutron activation analyses. The measured iodine values range from 26.56 to 239.48 ng/g (dry weight). The geometric mean by geometric standard deviation of the iodine concentration is 67.6 ng/g × 1.8, which equates to a daily iodine intake of 40.0 μg/day × 1.8. The iodine content of the Pakistani diet is considerably lower than the intakes recommended by the US Food and Nutrition Board (150 μg/day) and the International Commission of Radiological Protection Board (200 μg/day) [15].

Iron Deficiency Anaemia

IDA is another leading nutritional disease worldwide which reduces haemoglobin content of the red blood cells. The symptoms of iron deficiency include fatigue, rapid heart rate, palpitation, and rapid breathing on exertion. In children, reduced growth and decreased resistance to infections are serious effects. It also results in increased infant mortality. An iron-poor diet and rapid growth are primary causes of iron deficiency in infants and preschool children. After correction of this deficiency pregnancy outcome becomes optimal with decreased risks for mothers and their babies. The mental and motor development of very young children is enhanced with greater academic performance [16].

In many developing countries, one out of two pregnant women and more than one out of every three preschool children are anaemic. In countries where meat consumption is low, such as India and sub-Saharan Africa, up to 90 % women are or become anaemic during pregnancy [17]. WHO estimates that some 8,00,000 deaths worldwide are attributable to IDA and this disease remains among the 15 leading

contributors to the global disease burden. As measured in disability-adjusted life years (DALYs), IDA accounts for 25 million, or 2.4 % of the total [18]. Flour is now being fortified with iron in many countries including USA, Sweden, Saudi Arabia, Egypt, and the Islamic Republic of Iran [19].

IDA is a common micronutrient problem in Pakistan. Although regional variations exist, most anaemia prevalence estimates suggest that more than 40 % pregnant women and a high percentage of adolescents, children, and men suffer from anaemia. The high level of anaemia in pregnant and lactating women is a leading cause of Pakistan's tragically high maternal mortality rate estimated at between 350 and 400 deaths per 1,00,000 live births. More than 25 % maternal deaths are believed to be directly or indirectly related to IDA that also affect up to three million newborns per year. This is responsible for increased morbidity, and could prevent them from attaining additional 5–10 IQ points. Accumulated evidence suggests that such deficiencies are associated with adverse effects on child cognitive and motor development [20].

In Pakistan, the most vulnerable groups are preschool children and pregnant and lactating women. Iron deficiency in children accounts for 83 % of all anaemia. The situation among school-aged children in urban squatter settlements is not well known [21]. Age and gender is a primary factor which determines the iron requirement. Females require more iron than males, especially during the childbearing age [22]. The contributing factors include low purchasing power, low bioavailability of iron from cereal-based diets, poor dietary practices, and poor hygiene and sanitation with insufficient dietary intake.

In Pakistan, wheat flour can be used extensively for iron fortification because almost 80 % of total wheat production is consumed in the form of chapattis/roties. Fortified chapatti is also preferred over the non-fortified due to slightly salty taste imparted by the minerals contained in the fortified product. Two kinds of flours, roller mill flour (75 % extraction rate) and "chakki atta" (whole wheat flour), are used to prepare these products. Chakki atta contains less moisture; hence it can be stored for longer period than mill atta. The cost of fortification is less than 0.11 % GDP, while the losses due to non-fortification are calculated as 4.3 % GDP [7].

Progress Towards Control of IDA in Pakistan

In 2001, some organizations were called to jointly develop a concerted national strategy to tackle malnutrition in the population. Nutrition Cell in the Ministry of Health took over the coordinating role in 2002 and embarked on a process of national consensus building. Between 2002 and 2004, a multisector team from Pakistan participated in the ADB-supported Regional Technical assistance (RETA) project leading to the development in 2004 of an outline for investment in the fortification of atta (wheat flour), cooking oil, and complementary foods. In early 2005, these activities got importance in the release of a 10-year National Nutrition Strategic Plan (NNSP) paper, along with a National Plan of Action for the control of micronutrient malnutrition. An analysis of existing data revealed that in 10 years, 22,000 maternal reproductive deaths and US$ 4.6 billion lost productivity could be prevented by eliminating IDA, while further elimination of folic acid deficiency would prevent 40,000 deaths from birth defects and heart disease over the same period [23].

After the devastating earthquake of 2005 in parts of AJK and KPK, the MI and the World Food Programme (WFP) have undertaken a major project to implement fortification of wheat flour with iron and folic acid to address these deficiencies especially in the affected areas. The aim of this project is to improve the health of families by increasing awareness of the importance of flour fortification, facilitate fortification of wheat flour, and help in the production of fortified flour in flour mills in Pakistan [23].

National Wheat Flour Fortification Programme

Another project introduced by the Government of Pakistan, the "Global Alliance for Improved Nutrition Assisted National Wheat Flour Fortification Project (KPK)", is a 3-year programme. An estimated 48 million (32 % of Pakistan's population) are expected to benefit from this programme [23]. The primary goal is to reduce the prevalence of IDA among preschool children (from 30 to 10 %) and women of reproductive age (from 50 to 18 %), and half the occurrence of neural tube defects among newborns (from 0.4 to 0.2 % of live births), through universal fortification of wheat flour with iron and folic acid. The iron fortification in roller milled wheat flour followed by chakkies has been an agreed strategy for addressing the iron and folic acid deficiencies in the country. This project also aims to recruit, train, and equip an additional 275 flour mills nationwide to initiate fortification and increase consumer awareness about anaemia and means of prevention from 35 to 50 % [22].

Bioavailability of Iron Compounds

The success of any fortification programme depends on the stability of fortificants and food to which these are added. Exposure of the fortificant to any of the physical and chemical factors including heat, moisture, air or light, and acid or alkaline environments during processing, packaging, distribution, or storage affects its stability. Mineral elements are more stable during manufacturing processes than vitamins. Such mineral elements as copper, iron and zinc are adversely affected by moisture and may react with food components like proteins and carbohydrates [24].

The bioavailability of iron compounds depends, to a large extent, on their solubility in the gastric juice. On the basis of solubility, iron compounds are divided into three categories, viz., (a) water soluble, (b) poorly soluble in water but soluble in dilute acid, and (c) water insoluble and poorly soluble in dilute acid. Ferrous sulphate is a common water-soluble compound that readily dissolves in the gastric juice. It is common to rank the absorption of iron compounds relative to that of ferrous sulphate (relative bioavailability value (RBV) = 100). Other water-soluble compounds and compounds readily soluble in dilute acid (ferrous fumerate) also have a RBV of 100. In contrast, compounds poorly soluble in dilute acid (elemental iron and iron phosphates) have a lower and variable RBV (e.g. ferric pyrophosphate RBV = 25–75) [25]. Encapsulation of water-soluble compounds with hydrogenated oils with a capsule-to-iron compound ratio ≤60:40 can prevent sensory changes with little or no effect on absorption [9].

Effect of Packaging Materials on the Quality of Iron-Fortified Wholemeal Flour During Storage

In Pakistan, flour mills pack wheat flour in jute, cotton, and polyethylene bags. The consumer may store it in the same packing or transfer it to tin canisters; hence suitability of packaging materials for packing of iron-fortified flour was determined. Wholemeal flour (WMF) was fortified with $FeSO_4$ + folic acid (FSF) (30 + 1.5 ppm), $FeSO_4$ + EDTA + folic acid (FSEF) (20 + 20 + 1.5 ppm), and elemental iron + folic acid (EIF) (60 + 1.5 ppm) and packed in polypropylene bags (30 SWG) and tin boxes (22 gauges) of 20 kg capacity and stored at ambient temperature (30–35 °C) and relative humidity (45–80 %) for 42 days [26]. Flour stored in cotton bags lost more moisture than in tin boxes. However, there are less changes in chemical characteristics of iron-fortified WMF packed in cotton bags than in tin boxes [27, 28].

Packaging materials have no effect on total iron content of fortified samples. Freshly prepared iron-fortified flour contains higher concentration of phytate which decreases significantly with the length of storage. Significant effect of storage but non-significant effect of packaging materials on phytate content occurs. Phytic acid decreases in WMF during storage at ambient temperature. Other treatments such as soaking of flour activates the phytase and thus increases phytic acid hydrolysis, thereby reducing phytate in the final products [29].

Effect of Storage on the Conversion of Fe_2 into Fe_3 in Iron-Fortified Flour

For an efficient fortification programme, both iron fortificant and food vehicle must be stable, safe, and acceptable during storage and consumable by the target population. In iron-fortified flour, conversion of Fe_2 into Fe_3 is recorded as a consequence of storage. This conversion is higher in tin boxes than in polypropylene bags. More conversion of Fe_2 into Fe_3 is observed in flour containing FSF followed by flour fortified with FSEF and EIF. This is due to EDTA which controls the conversion of Fe_2 into Fe_3 during storage [28].

Chemical changes during storage in flour badly affect its chapatti-making properties. The changes in physico-chemical and baking properties of stored flours are prevalent in tropical countries. Stored flours either release or absorb moisture until atmospheric equilibrium is reached. Proteins, crude fat, free amino acids, proteolytic activity, diastatic activity, and damaged starch decrease with an increase in the length of storage [30]. However, incidence in lipid rancidity in stored flour depends on type of flour, level of iron, storage temperature, fat content, and its moisture level [24]. Fat deterioration occurs at faster rate in flour containing 12 % moisture than that containing 7.5 %. Lipid hydrolysis affects the sensory characteristics which decreases product acceptance. Moreover, catalytic effect of iron on fat oxidation during storage is another major problem when cereal foods are used as vehicles for iron fortification [28].

The extent to which fat is least hydrolysed by lipases is a suitable criterion of flour soundness. This is usually determined by measuring peroxide value that almost doubles during storage of flour. The values in flour containing FSF and EIF are higher than those containing FSEF and unfortified flour. Rancidity develops in WMF during storage, which adds bitter taste and musty odour. Rancidity in fortified flour also doubles (from 1.4 to 2.9 %) after 6 weeks of storage since oxidative rancidity is catalysed by metals such as Fe and Cu [35]. Total acidity in flour increases from 0.2 to 0.4 % during storage. The increase in acidity is attributed to the lipase action on the triacylglycerols and other acylated lipids and production of free fatty acids. However, unfortified flour exhibits significantly more acidity when other factors are pooled [31].

Effect of Storage on the Mould Load in Iron-Fortified Flour

The major hazards to stored flour are bacterial attack, insect infestation, and mould growth. Mould growth is the main cause of flour deterioration during storage under humid conditions. Higher initial moisture contributes more towards the spoilage of grains and flour. Moisture, storage length, and temperature control the rate of fungal growth that occurs in stored flour with about 14 % or slightly higher moisture. As the microorganisms grow, they produce both moisture and heat, which then leads to damage to the stored flour. The insects consume flour, increase fat acidity, and also contaminate the product [32].

Flour containing FSEF and stored in polypropylene bags shows low load of colony-forming units (cfu) (5×10^2 cfu/g). Flour stored in tin boxes contains higher cfu of moulds as compared to that stored

Table 27.2 Iron status of females [22]

Variables	Overall mean	16–25 Years mean	26–35 Years mean	36–45 Years mean
Serum ferritin (ng/mL)	18.15	17.72	15.39	21.87
Haemoglobin (g/dL)	9.85	9.65	9.84	10.14
Iron deficient (SF <16 ng/mL)	9.65	10.42	7.92	11.10
Non-iron deficient (SF >16 ng/mL)	26.49	25.90	24.43	27.02
Iron deficient and anaemic (SF <16 ng/mL and Hb <12 g/dL)	SF 9.49 Hb 9.52	SF 10.27 Hb 9.50	SF 7.58 Hb 9.67	SF 11.27 Hb 9.22
Non-iron deficient and anaemic (SF >16 ng/mL and Hb <12 g/dL)	SF 26.18 Hb 9.89	SF 25.89 Hb 9.42	SF 24.43 Hb 10.0	SF 27.58 Hb 10.24
Iron deficient and non-anaemic (SF <16 ng/mL and Hb >12 g/dL)	SF 12.25 Hb 12.36	SF 13.67 Hb 12.3	SF 13.37 Hb 12.0	SF 9.73 Hb 12.8
Non-iron deficient and non-anaemic (SF >16 ng/mL and Hb >12 g/dL)	SF 21.26 Hb 12.85	SF 26.05 Hb 13.6	Nil	SF 16.47 Hb 12.1
Anaemic (Hb <12 g/dL)	9.73	9.50	9.80	9.94
Non-anaemic (Hb >12 g/dL)	12.56	12.95	12.0	12.45

in polypropylene bags. Unfortified flour has more mould count than the fortified flour. The pH of flour also has substantial effect on the development of moulds during storage [33]. Less changes in colour are observed in flour stored in polypropylene bags as compared to tin boxes [22].

Effect of Storage on Rheological Characteristics of Iron-Fortified Flour

Rheological characterization of wheat flour provides information to quality of raw material, textural characteristics of the finished products, and properties needed for the design and development of new equipment. These characteristics are significantly affected by fortificants, packaging materials, and storage period, except for dough stability on which storage period and fortificants have no effect [26]. There is an increasing trend in water absorption, dough development time, tolerance index, softening of dough, and amylase units during storage. Addition of fortificants decreases the water absorption, tolerance index, and softening of dough. The water absorption capacity, dough development time, dough stability, and softening of WMF containing $FeSO_4$, EDTA, and folic acid increase during 3 months of storage [27].

Stability of fortified WMF also depends on the nature of the fortificant. Flour containing elemental iron and ferrous sulphate with EDTA remains stable for up to 42 days. However, unfortified flour and flour containing ferrous sulphate remain stable for only 21 days in tin boxes and 28 days in polypropylene bags. The flour stored in polypropylene bags proves more stable than that stored in tin boxes [26].

Efficacy Studies on Iron-Fortified Wholemeal Flour

The final effect of an iron fortification programme can only be judged through efficacy studies (Table 27.2). Haemoglobin (Hb) and serum ferritin (SF) levels are key factors for this purpose (Fig. 27.1). Hb level helps to determine the degree of anaemia. SF indicates a change in iron store and also identifies individuals at risk of developing an iron overload [34].

The effect of $FeSO_4$ (FS) and $FeSO_4$+EDTA (FSE) in combating IDA was studied among the rural masses in Pakistan using 200 volunteer families from a village (Fig. 27.2) [22]. A few subjects had Hb

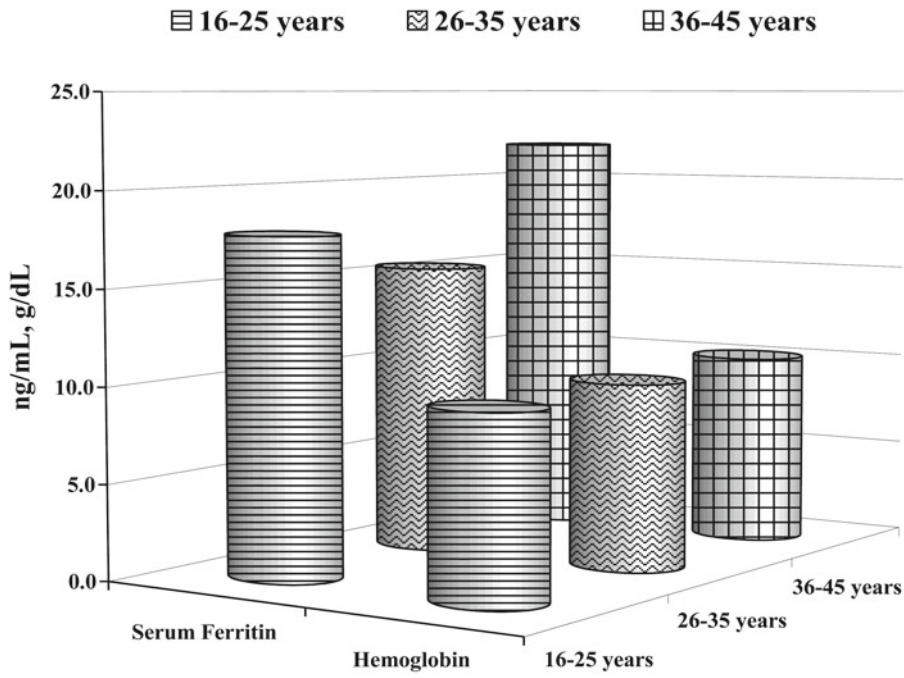

Fig. 27.1 Comparison in iron status of females from different age groups [22] (unpublished)

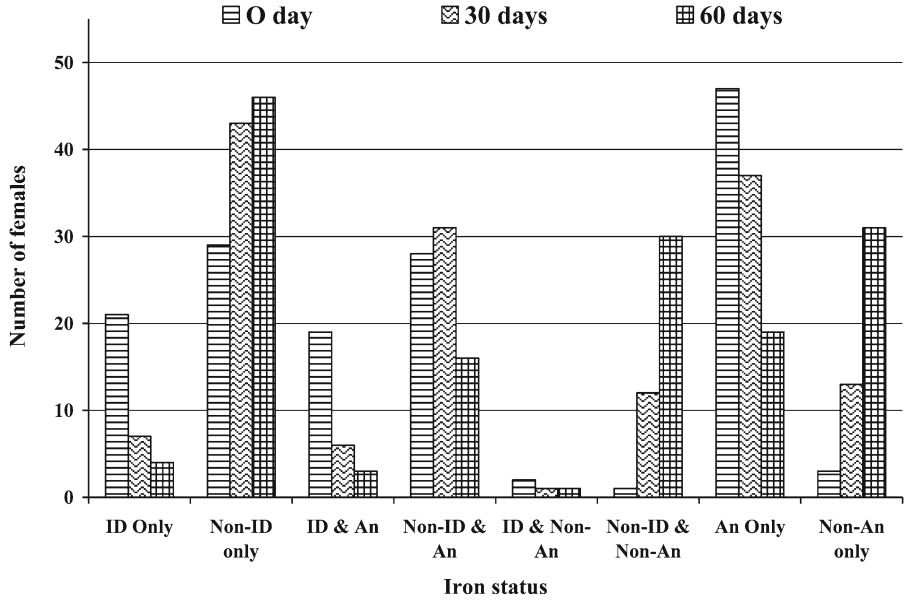

Fig. 27.2 Effect of $FeSO_4$ on iron status of females [22] (unpublished)

level <8 g/dL, while highest prevalence (43 %) of anaemia (Hb <12 g/dL) was in females of 16–25 years age group. Anaemia in age groups 25–35 years and 36–45 years was 29 % and 28 %, respectively (Fig. 27.2). FSF-fortified flour increases SF from 20.69 to 40.83 ng/mL and Hb level from 9.69 to 12.4 g/dL after 3 months in female subjects. The number of iron-deficient subjects (SF <16 ng/mL)

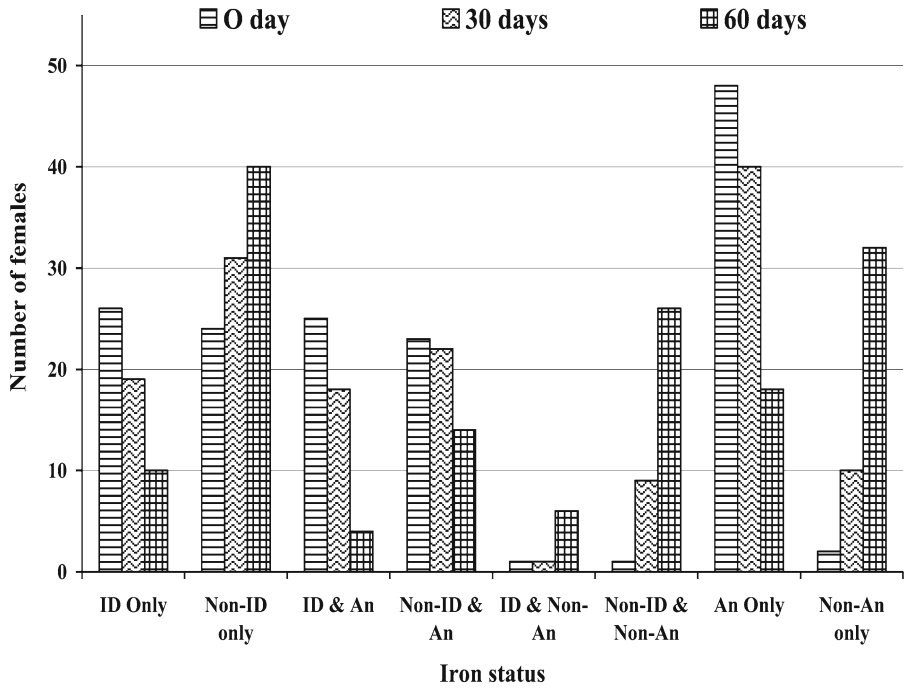

Fig. 27.3 Effect of $FeSO_4$ + EDTA on iron status of females [22] (unpublished)

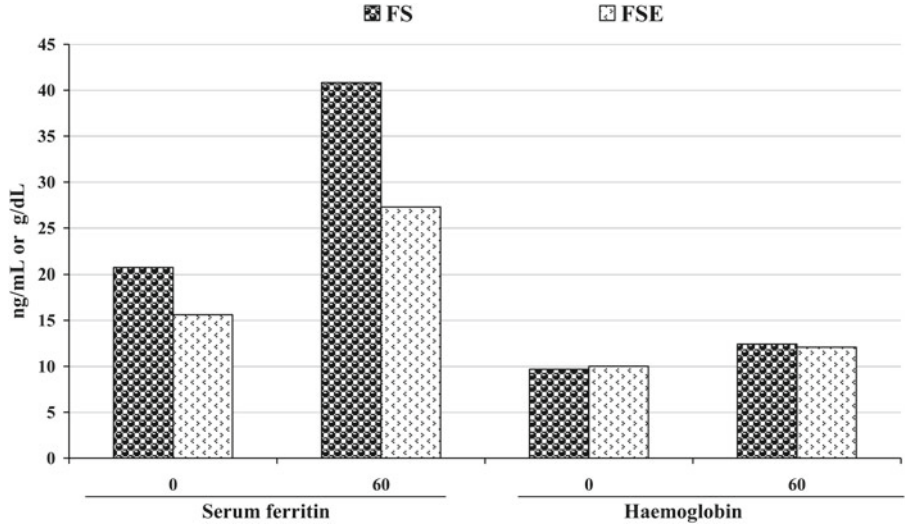

Fig. 27.4 Comparison of $FeSO_4$ and $FeSO_4$ + EDTA's effect on iron status [22] (unpublished)

decreases from 21/50 (42 %) to 4/50 (8 %). Increase in serum ferritin was 20 and 12 ng/mL in groups of FS and $FeSO_4$ + EDTA, respectively. More increase occurs in $FeSO_4$ as compared to $FeSO_4$ + EDTA (Fig. 27.3). Hb in females consuming FSF-fortified flour increases from 10 to 12 g/dL, while in those consuming $FeSO_4$ + EDTA-fortified flour from 9.69 to 12.41 g/dL. The SF level increases from 15.62 to 27.28 ng/mL after consuming fortified flour. Highest increase occurs in age group 36–45 years in which SF level increases from 19.78 to 32.97 ng/mL after 2 months. The increase in SF also appears in other age groups. The reduction in anaemia is 56 % in $FeSO_4$ and 60 % in $FeSO_4$ + EDTA (Fig. 27.4) [22].

Enhancers and Inhibitors in Bioavailability of Non-Haem Iron

The absorption of non-haem iron depends upon its form as well as presence of enhancers and inhibitors in foods. Fortification with non-haem iron is a good strategy to overcome iron deficiency, since it is absorbed at a lower rate than haem iron depending upon ligands and nutritional status of an individual.

The iron absorption enhancers include meat, fish, poultry, organic acids, EDTA, lactoferrin and vitamin A which increase the absorption of both heme and non-haem iron [35]. Lactic acid is identified as a promoter of iron absorption [36]. Ascorbic acid is also used in commercial food products as Fe absorption enhancer. Its effect is related to both reducing power of chelating action and forming soluble complexes with iron at low pH. Ascorbic acid activates the duodenal ferric reductase; therefore, its addition is directly related to the absorption of ferric iron from the food [37]. Ferrous sulphate, the most bioavailable iron compound, is subject to oxidation during storage at higher temperature and moisture levels. During oxidation, ferrous form is converted into the ferric form. Ferric complexes cause food product to turn brown and result in the formation of brown precipitates [26, 27]. These formed ferric complexes are insoluble and, therefore, poorly absorbed in the intestinal lumen [38]. The stability can be enhanced by adding EDTA to ferrous sulphate [24]. Lactoferrin has the ability to bind and transport iron, and release it again into specific receptor cells in the intestines. Therefore, it offers the benefit of enhanced iron absorption if added to food products [38].

The factors contributing to poor bioavailability of iron are tannins in tea and coffee, polyphenols in vegetables and legumes, oxalic acid in certain vegetables, calcium in dairy products, and fibre and phytic acid in cereals. These bind non-haem iron to form insoluble complexes that are poorly absorbed. There is drastic reduction in iron absorption (60 %) from foods when subjects consume just one cup (200–250 mL) of normal-strength tea [39]. The polyphenols in vegetables, legumes, and condiments have also strong inhibitory effect on iron absorption. The low bioavailability of iron from green vegetables, such as spinach, is partly due to their high oxalic acid content. Calcium salts lower iron absorption from a typical breakfast meal with low iron availability and high calcium content [40]. Proteins from plant foods, including soy beans and nuts, significantly inhibit non-haem iron absorption [41].

Phytic acid (PA) constitutes about 1–2 % by weight of many cereals, nuts, seeds, and legumes and forms very stable complexes with mineral ions, rendering them unavailable for intestinal uptake. As PA content in the diet increases, the intestinal absorption of zinc, iron, and calcium decreases. Phytates inhibit the absorption of iron and zinc, and hence, iron deficiency may be accompanied by zinc deficiency [42].

Treatments such as germination or soaking have been used to enhance phytase activity [43]. Wheat and rye flour can be treated in this manner to destroy practically all their phytate and enhance in vitro iron availability. Fermentation of raw as well as autoclaved wheat flour with buttermilk significantly decreases the level of phytic acid. Fermentation hydrolyses most of the phytate in wheat, resulting in improvement in iron absorption. Food processing, such as milling, soaking, heat treatment, baking, and fermentation, may enhance or reduce iron availability [24].

Toxicological Effects of Iron Overload

Excessive dietary Fe causes neurobiological dysfunction in weanling rats. It significantly decreases activity and causes habituation, reflex startle, and conditioned response performance. Body weights also decrease markedly. A higher dose of iron (200 mg/kg as ferrous sulphate) increases acute iron toxicity that significantly depresses the myocardial contractility in rabbits [44]. Daily oral administration of dicyclopentadienyliron (ferrocene) at dosage of 30, 100, and 300 mg/kg for 6 months and 1000 mg/kg for 3 months produces hemosiderosis in dogs [45]. Further, iron dextran, iron dextrin, folic acid, colchicines, and chlorpromazine hydrochloride are toxic in pregnant mouse following

intravenous administration [46]. However, reduced glutathione and vitamin E help to decrease the peroxidation of lipids and increase cell viability in rats. Free extracellular iron stimulates lipid peroxidation in hepatocytes [47].

Exposure to excessive doses of iron supplementation commonly occurs in children less than 6 years of age. Vomiting and diarrhoea characterize initially, followed by involving five organ systems; cardiovascular, central nervous system, kidney, liver, and hematologic [48]. To reduce the potential seriousness of iron overload exposures, carbonyl iron has been suggested as a possible replacement for ferrous sulphate. With $FeSO_4$ and NaFeEDTA, total liver non-haem iron rises with increasing dose, but the response is approximately 50 % lower as compared to $FeSO_4$. Diets containing as much as 5 % EDTA have been found to possess no adverse effects [49].

Dual Fortification of Salt with Iron and Iodine

Micronutrient interventions benefit the health and development of school-age children and multiple micronutrients are more effective than a single one. Dual fortification of salt (DFS) could be effective in reducing morbidity from diarrhoea and respiratory infections and have positive effects on child growth and cognition, particularly those related to memory [50]. DFS has positive impact on haemoglobin status in anaemic children and improves their urinary iodine concentration without adverse effects [51].

Adding iron and iodine concurrently to salt has its challenges. Iron and iodine compounds tend to react with each other and impurities normally present in the salt lead to conversion of iodide or iodate to iodine in its elemental state. The free iodine easily sublimes. Iron compounds also undergo reactions that lead to discolouration of the salt and amplified metallic taste [12]. To cope with this problem several modifications have been made and formulation for premix for fortification formed on the basis of salt. The dilution ratio required for making DFS containing 1,000 ppm of iron is 1 part of iron premix to 150 parts of salt. DFS prepared by dry blending 150 parts of iodized salt with 1 part of iron premix results in a fortified salt containing 50 ppm iodine and 1,000 ppm iron. The ratio of premix to salt can be adjusted depending on the salt consumption [52].

Recommendations

Fortification programmes for improvement of micronutrient deficiency need to be launched at national level to reduce the incidence of nutrition-related disorders and mortality. Staple food should preferably be used as vehicle for fortification. Economical and less investigated fortificants should be explored to cope with the malnutrition problems in developing countries like Pakistan.

Conclusion

Malnutrition is a major public health problem in Pakistan and developing countries. Vitamin A, iodine, and iron malnutrition contribute to about half of the 7,40,000 child deaths and many nutritional disorders. VAD is a common cause for childhood blindness and also affects growth and development among children and even leads to increased infection and mortality. Iodine scarcity is mainly related with goitre and IDD. Most Pakistanis in the Northern areas, KPK, and some parts of Punjab are iodine deficient with one of the highest reported rates of IDD in the world. Fortification of nutrients in staple diet can solve the problem of large-scale deficiency diseases. Table salt is an ideal vehicle for iodine fortification. Pakistan already has a USIP.

IDA in Pakistan is a major micronutrient problem among all age groups, especially preschool children and pregnant and lactating women. Iron deficiency is being addressed by fortification of wheat flour. Wheat flour is a good vehicle for iron fortification in Pakistan because almost 80 % of total wheat produced is consumed in the form of chapattis/roties. The cost of fortification is quite less, while the losses due to non-fortification are much high. The project "Global Alliance for Improved Nutrition" has been introduced in Pakistan. MI and the World Food Programme have undertaken a major project to implement fortification of wheat flour with iron and folic acid.

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Chapter 28
Neural Tube Defects in Australia and Food Fortification with Folic Acid

Carol Bower and Jane Halliday

Key Points

- Historically, neural tube defects in Australia have affected around 2 per 1,000 births
- Promotion of periconceptional folic acid supplement use began in Australia following publication of randomised trials showing effectiveness
- 30–50 % of Australian women take periconceptional folic acid supplements
- Voluntary fortification of selected foods (mainly breads, breakfast cereals) with folic acid was permitted in 1995 in Australia, but uptake was limited
- There was a 20–30 % fall in NTD in association with supplement use and voluntary fortification
- Mandatory fortification of wheat flour for bread-making was legislated in 2007 in Australia and required by September 2009
- A framework for monitoring mandatory fortification has been established
- There is evidence of an increase in serum and red cell folate status following mandatory fortification, but it is too soon to observe an effect on NTD
- In order to monitor trends in NTD, it is critical to have complete ascertainment from livebirths, stillbirths and terminations of pregnancy

Keywords Neural tube defects • Folate • Folic acid • Fortification • Australia

Abbreviations

FSANZ	Food Standards Australia New Zealand
NTD	Neural tube defects
NHMRC	National Health and Medical Research Council

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V.R. Preedy et al. (eds.), *Handbook of Food Fortification and Health: From Concepts to Public Health Applications Volume 2*, Nutrition and Health, DOI 10.1007/978-1-4614-7110-3_28,

Introduction

A review of Australian data on neural tube defects (NTD) from 1942 to 1981, published in 1984 [1], reported total NTD rates ranging from 0.8 per 1,000 births to 2.7 per 1,000, with the rates for anencephaly similar to those for spina bifida. For the four states that provided data by year of birth from 1975 to 1981 (New South Wales, Tasmania, Victoria and Western Australia), there was a decline in NTD rates from around 2 per 1,000 or more to 1.6 per 1,000 or less [1].

In the 1980s, three states, Western Australia, Victoria and South Australia, established birth defects registers and rates of NTD from these registers (which include terminations of pregnancy for NTD) have varied between 1.3 and 2.4 per 1,000 births from 1980 to 1989 [2, 3]. Data for Tasmania from birth notifications (plus terminations of pregnancy for 1987–1989) showed wider yearly fluctuations (0.8–2.1 per 1,000), but was based on a small number (~7,000) of births annually [2]. In none of these states was there a consistent trend over time and the earlier decline seen in Tasmania, Victoria and Western Australia was not sustained [2].

An Early Aetiological Study in Australia on Folate and NTD

A case-control study in Western Australia from 1982 to 1984, examining dietary folate and NTD, found a reduction in odds ratios for NTD with increasing dietary folate (odds ratio of 0.16; 95 % CI 0.06–0.49, comparing the highest quartile of dietary folate intake with the lowest quartile) [4] and small and imprecise reduction in risk with supplemental folic acid periconceptionally (OR 0.7; 95 % CI 0.3–1.8) [5]. Periconceptional use of folic acid supplements was uncommon at the time of the study—only 1.3 % of women took folic acid supplements before pregnancy and only 15 % took them in the first 6 weeks of pregnancy.

Promotion of Folate to Prevent NTD in Australia

In the 1980s, several centres in Australia contributed to a multicentre randomised controlled trial of multivitamins for the prevention of recurrent NTD [6]. Following the publication of this trial, which showed a reduction in recurrent NTD with periconceptional folic acid supplementation, the National Health and Medical Research Council (NHMRC) in Australia issued a statement on the relationship between dietary folic acid and NTD in 1992. The statement was revised slightly in 1993, after consideration of the results of two further trials (Hungary and Ireland) [7]. The revised statement included the recommendations shown in Box 28.1. Box 28.2 contains a timeline of activities relating to folic acid and the prevention of NTD in Australia.

Box 28.1. National health and medical research council revised statement on the relationship between dietary folic acid and neural tube defects such as spina bifida [7]

1. All women planning a pregnancy or likely to become pregnant should be offered advice about folate in the diet, and encouraged to increase their dietary intake of folate-rich foods, particularly in the month before and in the first 3 months of pregnancy.
2. Low risk women (i.e., no family history of neural tube defects, not on anticonvulsants) should be offered periconceptional folic acid supplementation (0.5 mg daily). Generally,

(continued)

Box 28.1. (continued)

periconceptional supplementation with other vitamins is not necessary. When supplements are used the potential risks of vitamin overdose should be considered. In particular, large therapeutic doses of vitamin A may predispose to birth defects.

3. Women with a close family history of neural tube defects (e.g., they or their partner has spina bifida, they already have an affected child, they have a sibling or other close relative with a neural tube defects) should be (a) referred for genetic counselling, (b) advised to take periconceptional folic acid supplementation (5 mg daily; 4 mg formulation not available in Australia) and (c) continue to be offered prenatal diagnosis with alpha-feto-protein estimation and tertiary level ultrasound, by an operator experienced in anatomical scans, at 16–18 weeks gestation. Although the risk of recurrence is significantly reduced if folic acid supplementation is used appropriately, there is a residual risk of about 1 % in women taking supplements who have had a previously affected child.
4. Women on anticonvulsant drugs should: (a) take folic acid supplementation only under the supervision of and close monitoring by their physician, and (b) because of the increased risk of neural tube defects in the offspring of women taking some anticonvulsants (notably sodium valproate), these women should also be counselled and offered prenatal diagnosis.
5. Fortification of staple foods, such as bread and cereals, with folic acid, as is recommended in the USA and Britain, should be introduced in Australia.
6. Education, research and monitoring: (a) there should be education programmes, for health professionals and the public, on how to achieve adequate folate intake with diet and supplementation to prevent neural tube defects; (b) there should be continued research into the mechanisms of action of folic acid and the minimum dose of folic acid required for prevention; (c) close monitoring of both the prevalence of neural tube defects (including terminations of pregnancy) and the increase in folate intake should be undertaken to evaluate the effectiveness of any health promotion campaigns and (d) further research should be monitored, and these recommendations reviewed in the light of any developments.

Box 28.2. Timeline of activities related to folic acid and ntd prevention in Australia

1980s	Birth defects registers established in Australia
1991, 1993	Randomised controlled trials confirming NTD prevention with periconceptional folic acid
1992	National Health and Medical Research Council Statement
	Health promotion campaign in Western Australia
1993	Revised National Health and Medical Research Council Statement (see Box 28.1)
1994	Health promotion campaign in South Australia
	Expert Panel to consider food fortification with folic acid
1995	Voluntary fortification of some foods with folic acid approved
	National Nutrition Survey
1997	Randomised controlled trial on consumer-directed information in Victoria
2001	Interim evaluation of voluntary fortification
2004	Food Standards Australian New Zealand (FSANZ) Initial Assessment Report
2006	FSANZ Draft Report
	FSANZ Final Report
2007	FSANZ First Review Report
2009	Mandatory fortification of flour for bread-making implemented
2011	Baseline Monitoring Report

As a consequence of recommendation 5 (see Box 28.1), and expert Panel was convened in 1994 to consider food fortification with folic acid in Australia [8]. Folic acid was not permitted to be added to foods in Australia at that time. The Expert Panel concluded that increasing the amount of folate in the Australian diet would reduce the rate of NTD and that there was sufficient evidence to recommend, as a public health measure, the mandatory fortification of flour and voluntary fortification of breakfast cereals, rice, pasta, yeast products and fruit and vegetable juice. However, they also agreed, as a practical first step, to recommend voluntary fortification only, to be reviewed 3 years after introduction, to determine whether it had been effective or whether mandatory fortification of flour was necessary. The Expert Panel also agreed that at the levels of increased folate that were proposed, there would be no foreseeable risk to any population group [8].

Voluntary Fortification of Food with Folic Acid

Following the recommendations of the Expert Panel, voluntary fortification of food with folic acid was permitted in Australia. In June 1995, changes to *Food Standard A9* of the Food Standards Code were made, to permit voluntary fortification with folic acid of the following foods at a level of up to 50 % of the Australian Recommended Dietary Intake (100 μg per reference quantity): flour, bread, savoury biscuits, breakfast cereals, pasta, yeast extracts, fruit and vegetable juices and meal replacements. In 1998, supplemental foods such as folate-fortified milk products were added to the list. Rice was not included because of technical difficulties in fortifying such that sufficient folic acid would be retained once the rice was cooked. By June 1999, 104 products had been fortified: 38 breads, representing 18 % of breads on the market and 34 breakfast cereals, representing 49.6 % of market share [9].

Monitoring of Periconceptional Folate Supplement Use and Voluntary Fortification in Australia

Two national population nutrition surveys carried out on Australian households during 1995 and 1996 obtained data on knowledge about folate and folic acid supplement intake; this was done in response to the changes to the 1995 *Food Standard A9* permitting voluntary fortification with folic acid [10].

Of the 5,400 adults surveyed, 45 % had not heard of folic acid or folate and only 11 % knew that it helped prevent birth defects. Twice as many men had not heard of it than women. Overall, 10 % of Australian women were taking a folic acid supplement, with this proportion being higher in WA and Queensland and lower in Tasmania. Knowledge appeared to be socially patterned and increased with the respondents' education and income and was higher in married women.

Western Australia

A health promotion research project began in Western Australia in mid-1992, with the aims of informing health professionals about the prevention of NTD with folate and to ask them to advise women of childbearing age to increase their intake of folate, and informing women about folate and spina bifida and to encourage them to increase their folate intake [11]. Recommendations for use of folic acid supplements were based on the NHMRC recommendations [7]. A wide range of health promotion activities were undertaken, including the distribution of posters, pamphlets and information sheets to general practitioners, pharmacists and other health professionals before the public launch of the project in November 1992, and again in 1993 and 1994. In addition, there were paid and unpaid media items, professional and public presentations, advertisements on taxis and distribution of promotional materials to schools, child care centres,

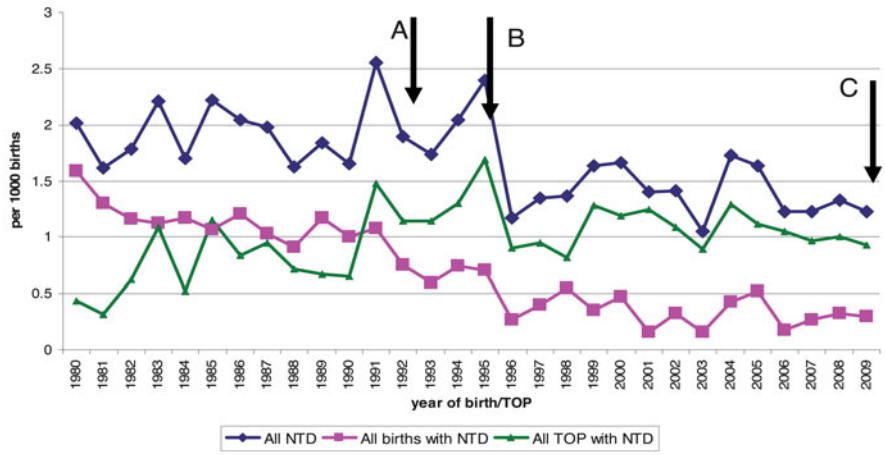

Fig. 28.1 Neural tube defects (NTD) in Western Australia, 1980–2009

family planning facilities and public libraries. Surveys of women of childbearing age revealed an increase in knowledge of the folate-spina bifida link from 8.2 % before any promotion to 67.5 % two and a half years later. Around two-thirds of women surveyed had seen the project pamphlet and 72 % had seen the poster. By March 1995, 70 % of general practitioners always offered folic acid supplements to women planning pregnancy (up from 15 % in 1992) and wholesale sales of 0.5 mg folic acid produced by the main supplier in Western Australia increased markedly following the promotional activities [11]. This project predated voluntary fortification.

A case–control study conducted 1997–1999 examined folate intake from supplements as well as folate from naturally occurring and fortified foods [12]. Amongst women not taking folic acid supplements, dietary sources of folate were associated with a reduction in risk of NTD and about half the women obtained over 100 μg dietary folate equivalents from voluntary fortified foods (46 % of case mothers; 55 % of control mothers).

A study of trends in NTD in Western Australia [13] showed that between 1980 and 1995 the rate of NTD (births plus terminations) was 1.96 per 1,000 births, whilst from 1996 to 2000 it was 1.38 per 1,000; a 30 % fall. A further study found that the reduction in NTD was confined to non-Indigenous births. Prior to promotion of folate (1980–1992), Indigenous infants had a rate of 2.55 per 1,000—42 % higher than for non-Indigenous infants [14]. In the period when there was promotion of supplements but no voluntary fortification, the Indigenous rate was 3.19 per 1,000 (68 % higher than non-Indigenous) and, during the supplement plus voluntary fortification period 1996–2000, it was 2.56 per 1,000, almost double the non-Indigenous rate at that time, suggesting that health promotion and voluntary fortification was failing to reach Indigenous women.

The rate of NTD in Western Australia has remained stable at around 1.3 per 1,000 up to 2009, with most NTD being diagnosed prenatally and the pregnancy terminated (Fig. 28.1).

South Australia

A population-based health promotion campaign was undertaken in South Australia between October 1994 and August 1995, targeting women of childbearing age and health professionals (general practitioners, pharmacists, dieticians and nursing and medical staff of the child health service in the state) [15].

Information leaflets for health professionals, posters and pamphlets were translated into seven other languages and widely distributed through pharmacies, doctors' practices, hospitals, shopping centres, libraries, childcare centres and schools. A before-after evaluation was conducted using surveys of women and health professionals. More limited promotion of folate continued beyond the period of the campaign.

Knowledge about the protective effect of folate increased from 25 to 45 % amongst women of childbearing age in the general population and from 48 to 92 % in women surveyed postnatally. Amongst the women surveyed postnatally in 1995, 10 % took folic acid supplements in the periconceptional period, whilst by 1998, this proportion had increased to 46 %. Knowledge amongst health professionals of folate for the prevention of NTD and of the NHMRC recommendations, increased over the study period. The proportion of health professionals who always recommended folic acid supplements to women planning a pregnancy increased from 14 % before the campaign to 35 % after. Sales of folic acid supplements doubled over this period and the prevalence of NTD fell from a baseline of 2.2 per 1,000 to 1.1 per 1,000 [15].

Victoria

A study to determine and raise awareness and use of folate was first undertaken in Victoria in the form of a randomised trial of a consumer-directed informational campaign [16]. This was a cluster community-based trial where three pairs of communities were randomised to receive an intervention aimed to raise awareness (information kit, posters, leaflets) or to have no additional information supplied. The intervention was implemented in 1997 for 2 months, disseminated to maternal and child health nurses, supermarkets, schools, hospitals, infant-related shops, libraries, hairdressers, fitness centres and more, targeting women of childbearing age. Through 1,200 telephone surveys done in the study areas before and after the intervention, it was shown that 12 % of respondents were aware of folate and its association with NTD at baseline and this significantly increased in the intervention areas to 20 %. The nonintervention area increased to 16 % in the study period probably because of a background change over time. There were marked differences in awareness across the age groups with younger women (15–24 years) less aware than older women and not affected by the intervention. This lower awareness was also seen in women with less education and less skilled employment.

The Victorian Department of Human Services stepped up to the mark in 1999 and set out to increase awareness of folate through a campaign, entitled 'Little Things Make Big Differences', that had two approaches, one for women planning a pregnancy (the 'not averse' group) and one for women who were not necessarily planning to do so (the 'not planners'). Extensive qualitative research guided the text message and visual imagery and the communication techniques used. Evaluation of the 6-month campaign was limited to sales data supplied by three folate supplement manufacturers/retailers indicating an increase in sales of folate during media activity. It also included qualitative evaluation of resources with health professionals who showed high approval for the materials used. Unfortunately there was only enough funding to survey 350 women of childbearing age to determine whether there had been an increase in awareness and use of folate. From these small numbers (230 responded), it appeared that use of a folate supplement had increased from 8 to 13 %. There was no peer-review publication of the campaign content or results and there was no further follow up in subsequent years.

There was, however, another contemporaneous attempt to monitor awareness in Victoria, using a retrospective postal survey of 1,600 mothers who gave birth in a 2 week period in September 1999 [17]. The same research team also surveyed and reported results of 647 mothers in the NSW Child Health Survey in 2001, recruited from area health services. This showed that 36 % of recent mothers in Victoria reported taking periconceptional supplements compared with 46 % in NSW.

Two more recent surveys in Victoria have added to our picture of use of folate supplements in the voluntary fortification period. The Victorian Population Health surveys in 2005 and 2006 included specific questions for women aged 18–50 years on use of and knowledge of the reasons for taking folic acid supplements. These data were published in 2008 and reported a much lower proportion of women taking a folic acid supplement than that seen in the 1999 survey of recent mothers, 18 % in 2005 and 23 % in 2006 [18]. Younger women (18–24 years old) had the lowest use of folate supplements (15.9 % in 2006). NTD declined from 1.7 per 1,000 births in 1997 to 1.4 per 1,000 in 1999 but remained static between 1999 and 2006.

Although there were also some national and other state-based health promotion activities, none of them has been formally reported or evaluated.

In 2001, an interim evaluation was reported of the voluntary folate fortification policy in Australia since its gazettal in 1995 [9]. This evaluation found that relatively few of the recommended foods (mainly different varieties of breakfast cereals) in Australia had been fortified with folate. Most fortified products appeared to meet the recommended level of folate fortification, the availability of fortified products varied only slightly between cities and regional, rural and remote areas and there was little difference in price between similar fortified and non-fortified products. Based on dietary modelling, it was estimated that voluntary fortification had had little effect on folate intake amongst women of childbearing age. The majority of women of childbearing age were reported to be unaware of the folate-NTD link and few of them knew which products were fortified. There was no evidence of an effect of voluntary fortification on reducing NTD. The authors concluded that in Australia, until the programme was implemented with an adequate number of fortified products, it was too early to assess the impact of folate fortification on the occurrence of NTD, and that mechanisms were not in place to monitor other health outcomes. The authors recommended improving the implementation of the programme, and developing and implementing a coordinated monitoring and evaluation plan [9].

Introduction of Mandatory Fortification in Australia

Following the interim evaluation report of voluntary fortification [9], the Australia and New Zealand Food Regulation Ministerial Council also asked Food Standards Australia New Zealand (FSANZ) to determine the most effective mechanism to increase total periconceptional folate intake in women to reduce NTD [19]. FSANZ initially considered four options:

1. Maintain the current situation at the time (voluntary folate fortification permitted for some foods).
2. Increase permissions for voluntary folate fortification.
3. Mandatory folate fortification.
4. Increase health promotion and education strategies either as a sole strategy or in conjunction with any of the other three options.

Submissions were encouraged from consumers, industry, health professionals and government [19].

Responding to Ministerial advice in 2005, FSANZ reduced the number of regulatory options to two: maintenance of the *status quo* and mandatory folic acid fortification [20]. An assessment of the risks and benefits of increased folate intake in relation to cancer, cardiovascular disease, cognitive function, vitamin B12 deficiency and twinning were included in this report, along with an assessment of the potential effect of incremental increases in folic acid intake on NTD in Australia and New Zealand. Both the twinning data and the latter analysis were subsequently published [21, 22]. Following consideration of these assessments and also an assessment of feasibility and cost analysis, FSANZ concluded that mandatory fortification of all bread-making flour with folic acid was the preferred approach in Australia and New Zealand to further reduce the incidence of NTD. A level of 230–280 μg of folic acid per 100 g of bread-making flour was proposed, to achieve an average residual level of

approximately 200 μg folic acid in the flour component of the final food. FSANZ also proposed that current voluntary folic acid permissions be maintained (except for bread) [20]. Further community submissions were invited on the Draft Assessment Report.

A Final Assessment Report [23] included an assessment of the potential health benefits and risks of increased dietary intakes of folic acid, technical issues of fortification, a consideration of alternative approaches to mandatory fortification (the topic of several of the submissions to the Draft Assessment Report), a further cost-benefit analysis, communication and education strategies, monitoring and implementation issues, and presentation of a regulatory approach. The decision reached in the Final Assessment Report was that mandatory fortification of bread with folic acid was the preferred approach, with a proposed level of 80–180 μg of folic acid per 100 g of bread and voluntary folic acid permissions remaining except for bread.

Following consideration of the Final Assessment Report by the Ministerial Council, a First Review Report was requested and, based on the proposals in this report [24], the Australia and New Zealand Food Regulation Ministerial Council affirmed, on 22 June 2007, the mandatory fortification of wheat flour for bread-making (200–300 μg folic acid per 100 g of flour). There was to be an exemption from fortification for organic wheat flour for bread-making; the retention of voluntary folic acid fortification; and the need for a monitoring system was emphasised. Mandatory fortification was to be implemented in Australia by September 2009. In September 2012, the New Zealand government ruled against mandatory folic acid fortification of bread [25].

Monitoring the Effects of Mandatory Fortification in Australia

A framework for monitoring mandatory fortification in Australia has been developed, covering five areas: food composition and food industry compliance, nutrient intake, nutrient status, health benefits, and adverse health effects [26, 27]. Key baseline data available to inform future monitoring are presented in the original report (May 2011) and a supplement published more recently (October 2011).

1. Based on levels determined during the voluntary folic acid fortification period, women of childbearing age (16–44 years) consumed an estimated average of 108 μg of folic acid a day, with a 95th percentile intake of 283 μg/day (excluding supplements).
2. Analysis of the 2007 Children's Survey showed that only 4 % of Australian children exceeded the upper level of intake for folic acid and only a small proportion (6 %) took supplements containing folic acid. Children aged 2–16 years consumed an estimated average of 114 μg of folic acid a day, with a 95th percentile intake of 279 μg/day.
3. During 2001–2008, the main food categories contributing to folic acid intake consumed by the highest proportion of the population were breads, followed by breakfast cereals and then fruit juices. During this time period, the proportion consuming bread and cereals remained relatively stable. Between the sexes, more females consumed breads and cereals than males.
4. Population health surveys in three states (New South Wales: 2007–2008, Australian Capital Territory: 2007–2008 and Tasmania: 2009) showed that about half of all Australian mothers took folic acid supplements just prior to and during their first trimester of pregnancy, as recommended. Supplement usage was lower in mothers without tertiary qualifications and in those living in more disadvantaged and remote areas.
5. In 2005, the overall prevalence of NTD in the three states monitoring both births and pregnancy terminations, was 13.3 per 10,000 births, representing an absolute number of 149 pregnancies affected by NTD [28].

Nutrient status will further be monitored by measurement of serum and red blood cell (RBC) folate levels. There are, however, no accepted standards for folate deficiency based on these concentrations and limited data are available on pre-fortification levels.

In terms of adverse outcomes, the monitoring process also addresses the speculation that folic acid may increase the risk of certain cancers, noting that all possible causes for any increase or decrease in cancer incidence and/or mortality following the introduction of mandatory fortification must be considered. The baseline report contains incidence and mortality data on bowel and prostate cancer allowing for changes to be monitored over time.

Importance of Monitoring NTD

We have been fortunate in Australia to have several registers that collect detailed data on NTD, both at birth (20 weeks gestation and later) and in terminations of pregnancy before 20 weeks gestation. The latter collection is vital to the monitoring process as approximately 60 % of all NTD may be missed if this was not done [18, 28]. The effect of not fully accounting for terminations of pregnancy for NTD is demonstrated in Fig. 28.2. Victoria, South Australia and Western Australia have excellent ascertainment of terminations, whilst New South Wales does not, resulting in similar birth prevalence of NTD in the four states, but much lower total prevalence (which includes the termination data) in New South Wales.

The aim in Australia is to routinely collect data on NTD using a centralised monitoring base encompassing all jurisdictions, the Australian Congenital Anomalies Monitoring System. This is contingent on funding from the Commonwealth and various jurisdictions to enable national data development and is not assured at this stage.

Methodological issues abound in collection of NTD data. Firstly, new technologies will enable earlier prenatal diagnosis and detection of affected pregnancies that may have never entered the statistics before, having been missed in spontaneous miscarriages occurring early in pregnancy. This has the potential to inflate the NTD numbers reported. Secondly, NTD-affected pregnancies can vary substantially in duration and the critical exposure time for effective folic acid prevention is in the first weeks of pregnancy. It must therefore be determined whether analysis of NTD figures should be on the basis of the year in which the exposure occurred or on the expected date of delivery.

Fig. 28.2 Birth prevalence and total prevalence of NTD in four Australian states, 2002 [29]

Evidence of Effect of Mandatory Fortification

A retrospective analysis of serum and red cell folate levels in 20, 592 blood samples collected 2007–2010 from patients attending a large public hospital in New South Wales found a reduction in low levels of serum and red cell folate following the introduction of mandatory fortification, including amongst women of childbearing age. This sample is unlikely to be representative of the Australian population, as it consists of patients requiring blood tests and may include patients specifically being investigated for folate deficiency, and hence may be biased towards low folate status [30]. Confirmation in more representative population samples are needed.

With respect to NTD, data available for monitoring from most registers are usually collected annually, so will therefore be a year or so behind the current situation. As fortification was not introduced until September 2009, the impact on conception and therefore on incidence of NTD would not have begun until mid to late 2010 at the earliest. In turn, data on these births and terminations will probably not be available from the registers until 2012. The timeliness of the registry collections may improve with use of electronic health records and updated systems for collection accordingly. But again, it is not clear that Australia is supporting such registers in many jurisdictions and this will compromise adequate public health monitoring of the primary outcome of the mandatory folate fortification.

Recommendations

Coordinated and appropriate monitoring of mandatory fortification, including collection of data on NTD, particularly in terminations of pregnancy, should be adequately supported.

Conclusions

After a lengthy process involving attempts to raise folate awareness amongst women of childbearing age and the introduction of voluntary food fortification with folic acid, it was deemed necessary to introduce mandatory fortification of bread flour if the formation of NTD were to be prevented in Australia. This regulation occurred in September 2009 and a monitoring framework has been developed to determine its effectiveness. There are early indications of an increase in folate status in the general population, but not yet whether there have been any changes in nutrient status of women and children, a reduction in NTD or any other population health changes.

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Chapter 29
Vitamin D Supplementation in Children: Indian Perspectives

Anuradha V. Khadilkar and Shashi A. Chiplonkar

Key Points

- Over the last several years there are increasing reports of vitamin D insufficiency from India.
- Poor sunlight exposure due to social reasons, pollution, accelerated degradation of vitamin D due to genetic factors, and poor calcium intake are believed to be possible reasons.
- Specific recommendations have not been laid down for vitamin D intake in Indian children; however, 400 IU/day for exclusively breast fed infants and 400 IU/day for toddlers and older children along with calcium supplementation as necessary for low calcium intakes should be considered.

Keywords Vitamin D • India • Sun-rich • Deficiency • Supplementation

Abbreviations

25(OH)D	25-Hydroxyvitamin D
PTH	Parathyroid hormone
IU	International unit
μg	Microgram
VDR	Vitamin D receptor

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V.R. Preedy et al. (eds.), *Handbook of Food Fortification and Health: From Concepts to Public Health Applications Volume 2*, Nutrition and Health, DOI 10.1007/978-1-4614-7110-3_29,

Introduction

The crucial role played by vitamin D in musculoskeletal development is very well recognized. In infants and toddlers poor vitamin D status may lead to rickets while in children and adolescents it affects the acquisition of peak bone mass. Maternal vitamin D status has been shown to have a significant positive effect on neonatal bone status, and this effect appears to track up to the prepubescent period [1]. There is also a growing body of evidence which links vitamin D to various nonskeletal disorders including autoimmune disorders (Crohn's disease, multiple sclerosis, rheumatoid arthritis, and type 1 diabetes), infections, and risk of developing cancers. However, in this chapter, the discussions on vitamin D supplementation mainly focus on its role for the improvement of childhood bone health [2].

Prevalence of Low Vitamin D Status in Indian Children and Adolescents

Over the last several years there are increasing reports of vitamin D insufficiency from many sun-rich countries, including India [3]. It was believed that populations residing in countries with adequate sunlight derive most of their vitamin D from synthesis in the skin by conversion of 7 dehydro cholesterol to vitamin D3 in the presence of UVB rays. However, vitamin D deficiency/ insufficiency has been reported in pregnant women as also in apparently healthy teenagers, and in individuals from both rural as well as urban areas [3, 4]. A study from Lucknow describes a high prevalence of physiologically significant hypovitaminosis D among pregnant women and their newborns, the magnitude of which, authors feel warrants public health intervention [5]. Further, studies in toddlers and adolescents from both urban and rural areas also describe a high prevalence of hypovitaminosis D [4] (Table 29.1, Fig. 29.1).

Table 29.1 Indian studies reporting concentrations and prevalence of vitamin D deficiency from various locations

Study population	Location and reference	Vitamin D 25-(OH)D (nmol/L)	% Prevalence of vitamin D deficiency
Urban and rural children (11–13.6 years)	Andhra Pradesh [6]	17.5	17.2
Urban girls	Delhi [7]	31	93.7
Toddlers from lower socioeconomic class	Pune [8]	14	77

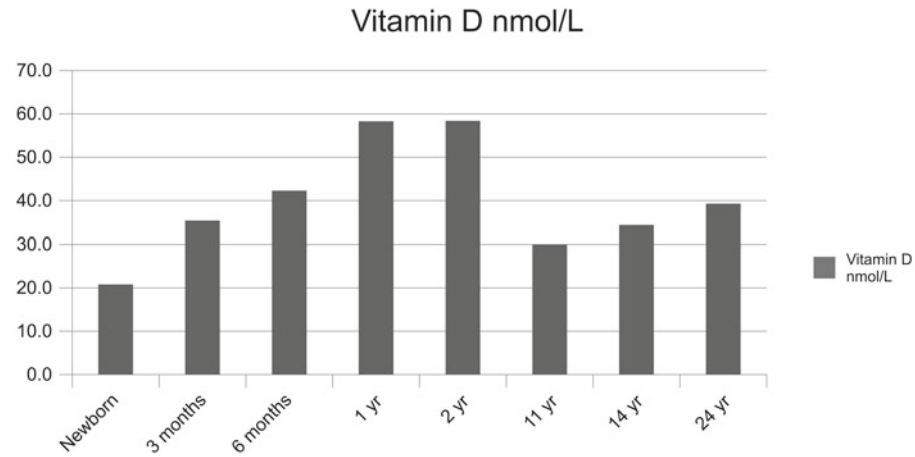

Fig. 29.1 Vitamin D concentrations as reported by various Indian studies

Causes for Vitamin D Deficiency in India

Various reasons have been suggested for poor vitamin D status in Indians. Poor sunlight exposure due to social reasons, pollution, and accelerated degradation of vitamin D due to genetic factors are some reasons being put forth [9]. In a study carried out to determine whether the vitamin D-endocrine system is altered in Asians, Indian immigrants living in southern United States were studied. Results of the study suggest that in South Asians 25(OH)D-24-hydroxylase (an enzyme which degrades 25(OH)D to inactive metabolites) activity in cultured skin fibroblasts is markedly reduced, thus resulting in lower concentrations of vitamin D [10]. More recently, it has been shown that the increment in serum 25OHD in response to treatment depends on the heritability of vitamin D binding protein [11]. Poor intake of calcium, as is reported from many studies is likely to result in raised serum PTH concentrations due to reversible end organ resistance to the actions of PTH [3, 12]. Further, calcium is derived in many cases from non-milk sources, thus reducing its absorption [13]. A diet rich in cereals and pulses, which is a characteristic of the Indian lifestyle, also contributes more phosphates, thus disturbing the calcium: phosphorous ratio which may again lead to secondary hyperparathyroidism [14]. Thus, on the background of widespread vitamin D deficiency, it is crucial to develop policies to combat the same.

Strategies to Improve Vitamin D Status in Children and Adolescents

The National Institute of Nutrition (India) advises increasing sunlight exposure as a strategy for achieving adequate vitamin D concentrations [15]. However, studies in rural adults from South India (latitude) suggest that 44 % men and 70 % women who were farm workers having plenty of sunlight exposure had vitamin D concentrations < 50 nmol/L [6]. Further, there is no consensus on the duration of sunlight exposure which would be appropriate for the maintenance of adequate vitamin D concentrations in darker skinned individuals. To alleviate vitamin D status, other choice would be nutrition intervention strategies, which mainly include: supplementation, fortification, and dietary diversification/modification. Supplementation can be especially useful for vulnerable groups, such as growing children and pregnant women, whose nutritional status needs to be improved over a relatively short period of time. Fortification of foods such as iodized salt, vitamin A-fortified oil, is useful for implementation at a larger scale. Vitamin D can be obtained only in very small amounts from dietary sources such as from oily fish (trout, salmon, mackerel, herring, sardines, anchovies, pilchards, and fresh tuna), cod liver and other fish liver oils, egg yolk, and from vegetarian sources such as mushrooms [16] (Table 29.2). Very few foods in India, mainly oils, are fortified, and these fortified products are not commonly consumed. Thus, reports suggest that, in Indians, only one tenth of the body's requirements for vitamin D are derived from dietary sources [17]. Hence, dietary diversification may not be a viable option for prevention of vitamin D deficiency in vulnerable groups in India. Providing vitamin D through supplementation or fortification to vulnerable groups may thus be the strategy of choice.

Pregnant, lactating mothers and their infants have been shown to have vitamin D deficiency in the Indian subcontinent [18]. However, studies suggest that doses exceeding 1,000 IU of vitamin D daily are necessary to achieve 25-OH-D concentrations of >50 nmol/L in pregnant women and there is still no consensus on the requirement of vitamin D during pregnancy and lactation [19]. Infants who are exclusively breastfed and do not receive supplemental vitamin D or adequate sunlight exposure are at increased risk of developing vitamin D deficiency, hence supplements to infants are necessary [20]. Balakrishnan et al. suggest that a routine vitamin D supplementation program starting from neonatal period should be instituted [20]. Though the Indian Academy of Pediatrics and the National Institute of Nutrition, Hyderabad, do not lay down specific recommendations, considering the various reports on vitamin D deficiency in mothers and infants from all parts of India, and that babies and mothers

Table 29.2 Sources of vitamin D in Indian foods

SrNo.	Brand	Type	Approximate amount of vitamin D/100 g
Natural sources			
No official data is available for vitamin D content of Indian raw or cooked foods			
Fortified foods			
1	Sweekar	Refined sunflower oil	5 μg (200 IU)
2	Nutrela	Refined sunflower oil	5 μg (200 IU)
3	Fortune	Soyabean oil	5 μg (200 IU)
4	Gemini	Refined sunflower oil	5 μg (200 IU)
5	Gemini	Groundnut oil	5 μg (200 IU)
6	Fortune plus	Refined sunflower oil	5 μg (200 IU)
7	Sundrop nutrilite	Blended edible vegetable oil (soyabean 80 % + sunflower 20 %)	15 μg (600 IU)
8	Sundrop superlight advanced	Refined sunflower oil	15 μg (600 IU)
9	Nutralite butter (zydus cadila)	Nutralite butter	5 μg (200 IU)

Table 29.3 Recommendations for vitamin D supplementation by various committees

American Academy of Pediatrics [21]	Endocrine Society Clinical Practice Guideline [22]	Indian recommendations [15]	Recommendations
Infants and children—400 IU/day	For infants upto 1 year of age—400 IU/day	Under situations of minimal sun exposure—400 IU/day	400 IU/day for exclusively breast fed infants 400 IU/day for toddlers + consider calcium for low calcium intakes
Adolescents— 400 IU/day	For 1–18-year-old children and adolescents—600 IU/day		400 IU/day + consider calcium for low calcium intakes
Higher doses and monitoring of vitamin D for children at increased risk of vitamin D deficiency			

from the Indian subcontinent are dark skinned, it would be prudent to follow the American Academy of Pediatrics recommendations which suggest that all breastfed and partially breastfed infants should be supplemented with 400 IU/day of vitamin D /day and that supplementation should be continued unless the infant is weaned to at least 1 L/day of vitamin D-fortified formula [21] (Table 29.3). Since vitamin D-fortified milks may not be affordable to a large section of the Indian population, continued supplementation with vitamin D drops is advisable. Several studies have reported the effectiveness of such regimens. In a study designed to compare the supplementation of breastfeeding women and their infants with vitamin D, infants who received 300 IU vitD3/day maintained vitamin D concentrations above 50 nmol/L throughout the study period [23]. Similar results are reported by studies from other countries [24, 25]. Though Indian supplementation studies in full term exclusively breast fed infants are scarce, a study was conducted in Delhi to investigate whether vitamin D supplementation could decrease the mortality and morbidity of low birth weight infants in low income countries. Low birth weight infants born at term (>37 weeks' gestation) were given weekly vitamin D supplements for 6 months at a dose of one recommended nutrient intake per day (35 μg/week). Authors found that vitamin D supplementation resulted in better vitamin D status as assessed by plasma calcidiol levels at 6 months [26]. Further, vitamin D supplementation significantly increased standard deviation (z) scores at 6 months for weight, length, and arm circumference and decreased the proportion of children

Table 29.4 Indian studies reporting vitamin D and calcium intake in children and adolescents

Age	Location	Study population	Vitamin D intake (μg/day)	Calcium (mg/day)
6–17-year-olds	Delhi [28]	LSES, girls	1.6 ± 1.2	468 ± 180
		HSES, girls	2.9 ± 6.4	690 ± 170
14–15 years	Pune [3]	LSES, girls	0.1 ± 0.4	450 (356–538)
14–15 years	Pune [29]	LSES, girls	5.1 (3–12)	450 (350–536)
9–15 years	Delhi [7]	LSES, girls	1.5 ± 1.3	454 ± 187
		HSES, girls	2.8 ± 1.4	685 ± 184

with stunted growth (length for age z score ≤ −2) or with arm circumference z scores of 2 or less. The authors thus concluded that a weekly dose of vitamin D resulted in better vitamin D status and benefited the bone growth among young low birth weight infants [26].

The toddler age group is also very vulnerable to vitamin D deficiency. In developing countries such as India and Africa, vitamin D deficiency is coupled with calcium deficiency, thus further compromising their bone health. Our studies in underprivileged toddlers in Pune suggest that low habitual dietary calcium intake (calcium intake between 55 and 75 % of the Indian recommendations of 200 mg/day) and vitamin D deficiency are common among Indian children [27] (Table 29.4). Thus, it is advisable that toddlers and children who are not ingesting 1,000 mL/day of vitamin D-fortified formula or milk should be supplemented with 400 IU of vitamin D daily and also with 500 mg of calcium to combat dietary calcium deficiency. Long-term compliance being poor, we used a 'laddoo', an Indian snack, as a vehicle for administering calcium and vitamin D supplements in toddlers (mean age 2.7 ± 0.52 years), in a randomized double-blind controlled trial for a period of 12 months [27]. All toddlers ($n = 60$) received a laddoo fortified with 30,000 IU of vitamin D3 per month. One group of 30 children received a calcium fortified laddoo (cereal–legume snack) containing 405 mg calcium per day, the other group received a non-fortified laddoo, containing 156 mg of indigenous calcium. Post-supplementation, vitamin D concentrations increased significantly in both groups; further in the first group, where children received both calcium and vitamin D, there was a significant increase in total body bone mineral content. Thus, such strategies may have the potential of addressing nutritional problems in developing countries [27].

Various supplementation studies have been carried out in adolescents, since almost 40 % of peak bone mass is accrued during puberty. A list of commonly available Indian vitamin D preparations is given in Table 29.5. In a pilot, double-blind randomized controlled trial to investigate the effect of vitamin D supplementation on bone mineral content in underprivileged adolescent girls, in Pune, India, 50 post-menarcheal girls aged 14–15 years were randomized to receive 300,000 IU (7.5 mg) of Ergocalciferol or placebo orally, 4 times in a year. All participants received 250 mg elemental calcium (Calcium carbonate, Calcium Sandoz, Novartis) daily [29]. Post-supplementation, the median serum concentration of 25-hydroxyvitamin D was significantly higher in the intervention group ($p < 0.05$) although the increment in bone outcome measures was not different in the two groups ($p > 0.1$). However, there was a positive effect of intervention in the size adjusted total body bone area ($p < 0.05$), total body bone mineral content ($p < 0.05$) and lumbar spine bone mineral content ($p < 0.05$), and positive trend in lumbar spine bone area ($p = 0.07$) in girls who were within 2 years of menarche [29]. In line with these results, in a study on 10–17-year-old girls from Lebanon who were randomly assigned to receive weekly oral vitamin D doses of 1,400 IU (equivalent to 200 IU/day) or 14,000 IU (equivalent to 2,000 IU/day) in a double-blind, placebo-controlled, 1-year study, authors found that only in premenarcheal girls, lean mass increased significantly in both treatment groups, and there were consistent trends for increments in BMD and/or BMC at several skeletal sites, reaching significance at lumbar spine. There was no significant change in lean mass, BMD, or BMC in post-menarcheal girls [30]. Thus, the positive impact of vitamin D replacement is more likely to be seen before the period of peak mineral and muscle mass accretion is complete [31]. In a study from Delhi, which aimed to determine the efficacy of supplementation with oral vitamin D3 (cholecalciferol) on bone mineral

Table 29.5 Some common vitamin D preparations available in India

Sl No	Name	Content	Dose
1	Arachitol	Cholecalciferol	3 lac IM
			6 lac IM
2	Calcirol	Cholecalciferol	60,000 IU sachet
			1 g granules
3	Ostocalcium	Calcium	400 mg/tab
		Vitamin D3	200 IU
4	Ostocalcium syrup	Calcium	82 mg
		Vitamin D	200 IU/5 ml
5	Calcimax	Calcium	400 mg
		Vitamin D	200 IU
6	Shelcal	Calcium	250 mg
		Vitamin D	125 IU/5 ml
7	Ossopan	Calcium	125
		PO4	55
		Vitamin D	125 IU/5 ml
8	Gemcal	Calcium	500 mg
		Vitamin D3	0.25 µg/g ml
9	Alfa D3	Vitamin D3	0.25, 1 µg capsule
10	Rocaltrol	Vitamin D3	0.25, 1 µg capsule

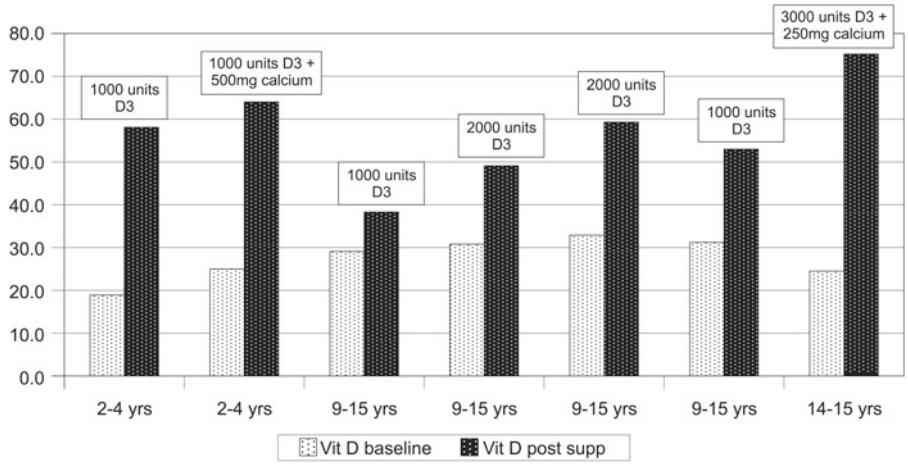

Fig. 29.2 Rise in vitamin D after 1-year supplementation in vulnerable groups

biochemical parameters of school-going girls, researchers found that at baseline, 93.7 % schoolgirls were vitamin D deficient. They supplemented the girls with two monthly or one monthly 60,000 units of vitamin D3. While a significant increase was seen in serum calcium and vitamin D and decrease in alkaline phosphatise levels in both groups with both interventions, only 47 % girls were vitamin D sufficient at the end of 1 year [7] (Fig. 29.2).

Thus vitamin D supplementation together with calcium in girls who are younger, having poor habitual calcium intake, and supplementation of girls with hypovitaminosis D seems to confer the maximum advantage of supplementation with vitamin D. Certain subgroups, for example, with the FF VDR genotype may also benefit more from supplementation. Studies also suggest that higher doses may be beneficial [32]. Most of these studies have supplemented vitamin D for a period of a year. Longer supplementation studies are required.

Tolerable Upper Limits

Though there are no Indian recommendations on the maintenance of tolerable upper limits of vitamin D, studies suggest doses up to 1,000 IU/day for infants up to 6 months, 1,500 IU/day from 6 months to 1 year, 2,500 IU/day for children aged 1–3 years, 3,000 IU/day for 4–8-year-olds, and 4,000 IU/day for everyone over 8 years [22].

Recommendations

For musculoskeletal health of Indian children, a supplement of 400 IU/day of vitamin D for exclusively breast fed infants and 400 IU/day for toddlers and older children with calcium supplementation (as necessary) for low calcium intakes is recommended.

Action Plan for Alleviating Vitamin D Status

1. Promote role of vitamin D status in health:
   (a) Contact/discuss with parents, educators, and caregivers and stress the importance of vitamin D adequacy for healthy bone growth in childhood and adolescence.
   (b) Create awareness among parents about health risks of vitamin D deficiency in children.
   (c) Support education for parents, caregivers, and individuals who provide services for children, e.g., crèche, to enhance their knowledge of dietary needs and food sources of vitamin D.
   (d) Prepare fliers, material for disseminating importance of vitamin D in child health.
   (e) Better access to information on vitamin D; daily requirements, sources, and health risks from excessive vitamin D supplements.
2. Early detection of vitamin D deficiency:
   (a) Identify at-risk individuals. Then detection of vitamin D deficiency needs to be carried out by clinical methods and serum concentrations of vitamin D.
3. Corrective measures:
   (a) Advice 30 min of sunlight exposure daily.
   (b) Impart nutrition education about sources of vitamin D in food. Impart the knowledge that obtaining sufficient vitamin D from natural food sources alone is difficult. For many people, especially vegetarians, consuming vitamin D-fortified foods or vitamin D supplements are essential to meet the daily need for vitamin D.
   (c) Provide knowledge about vitamin D and bone health in school curriculum implementation as part of nutrition and physical education, based on effective learning strategies.
   (d) Involve various professional bodies in developing and disseminating collaborative guidelines for alleviating vitamin D status of children and adolescents.
4. Precautions for taking vitamin D supplements:
   Supplements of vitamin D should be taken under clinical supervision.

Conclusions

Vitamin D plays a crucial role in musculoskeletal development. Although India is a sun-rich country, vitamin D deficiency has been reported in all age groups from pregnancy to late adolescence. Vitamin D deficiency is often accompanied by low calcium intakes. Very few foods fortified with vitamin D are available in the Indian market, and these are not commonly consumed. As there is very little vitamin D in Indian children's diet, providing vitamin D through supplementation or fortification to vulnerable groups may thus be the strategy of choice. Thus, a supplement of 400 IU/day of vitamin D for exclusively breast fed infants and 400 IU/day for toddlers and older children with calcium supplementation (as necessary) for low calcium intakes is recommended.

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Chapter 30
Use of Fortified Foods for Indonesian Infants

Umi Fahmida

Key Points

- Complementary feeding of Indonesian infants is characterized by rice-based diet with predominantly plant rather than animal source foods and thus is associated with low nutrient density particularly for iron, zinc, and calcium.
- Intakes of fortified foods (FF) among Indonesian infants are increasing within the past 5 years with trend of higher intakes in urban than rural area. Fortified infant cereals are only typically consumed by younger (6–8 months) rather than older (9–11 months) infants, putting older infants at higher risk of inadequate micronutrient intakes.
- Fortified foods can increase intakes of iron, zinc, and calcium by 31–45 % of the estimated needs from complementary feeding. In addition fortified foods can improve energy balance of the diet toward higher proportion of energy from fat and protein and lower proportion of energy from carbohydrate.
- Level of fortification should consider the problem nutrients in this age group (iron, zinc, calcium); the recommended proportion of fatty acids to optimize growth, development, and long-term health; and the actual portion infants actually consume. Multiple nutrients added at moderate amount are preferred over single nutrient added in high amount.
- Fortification of staples—in addition to the existing manufactured infant cereals, noodle, and biscuits—is promising alternative and may be a more feasible one under poorer community in which intake of problem nutrients especially iron is difficult to be met with nutrient-dense foods or fortified foods.

Keywords Indonesia • Infant • Fortified foods • Nutrient-dense foods • Iron • Zinc • Calcium • Fatty acids • Linear/goal programming

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V.R. Preedy et al. (eds.), *Handbook of Food Fortification and Health: From Concepts to Public Health Applications Volume 2*, Nutrition and Health, DOI 10.1007/978-1-4614-7110-3_30,

Abbreviations

CF	Complementary foods
CFR	Complementary feeding recommendations
EFA	Essential fatty acids
FF	Fortified foods
LA	Linoleic acids (18:2(n-6), an omega-6 fatty acid)
LNA	α-linolenic acid (18:3(n-3), an omega-3 fatty acid)
LP	Linear/goal programming
NDF	Nutrient-dense foods
PUFA	Polyunsaturated fatty acids
RE	Retinol equivalent
SNI	*Standar Nasional Indonesia* (The Indonesian National Standard)

Introduction

Adequate nutrition during the first 2 year of life is important to ensure optimal physical and mental development. Malnutrition, which prevents infants/young children from growing to their full genetic potential, remains problematic in developing countries including the Southeast Asian countries. It occurs primarily during the first 2 year of life [1, 2] making it essential to provide a nutritionally adequate complementary diet. During the complementary feeding period, breast milk provides less than 50 % of an infant's high nutrient needs for iron, zinc, calcium, thiamin, and riboflavin [3]. Given the limited gastric capacity of infants to consume foods, consequently, complementary foods (CF) with a high nutrient density should be provided (WHO, 1998). Animal source foods have been cited in particular as essential to achieve micronutrient requirements [4]. However in developing countries where significant numbers of households still live below poverty level such as in Indonesia, promoting intakes of animal source foods will not be an easy task. Fortification of food vehicles which are consumed predominantly by lower-socioeconomic population can contribute to increase micronutrient intakes.

Fortification of foods which commonly consumed by infants provides an opportunity to improve nutrient density of the complementary foods especially in infants <12 months with more limited gastric capacity. The success of this strategy, however, depends on the availability of fortified foods at the community level (distribution), accessibility, intake (consumption), and levels of fortification. The objective of this paper is to present evidence on the use of fortified foods among Indonesian infants, its contribution to nutrient adequacy of the infants, and potential implication on relevant programs.

Profiles of Nutrient Intakes of Indonesia Infants

Complementary feeding intakes of Indonesian infants are characterized by rice-based diet with low intake of both plant and animal protein and moderate intake of fruits and vegetables. There is only little difference in complementary feeding pattern between rural and peri-urban area [5, 6] (Table 30.1). As a result the complementary food has very low nutrient density of iron, zinc, calcium (<50 % recommended levels). In older infants (9–11 months of age), there is indication that vitamin A may also be inadequate based on data from rural area in West Nusa Tenggara (Table 30.2). In addition to the very low iron density, folate and other B-vitamins are still inadequate (<70 % recommended levels);

Table 30.1 Population food patterns (frequency of intake per week) of typical Indonesian infants, by age group and area[a]

	Rural[b]		Peri-urban[c]
	6–8 Months	9–11 Months	9–11 Months
Cereals staples	21 (7–28)	21 (11–28)	19 (11–21)
Animal protein	2 (0–7)	2 (0–6)	3 (0–12)
Plant protein	2 (0–7)	2 (0–7)	1 (0–5)
Vegetables	4 (0–11)	4 (0–10)	5 (0–14)
Fruits	4 (0–7)	4 (0–11)	2 (0–9)
All snacks	14 (0–25)	14 (4–27)	16 (7–29)

[a]Median (min–max)
[b]*Source*: Harper (2006)
[c]*Source*: Santika et al. (2009)

all of these might have contributed to the high prevalence of anemia among this age group in Indonesia. Anemia prevalence data in infants reported in previous studies in Indonesia ranged from 41 % [7] to as high as 71–82 % of infants within the first 6 month of age [8, 9] and by the second 6 month of infancy the reported prevalence ranged from 58 % [10] to as high as 90 % in rural area with dietary profile as reported in this paper [9].

Intakes of Fortified Foods by Indonesian Infants

Food fortification in Indonesia was initiated in 1994 with mandatory fortification of iodized salt [11]. In 1997 trials on wheat flour fortification with iron, zinc, folate, vitamin B1 and B2 were started and as a result wheat flour fortification was specified in National Standard of Indonesia (*Standar Nasional Indonesia*, SNI) and became mandatory in 2001. Both the iodized salt and fortified wheat flour fortification were developed by National Planning Board, Ministry of Health, Ministry of Industry, and Ministry of Trade with the support of UNICEF. In 2009/2010 feasibility study to fortify cooking oil with vitamin A was launched and in 2011 vitamin A fortification in cooking oil was encouraged as voluntary fortification. In the near future vitamin A fortification in cooking oil will become mandatory fortification.

The Indonesian National Standard for Complementary Foods (SNI MP-ASI) defines complementary foods as "*nutritious foods given in addition to breastmilk to infants aged 6 months and older or on medical indication, until the age of 24 months*" [12]. Commercially available food products falling under this category for infants and young children are instant cereals, biscuits, and a few ready-to-cook products. All of these products must comply with the national Codex standards regarding the content—which is a prerequisite for the registration and marketing—and all are currently fortified to some degree [13].

The availability of fortified foods including fortified complementary foods in the Indonesian market has been growing in the past 5 years. Fortified foods have entered not only urban but also rural markets, which make potential contribution given that approximately 70 % of the Indonesian population living in rural areas. For instance, our study conducted in rural West Nusa Tenggara province in 2005 showed that only 42 % of the infants consumed biscuits, whereas our recent study in 2010 conducted in the same area showed that as many as 82 % of infants aged 9–11 months in the area consumed biscuits. Based on observation in the local markets, more brands of biscuits (and similar type such as wafer, crackers), noodles, and drinks found in 2010 were fortified than were found in 2005 surveys. There is a tendency that intakes of fortified foods are higher in urban than rural. For instance,

Table 30.2 Nutrient densities of the complementary feeding diets consumed by Indonesian infants aged 6–8 and 9–11 months in comparison with WHO recommendation[a]

	Recommended levels (WHO, 2003)	6–8 Months ($n=72$)	% Recommended levels	Recommended levels (WHO, 2003)	9–11 Months ($n=67$)	% Recommended level
Protein (g/100 kcal)	1.0	2.5	250	1.0	2.4	240
Iron (mg/100 kcal)	4.5	0.55	12	3.0	0.38	13
Zinc (mg/100 kcal)	1.6	0.37	23	1.1	0.33	30
Calcium (mg/100 kcal)	105	22	21	74	12	16
Magnesium (mg/100 kcal)	15	13	87	11	13	118
Thiamin (mg/100 kcal)	0.08	0.03	38	0.06	0.03	50
Riboflavin (mg/100 kcal)	0.08	0.04	50	0.06	0.03	50
Niacin (mg/100 kcal)	1.5	0.40	27	1.0	0.39	39
Pantothenic acid (mg/100 kcal)	0.29	0.29	100	0.23	0.25	109
Vitamin B6 (mg/100 kcal)	0.12	0.06	50	0.08	0.06	75
Folate (μg/100 kcal)	11	6.8	62	9.0	6.1	68
Vitamin B12 (mg/100 kcal)	0.08	0.05	63	0.03	0.02	67
Vitamin C (mg/100 kcal)	1.5	2.1	140	1.7	1.5	88
Vitamin A (μg RE/100 kcal)	31	16	52	30	8.4	28

[a]*Source*: Harper (2006)

Table 30.3 Percentage of infants who consumed fortified products and the portion size, by area and age group

	Rural[a]				Peri-urban[b]	
	6–8 Months		9–11 Months		9–11 Months	
Fortified foods	%	Portion (g)[c]	%	Portion (g)[c]	%	Portion (g)[c]
Infant cereal	20	19 (11–32)	0	–	29	20 (3–40)
Noodle	2	49 (24–75)	14	15 (8–29)	4	22 (5–34)
Biscuits	44	9 (7–11)	41	7 (5–10)	20	6 (1–30)

[a]*Source*: Harper (2006)
[b]*Source*: Santika et al. (2009)
[c]Median (min–max)

national data shows that the proportions of children (6–59 months of age) who received fortified milk and fortified noodles were 30.1 % and 22.6 %, respectively, in rural families and 40.1 % and 48.9 %, respectively, in urban families [14]. Nevertheless more studies are required to provide information on intakes of fortified foods and their portion sizes for different parts of Indonesia.

Intakes of fortified foods in rural Lombok mainly came from manufactured infant cereals which are powdered cereal sold in one portion package to make one portion soft porridge. Due to its too soft texture, however, it is more consumed by younger infants aged 6–8 months and is less consumed by older infants aged 9–11 months [5, 6] (Table 30.3).

Contribution of Fortified Foods to Nutrient Intakes of Indonesian Infants

Table 30.4 shows the expected contribution which can be delivered in each portion of fortified foods. It is obvious that the contribution to calcium, iron, and zinc intakes made by fortified foods is still quite small, except for fortified infant cereal.

To demonstrate how much the contribution of fortified foods in infant's diet, we illustrate four types of diet which have been optimized as complementary feeding recommendation (CFR) using linear/goal programming (LP) approach [15] based on actual data of rural Indonesian infants. The food patterns used in this illustration are detailed in Table 30.5. The *first* type of diet (*local diet*) is optimized diet without specifying locally available specific nutrient-dense foods (NDF). The *second* type (*local diet*+*NDF*) is diet which was optimized with inclusion of NDF which had been identified as potential to fill the gap of problem nutrients—defined as nutrients in which the worst-case scenario of the CFR is below 70 %—in the diet, i.e., calcium, iron, and zinc. Addition of fortified foods available in the local market is shown by the *third* type of diet (*local diet*+*NDF*+*FF*). Finally we also illustrate the situation when rice fortified with iron and other nutrients based on the level reported in previous report [16] is included in the diet (*local diet*+*NDF*+*FF*+*Fe-rice*). The fortified rice is based on the following composition (per 100 g dry weight): 180 μg retinol equivalent (RE) vitamin A, 0.36 mg vitamin B1, 4.8 mg vitamin B3, 1.26 mg zinc, 2.73 mg iron, and 120 μg folate.

As can be shown by the illustration (Fig. 30.1), fortified foods can increase adequacy of calcium, iron, and zinc by 31–45 % of the estimated needs from complementary feeding. Adequacy of iron is the most difficult to meet, followed by zinc and calcium.

While effort to meet adequacy of the key problem nutrients (iron, zinc, calcium) is the most important, this should not discourage from targeting toward favorable dietary diversity and balance of energy contributed from carbohydrate, protein, and fat. As infant is the period of rapid growth, fats particularly essential fatty acids (EFA) are very important in the diet for the normal neurodevelopment. In infant and young children above 6 months old total fat in the diet should meet 30–35 % of

Table 30.4 Fortified foods consumed by Indonesian infants in rural West Nusa Tenggara province and their nutrient contributions on calcium, iron, and zinc intakes

Fortified foods	Average portion (g)	Frequency per week[a]	Nutrient intake per portion		
			Calcium (mg)	Iron (mg)	Zinc (mg)
Manufactured infant cereals	14	1 (0–21)	105.0	2.28	1.00
Noodles	13	2 (0–17)	2.7	0.22	0.06
Biscuits					
Calcium fortified	10	0 (0–5)	15.0	0.07	0.07
Multi vitamins-minerals	3–5	5 (0–21)	8.6–12.6	0.13–0.48	0.08–0.44
Crackers	7	5 (0–21)	0	0.10	0.27
Wafer					
Calcium fortified	5	1 (0–10)	127.1	0.11	0.02
Multi vitamins-minerals	4	1 (0–10)	0.4	0.03	0.02
Drinks	45–70	0 (0–8)	75.1–80.0	0	0

[a]Median (min–max)

Table 30.5 Food pattern used in the illustration of the four types of optimized diets based on the result of goal/linear programming

Weekly	Group	Food	Local diet	Local diet + NDF	Local diet + NDF + FF	Local diet + NDF + FF + Fortified rice
21	*Staples*		*21*	*21*	*21*	*21*
		Rice	21	21	14	
		Infant cereal-fortified[a]			7	7
		Rice-fortified[a]				14
14	*Animal protein*		*14*	*14*	*14*	*14*
		Chicken liver	2	2	2	2
		Fish	4	2	2	2
		Egg-chicken	4	2	2	2
		Egg-quail	4	2	2	2
		Shredded chicken liver[b]		2	2	2
		Shredded fish[b]		2	2	2
		Anchovy powder[b]		2	2	2
7	*Plant protein*		*7*	*7*	*7*	*7*
		Tahu	4	4	4	4
		Tempe	3	3	3	3
7	*Vegetable*		*7*	*7*	*7*	*7*
		Bayam	7	4	4	4
		Stir-fried vegetable mixed with protein[b]		3	3	3
21	*Snacks*		*21*	*21*	*21*	*21*
	Fruit	Banana	5	5	5	5
	Other snacks	Chips	8	5	0	0
	Biscuits	Biscuits	8			
		Anchovy crackers[b]		6	6	6
		Fish meatball[b]		5	5	5
		Biscuits-fortified[a]			5	5

[a]Fortified with at least one of the problem nutrients (iron, zinc, calcium)
[b]Nutrient-dense foods

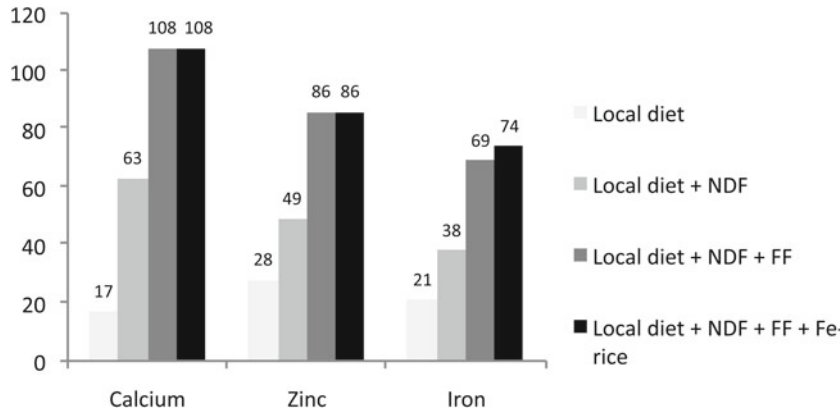

Fig. 30.1 Intakes of problem nutrients as percentage of estimated nutrient needs (WHO, 2008). *NDF* nutrient-dense foods, *FF* fortified foods, *Fe-rice* rice fortified with iron and other nutrients (zinc, vitamin A, B2, B3, folate)

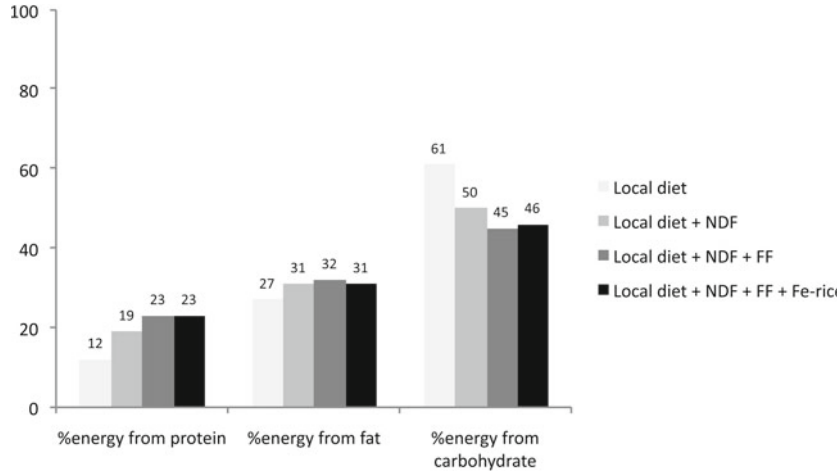

Fig. 30.2 Percentage energy contributed from protein, fat, and carbohydrate from the four types of diets. *NDF* nutrient-dense foods, *FF* fortified foods, *Fe-rice* rice fortified with iron and other nutrients (zinc, vitamin A, B2, B3, folate)

total energy, with 3–4.5 % of total energy from linoleic acid (LA) and at least 0.5 % energy from linolenic acid (LNA) to meet EFA requirements [17]. As illustrated in Fig. 30.2, fortified foods and nutrient-dense foods can improve proportion of energy from fat and protein and thus decrease the proportion of energy from carbohydrate source foods. More specific information regarding the contribution of energy from LA and LNA, saturated fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids (PUFA), and *trans* fatty acids from the fortified foods—such as those with added omega 3 and omega 6—and to the total diet cannot be ascertained due to lack of detailed information on the label as well as incomplete food composition database on specific types of these fatty acids.

It is important to note that this proportion is obtained from combination of fortified foods and other non-fortified foods, including nutrient-dense foods. Effort to meet the entire micronutrient requirement from fortified foods is not recommended as it will interfere with dietary diversity and there is a concern on the caloric content of the fortified infant cereals if consumed following the recommended

portion size and the recommended frequency of feeding [13]. The actual average portion size from our data (20 g/portion) is only half of the recommended portion per serving (40–50 g/portion) [13], yet this amount if consumed twice by 6–8 month and 9–11 month infants will provide up to 190 and 275 kcal/day, respectively. These amounts if combined with other foods including the snacks consumed on the same day will likely exceed the energy needs from complementary foods of 200 and 300 kcal/day, for 6–8 month and 9–11 month breastfed infants, respectively [18].

Small Subsection "Recommendations" (for Policy Planners, Manufacturers, etc.)

The current trend of the fortification in manufactured complementary foods and biscuits targeted for infants and children includes fortification with vitamin-minerals and fatty acids, mainly as omega 3, omega 6, and DHA.

While manufactured infant cereal is one of the most contributing fortified foods in infants, the level of iron fortification may need to be increased. Gibbs et al. analyzed samples of 14 manufactured infant cereal sold in Indonesia [19] and found that while phytate:zinc and phytate:calcium molar ratios were desirable for all (<18 and <0.17 for zinc and calcium, respectively) only one sample met desirable phytate:iron molar ratio of <1 (Table 30.6). Assuming daily intake of 40 g dry weight (20 g dry weight per portion, twice daily) the contribution to the daily nutrient intakes (as percentage of WHO estimated needs) were 10–45 % for iron, 20–65 % for zinc, and 50–90 % for calcium. This suggests that the levels of fortification may need to be increased in order to meet the requirements. The increment in the fortification levels should be highest for iron, followed by calcium and zinc. This consideration is supported by evidence on: (1) nutrient density in typical home-made complementary foods as illustrated above and (2) molar ratios of phytate and the respective nutrients in the fortified complementary foods.

On the other hand, levels of fortification used in several efficacy studies [20, 21] may be too high if applied in the real setting. For instance, the simulation of LP-optimized diet using iron-fortified rice (24 mg iron per 100 g of rice) will result in increase of iron intake to as high as 160 %, which may interfere the absorption of zinc, another problem nutrient. In addition, iron and zinc are among those nutrients which exhibit a narrow safety margin, therefore risk of excessive intake is potentially high [22]. Multiple vitamin-minerals added at moderate level are preferred rather than high level of a single nutrient.

Levels of fatty acids particularly EFA should also be given more attention in the fortified products as very high EFA may have no advantage and are associated with potential health risks [17]. Uauy and Castillo (2003) recommend that intake of LA and other (n-6) fatty acids should be limited to <10 % of energy, that of total PUFA should be limited to <15 % energy, saturated fatty acid intake should not exceed 10 % total energy, and *trans* fatty acids should be avoided. While PUFA is beneficial, its intake should contribute <6–10 % of energy as the remaining fat energy should come from monounsaturated fatty acids. Detailed information on the food label regarding composition of the fatty acid content in the fortified foods targeted for infants and young children is often incomplete in order for the consumers to be better informed on the favorable (e.g., omega 3) and unfavorable (e.g., *trans* fatty acid) fat content in the food products.

It is recommended that manufacturer of fortified foods targeted for infants considers the actual nutrient intake, portion size, and frequency of this age group, so that types and levels of the nutrient fortification will address to the problem nutrients. In addition, providing sufficient information on the food label is important and should be reinforced by the relevant government and nongovernment institutions.

Table 30.6 Some manufactured infant cereals in Indonesian market and their profiles of the key nutrients iron, zinc, and calcium

Code	Ingredients	Fortification		Iron	Zinc	Calcium	Phy:Fe	Phy:Zn	Phy:Ca
		Vitamins-minerals	Omega 3 and 6						
I1	Rice, soya, mixed fruit, lecithin, FOS	√	√	5±0.07	3.4±0.08	357±2	1.7	4	0.02
I2	Rice, soya, chicken, vegetables, lecithin, FOS	√	√	7.7±0.09	2.2±0.05	440±4	2.2	9	0.03
I3	Mung bean, rice, soya, corn, lecithin, FOS	√	√	9.2±0.01	1.9±0.03	456±4	2.1	12	0.03
I4	Rice, brown rice, red kidney beans, soya, corn, lecithin, FOS	√	√	9.0±0.16	2.9±0.24	365±2	2	7	0.04
I5	Rice, beef, broccoli, lecithin, FOS	√	√	2.9±0.03	2.1±0.07	277±2	2.2	4	0.02
I6	Mung bean, spinach, rice, soya, lecithin, FOS	√	√	9.5±0.07	6.8±0.12	474±6	2.7	4	0.04
I7	Mung bean, rice, soya, FOS	√		7.1±0.22	4.5±0.41	413±2	1.1	2	0.01
I8	Rice, corn, chicken, vegetables (tomato, carrot, onion, beans)	√		6.3±0.26	5.2±0.32	456±9	0	0	0
I9	Rice, mung bean, lysin, FOS	√		3.9±0.25	5.1±0.68	364±1	1.3	1	0.01
I10	Brown rice, rice, lysin, FOS	√		10.4±0.18	4.5±0.13	399±7	2.3	6	0.04
I11	Brown rice, rice, chicken, vegetables	√		8.2±0.09	2.6±0.06	508±9	2.6	10	0.03
I12	Mung bean, rice, lysin, FOS	√		8.3±0.09	4.4±0.07	408±1	1.7	4	0.03
I13	Brown rice, rice, lysin, FOS	√		8.6±0.08	5.1±0.06	349±3	1.8	3	0.03
I14	Rice, corn, banana, lysin, FOS	√		6.1±0.03	5.0±0.10	348±3	1.1	1	0.01

Source: Gibbs et al. (2011)

Conclusions

- Complementary feeding of Indonesian infants have very low nutrient density particularly for iron, zinc, and calcium. Fortified foods can increase intakes of iron, zinc, and calcium by 31–45 % of the estimated needs from complementary feeding. In addition fortified foods can improve energy balance of the diet toward higher proportion of energy from fat and protein and lower proportion of energy from carbohydrate
- Level of fortification should consider the problem nutrients in this age group (iron, zinc, calcium); the recommended proportion of fatty acids to optimize growth, development, and long-term health; and the actual portion infants consume. Multiple nutrients added at moderate amount is preferred over single nutrient added in high amount.
- Fortification of staples –in addition to the existing manufactured infant cereals, noodle and biscuits—is a promising alternative and may be a more feasible one under poorer community in which intake of problem nutrients especially iron is difficult to be met with nutrient-dense foods or fortified foods.

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Chapter 31
Micronutrient Fortification of School Lunch Meals in Himalayan Villages

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Key Points

- Micronutrient deficiencies affect millions of schoolchildren in developing countries.
- In this review, we describe the effectiveness of fortification of cooked lunch meals with multiple micronutrient powder at school on anemia and micronutrient status of primary schoolchildren in Tehri Garhwal District of Uttarakhand, India.
- The micronutrient fortified school lunch meal was well accepted by the schoolchildren.
- Schoolchildren who consumed the micronutrient fortified school lunch meals for 8 months had 43 % reduced odds of vitamin A deficiency [OR (odds ratio) (95 % CI): 0.57 (0.33–0.97)], folate deficiency of 53 % [OR (95 % CI): 0.47 (0.26–0.84)], and vitamin B_{12} deficiency of 59 % [OR (95 % CI): 0.41 (0.22–0.86)] compared to placebo.
- Iron status of schoolchildren also improved significantly after consuming the micronutrient fortified school lunch meals. The mean total body iron (TBI) increased from 148.6 ± 0.1 μmol/kg to 220.5 ± 0.1 μmol/kg from baseline to post-intervention in the children who consumed the micronutrient fortified lunch meal compared to an increase from 148.1 ± 0.1 μmol/kg to 197.6 ± 0.1 μmol/kg in the placebo group ($p < 0.05$).

Keywords Micronutrient powder • Home fortification • Effectiveness • Schoolchildren • India • Anemia • Iron • Vitamin A • Zinc • Folate • Vitamin B_{12}

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V.R. Preedy et al. (eds.), *Handbook of Food Fortification and Health: From Concepts to Public Health Applications Volume 2*, Nutrition and Health, DOI 10.1007/978-1-4614-7110-3_31,

Abbreviations

CI	Confidence interval
IQR	Interquartile range
OR	Odds ratio
RDA	Recommended dietary allowance
RNI	Recommended nutrient intake
SD	Standard deviation
SEM	Standard error of the mean
TBI	Total body iron
UL	Tolerable upper intake level

Introduction

Micronutrient deficiencies affect millions of schoolchildren in developing countries [1]. Anemia is by far the most widely prevalent and affects about 46 % of schoolchildren in low income countries, with the highest prevalence in South Asia (50 %) [1, 2]. Vitamin A deficiency (serum retinol < 0.7 μmol/L) also affects 23 % of South Asian schoolchildren [3]. Children with micronutrient deficiencies may experience retarded growth [4], reduced immune function [5], impaired motor and cognitive development [6], and poor school attendance, academic performance, and achievement [7].

Inadequate dietary intake, poor bioavailability, and excessive losses from the body are considered the major direct causes of micronutrients deficiencies [1]. These conditions are widespread in developing countries, mainly because the diet in most parts consists mainly of plant-based staples (with high amounts of micronutrient absorption inhibitors) and a relatively limited intake of animal source foods. Infections are also widespread in most developing countries, particularly in rural areas. Nevertheless, the timely provision of micronutrients through nutrition, in addition to adequate care and other health-related interventions, is essential in preventing and often reversing such deficiencies and their associated developmental impairments [8]. Fortification of cooked food just before consumption, using micronutrient powders (also called "home fortification"), is considered one of the most cost-effective interventions for improving micronutrient status of young children. Studies from several countries, including India, have demonstrated that fortification of cooked food with micronutrient powders just before consumption is efficacious in improving micronutrient status of preschool and schoolchildren [9–11]. In addition, a recent systematic review found that home fortification of cooked food using micronutrient powders (containing at least iron, vitamin A, and zinc) significantly reduced anemia by 31 % and iron deficiency anemia by 51 % among children less than 2 years old [12, 13]. However, there is still limited evidence of the effectiveness of this intervention, particularly among schoolchildren. In this chapter, we present the fortification of cooked lunch meals with a multiple micronutrient powder (whereby the cooking and fortification are done at the school); the acceptance of the fortified meal by schoolchildren; and the effectiveness of the fortified meal on anemia and micronutrient status of schoolchildren in Tehri Garwal district in Uttarakhand State, India. Tehri Garhwal district is a hilly agrarian community located in the mid-Himalayan ranges of Uttarakhand State, about 250 km northeast of Delhi, the capital city of India (Fig. 31.1).

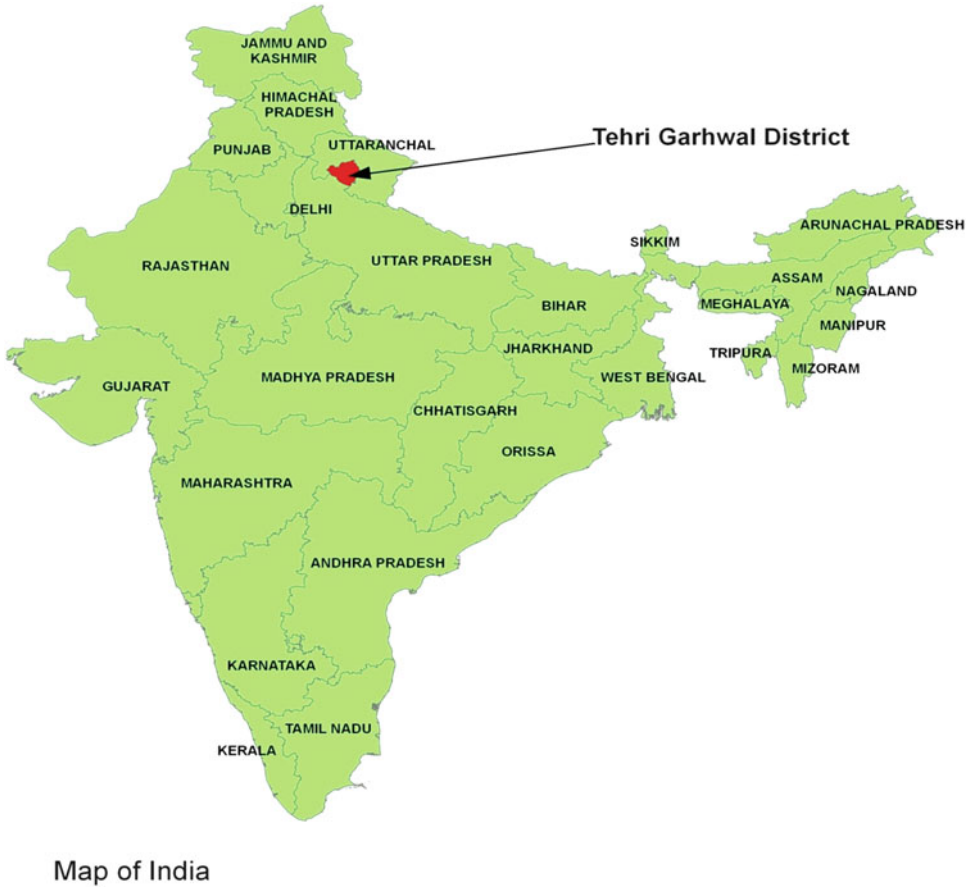

Fig. 31.1 Map of India showing Tehri Garhwal District, the study location. *Source*: Created with ArcGIS Desktop software, version 9.0

The School Feeding Program in India

Since 2001, an order from the Supreme Court of India directs all State governments of the country to implement a school feeding program in all public primary schools. Under this program, commonly known as the "midday meal" scheme, primary schoolchildren receive free lunch meals, cooked and served at school for at least 200 days in a school year. In most States, the program has a standardized menu that consists mainly of rice and dhal (sauce prepared using pulse/lentils) or vegetables. In all the States, the aim of the midday meal scheme is to provide each child with at least 1,884 kJ and 12 g of protein per day. This program was mainly designed to address protein-energy malnutrition and not micronutrient deficiencies, therefore the micronutrient content of the lunch meal in most schools is considered less than adequate. Although a revision of the guidelines of the program in 2006 recommended that the lunch meal should provide adequate quantities of micronutrients such as iron, vitamin A, and folic acid, little has been done to ensure the successful achievement of this objective. In addition, implementation of the midday meal program has not been uniform across all States and has been faced with logistical challenges including limited funding and shortage of supplies including cereals and cooking fuel. In August 2007 (at the beginning of the research that provided data for the

review presented in this chapter), there were 1,350 public primary schools with 71,423 children enrolled in all the 9 blocks (subdistrict) in Tehri Garwal district of Uttrakhand. The midday meal program was operational in all the schools, which provided schoolchildren with cooked lunch meals at school 6 days per week. No micronutrient intervention or deworming program was active among schoolchildren in the district. This was not surprising since to date programs for addressing micronutrient malnutrition in India have focused mainly on preschool children and women of reproductive age. With the exceptions of deworming, iodization of salt, and fortification of commercially available foods, no clear policies exist on addressing micronutrient malnutrition among schoolchildren in most parts of India. Even in places where these policies exist, implementation and coverage of micronutrient programs is usually poor in rural areas [13]. Therefore, there is a need for a simple and easy-to-transfer strategy that can be used to address micronutrient deficiencies among schoolchildren in rural communities of India.

Formulation of a Micronutrient Powder for Fortification of the School Lunch Meal

To improve the micronutrient content of the lunch meals served to schoolchildren in Uttarakhand, the World Food Program (Delhi, India) with the input of the lead author (A.K. Osei) decided the contents of a micronutrient powder to be added to lunch meals after preparation and just before consumption (Table 31.1). The micronutrients were carried in dextrose anhydrous powder and composed such that every 0.25 g of the powder (daily dose per child) provided approximately 75 % of the recommended dietary allowance (RDA) of the vitamins and minerals in the powder for each child. These levels were based on the recommendations of the Indian Council of Medical Research, Delhi. The micronutrient powder and placebo were produced by Nicholas Piramal India Limited, Thane, Maharashtra. The placebo was dextrose anhydrous powder with no added micronutrients. It was considered necessary to use a placebo in some schools in order to assess the effectiveness of fortifying school lunch meals with micronutrient powder on the micronutrient status of schoolchildren in the district. The micronutrient and placebo powders were provided as 500 g packs, accompanied by two sets of standardized plastic spoons that measure 0.5 and 2.5 g of the powder, respectively (the serving sizes for two and ten schoolchildren, respectively). From August 2007 to April 2008, all the public primary schools in Tehri Garhwal district (except ten schools) were provided with a monthly supply of the micronutrient

Table 31.1 Composition of the micronutrient powder

Micronutrient	Chemical form	Amount per 0.25 g of powder
Iron	NaFeEDTA	10 mg
Vitamin A	Retinyl acetate	375 μg
Zinc	Zinc gluconate	4.2 mg
Folic acid	Folic acid	225 μg
Iodine	Potassium iodide	90 μg
Vitamin C	Ascorbic acid	26.25
Thiamine	Thiamine mononitrate	0.68 mg
Riboflavin	Riboflavin 5-phosphate sodium	0.68 mg
Niacin	Nicotinamide	9 mg
Vitamin B_{12}	1 % on manitol, as carrier	1.35 μg
Vitamin B_6	Pyridoxine hydrocholoride	0.75
Vitamin D	Ergocalciferol	3.75 μg
Vitamin E	all-rac-α-tocopherol	5.25 mg
Copper	$CuSO_4 \cdot (H_2O)_5$	0.45 mg

powder to be added daily to the lunch meals. The remaining ten schools were given the placebo powder during this period, but were switched to receive the micronutrient premix after April 2008. All schools were given a dark brown plastic container for storage after opening the packet of the powder to prevent breakdown of light-sensitive vitamins in the micronutrient powder. Prior to the start of the intervention, the cook and one teacher from each school in the district participated in a half-day training session on the importance of micronutrient fortification, standard procedures of fortifying the school meals (including dosage), and proper handling and storage of the micronutrient and placebo powder.

Fortification of the School Lunch Meals

At each school, a trained school cook prepared and fortified the lunch meals. On each day, the amount of meals prepared was based on the total number of students present at school. After meal preparation, the cook counted the number of students present in school and measured the appropriate number of spoons of the assigned powder (either micronutrient or placebo) to be added to the food. The measured powder was then mixed thoroughly with a small quantity of water before addition to the meal at "room temperature," in order to reduce the extent of breakdown of heat-sensitive micronutrients.

Retention of the Vitamin and Mineral Content of the Micronutrient Powder

For any fortification intervention to be successful, the target population group for such intervention needs to consume adequate amounts of the intended micronutrients from the fortified food. Therefore there was a need for proper monitoring of the school lunch meal fortification in Tehri Garhwal district to ensure adequate levels and quality of vitamins and minerals in the micronutrient powder and the fortified school lunch meals, as well as ensuring the fortified meal was well accepted and adequately consumed by the schoolchildren. The vitamin and mineral content of the micronutrient powder was measured at the factory to ensure adequate levels before distribution to schools. Our team conducted an assessment in four of the intervention schools to check the retention of the vitamins and minerals in the micronutrient powder during storage and whether the fortification resulted in adequate amounts of the micronutrients in the lunch meal. The results of this assessments showed that the levels of the micronutrients of greatest interest in this intervention: iron, vitamin A, zinc, folate, and vitamin B_{12} did not change significantly after 20 days of storage at the school (Table 31.2). It also showed that micro-

Table 31.2 Iron, vitamin A, zinc, folic acid, and vitamin B_{12} content of micronutrient premix and of school meals

	Micronutrient premix		Food sample[a]	
	Factory[b]	School[c]	Non-fortified	Micronutrient fortified
	$n=1$	$n=4$	$n=4$	$n=4$
Iron (mg)	10.1 (0.0)	10.1 (0.6)	4.1 (4.0)	12.1 (4.9)[d]
Vitamin A (μg)	410.0 (0.0)	398.5 (16.0)	6.7 (24.1)	381.6 (21.3)[d]
Zinc (mg)	4.24 (0.0)	4.2 (0.1)	0.6 (0.2)	4.5 (0.7)[d]
Folic acid (μg)	223.8 (0.0)	235.5 (3.3)	0.0 (0.0)	224.5 (6.6)[d]
Vitamin B_{12} (μg)	1.4 (0.0)	1.4 (0.0)	0.0 (0.0)	1.40 (0.0)[d]

All values are median (IQR)

[a]Micronutrient content per 150 g of school lunch meal taken from 4 schools, before (non-fortified) and after (micronutrient fortified) micronutrient fortification

[b]Micronutrient content before premix left the factory

[c]Micronutrient content after 20 days of storage of micronutrient sample in four treatment schools

[d]Different from values of non-fortified food ($p<0.05$)

nutrient content of the unfortified school meal was very low, but the addition of the micronutrient powder significantly improved the level of micronutrients in the fortified food (Table 31.2). These findings were encouraging as it suggests that storing the micronutrient powders under school conditions and fortification of the lunch meals by trained school personnel were feasible.

Acceptance of Micronutrient Fortified Lunch Meal by Schoolchildren

To determine the acceptance of the lunch meals fortified with micronutrient powders by schoolchildren, we conducted a cross-sectional randomized controlled study among 181 schoolchildren in grades 1–5 in four primary schools in July, 2007. This study has been described in detail elsewhere [14]. In brief, 91 children were randomized to receive a weighed amount of cooked lunch meal fortified with the micronutrient powder (treatment group) and 90 children received the regular unfortified lunch meal (control group). Just before eating, the children were interviewed to determine if they were hungry, their desire to eat the school meals, and whether they took breakfast at home before coming to school. After having eaten, the children were asked to rate, on a three point Likert scale using "smiley" faces, the pleasantness of smell and taste with the scale ranging from 1 (dislike very much), 2 (like it), to 3 (like it very much). Overall, the micronutrient fortified food was well accepted by the schoolchildren. The addition of a micronutrient powder to cooked lunch meals at the school did not affect the weight of food consumed and therefore acceptance of the meal by the schoolchildren. The mean weight of food consumed by children in the treatment (345.0 ± 114 g) and control (360.0 ± 102.4 g) groups was similar. A fifth of the children did not finish the plate of food served to them and this was not significantly different between the treatment (25.6 %) and control (16.9 %) groups. Almost one out of every five children in each of the two groups requested more food after finishing their first servings (treatment = 21.1 % and control = 20.2 %). Children also highly rated the taste, smell, and general acceptability of the micronutrient fortified and unfortified school meals with no significant difference between the 2 ratings. No child showed dislike for the taste or smell or general appeal of the micronutrient fortified school lunch meal. Only one child disliked the smell of the unfortified lunch meal. These findings are consistent with results of several studies which have demonstrated that foods fortified with a micronutrient powder just before consumption are well accepted by children [15]. These data also suggest that fortification of school meals has the potential to improve the micronutrient status of schoolchildren in the Himalayan villages of India, because a fortified food needs to be well consumed for it to have impact on micronutrient status of the target population.

Effectiveness of the Micronutrient Fortified School Lunch Meal on Micronutrient Status of Schoolchildren

The impact of consumption of the micronutrient fortified lunch meal on micronutrient status of schoolchildren was assessed through an effectiveness trial. This took place from August 2007 to April 2008 (8-months) and used a single-blind, placebo-controlled, cluster-randomized design, in which schoolchildren received either a micronutrient powder (treatment) or placebo (control) as part of their daily school meals for 8 months ("one school year"). The effectiveness trial involved 499 children aged 6–10 years old in grades 1–4 in 20 primary schools selected from across all the nine blocks in Tehri Garhwal district (Fig. 31.2). Ten schools were assigned randomly to treatment and the remaining ten schools to control. Within each selected school, 25 children in grades one to four were randomly selected for anthropometric, biochemical, and parasitological assessments prior to and

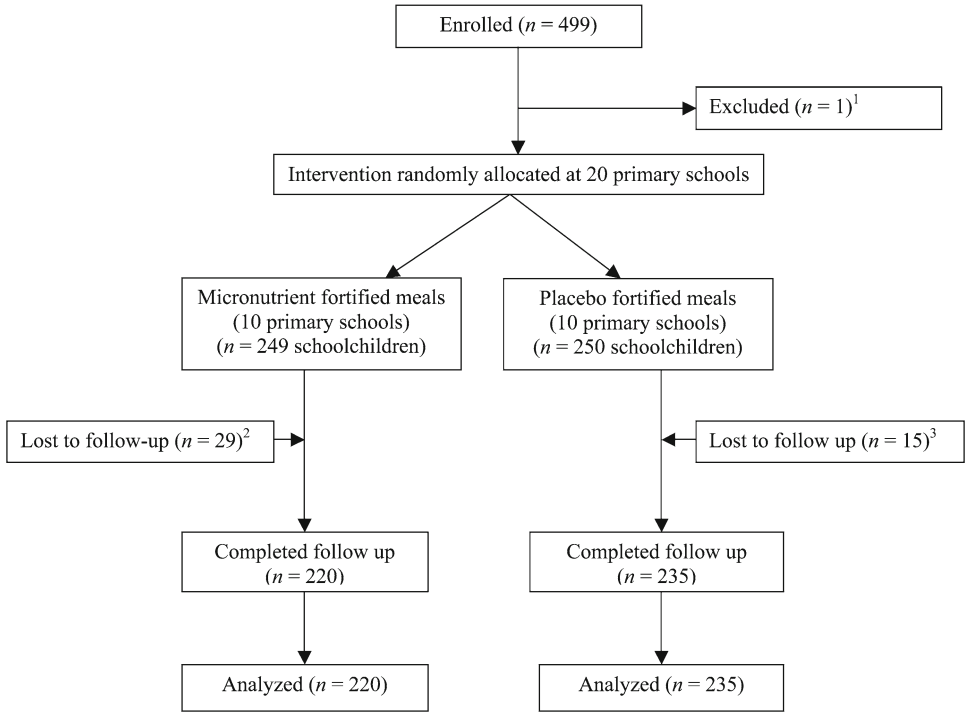

Fig. 31.2 Trial Profile for the effectiveness trial on micronutrient fortification of school lunch meals. [1]One child was excluded (did not meet inclusion criteria for the study ($n=1$)). [2]Twenty-nine children were lost to follow-up since baseline [changed school ($n=22$); travelled out of village ($n=7$)]. [3]Fifteen children were lost to follow-up since baseline [changed school ($n=6$); travelled out of village ($n=7$); sick ($n=1$); parents refused blood draw ($n=1$)]

after 8 months of the intervention. The primary caretakers were asked about the child's morbidity related to occurrence of diarrhea, fever, cough, runny nose, and vomiting in the past 2 weeks. All treatment and control group children who were involved in the assessments were administered an oral dose of 500 mg albendazole before beginning the fortification for the effectiveness trial. All schools in the district, except the ten control schools, were given a micronutrient powder for fortification of lunch meals. The control schools were switched from placebo to micronutrient powder at the end of the trial.

Compliance with Intake of Lunch Meals by Schoolchildren Over the 8-Month Period

In an effort to ensure children in this effectiveness study adequately consumed the fortified lunch meal, school headmasters were given a notebook to document the school attendance of children and the number of days that school meals were cooked and fortified. From these registers, it was noted that lunch meals were served on all days that schools were opened during the 8 months of intervention (165 and 166 days for treatment and control schools, respectively). However, in one control school meals were not prepared for 4 days because of shortage of rice. Overall, estimated compliance with intake of the fortified lunch meal, assessed as the ratio of total days of school attendance to the number of days school meal was cooked and fortified during the 8 months of intervention, was 91.2 %, with no difference between children in the treatment (90.7 %) and control groups (91.7 %). This confirms the acceptance and adherence of the micronutrient fortified meals by schoolchildren in the district.

Table 31.3 Concentrations of hemoglobin and iron status indicators of children at baseline and after 8 months of intervention

	Micronutrient		Placebo	
	n = 220		*n* = 235	
	Baseline	8 months	Baseline	8 months
Hemoglobin (g/L)	122.0 ± 0.1	123.2 ± 0.1	121.7 ± 0.1	122.5 ± 0.1
Ferritin (μg/L)	28.6 (25.1–32.7)	34.7 (32.8–36.7)[a]	30.3 (26.3–34.8)	32.8 (30.9–34.8)
sTfR (mg/L)	2.0 ± 0.1	1.2 ± 0.1[a]	2.2 ± 0.1	1.3 ± 0.1[a]
TBI (μmol/kg)	148.6 ± 0.1	220.5 ± 0.1[a,b]	148.1 ± 0.1	197.6 ± 0.1[a]

Values are adjusted mean ± SEM (standard error of the mean), adjusted geometric mean (95 % CI), or percent. Means and geometric means are adjusted for age, sex, and C-reactive protein

Anemia was defined as hemoglobin <115 g/L and iron deficiency anemia as: hemoglobin <115 g/L and serum ferritin <15 μg/L

[a]Different from baseline value, $p < 0.05$

[b]Different from corresponding placebo value, $p < 0.05$

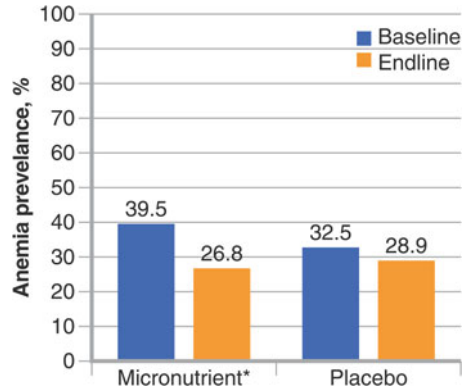

Fig. 31.3 Effect of consumption of the fortified school lunch meals on anemia (hemoglobin <115 g/L) prevalence. The histogram shows the proportion of children with anemia (hemoglobin <115 g/L) in the micronutrient and placebo fortified groups, before and after 8 months of the intervention. *8 months value is different from corresponding baseline value, $p < 0.05$

Impact of the Intervention on Hematologic Indicators and Anemia

Consumption of the fortified school lunch meals for 8 months was not associated with any significant change in mean hemoglobin concentration within or among the children who received micronutrient fortified and placebo fortified lunch meal. However, iron status of the children improved as a result of the intervention as demonstrated by a significant improvement in geometric mean serum ferritin concentrations and increased mean total body iron (TBI) (estimated using the formula by Cook and others [16]) in the treatment relative to the control group (Table 31.3). Consumption of the micronutrient fortified meals was also associated with a significant reduction in the prevalence of anemia (hemoglobin <115 g/L [17]) in bivariate analysis (Fig. 31.3), but not in multivariate analysis (see Table 31.4). Prevalence of low serum ferritin (<15 μg/L [17]) (Fig. 31.4) and iron deficiency anemia (both hemoglobin <115 g/L and serum ferritin <15 μg/L [17]) (Fig. 31.5) decreased in the children over the study period, with no significant effect of the intervention.

The lack of a significant effect on hemoglobin concentration and anemia in multivariate models despite an improvement in iron status in the treatment group can be explained in several ways. The micronutrient fortification is expected to reduce anemia mainly through improvement in iron status,

Table 31.4 Repeated measures logistic regressions for assessing the effect of the micronutrient fortification on changes in prevalence of anemia and low serum concentrations of ferritin, retinol, zinc, folate, and vitamin B_{12} after 8 months of intervention

	Binary outcome variable					
	Anemia	Low serum ferritin	Vitamin A deficiency	Zinc deficiency	Folate deficiency	Vitamin B_{12} deficiency
Treatment	0.31 (−0.08–0.70)	−0.02 (−0.42–0.46)	0.16 (−0.21–0.53)	−0.12 (−0.50–0.25)	0.43 (0.03–0.83)[a]	0.19 (−0.29–0.66)
Time (8 months)	−0.18 (−0.52–0.16)	−1.63 (−2.23 to 1.03)[a]	−0.64 (−1.01 to 0.28)[a]	−0.74 (−1.09 to 0.40)[a]	0.06 (−0.35–0.46)	2.46 (2.03–2.90)[a]
Treatment × time	−0.40 (−0.90–0.09)	−0.82 (−1.85–0.22)	−0.57 (−1.10 to 0.03)[a]	0.19 (−0.30–0.68)	−0.75 (−1.34 to 0.17)[a]	−0.89 (−1.49 to 0.28)[a]
Constant	0.31 (−0.84–1.45)	0.87 (−0.65–2.39)	0.32 (−0.69–1.32)	−0.19 (−1.22–0.83)	1.21 (0.26–2.16)[a]	−1.20 (−2.32 to 0.07)[a]

Values are regression coefficients (95 % CI), $n = 455$ for each model
Cut-offs for definition of each dependent variable is given in the methods section
All the models were adjusted for the child's age, sex, and CRP
[a]Statistically significant, $p < 0.05$

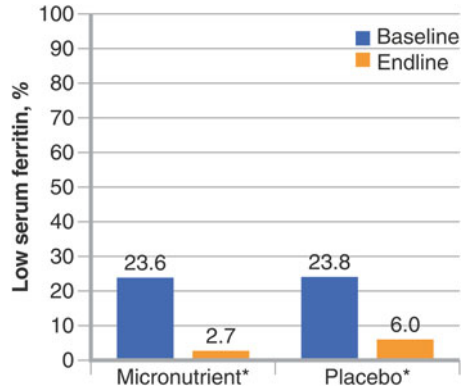

Fig. 31.4 Effect of consumption of the fortified school lunch meals on prevalence of low serum ferritin concentration (<15 μg/L). The histogram shows the proportion of children with low serum ferritin concentration (<15 μg/L) in the micronutrient and placebo fortified groups, before and after 8 months of the intervention. *8 months value is different from corresponding baseline value, $p < 0.05$

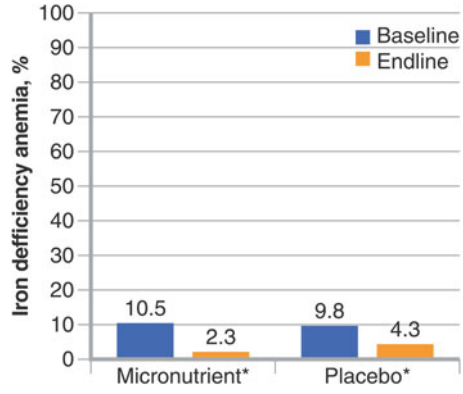

Fig. 31.5 Effect of consumption of the fortified school lunch meals on prevalence of iron deficiency anemia (hemoglobin <115 g/L and serum ferritin concentration <15 μg/L). The histogram shows the proportion of children with iron deficiency anemia (hemoglobin <115 g/L and serum ferritin concentration <15 μg/L) in the micronutrient and placebo fortified groups, before and after 8 months of the intervention. *8 months value is different from corresponding baseline value, $p < 0.05$

because iron is perceived as the most important contributor to anemia among children in developing countries [1, 2]. The baseline analysis of our sample revealed that anemia in these schoolchildren was related to multiple factors besides iron deficiency [18]. The prevalence of iron deficiency anemia in our sample at baseline was low (10.2 %) compared to total anemia prevalence of 36.7 % [18]. Similar findings, of a high prevalence of anemia but little iron deficiency, have been reported in other studies of schoolchildren in Asia [19, 20]. In populations like this, detecting a larger decrease in total anemia prevalence as a result of improvement in iron status is less likely, since the majority of anemia is not due to iron deficiency. Nevertheless, there was some evidence of a high rate of resolution of anemia associated with the intervention in our sample. A subgroup analysis involving only the children who were anemic at baseline (treatment = 87 and control = 77) revealed that 35.6 % of such children in the treatment group remained anemic after 8 months of consuming the fortified food compared to 49.4 %

Table 31.5 Serum concentrations retinol, zinc, folate, and vitamin B_{12} of children before and after 8 months of intervention

	Micronutrient		Placebo	
	n = 220		*n* = 235	
	Baseline	8 months	Baseline	8 months
Retinol (μmol/L)	1.03 ± 0.03	1.29 ± 0.03[a]	1.10 ± 0.02	1.19 ± 0.03
Zinc (μmol/L)	9.9 ± 0.2	10.7 ± 0.2[a]	9.7 ± 0.2	10.8 ± 0.2[a]
Folate (nmol/L)	6.4 (5.3–7.7)	9.5 (8.3–10.8)[ab]	7.9 (6.8–9.2)	6.3 (5.2–7.6)
Vitamin B_{12} (pmol/L)	454.6 (395.8–522.2)	277.8 (261.2–295.4)[ab]	477.7 (454.8–511.9)	222.9 (207.5–239.5)[a]

Values are adjusted mean ± SEM, adjusted geometric mean (95 % CI), or percent. Means and geometric means are adjusted for age, sex, and CRP

[a]Different from baseline value, $p < 0.05$

[b]Different from corresponding placebo value, $p < 0.05$

in the control group (relative risk: 0.74, 95 % CI: 0.54–1.03). This suggests that the risk of continuing to be anemic was reduced by 26 % in the treatment group compared to control group, during the period of the intervention. However, anemia still persisted in the treatment group because for the children who were not anemic at baseline (treatment = 133 and control = 158), the cumulative incidence of anemia over the 8 months of intervention was 21.1 % in the treatment group compared to 19.0 % in the control group (relative risk: 1.06; 95 % CI: 0.81–1.40). Thus, despite the high cure rate for anemia, potentially new cases of anemia were about 6 % more likely to happen in the group of children receiving the micronutrient fortified food compared to the group receiving placebo fortified food. All these data suggests that factors other than iron status may influence anemia among schoolchildren in Tehri Garhwal district of Uttarakhand. Our analysis also revealed high prevalence rates of helminth infections including hookworm, *Ascaris lumbricoides*, *Trichuris trichiura,* and *Taenia saginata* among children at both baseline and post-intervention (despite the mass treatment of all the children with albendazole at baseline). Details of these findings are presented elsewhere [18, 21]. About a third of anemia among schoolchildren in the baseline sample were attributable to helminth infection (attributable fraction of 28 %) [18]. Thus, the resurgence of helminth infection during the intervention period may have contributed to new cases of anemia and therefore a lack of a significant effect of the micronutrient fortification of the school lunch meal on total anemia prevalence. As explained below, there was also a high prevalence of other micronutrient deficiencies such as vitamins A, B_{12}, and folate deficiencies among schoolchildren in the district, which may also contribute to anemia, since such deficiencies have been associated with anemia in other studies [22, 23].

Our data underscore the well-known fact that although micronutrient fortification of lunch meals for schoolchildren is important in reducing iron deficiency anemia, a significant reduction in total anemia prevalence can be realized only by a comprehensive approach which addresses all other potential causal factors of anemia. Of particular importance is routine mass deworming of schoolchildren in this district as part of strategies to control anemia.

Impact of the Intervention on Vitamin A, Zinc, Folate, and Vitamin B_{12} Status

The 8 months intake of the micronutrient fortified food by schoolchildren was also associated with a significant improvement in vitamin A, folate, and vitamin B_{12} status. The treatment group showed a significant increase in mean serum retinol and geometric mean serum folate concentrations relative to the control group (Table 31.5). The geometric mean serum vitamin B_{12} concentration decreased in both groups, but the decrease was smaller in the children who consumed micronutrient fortified lunch meal compared to placebo ($p < 0.05$). There was no significant effect of the intervention on mean serum zinc concentration (Table 31.5).

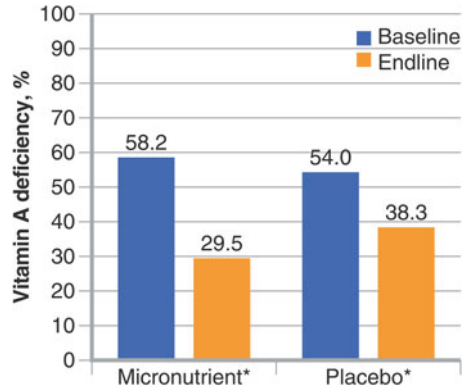

Fig. 31.6 Effect of consumption of the fortified school lunch meals on prevalence of vitamin A deficiency (serum retinol <1.05 μmol/L). The histogram shows the proportion of children with vitamin A deficiency (serum retinol <1.05 μmol/L) in the micronutrient and placebo fortified groups, before and after 8 months of the intervention. *8 months value is different from corresponding baseline value, $p < 0.05$

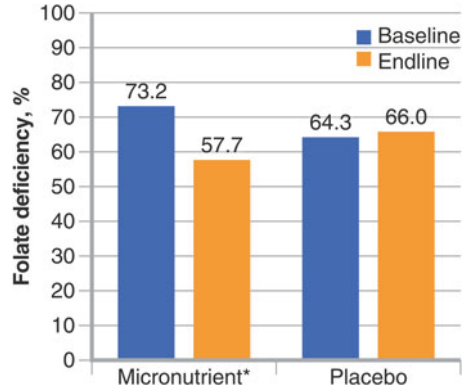

Fig. 31.7 Effect of consumption of the fortified school lunch meals on prevalence of folate deficiency (serum folate <13.6 nmol/L). The histogram shows the proportion of children with folate deficiency (serum folate <13.6 nmol/L) in the micronutrient and placebo fortified groups, before and after 8 months of the intervention. *8 months value is different from corresponding baseline value, $p < 0.05$

In bivariate analysis, the percentage of children with vitamin A deficiency (serum retinol <1.05 μmol/L [1]) decreased in both groups, with the treatment group showing a greater decrease in prevalence of vitamin A deficiency compared to the control group, although the difference was not statistically significant (Fig. 31.6). Prevalence of children with folate deficiency (serum folate <13.6 nmol/L [24]) declined in the treatment group ($p < 0.05$), whereas there was no significant change in the control group (Fig. 31.7). There were more children with vitamin B_{12} deficiency (serum vitamin B_{12} <300 pmol/L [25]) at the end of the intervention compared to baseline data in the two groups ($p < 0.05$). However, the increase in prevalence of children with vitamin B_{12} deficiency was significantly smaller in the treatment compared to the control group (Fig. 31.8). This significant decrease in vitamin B_{12} status of children in the treatment group was inexplicable. Vitamin B_{12} deficiency in our sample may be associated with the consumption of a primarily vegetarian diet because 98.2 % of our study subjects were Hindus. In spite of the decrease, the vitamin B_{12} status of the treatment group was not as low as that of the control group at the end of the intervention. This suggests that the micronutrient fortification of lunch meals had a

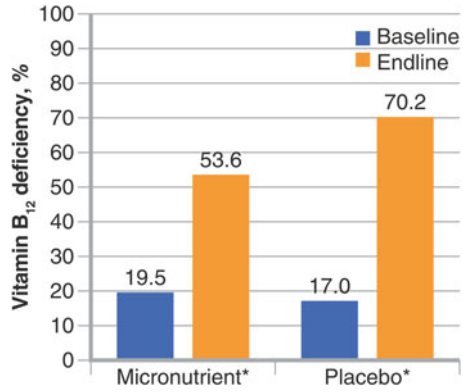

Fig. 31.8 Effect of consumption of the fortified school lunch meals on prevalence of vitamin B_{12} deficiency (serum vitamin B_{12} <300 pmol/L). The histogram shows the proportion of children with vitamin B_{12} deficiency (serum vitamin B_{12} <300 pmol/L) in the micronutrient and placebo fortified groups, before and after 8 months of the intervention. *8 months value is different from corresponding baseline value, $p < 0.05$

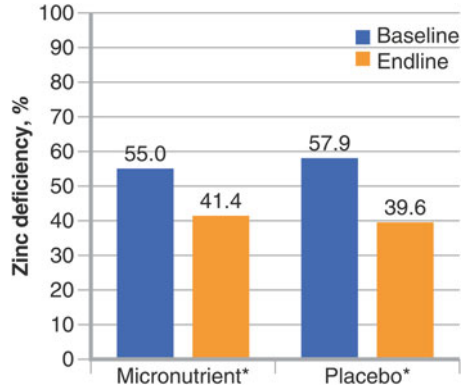

Fig. 31.9 Effect of consumption of the fortified school lunch meals on prevalence of zinc deficiency (serum zinc <9.9 µmol/L). The histogram shows the proportion of children with zinc deficiency (serum zinc <9.9 µmol/L) in the micronutrient and placebo fortified groups, before and after 8 months of the intervention. *8 months value is different from corresponding baseline value, $p < 0.05$

beneficial effect on vitamin B_{12} status of the children. In a multivariate analysis using repeated measures logistic regression models, only the models involving vitamin A, folate, and vitamin B_{12} deficiencies as dependent variables showed a significant treatment × time interaction, indicating that the change in log odds of children being deficient in vitamin A, folate, and vitamin B_{12} was different for the treatment and control groups (Table 31.4). At the end of the intervention, children in the treatment group were 43 % less likely to be vitamin A deficient [OR (95 % CI): 0.57 (0.33–0.97)], 53 % less likely to be deficient in folate [OR (95 % CI): 0.47 (0.26–0.84)] and 59 % less likely to be deficient in vitamin B_{12} [OR (95 % CI): 0.41 (0.22–0.86)] compared to children in the control group (Table 31.4).

There was no effect of the intervention on proportion of children with zinc deficiency (serum zinc <9.9 µmol/L [26]) either in bivariate (Fig. 31.9) or multivariate analysis (Table 31.4). This lack of impact of the fortification on zinc status of children could be due to several factors that might have

Table 31.6 Morbidity among children before and after 8 months of intervention

	Micronutrient	Placebo
	$n=178$	$n=176$
Morbidity		
Diarrhea (%)		
Baseline	12.9	15.3
After 8 months	3.4	5.1
Fever (%)		
Baseline	52.8	44.3
After 8 months	20.8	20.5
Couth (%)		
Baseline	25.3	19.9
After 8 months	12.9	10.8
Runny nose (%)		
Baseline	18.0	21.0
After 8 months	2.2	1.7
Vomiting (%)		
Baseline	7.9	10.2
After 8 months	0.6	2.3

Values are percent

hindered zinc absorption, including the potential interference of iron with zinc and potentially high phytate content of the school meals (since it consist of mainly rice and lentils) [26, 27], and the fact that serum zinc is not an adequate indicator of individual zinc status [2].

Impact of the Intervention on Infections

There was no beneficial effect of the fortification on the prevalence of infectious morbidities among children that was assessed through recall by mothers. The prevalence of diarrhea, fever, cough, runny nose, and vomiting decreased similarly in the treatment and control children between baseline and post-intervention surveys (P, 0.05) (Table 31.6). This was not surprising as the limited evidence that exists on impact of home fortification on morbidity outcomes such as diarrhea has been mixed.

Impact of the Intervention on Growth

There was no significant benefit of the lunch meal fortification on the anthropometric indicators of the children. The prevalence of wasting and underweight decreased similarly from baseline to post-intervention in the treatment and control groups. Wasting decreased from 12.7 to 8.4 % in the treatment group and control 11.9–7.4 % in the control groups. The prevalence of underweight also decreased from 60.5 to 51.8 % in the treatment group and from 59.6 to 51.5 % in the control group. Again, the lack of impact of the fortified lunch meals on anthropometric indicators of the schoolchildren was not surprising. A systematic review conducted on the impact of home fortification of foods using micronutrient powders (containing at least iron, vitamin A, and zinc) on anthropometric indicators of preschool children, showed no effect of such interventions on the weight-for-age, length-for-age, and weight-for-length Z-score of these children [12].

Conclusion

In summary, micronutrient fortification of cooked school meals by trained school authorities was effective in improving iron, vitamin A, and folate status and reducing the magnitude of a decrease in vitamin B_{12} status of schoolchildren in Himalayan villages of India. School feeding programs can therefore serve as a suitable vehicle for addressing micronutrient malnutrition among rural schoolchildren. Our findings also add important information to the growing body of evidence that fortification of cooked foods just before consumption is a feasible approach to reduce micronutrient deficiencies among children in rural communities.

Guidance on Levels of Micronutrients to be Added in the Powder

The types and amounts of specific nutrients in a micronutrients powder for home fortification interventions are decided based on several factors including the usual nutrient intake, micronutrients status and the recommended nutrient intake (RNI) of the various micronutrients as specified for the target group, the safety of consuming the fortified food, and the form and levels of the nutrient that will not result in significant organoleptic changes in the fortified food that can potentially hinder its acceptance and consumption by the target population [28]. In general, the nutrient levels in the micronutrient powder formulations are composed such that a serving dose of the powder provides at most one RNI of each of the vitamins and minerals per child. It is anticipated that addition of the micronutrient powder to the diet will address the nutrient gap between the usual dietary intake and the RNI of the various micronutrients for individuals in the target population. The combination of the diet and micronutrient powders is also expected not to exceed the Tolerable Upper Intake Level (UL) of each micronutrient, a level beyond which there is likelihood for potential safety concerns. For almost all the vitamins and minerals in the micronutrient powder formulations for home fortification interventions, the UL is well above the RNI [28]. Therefore the Home Fortification Technical Advisory Group (HF-TAG) suggests providing a dosage of one RNI of these nutrients solely from the micronutrient powder, in addition to whatever amount being obtained from the diet, is safe for the target populations in home fortification interventions.

Recommendations

Policy makers should therefore consider micronutrient fortification of cooked meals just before consumption by trained school authorities as one of the feasible public health strategies for addressing micronutrient deficiencies among rural schoolchildren. The addition of micronutrient powder to meals at school has potential advantages of being locally acceptable, sustainable, and has lower implementation cost because it uses the infrastructure of an already existing program. Similar intervention can be considered at home for children not attending schools. However, adoption of this fortification strategy in new areas will require adjustment to the micronutrient levels of the fortificant based on the micronutrient status and/or consumption profile of the population to avoid excessive intake. Further research is needed to assess the cost-effectiveness of delivering micronutrients through this strategy to schoolchildren.

Acknowledgements We are grateful to Dr. Saraswati Bulusu, Tripti Pantajoshti, Anil Bhutani, and Dr. Rajesh Singh, for assistance with implementation of the intervention and data collection; and to Dr. Ujwala Godbole for laboratory analysis. We appreciate the inputs of Dr. Nevin Scrimshaw and Dr. Gary Gleason for their comments in improving the quality of this work.

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Chapter 32
Iron Fortification Strategies in Brazil

Joel Alves Lamounier, Flávio Diniz Capanema, José Eduardo Dutra de Oliveira, Daniela da Silva Rocha, and Carlos Alberto Nogueira de Almeida

Key Points

1. Iron deficiency anemia represents a serious nutritional problem worldwide, and it especially affects children and pregnant women in developing countries, like Brazil.
2. The objective of food fortification programs is to increase the dietary iron in foods to prevent and control iron deficiency in at-risk groups.
3. Food fortification is highlighted as one of the most cost-effective health solutions to fight malnutrition and iron deficiency among children and women.
4. The direct costs of fortification are extraordinarily low compared with the social costs of disability.
5. The addition of iron to potable drinking water is one alternative to the control and prevention of iron deficiency and anemia.
6. The use of drinking water as a vehicle for the control and prevention of iron deficiency and anemia is an effective and efficient model, which can be used in targeting preschool children enrolled at daycare facilities, and/or at the household level, which will include all family members.

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V.R. Preedy et al. (eds.), *Handbook of Food Fortification and Health: From Concepts to Public Health Applications Volume 2*, Nutrition and Health, DOI 10.1007/978-1-4614-7110-3_32,

7. The use of iron-fortified orange juice is a promising strategy as a complimentary vehicle for ingestion of iron in children.
8. In Brazil, studies on iron food fortification showed a positive response, both in relation to acceptance of fortified food and prevention, as in the recovery of hemoglobin levels in both groups.

Keywords Anemia • Iron deficiency • Fortification • Drinking water • Brazil

Abbreviations

CDC	Centers for disease control
EDTA	Ethylenediamine tetraacetic acid
PNDS	Política Nacional de Desenvolvimento Social
RDA	Recommended dietary allowance
RNI	Recommended nutrient intake

Introduction

Iron deficiency anemia represents a serious nutritional problem worldwide, and it especially affects children and pregnant women in developing countries, like Brazil. According to World Health Organization, anemia can be considered a major public health problem in Brazil, which requires effective public policies [1] regarding the short- and long-term effects anemia can have on growth in at-risk groups [2].

In 12 urban centers in five Brazilian regions with 2,715 children between 6 and 12 months, 65.4 % were identified with iron deficiency anemia [3]. The National Survey of Demography and Health of Children and Women—PNDS (Política Nacional de Desenvolvimento Social)—showed 20.9 % prevalence of anemia among children 6–59 months with the highest prevalence in the northeast [4]. According to epidemiological studies, data of different regions of Brazil have shown high prevalence rates of anemia in children and adolescents (Fig. 32.1). A systematic review study evaluated 256 publications from January 1996 to January 2007 which included articles indexed in Medline and Lilacs and languages Portuguese and English. The data included 20,952 children under 5 years and showed 53 % of anemia prevalence. The prevalences were 47.8–63.7 % south, 10.4–77.8 % southern, 55.1–84 % north, 35.7–89.1 % northeast, and 31–63.1 % midwest. In day care centers, a lot of studies have shown that iron deficiency anemia was the most common nutritional deficiency in childhood, with high prevalence [5–7].

Iron Food Fortification

The fortification of foods consists in the addition of complementary nutrients to foods *in natura*. The objective of food fortification programs is to increase the dietary iron in foods to prevent and control iron deficiency in at-risk groups [8]. A concern with nutritional deficiencies and a utilization of fortification as an intervention measure were extensively documented throughout the twentieth century. In 1910, for example, in Denmark, due to concern over vitamin A deficiency, which affected large numbers of children, health officials initiated large-scale industrial fortification of margarine with vitamin A, resulting in the elimination of xerophthalmia in the population [9].

Food fortification is highlighted as one of the most cost-effective health solutions to fight malnutrition and iron deficiency among children and women. Fortification of staple foods improves micronutrient

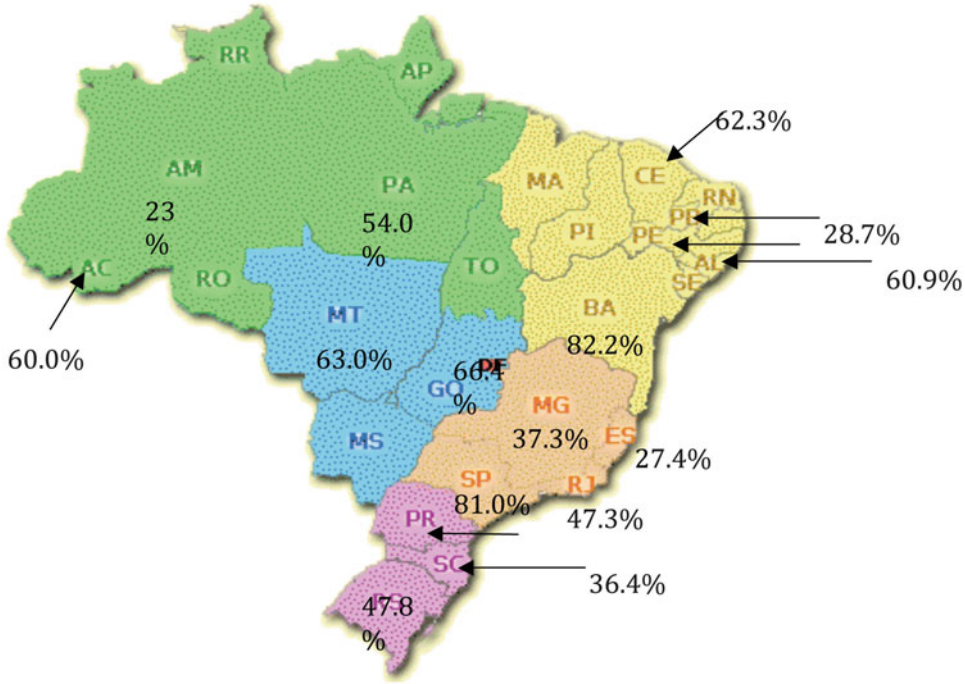

Fig. 32.1 Map of childhood anemia prevalence in Brazil

status by delivering small amounts of micronutrients on a daily basis. In addition, some other options, like drinking water, has also been a good alternative. Staple food fortification is routinely practiced around the globe in developed countries and contributed for decreasing childhood anemia. In developing countries, anemia is still a public health problems in some countries. Therefore, food fortification with iron have been considered, since it requires no change in eating habits and delivers benefit through the consumption of fortified staple foods and drinking water.

The fortification of foods with iron is a preferred strategy advocated by the World Health Organization. Iron added to foods has been shown to be the most efficient options to control iron deficiency, and studies have shown improvements over a period of 1–3 months in people suffering from this deficiency [9]. Once foods are enriched with micronutrients, such as iron, large, at-risk populations will be reached over long periods without the need of effective individual cooperation [10, 11]. Therefore, food fortification is considered highly effective and flexible, is socially acceptable, and furthermore, it does not interfere with the population's dietary habits. In addition, the risks of side effects and toxicity are minimal due to reduced doses of micronutrients added to foods [10].

In Europe, some countries have adopted a policy of distribution of infant formula and fortified cereals, which resulted in decreasing the prevalence of iron deficiency in last decades [12]. In the United States, the prevalence of anemia on 1988 and 1994 was 3 % and 9 % in children aged 1–2 years, and less than 1 % and 3 % for children aged 3–5 years, respectively [13]. The Centers for Disease Control and Prevention (CDC) in a cross-sectional study using data of five states reported the prevalence of anemia among children dropped by more than 50 % in last 2 decades. The reduction was attributed to better nutritional conditions related to large-scale consumption of fortified foods and possibly better iron bioavailability in some products [14].

In Central and South America, food fortification is widely practiced and can be classified in three program types: mandatory fortification of foods commonly consumed by the population, such as wheat flour and corn meal; fortification targeting specific groups, such as cereal, powdered milk, biscuits, and

other industrialized foods; and voluntary fortification, in which the food industry adds iron and other micronutrients to industrialized foods. The direct costs of food fortification are extraordinarily low when compared to the high social costs of micronutrient deficiencies. In most cases, according to the World Bank, the cost of fortification is less than 1 dollar per year to protect an individual against vitamin A, iron, and iodine deficiencies. The cost to prevent an iron deficiency alone has been estimated to be less than US$ 0.10 per year [9].

Type of Iron Salts for Food Fortification

The iron salts used in food fortification along with their characteristics are presented in Table 32.1. These salts are divided into four groups according to their solubility: (1) those that are easily soluble in water, (2) those that have a low solubility in water, but soluble in dilute acids such as gastric juice, (3) those insoluble in water and sparingly soluble in dilute acids, and (4) those protected by iron components [15]. Below there is some information about each one:

1. Among the iron salts soluble in water, the most common representative is ferrous sulfate. Its solubility is instantaneous in the stomach. The absorption can vary from about 1 to 50 % depending on the iron status of the individual and the presence of inhibitors or enhancers in diet, besides the iron content thereof. The disadvantage of ferrous sulfate is that it can react with substances that are naturally present in foods and may cause sensory changes (color, smell, and taste). The cost is relatively low compared to other iron salts [1].
2. The compounds included in the second group slowly dissolve in the stomach. The main component of this category is ferrous fumarate. Unlike ferrous sulfate, it undergoes little interaction with food, causing fewer sensory changes. Therefore, it is used in the fortification of infant cereals and chocolate drinks.
3. The iron salts insoluble in water and sparingly soluble in dilute acids are widely used in food industry in industrialized countries and they do not interfere in the sensory properties of foods.

Table 32.1 Sources of iron salts for food fortification

Salt	Iron content (%)	Mean relative bioavailability (%)	Relative cost approaching (U$)
Easily soluble in water			
Ferrous sulfate $7H_20$	20	100	1.0
Dry ferrous sulfate	33	100	0.7
Ferrous gluconate	12	97	5.1
Ferrous lactate	19	106	4.1
Ferric citrate amino	18	107	2.1
Poorly water soluble/soluble in dilute acids			
Ferrous fumarate	33	100	1.3
Ferrous succinate	35	119	4.1
Water insoluble/poorly soluble in dilute acids			
Ferric orthophosphate	28	25–32	4.1
Ferric orthophosphate amino	19	30–60	–
Ferric pyrophosphate	25	21–74	2.3
Reduced iron powder	97	13–148	0.2
Components chelates			
NaFeEDTA	14	28–416	6.0
Hemoglobin	0.34	100–700	–

Source: Hurrell, 1997

However, its contribution to the promotion of iron status is questionable due to its low solubility and absorption. The ferric pyrophosphate and ferric orthophosphate used in breakfast cereals and other fortified products in North America are also not recommended for fortification due to low solubility and absorption in humans [1].

4. In the group of salts of iron chelates, iron is protected from inhibitors present in food, and this is its biggest advantage in food fortification. The most common salt is NaFeEDTA. Its absorption is three times higher in the presence of inhibitors such as phytate in cereals compared to ferrous sulfate. However, it can cause unacceptable changes in color of some food vehicles and have higher cost compared to other iron salts. Another component chelate available for use in fortification programs is the amino acid chelate, also known as amino chelate iron, which exists in two forms: ferrous and diglicinate or triglicinate ferric. Absorption of ferrous diglicinate is 1.1–5 times that of iron, but less than the NaFeEDTA. The ferric triglicinate causes less reaction in foods, but the bioavailability is less than the ferrous diglicinate [1].

Costs of Fortification

The direct costs of fortification are extraordinarily low compared with the social costs of disability. In most cases, according to the World Bank, this cost is less than 1 dollar a year to protect the individual against vitamin A deficiency, iron, and iodine. With respect to iron deficiency cost is less than $ 0.10 [1]. In Thailand a study was conducted to test different iron sources (ferrous sulfate, NaFeEDTA, ferric ammonium citrate, ferrous lactate, and ferrous gluconate) for fortification of four types of soy sauce: naturally fermented in the traditional style, naturally fermented according to large-scale industrial formulas 1 and 5, and chemically hydrolyzed at 5 mg per serving (15 mL, per Thailand's food labeling regulations). The cost of fortification was US 0.22 to US 3.28 per bottle (700 mL). Both naturally fermented and chemically hydrolyzed soy sauces could be fortified with all five iron sources. Ferrous sulfate is the most appropriate source because of its low cost and acceptable sensory characteristics. Therefore, soy sauce is a promising vehicle for iron fortification [16].

Fortification of complementary foods at home is another alternative. Zlotkin developed a less costly alternative of the provision of micronutrients, which can be added to infant foods, named "home fortification," and used sprinkles, the multiple-micronutrient sachet containing iron, vitamin A, vitamin C, folic acid, and zinc [17]. Each child receives one sachet per day in one meal. Studies that looked at the costs and potential impacts of sprinkles concluded that the benefit/cost ratio of sprinkles interventions, containing iron as well as other micronutrients, can be as high as 37:1 if one assumes that a course of intervention for 4 months between the ages of 6 months and 1 year largely protects an infant against anemia throughout childhood [18].

Fortification Strategies Experiences in Brazil

Table 32.2 presents a summary of major studies of fortification of foods that have demonstrated positive results in combating iron deficiency and iron deficiency anemia in the Brazilian population.

Potable Drinking Water

The addition of iron to potable drinking water is one alternative to the control and prevention of iron deficiency and anemia. This rather simple method can reach a large part of the Brazilian population at each level of the social-economic stratum by the use of drinking water on a daily basis. Potable water,

Table 32.2 Impact of iron food fortification on the prevalence of anemia in Brazil

Reference	Duration Month	Food Nutrient	Ferrous salt Kind of food	Prevalence % Before	Prevalence % After
Nogueira et al. [36]	3	Biscuits	Bovine hemoglobin	75.0	0
Dutra de Oliveira et al. [20]	8	Drinking water	Ferrous sulfate	58.0	3.0
Torres et al. [24]	6	Powder milk	Ferrous sulfate + vitamin C	66.4	20.6
				72.8	18.0
Braga [27]	6	Infant formula	Ferrous sulfate	25.0	7.0
Torres et al. [26]	12	Cow's milk	Ferrous amino acid chelate	62.3	26.4
Fisberg et al. [37]	2	Biscuit and Bread	Ferrous amino chelate	32.0	11.0
Ferreira [8]	6	Cow's milk	Ferrous sulfate + vitamin C	63.2	33.8
Giorgini et al. [38]	6	Bread	Ferrous chelate	62.0	22.0
De Paula and Fisberg [44]	6	Sugar	Triglicinato chelate	38.1	19.7
				29.4	19.6
Tuma et al. [10]	4	Cassava flour	Ferrous amino chelate	22.7	8.0
Fisberg et al. [49]	4	Bean powder	Ferric pyrophosphate	13.0	0.0
Miglioranza et al. [29]	12	Cow's milk drink	Ferrous amino chelate	41,9	9,6
Almeida et al. [30]	4	Orange juice	Ferrous sulfate	60.0	20.0
Vellozo et al. [39]	1	Bread	Ferrous amino chelate	21,0	12,6
Beinner et al. [22]	8	Drinking water	Ferrous sulfate	43.2	21.0
de Almeida et al. [50]	6	Drinking water	Ferrous sulfate + vitamin C	45.9	31.1
Beinner et al. [43]	5	Rice	Ferric pyrophosphate	69.1	25.0
Bagni et al. [43]	4	Rice	Bisglicinato chelate	37.8	23.3
Rocha et al. [23]	5	Drinking water	Ferrous sulfate + vitamin C	29.3	7.9

besides been used for drinking, is commonly used for preparation of foods, which may contribute even more towards increasing iron ingestion [19].

Dutra de Oliveira et al. [20] evaluated 31 preschool children aged 2–6 years enrolled in daycare centers in Ribeirão Preto, São Paulo. During 8 months, children consumed iron-fortified drinking water (20 mg Fe/L) which resulted in a significant decrease in the prevalence of anemia. At baseline, anemia prevalence was diagnosed in 58 % of subjects. Four months after, 16 % continued anemic, but at 8 months post-study intervention, anemia virtually disappeared, since anemia was present in only 3 % of subjects. Mean hemoglobin levels at baseline (10.6 ± 1.1 g/dL) increased significantly to 12.1 ± 1.4 g/dL at 4 months, and 13 ± 1.1 g/dL at end study. In a later study, Dutra de Oliveira et al. [21] studied low-income families during 4 months in which 21 families with children aged 1–6 years were divided into experimental and control groups. In the experimental group, family members consumed iron-fortified drinking water containing 10 mg of ferrous sulfate plus 60 mg of ascorbic acid per liter of water. The control group consumed drinking water without the addition of iron and ascorbic acid. Results were very promising and showed that hemoglobin levels in children increased from 10.9 ± 1.1 to 11.7 ± 1.1 g/dL after 4 months of fortification intervention. Similar results were observed in the experimental adult group in which hemoglobin levels increased (12.9 ± 1.7 to 13.7 ± 1.7 g/dL). Results for ferritin were also positive in the experimental group in which ferritin levels increased in children, and significantly in adults. According to the authors, the iron fortification of drinking water is an effective, feasible alternative and practical way to distribute iron to low-income families and it is technically inexpensive and has the promising potential for the control and prevention of anemia in Brazil and in other countries.

In another study, 160 preschool children from eight municipal daycare facilities benefited from daily consumption of iron (12 g element iron/L) plus ascorbic acid (90 mg/L) prepared in 20-L plastic water jugs. Mean Hb at baseline and after 8 months of intervention increased significantly from

11.8 ± 1.3 to 12.4 ± 0.93 g/dL, respectively. The prevalence of iron deficiency determined by hemoglobin levels decreased from 43.2 to 21 % at 8 months post-intervention. Significant ($p < 0.05$) increase in anthropometric indicators (weight/age, height/age, and weight/height) was also observed during the study. Fundamentally important to the success of this study was education of the targeted population, which resulted in behavior change and greater awareness of the importance of combating iron deficiency and anemia by the use of iron-fortified drinking water [22].

The use of drinking water as a vehicle for the control and prevention of iron deficiency and anemia is an effective and efficient model, which can be used in targeting preschool children enrolled at daycare facilities, and/or at the household level, which will include all family members. Consumption of drinking water fortified with iron can contribute to increase iron intake to meet minimal Recommended Nutrient Intake (RNI) allowance of bioavailable iron acceptable to preschool children aged 6–59 months of age and fortified drinking water is easy to distribute and can be easily monitored [22].

In Belo Horizonte city, southern of Brazil, a longitudinal study was conducted to evaluate the effectiveness of fortification of drinking water with iron and vitamin C in the reduction of the anemia as well as to identify the prevalence of anemia in daycare centers. Three hundred and eighty children aged 6–74 months were evaluated. Since 55 did not participate in the second evaluation, a total of 312 children were assessed before and at the end of the intervention. To study the identification of risk factors, only children under 5 years old were evaluated, the group with the highest risk for anemia. A questionnaire was applied to parents or those responsible for the children, containing socioeconomic, maternal, and health information. Anthropometric measurements (weight and height) and finger-stick blood samples were collected in two moments: before and after 5 months of fortification. Children were considered as anemic with hemoglobin <11.0 g/dL for the group aged 6–59 months, and values <11.5 g/dL for those aged 60–74 months. Multivariate analysis was performed to evaluate the association between these variables and anemia. The total number of children evaluated before and after the fortification was 318, being 52.2 % male, with average of 45.4 ± 15.8 months. The prevalence of anemia decreased significantly from 29.3 % before the fortification, to 7.9 % at the end of the study ($p < 0{,}001$). Considering the prevalence by age group, a reduction of 62.5 %, 75 %, and 78.8 % was found for children of 24 months, 24–48 months, and >48 months, respectively. The hemoglobin median increased 10.2 %: from 11.8 to 13 g/dL, with a significant increase in all age groups. There was improvement in height-for-age and weight-for-age; however, only the first measurement showed a significant difference. The prevalence of anemia in this population was 30.8 %, and the prevalence was 71.1 % in children aged ≤24 months. The risk factors of anemia were age ≤24 months (OR: 9.08 CI: 3.96–20.83) and height-for-age <−1 z score (OR: 2.1, CI: 1.20–3.62). The fortification of water with iron and vitamin C significantly reduced the prevalence of anemia in children attending daycare centers, as well as it improved the nutritional status of them, being considered an important strategy to control this nutritional deficiency [23].

Milk and Infant Formulas

The Brazilian Association of Pediatrics has recommended the use of infant formula supplemented with iron for infants until the age of two as an alternative to breastfeeding, when it is not possible. However, cow's milk is an important food consumed by children especially those families of low socioeconomic status. Cow's milk presents a small concentration and low bioavailability of iron, and consumption of excessive amounts of fresh or pasteurized cow's milk may be associated with occult intestinal blood loss during infancy, which may also contribute towards increasing the occurrence of anemia in infancy [24]. The use of cow's milk, due to social-economic and cultural practices, is

frequent in Latin America, including Brazil, during infancy, and iron fortification of this vehicle is an inexpensive alternative to increase iron levels in children [25].

The impact of using powdered whole milk fortified with 9 mg of iron and 65 mg of vitamin C per 100 g of powdered milk during 6 months was measured in 107 children in municipal daycare centers, and another 228 children at public health clinics in the city of São Paulo [25]. At baseline intervention, 66.4 and 72.8 % of the children attending public daycare and public health clinics were diagnosed with anemia, respectively. At 6 months post-study, the percentage of children still anemic decreased to 20.6 % in daycare and 18 % in children seen at health clinics. In a later study, Torres et al. [26] evaluated the use of 3 mg of amino acid chelate in pasteurized cow's milk (3 mg/L). During the 12-month study, 239 children 6–42 months of age received, daily, 1 L of fortified cow's milk. The mean hemoglobin levels at baseline for children less than 12 months, 12–23 months, 24–35 months, and 36 months of age and older were 10.2 ± 1.3, 10.1 ± 1.6, 11 ± 1.3, and 11.8 ± 1.3 g/dL, respectively. At baseline, anemia prevalence was 62.3 % and, at 6 months, the percentage of children still anemic decreased to 41.8 % and after 1 year decreased to 26.4 %. Mean hemoglobin levels at 12 months were 11.1 ± 1.3, 11.6 ± 1.1, 12 ± 1.2, and 12.1 ± 1.0 g/dL, for 11, 12–23, 24–35, and 36 months of age, respectively. The increases were significant for the first three age groups, but not for the last group (36 months and older).

In Sao Paulo, in a poor, socioeconomic community 102 children aged 2–6 years of age from daycare centers were evaluated. An infant formula with 1.4 mg of iron and 100 mg of ascorbic acid was added to 200 mL of formula daily. During a 180 days intervention, iron deficiency was 25 % and after the nutritional intervention decreased to 7 %. A significant increase in anthropometric indicators was observed. Mean hemoglobin levels increased from 12.1 ± 0.66 to 12.7 ± 0.66 g/dL [27]. In another study to evaluate iron fortification of infant formula, Ferreira [28] randomly assigned 111 children, between the ages of 4 and 6 months, to two intervention groups during 6 months: the experimental group (68 infants) received iron-fortified (1.8 mg ferrous sulfate/200 mL) milk formula and the control group (43 infants) received milk formula (0.7 mg iron/200 mL). At baseline, anemia prevalence in groups 1 and 2 was 63.2 % and 67.4 %, respectively. Mean hemoglobin levels in group 1 increased from 10.6 to 11.3 g/dL; however, in group 2, mean Hb actually decreased from 10.6 to 10.1 g/dL at 6 months. Similar significant results were seen for mean ferritin values: at baseline, ferritin values increased from 34.8 to 44.8 μg/dL, but at the group 2, mean ferritin values decreased from 41.8 to 26.1 μg/dL. Results for Hb and ferritin were significant. Overall, the anemia prevalence decreased from 63.2 to 33.8 % at group 1 and increased from 67.4 to 72.1 % at group 2. The effectiveness of iron-fortified fresh or pasteurized cow's milk and infant formulas will depend on several factors such as iron compounds, quantity, bioavailability, iron enhancers and inhibitors likely to affect bioavailability, and overall added cost to the targeted consumer.

Miglioranza et al. [29] evaluated the effect of a cow's milk drink fortified with iron amino chelate (12 mg/100 mL) in Londrina, south of Brazil. Sample consisted of 468 children and adolescents 7–14 years old in public schools. Each child ingested around 100 mL/day. The prevalence of anemia dropped from 41.9 to 26.4 % after 6 months and to 9.6 % after 12 months. The hemoglobin levels increased significantly.

Orange Juice

Orange juice fortification studies have shown improvement in childhood anemia. Almeida et al. [30] evaluated iron fortification of this widely produced fruit rich in vitamin C which greatly facilitates iron absorption. Fifty preschool children consumed orange juice with iron (10 mg ferrous sulfate per 100 mL of orange juice) twice daily during 4 months. Anemia prevalence decreased from 60 to 20 % at end study, and mean hemoglobin level increased from 10.5 ± 1.7 to 11.6 ± 1.1 g/dL ($p = 0.00$).

The use of iron-fortified orange juice is a promising strategy as a complimentary vehicle for ingestion of iron in children. Orange juice is widely consumed by all levels of the social strata in Brazil. An iron compound can be added during processing without provoking organoleptic changes (color, flavor, and consistency) and even allow for much higher quantities of iron from 3–10 times more than in other targeted or mandatory foods. The added cost can be absorbed through advertising and processing.

Corn, Wheat, and Cassava Flour

In Brazil, since 2001, the Ministry of Health made mandatory the addition of iron [30 % Recommended Nutrient Intake (RNI) or 4.2 mg/100 g] and folic acid (70 % RNI or 150 μg) to milled wheat and corn flour. Federal law now dictates mandatory fortification of iron instead of voluntary fortification by the grain industry. This measure has its core objective of increasing the accessibility of milled cereal grains with iron and folic acid consumed by the Brazilian population to reduce the prevalence of iron deficiency and neural tube defects in Brazil [31].

However, iron-fortified wheat flour is not always available, or it is consumed in such small quantities that it could not be effective for children 6–60 months of age [32]. Fortification of specific foods, as part of a complementary diet, has shown to be more effective for the control and prevention of iron deficiency among infants [33]. In addition, and according to Hurrell [15] it is likely that the low levels of elemental iron (40 mg/Kg) added to wheat flour would have little impact on iron nutrition, but the much higher levels added to commercial infant cereals (200–550 mg/Kg) together with vitamin C could contribute substantially to the prevention of iron deficiency anemia. This measure becomes questionable in relation to infants, age of the greatest risk for anemia due to the fact that these foods are not recommended and regularly consumed in sufficient quantities to meet the iron needs of this particular group. Moreover, it is likely that the low level of elemental iron (40 mg/kg) added to wheat flour has little impact on nutritional status of children. A study has shown no effect of flour fortification in the hemoglobin levels of children less than 5 years in the city of Pelotas [34] and this could be due to insufficient consumption of flour and to the low bioavailability of dietary iron

The cassava flour enriched with ferrous bis-glycinate was studied during 4 months in 80 preschool children enrolled in a philanthropic institution in the city of Manaus. Anemia prevalence decreased significantly from 22.7 to 8 % after 4 months of intervention ($p < 0.05$). Since cassava flour is widely consumed in the north region of Brazil, it can be considered a promising food vehicle for control and prevention of iron deficiency [10].

Biscuits and Breads

Some studies were conducted on the effect of bovine hemoglobin-fortified cookies on the hemoglobin levels of 16 iron-deficient preschool children in northeast Brazil [35]. Each child was offered five cookies per day containing 1.25 mg of iron over 3 months as part of their normal school meal program. An evaluation of the total nutrients offered to the children showed an iron intake of just 4.0 mg/day. Baseline mean hemoglobin was 9.4 ± 2.6 g/dL, and at 3 months, mean hemoglobin increased to 13.2 ± 0.2 g/dL. Initial anemia prevalence was 73 % and disappeared at 3 months post-intervention. With the addition of bovine hemoglobin-fortified cookies to the children's diet, total iron intake increased to an average of 8.3 mg (83 % of iron RDA—Recommended Dietary Allowance) at a total cost of US$ 0.50 per child, with no measurable side effects or taste alterations reported.

A study with 1,500 children from daycare centers in the city of Barueri, Sao Paulo, also used cookies and breads fortified with iron aminoquelato at a dose of 2 mg/day during 2 months. This kind of intervention showed reduced levels of anemia from 32 to 11 % and a positive change on children's growth [36]. Other study evaluated 89 preschool children during 6 months using iron bis-glycinate chelate. Children received two sweet rolls twice daily each fortified with 2 mg iron bis-glycinate (4 mg/day) 5 days a week. At baseline, 28 % of the children had hemoglobin levels less than 11.0 g/dL and, at 6 months, 9 % of the children continued to be anemic. Mean hemoglobin at baseline was 11.5 g/dL, and at end, 12.6 g/dL. Mean hemoglobin increased 1.1 g/dL in non-anemic children and 1.4 g/dL in anemic ones. At the start of the study, mean ferritin level was 11.3 μg/L, and upon conclusion, mean ferritin increased significantly to 20.2 μg/L. Anthropometric indicators for weight/age and height/age also increased significantly [37]. A clinical research study has evaluated the effectiveness of hot dog bread enriched with 3 mg of iron aminochelate during 34 days in 275 children age 2–6 years in Sao Paulo daycare centers. After the intervention, a decrease of anemia in the group of children with bread fortified with iron from 21 to 12.6 % was observed. The average values of hemoglobin in this group increased from 16.5 times more than unsupplemented group [38].

However, the problem of fortification of breads and crackers is that these foods are not consumed in sufficient quantities to meet the needs of infants, and they, habitually, are not part of the food habits of this age group at highest risk for anemia. Despite the universal assumption that biscuits and sweet rolls are consumed by almost everyone, biscuits and sweet rolls consumption by infants, toddlers, and school children are quite different. As a consequence, the fractional iron intake contribution would be too low in a flour-based fortification program for infants. But these two vehicles—biscuits and sweet rolls—complement each other, resulting in a significant reduction of the population below the iron RDAs [39].

Rice and Bean

Rice is another alternative for food fortification. One study was conducted in four nurseries in Rio de Janeiro, with children in the intervention group ($n=180$) attending two nurseries and the control group ($n=174$) in the other two nurseries. An increase in hemoglobin concentration in both groups was observed. The reduction in the prevalence of anemia in the intervention group was 37.8 to 23.3 % and for the control group was 45.4 for 33 3 %, with no difference in reduction between the groups. According to the authors, the total amount of iron available was not sufficient to achieve more significant results in the intervention group, after 4 months of study [40].

In southeastern state of São Paulo, Brazil, studies were carried out, during 4 months, to evaluate bean flour enriched with iron in 85 anemic children 2–5 years of age. Results demonstrated a nonsignificant increase in anthropometric measurements and a significant reduction in the prevalence of anemia, which at baseline was 13 %, and at end study, anemia had disappeared in subjects that had received the iron-fortified bean flour [41]. Unfortunately, milled bean flour represents a greater cost burden, and in addition, is not widely consumed throughout Brazil.

In the metropolitan area of Belo Horizonte a study with families tested the rice fortified with iron. Initial study showed that there were no significant differences between the analyzed samples of conventional rice and iron-fortified rice. The iron did not alter the sensory characteristics of the final product, and the iron-fortified rice was well accepted [41]. A group of 84 children received iron-fortified rice (23 mg Fe/day) and another group received ferrous sulfate (25 g Fe/L). After 5 months of intervention, there was a reduction in the prevalence of anemia in both groups, with an initial prevalence of 100 % in both groups, decreasing to 61.9 % for the group receiving the fortified rice and 85.6 % for the group receiving ferrous sulfate, with a significant difference between groups [42]. However, regarding rice, more studies are needed to evaluate the timing and dose required for that

vehicle to achieve preventive effects and/or significant curative as well as assess the effect of simultaneous use with other supplements containing iron

Sugar

De Paula & Fisberg [43] evaluated the use of 20 g of iron-fortified sugar added to orange juice offered to 93 preschool children during 6 months. Children were divided into two groups: group 1 received 10 mg of iron per kilo of sugar, and group 2 received 100 mg of iron per kilo of sugar, both in the form of ferrous tris-glycinate. Anemia prevalence in both groups evaluated at baseline was 38.1 % and 29.4 %, respectively. At 6 months post-study intervention, anemia prevalence in both groups decreased to 19.7 % and 19.6 %, respectively ($p = 0.01$). Mean hemoglobin levels increased to 0.4 g/dL; in anemic children alone, mean Hb increased greatly to 1.3 and 1.5 g/dL in groups 1 and 2, respectively ($p < 0.001$). There was a positive trend towards normalization of ferritin values in iron-deficient children. It was suggested, in terms of cost, that use of 10 mg iron/Kg is preferred, as it leads to the same results, with low cost, when compared with 100 mg/Kg.

Safe Doses

The fortification of water with iron, for example, is a relatively new area, developed by our research group since 1994. For this reason, the amount of iron used in fortification has been modified since the first studies in order to find the lowest effective concentration, having begun with 20 mg/L and, in more recent studies, the attempt has been to use 5 mg/L use. Considering the daily requirement of iron 11 mg/day in pediatric patients and given to the question of bioavailability, most studies suggest that the fortified food provide around 5 mg/day of elemental iron.

Recommendations

Studies involving the fortification of drinking water with iron have shown that this strategy has three characteristics that make it an available option for the prevention of iron deficiency: the water is used indiscriminately for all people, the results are consistent, and it has very low cost. Additionally, the water is well accepted when fortified, and after a brief adjustment period, the logistics are relatively simple and one can even adjust the dose of iron in accordance with the epidemiological characteristics of the site.Therefore, we recommend the following strategy:

(1) Full time daycare center for preschool children.

- Prospect of using 1,000 mL of water during the period in the institution.
- The objective is to offer 5 mg of elemental iron per day.
- Dose of 5 mg/L which can be obtained with premix: 500 mg of $FeSO_4$-$7H_2O$ and 1,000 mg of ascorbic acid in 20 mL to be diluted in a gallon of 20 L of drinking water.

(2) Part time daycare center for preschool children.

- Prospect of using 500 mL of water during the period in the institution.
- The objective is to offer 5 mg of elemental iron per day.
- Dose of 10 mg/L which can be obtained with premix: 1,000 mg of $FeSO_4$-$7H_2O$ and 2,000 mg of ascorbic acid in 20 mL to be diluted in a gallon of 20 L of drinking water.

Conclusion

Food fortification is a public health strategy, and in order to be successful, several considerations should be kept in mind. First, the food vehicle of choice must be consumed regularly and in large scale by the targeted population. In addition, the selected food vehicle should be evaluated for potent absorption inhibitors, and if the added iron compound will have an impact on the iron status of the consumer. Secondly, it is important that the selected iron compound does not cause unacceptable changes in color and flavor when added to foods. Additionally, the food vehicle should be sufficiently stable during long periods of storage and during cooking in order to guarantee that true food consumption may be quantitatively capable of contributing significantly to the nutritional requirements of the population. Finally, the food vehicle must be centrally produced and proper technology is available for industrial-scale fortification [44, 45]. The food industries have used the enrichment of their products as a commercial appeal, focused on creating a quality attribute to further enhance the marketing of their products.

Iron deficiency is the most common and widely distributed nutritional clutter in the world, and it is a problem of public health in developing countries. Iron deficiency is the result of negative balance of this mineral throughout time. Iron deficiency anemia is the most serious type of iron deficiency, occurring after a long period of deficiency of this element, when supplies had already been depleted and after the reduction of biochemical iron. Fortification must be an instrument to not only correct deficiencies but also to guarantee the population an adequate supply of micronutrients, especially for the pediatric group, in which necessities are relatively higher due to growth. Regarding this, the World Bank mentioning food fortification as a strategy for combating micronutrient deficiency in the world says that “no other technology offers a chance of improving lives for so low a cost and in so short a time span” [46].

The high prevalence of iron deficiency and anemia in infancy in most regions of Brazil has called attention to an inadequate nutrition making this a serious public health problem leading to eventual losses in terms of future growth and productivity at all stages of human development. State and federal governmental health agencies must move forward to prioritize national nutrition agenda that will draft mandatory fortification of food staples for mass consumption. Fortified food is made available to vulnerable populations when industry is motivated to develop the logistics needed to fortify their products and when government is motivated to change policy requiring fortification.

In Brazil, studies on iron food fortification showed a positive response, both in relation to acceptance of fortified food and prevention, as in the recovery of hemoglobin levels in both groups [25, 47, 48]. Studies on iron food fortification, over the last 20 years, have shown promising results in the control and prevention of iron deficiency and anemia in infant and child populations. Unfortunately, only a small number of efficacy and effectiveness trails of iron fortification of foods and liquids conducted in Brazil have been published. Researchers have used various types of food vehicles as well as different iron compounds in an attempt to reduce nutritional deficiency, particularly an iron deficiency. However, there is no data in Brazil to assess the impact of these foods, fortified voluntarily by industry, on the prevalence of anemia.

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Suggested Sites and Sources

v. http://www.sbp.com.br
Associação Brasileira de Nutrologia. http://www.abran.org.br/
Governo do Brasil/Ministério da Saúde/Política Nacional de Alimentação e Nutrição. http://nutricao.saude.gov.br/
Governo do Brasil/Agência Nacional de Vigilância Sanitária. http://www.anvisa.gov.br/
Micronutrient initiative. http://www.micronutrient.org

Chapter 33
Iron-Fortified and Unfortified Nigerian Foods

Osaretin Albert Taiwo Ebuehi

Key Points

- The role of iron in human nutrition and current nutrition programs in Nigeria were highlighted.
- Iron fortification of staple Nigerian foods was discussed.
- Some staple Nigerian foods and crops rich and poor in iron were highlighted.
- The health consequences of iron deficiency were enumerated.
- The methods, types, prospects, and challenges in iron fortification of foods were highlighted.

Keywords Food fortification • Iron-fortified foods • Iron-unfortified foods • Nigeria • Prospects • Challenges

Abbreviations

WHO	World health organization
GAIN	Global alliance for improved nutrition
UNESCO	United Nations educational scientific cultural organization
MI	Micronutrient initiative
UNI	United Nations initiative
NAFDAC	National agency of food, drug, administration and control
CDC	Center for disease control
USAID	United States agency for international development
MOST	Museum of science and technology
SON	Standards organization of Nigeria
NIFST	Nigerian institute of food science and technology
NSN	Nutrition society of Nigeria
CHD	Coronary heart disease
UAC	United African company

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V.R. Preedy et al. (eds.), *Handbook of Food Fortification and Health: From Concepts to Public Health Applications Volume 2*, Nutrition and Health, DOI 10.1007/978-1-4614-7110-3_33,

WAMCO	West African milk company
RBC	Red blood cells
RDA	Recommended dietary allowance
PUFA	Poly unsaturated fatty acid
NDHS	National demographic health scheme
NaFe EDTA	Sodium iron (II) ethylene diamine tetra acetic acid
UNICEF	United Nations international children educational fund

Introduction

Nigeria is one of the world's most ethnically diverse countries. The Hausa and Yoruba make up around 21 % of the population; the Igbo/Ibo, 18 %; the Fulani, around 11 %; and Ibibio, 5 %. Various other ethnic groups, such as the Bini or Edo, Urhobo, Efik, Isoko, Ishan, Kwale, etc., make up the remaining 23 %. Nigeria has such a variety of people and cultures that it is difficult to pick one national dish. Each area has its own regional favorite that depends on customs, tradition, and religion. The different foods available also depend on the season: the "hungry season" is before the rains arrive in March, and the "season of surplus" follows the harvest in October and November [1, 2] (Fig. 33.1).

About 8 % of the population of Nigeria is classified as undernourished by the World Bank. This means they do not receive adequate nutrition in their diet. Of children under the age of 5, about 39 % are underweight and over 39 % are stunted (short for their age). Today Nigeria is among the ten countries in the world with the largest number of underweight children, with an estimated six million children under five who are underweight [1]. Apart from poor feeding practices and shortfalls in food intake, micronutrient deficiency is a direct cause of child morbidity and mortality. Micronutrients such as iron, iodine, vitamin A are necessary for the healthy development of children. Their absence in the diet causes serious disorders. A lack of iron causing anemia increases the risk of infants' death. Yet, according to the NDHS, 40 % of Nigerian pregnant mothers do not take any iron tablets, a recommended supplementation during pregnancy [1, 2] (Fig. 33.2).

Fig. 33.1 Nigeria, one of the world's great countries

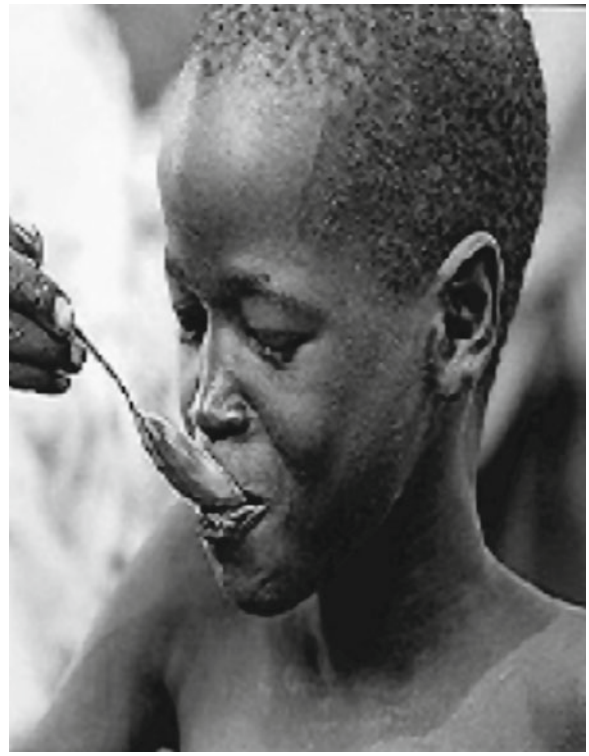

Fig. 33.2 A Nigerian child enjoying a fortified meal

Guidance on Safe Levels

The formation of national agency for food and drug administration and control (NAFDAC) in Nigeria was inspired by a 1988 World Health Assembly resolution requesting countries' help in combating the global health threat posed by counterfeit pharmaceuticals. As a governmental agency, NAFDAC has the mandate to regulate and control quality standards for *Foods*, Drugs, Cosmetics, Medical Devices, Chemicals, Detergents and packaged water imported, exported, manufactured locally, advertised, sold, and distributed in Nigeria [2].

The establishment of NAFDAC and standards organization of Nigeria (SON) is an expression of government's desire to ensure the well-being of the generality of the society in Nigeria. The human body is composed of certain combination of molecules and entities, which it is familiar with and which do not cause any harm when introduced from outside the body in small or reasonable amounts [1, 2].

Functions of Iron

1. Iron is present principally as hemoglobin of the RBC. Hemoglobin acts as a carrier of oxygen from the lungs to the tissues and indirectly helps in the return of carbon dioxide to the lungs. Hemoglobin is composed of heme, which contains iron, and globin, a simple protein. After the life span of the RBC is complete (120 days), the iron from it is removed and sent to the bone marrow where it is used for synthesis of new RBCs (haemopoiesis) [3, 4].
2. Iron is also a component of myoglobin, a protein located in the muscle tissue. Myoglobin stores oxygen within the muscle cells. When the body needs immediate supply of oxygen, as during strenuous exercise, myoglobin releases its stored oxygen.

3. Iron is also present in enzymes that permit the oxidation of glucose to produce energy. Several oxidase enzymes such as catalase, cytochrome oxidase, xanthine oxidase contain iron as an integral part of their molecular structures.
4. Because the brain has the highest metabolic rate of any organ, it requires high levels of iron and oxygen. Iron is required for the synthesis of myelin and of the neurotransmitters: serotonin and dopamine.
5. Transferrin or siderophilin is the circulating form of iron.

Sources of Iron

All foods are not equal in nutrient contents. Many foods are fortified with iron but its bioavailability depends on the compounds used. Iron is pervasive, but particularly rich sources of dietary iron include liver, lean meats, red meat, lentils, beans, poultry, legumes, dry fruits, fish, whole-grain cereals and hand-pounded cereals, green leafy vegetables, tofu, chickpeas, black-eyed peas, blackstrap molasses, fortified bread, and fortified breakfast cereals. Iron in low amounts is found in molasses, teff, and farina. Iron in meat (heme iron) is more easily absorbed than iron in vegetables [5].

Recommended Dietary Allowances of Iron

The recommended dietary allowance (RDA) for iron varies considerably based on age, gender, and source of dietary iron [4, 5]. Infants may require iron supplements if they are bottle-fed cows milk. Blood donors and pregnant women are at special risk of low iron levels and are often advised to supplement their iron intake [5].

The values set for the 1989 RDA are based on the assumption that only 10–15 % of iron in ingested foods is consumed. The RDAs of iron are 10 mg for men, children aged 6 months to 10 years, and women over the age of 51 years; 15 mg for women of reproductive age and lactating women; and 30 mg for pregnant women [6].

Iron Deficiency

Anemia is found to be due to different causes such as iron deficiency, folic acid deficiency, vitamin B_{12} deficiency, and deficiency of vitamin C. However, the most common type is iron deficiency. Anemia is a condition of insufficient hemoglobin to provide oxygen to the parts of the body. This deficiency is found commonly in infants, preschool children, adolescent girls and pregnant women. Characteristic symptoms are a low serum level of iron, high iron-binding capacity, low hemoglobin, lowered cell volume, low mean corpuscular hemoglobin, and small and pale cells (microcytic, hypochromic). The person suffers from weakness, frequent headaches, pallor, breathlessness, and dislike for work and exertion. There is sleeplessness, heartburn, palpitation, blurred vision, and swelling of the feet [5, 6] (Fig. 33.3).

Women suffer from anemia more than men or children. This is due to the loss of menstrual blood every month. Delivery of every child means heavy blood loss. Therefore, women must make every effort to consume iron-rich foods in their diets every day [5, 6].

Fig. 33.3 The eye logo of all fortified foods

Basic Food Commodities in Nigeria

The various diverse food commodities have great importance in Nigerian dietaries since they comprise the basic foods on which generations of Nigerians have thrived. Probably no other country can boast of a range of foods from which a multitude of recipes having such varied taste, aroma, color, and gastronomic appeal can be made [6, 7].

Food commodities are of two categories:

1. *The major food commodities*: These are used in ample amount at household and industrial level. For example, cereals and cereal products.
2. *The minor food commodities*: These food commodities are required in smaller quantities either accompaniments or as adjuncts in our diet. For example, preserves, sauces, etc.

Rice

Rice is the most popular cereal worldwide serving as staple food for 39 countries and nearly half of the world's population. Globally, rice accounts for 22 % of total energy intake [6–8]. There are two species of rice, namely *Oryza sativa* and *Oryza glabberima* which are commonly cultivated varieties in Nigeria. Several indigenous cultivars exist in Nigeria, such as Ofada and Abakaliki, and are widely consumed by the Nigerians as a major staple [7, 8].

Wheat

Wheat is another staple food of Nigerians. It is widely used in Nigerian dietaries besides its various products like semolina, semovita, and refined flour. As compared to rice, wheat has greater protein content. A variety of nutritious foods have been developed using wheat flour.

Millets

Besides the fine cereals rice and wheat, there are coarse cereals or millets and maize. The flours are used for making puffed products and snacks. For generations, it is been used as weaning food for children and a nutritious drink for pregnant and lactating women.

Bread and Biscuits

Bakery products that fall under the category of flour confectionery are wafers, waffles, pancakes, sponge rolls, bread, tea cakes, butter buns, cakes, plain biscuits, butter sponge and sponge cakes, short pastry, sweet biscuits, and so on. All bakery products are gaining extreme popularity as processed foods, which offer ready-to-eat convenience, as well as have comparatively long shelf life. Of these, biscuits are the most convenient and compact in form and are ideally suited for storage and distribution to a larger number of people. They are also capable of being enriched with additional proteins, vitamins, and minerals to enhance their nutritive value. Bread and biscuits contain calcium and iron [8, 9].

Pulses

Pulses are an important food item and are used in wide variety of forms. They may be cooked into thick or thin gruels or combined with cereals.

Milk and Milk Products

In spite of the theories for and against the consumption of milk, it remains the most common commodity of any Nigerian household, rich or poor. Cow milk is preferred although other type of milks such as standardized milk, toned milk, and homogenized milk are also available. Powdered milks or milk powders, either skimmed, partly skimmed, or whole milk powders are also available. They are used to impart taste and in various preparations like fruit salad and suitable to be used in place of fresh milk. It is suitable for the baby's digestive system. Weaning food, which may be introduced in the child's diet at the age of 6 months or more, usually contains both cereal and milk. Milk also contains two important minerals: calcium and phosphorus, although it lacks iron and vitamin C. It contains protein, lactose, and sometimes fat [9, 10].

Vegetables, Fruits, and Their Products

A vast variety of fruits and vegetables are grown and harvested all year round. Since there have been advances in water management and irrigation as well as hybridization techniques, a number of vegetables and fruits flood into the market every season. Among these are the relatively dark green leafy vegetables, such as spinach, amaranth, and cabbage. Their contribution to the diet in terms of vitamins, especially A, C, folic acid and B_{12}, minerals such as calcium and iron, water and above all the fiber content, cannot be underestimated by the nutritionist. The fruits and vegetables comprise of

carrots, mango, papaya, orange, lemon, sweet lime, etc. Roots and tubers like potato, onion, yam, sweet potato and other vegetables and fruits, namely, beans and peas, apple, pear are used in a wide variety of dishes in the Nigerian dietaries. Their contribution is significant. Although green leafy vegetables are abundant in Nigeria and several are available throughout the year, they are not often consumed. This results in a loss of an important nutrient source due to ignorance [7–10].

Eggs, Meat, Fish, and Poultry

Eggs are composed of 12 % shell, 30 % yolk, and 58 % white. Nutritionally, eggs are one of the most complete foods. Eggs contain lipids mainly as linoleic acid and arachidonic acid, 14 vitamins and 12 minerals, mainly calcium and phosphorus. Iron is not present in large amounts yet the body wholly absorbs it. The only nutrient absent in eggs is vitamin C. The egg yolk is rich in cholesterol. Eggs contain biotin (vitamin B complex), which is bound by avidin and rendered useless to the body. Fish furnishes first class proteins which are superior to meat and almost equal to milk. Minerals are present in fair amounts. Fish livers are rich in vitamins A, D, and E and poly unsaturated fatty acids (PUFA) [11].

In Nigeria, meat symbolizes pig meat, goat meat, ram meat, sheep mutton, and cow beef. This contains between 40 and 45 % proteins. Poultry such as chickens, turkeys, ducks, and geese are available in the frozen and in the dressed forms.

Iron Fortification of Staple Nigerian Foods

The WHO recently ranked iron deficiency seventh out of ten global preventable risks for disease, disability, and death that together account for 40 % of the 56 million deaths that occur worldwide each year and for one-third of the global loss of healthy life years. It is estimated that two billion people are iron deficient. Most of them live in developing countries [11, 12].

The five most important health consequences of iron deficiency are:

1. Suboptimal pregnancy outcome including lower birth weight, increased morbidity in mothers and neonates, increased infant mortality, and a greater risk of developing iron deficiency for the infant after 4 months of age.
2. Delayed mental and motor development in young children with effects on behavior and cognitive performance when the child reaches school age.
3. Reduced physical work capacity.
4. An increased risk for goiter and a suboptimal response to iodine in iodine deficient populations.
5. Increased frequency and duration of upper respiratory infections in young children.

The process of fortification is the addition of small quantities of vitamins and minerals to foods and condiments which are regularly consumed by a significant proportion of the Nigerian population [11–13]. Simply adding micronutrients such as iodine, iron, and vitamin A to commonly eaten foods such as salt, cereal, flours, and vegetable oils can make a positive difference to the well-being of the population. It is also interesting to note that the cost of fortification can be so little. Fortification is a simple and effective way of delivering iron. In more developed countries, the milling industry has been adding iron to flour as standard practice for many decades now and the effectiveness of flour fortification in reducing iron-deficiency anemia has been proven to reduce healthcare costs and allow for a more productive and prosperous society [14, 15].

There are three stages in the development of an iron-fortified food; these are optimization of the iron compound to obtain the highest relative bioavailability with no sensory changes, optimization of iron absorption to meet the consumer's needs, and demonstration of efficacy. Fortification with iron is technically more difficult than with other nutrients, because bioavailable forms of iron are chemically reactive and often produce undesirable effects when added into the diet [15, 16]. WHO guidelines [6] recommend the following fortificants in order of preference: ferrous sulfate, ferrous fumarate, encapsulated ferrous sulfate, encapsulated ferrous fumarate, electrolytic iron (added at twice the level of ferrous sulfate) ferric pyrophosphate (added at twice the level of ferrous sulfate) and NaFeEDTA. The Guidelines also recommend that the iron fortification level for a given population should be based on dietary iron intake, intake of vehicle, requirements of the most at-risk group, and the desired probability of inadequacy [16, 17].

Iron-fortified Nigerian Foods

Salt, wheat flour, sugar, milk, rice, fish sauce, curry powder, and all soy-based formulas are common examples of iron-fortified Nigerian foods. All complimentary foods from 6 months formula fed preterm infants; all commercial baby food—meat based, vegetable based, and cereal based; baby food brown rice—cereal, oat meal, and rice cereal; whole wheat bread, macaroni, nuts, legumes, and soy flour are also iron fortified in Nigeria.

Nigerian Foods Not Fortified

Some Nigerian foods not yet fortified include Breaker's yeast, soy bean, sesame seed, white beans; sun flower seeds, oats, almonds, wheat, pine nuts, spinach, peas, mushrooms, green beans, avocados, potatoes, carrots, grapes, apples, peaches, prunes, liver, beef, lamb, eggs, chicken, and pork.

Current Nutrition Programs in Nigeria

Nigeria launched its National Policy on Food and Nutrition in 2002, with the overall goal of improving the nutritional status of all Nigerians. The federal ministry of health, the federal ministry of industry, NAFDAC, SON, and the National Primary Health Care Development Agency are also involved in the government nutrition programs.

To tackle malnutrition, Nigeria has identified the following strategies:

1. Improving Food Security through programs and projects in the agricultural and nonagricultural sectors to increase household income especially in the poorer segment of the population.
2. Enhancing care-givers' capacity by promoting optimal infant feeding practices and reducing the workload of women to create more time for childcare, through the development of labor-saving technologies.
3. Improving Health services to provide essential maternal and child health care.
4. Controlling micronutrient deficiency and anemia through a strategy comprising vitamin and mineral supplementation, food fortification, and dietary diversification.
5. Eliminating Iodine Deficiency Disorder through salt iodization program.
6. Institutionalizing general consumer protection measures to safeguard food quality and consumer health.

GAIN (Global Alliance for Improved Nutrition) is supporting the NAFDAC to fortify wheat and maize flour with iron, vitamin A and B vitamins and vegetable oil and sugar with vitamin A through a second project phase. The goal of the project is to improve the nutrition of children between 2 and 5 years of age and of women of reproductive age by reducing micronutrient deficiencies, in particular vitamin A deficiency and anemia. The project began in November 2008 and is funded through November 2011 [17–19].

About 8 % of the population of Nigeria is classified as undernourished by the World Bank. Food is supposed to be enjoyable. Nigeria is one of the world's most ethnically diverse countries. Probably no other country can boast of a range of foods from which a multitude of recipes having such varied taste, aroma, color, and gastronomic appeal can be made. Throughout history, our ancestors survived on a variety of diet. Although the foods our ancestors ate may have been free of pesticides and additives, it was not always safe.

UNICEF is also supporting an integrated approach to early childhood development, involving the interplay between health, nutrition, sanitation, and education. Some professional bodies, such as Nigerian Institute of Food Science and Technology (NIFST) and Nutrition Society of Nigeria (NSN), are promoting food and nutrition programs in the country.

A nutrient with functions as vital as those of iron is indispensable to the human body. Although green leafy vegetables are abundant in Nigeria and several are available throughout the year, they are not often consumed. This results in a loss of an important nutrient source due to ignorance. A lack of iron causing anemia increases the risk of infants' death. Simply adding micronutrients such as iodine, iron, and vitamin A to commonly eaten foods such as salt, cereal, flours, and vegetable oils can make a positive difference to the well-being of the population [19, 20].

Food Fortification

Fortification of food with micronutrients is a valid technology for reducing micronutrient malnutrition as part of a food-based approach when and where existing food supplies and limited access fail to provide adequate levels of the respective nutrients in the diet [20, 21]. Food fortification has a long history of use in industrialized countries for the successful control of deficiencies of vitamins A and D, several B vitamins (thiamine, riboflavin and niacin), iodine, and iron. Salt iodization was introduced in the early 1920s in both Switzerland and the United States of America and has since expanded progressively all over the world to the extent that iodized salt is now used in most countries, including Nigeria [6, 7, 22].

From the early 1940s onwards, the fortification of cereal products with thiamine, riboflavin, and niacin became common practice. Margarine was fortified with vitamin A in Denmark and milk with vitamin D in the United States. Foods for young children were fortified with iron, a practice which has substantially reduced the risk of iron-deficiency anemia in this age group. Currently, the first sugar fortification experience in sub-Saharan Africa is taking place in Zambia, and if successful will be emulated elsewhere [20–23].

Iron Fortification

As reported by Hopkins Technology, iron fortification is done to many items made from refined grains as iron is lost in processing. Pasta, white rice, enriched breads, ready-to-eat breakfast cereals, oatmeal, and enriched grits are typically iron fortified. Iron-enrichment levels vary from brand to brand, but most products contain at least 25 % of the recommended dietary allowance for iron.

Choice of Iron Fortificant

Technically, iron is the most challenging micronutrient to add to foods, because the iron compounds that have the best bioavailability tend to be those that interact most strongly with food constituents to produce undesirable organoleptic changes. When selecting a suitable iron compound as a food fortificant, the overall objective is to find the one that has the greatest absorbability, i.e., the highest relative bioavailability (RBV) compared with ferrous sulfate, yet at the same time does not cause unacceptable changes to the sensory properties (i.e., taste, color, texture) of the food vehicle. Cost is usually another important consideration.

A wide variety of iron compounds are currently used as food fortificants. These can be broadly divided into three categories: water soluble, poorly water soluble but soluble in dilute acid, and water insoluble and poorly soluble in dilute acid.

Common Nigerian Foods

Iron fortification of foods in Nigeria is an integral part of the government effort in reducing micronutrient malnutrition among the citizens. This program is spearheaded by the key regulatory organizations like NAFDAC, who is always at the forefront in ensuring a healthy living for the citizenry. Ironically, most of our food items in Nigeria are naturally rich in iron, but, owing to the gross loss of iron during processing, the need for iron fortification becomes necessary.

Dairy Products

Dried whole milk powders and dried or ready-to-feed milk-based infant formulas have been successfully fortified with ferrous sulfate (together with ascorbic acid to enhance absorption). For example, ascorbic acid (700 mg/kg) and iron (100 mg as ferrous sulfate/kg) are routinely added to dried milk powders consumed by infants. In the case of soy formulas, it has been found necessary to use ferrous sulfate encapsulated with maltodextrin in order to prevent unwanted color changes (i.e., darkening). Ferrous sulfate, and many other soluble iron compounds, cannot be used to fortify liquid whole milk and other dairy products because they cause rancidity and off-flavors. Ferric ammonium citrate, ferrous bisglycinate, and micronized ferric pyrophosphate are generally more suitable for this purpose.

Iron fortificants are best added after the milk has been homogenized and the fat internalized in micelles, so as to help protect against oxidation. An example is Peak 123 milk powder manufactured by FrieslandCampina, West African Milk Company (WAMCO) Nig. Plc, which is fortified with 9.1 mg/100 g milk and is meant for babies of 1–3 years of age.

Rice

The fortification of rice grains which presents a number of technical challenges has been achieved in Nigeria, as is done in the United States, by coating the grain with an appropriate formulation. Alternatively, a rice-based extruded grain that contains a high concentration of iron can be mixed with normal rice grains (usually at a ratio of 1:200). Ferric pyrophosphate, added at a twofold higher level, and micronized, ferric pyrophosphate (0.5 μm) have recently been recommended for adding to extruded artificial rice grains. Technical difficulties, combined with cultural preferences for specific types of rice, mean that mass fortification of rice, although desirable, remains problematic in Nigeria.

The fact that in most of the rice-producing areas in the country, production takes place in thousands of small mills, also creates problems for mass rice fortification. Not only are smaller mills sensitive to small increases in costs, the sheer number of them makes it difficult to maintain adequate quality control programs. Although the extruded grains have found some application in targeted food fortification programs, such as school feeding programs, much more research and development is required before mass rice fortification programs can be implemented on a wider scale. However, iron-fortified rice products in Nigeria are mainly seen in smaller packaged rice products, unlike the bigger ones.

Cocoa Products

Cocoa products in Nigeria have been successfully fortified with iron. For instance, the Nestle Nigeria Plc cocoa product, "Nestle Milo" is fortified with 13 mg iron/100 g, while *Bournvita* by Cadbury Nigeria Plc fortified with 15 mg iron. As cocoa is naturally high in phenolic compounds, the addition of ferrous sulfate and other water-soluble iron compounds tends to cause color changes in cocoa-based products. Ferrous fumarate is a useful alternative for some products, but grey or blue/grey colours are still a problem for chocolate drinks, especially if boiling water is used to make up the drink.

Bread

The recent launching of NAFDAC into the Bakery Industry is an applaudable one, as it has brought a significant change in the quality of the products. In adherence to the Agency's directive, most brands of Bread are now well packaged and fortified with some food nutrients such as iron. "Butterfield" bread, for example, manufactured by Butterfield Bakery, is fortified with 25 % iron. However, so many have remained adamant to this directive as we can see in the different manufacturers of bread in Nigeria.

Soy Sauce and Fish Sauce

Even though some companies in Nigeria have made great effort in producing some of these sauces within the country, others are mostly imported from the Western and Asian counties, even though registered and recommended by NAFDAC. Some of them come in the forms of Geshiers, Sardines, and so on. Most of these products come out iron fortified while others do not. Sodium iron EDTA has proved to be a useful fortificant for both fish sauce and soy sauce. Studies have demonstrated that absorption of iron by human subjects fed NaFeEDTA-fortified fish or soy sauce added to rice meals is similar to that from the same meals to which ferrous sulfate-fortified sauces had been added.

Salt

The success of salt iodization programs has led several countries, including Nigeria to consider using salt as a vehicle for iron fortification. In practice, this means the double fortification of salt, i.e., with iron and iodine. Promising approaches that are already being tested include the addition of encapsulated ferrous fumarate, encapsulated ferrous sulfate, or ferric pyrophosphate (at twice the concentration).

Encapsulation is necessary as ferrous sulfate, ferrous fumarate, and other soluble iron compounds very quickly cause a yellow or red/brown discoloration in the moist, low quality salt that is currently used in many developing countries. The main disadvantage of the encapsulation options is the increase in the price of the fortified product, which can be as much as 30 %. Unfortunately, in the local villages where this salt is produced (e.g., Uburu in Ebonyi state, Nigeria), people consume it unfortified, restricting the benefit of iron fortification to only the urban dwellers who consume the fortified packaged salt. Also, some salt processing and packaging companies in Nigeria are yet to adhere to this double fortification of salt.

Noodles

The excessive influx of noodles in Nigeria took effect between 1980s and 1990s. Because of easy availability and affordability, fast preparation and flavory and sweet taste, they are preferred by many, especially children. Today, they are used as a salient tool in reducing micronutrient malnutrition by getting them fortified with minerals and vitamins. For example, "Indomie Noodles" produced by De United Foods Industries Limited, is fortified with iron. Some of the other available fortified brands are Mimee Noodle by May & Baker Plc, Chikki Noodles (16 %) by Chikki Foods Ind. Ltd, O! Noodles and so on.

Cereal Products

Most processed cereal products in Nigeria are fortified with iron. These products come in forms of Corn Flakes, Custard, Oat meal, Flours, etc. Some of the available iron-fortified cereal products are *Gold Custard* manufactured by Lisabi Mills Nig Limited, *Nestle Cerelac* manufactured by Nestle Nigeria Plc (fortified with 7.5 mg/100g), *Honeywell Semolina* (40.7 mg iron) manufactured by Honeywell Flour Mills and Semovita manufactured by Flour Mills Nig. Plc.

Safety Issues

Concern has been raised about increased iron intakes, particularly in terms of the potential effects on infection rates and on the risk of cardiovascular disease and cancer. Much of this concern, however, relates to the use of pharmaceutical iron supplements and not to fortified foods. A review of intervention studies with iron-fortified milk or cereals concluded that iron fortification did not increase infectious morbidity in children under 18 months of age. Studies in Chile, Hungary, and South Africa reported that iron added to milk formula had no influence on infectious outcome [23–25].

Studies have indicated that iron fortification of milk formula is safe. It has been suggested that higher levels of iron intake and elevated body stores are potential risk factors for both coronary heart disease (CHD) and cancer. Results from studies carried out over the last 10 years to test this hypothesis are, however, inconclusive and unsubstantiated [24–26].

Iron-Unfortified Nigerian Foods

Iron fortification of food in some cases are however, considered unnecessary due to the high iron content of some food items. These foods can be of animal origin as well as plant. When properly cooked or processed in the absence of iron inhibitors, they retain their iron contents. Their high concentration in some of our packaged food products make those products iron unfortified. Unlike in Western countries, majority of these foods in Nigeria does not undergo industrial processing. They are rather locally processed or taken raw as in fruits.

On the other hand, some other reasons for iron unfortification can be merely due to noncompliance with the regulatory authorities or nonchalance where there is no mandatory directive. Below are some examples [5–7, 19, 25].

Cassava Products ("Gari and Fufu")

"Gari" and "Fufu" are among the most staple foods in Nigeria, obtained by the fermentation of cassava. Their processing is mainly carried out in the rural areas, but then, they are widely accepted and consumed in every part of the country. Iron fortification of these products in Nigeria has not been commercially recommended by any regulatory body. However, some researchers have recorded a successful iron fortification of these products, for example "gari" and their positive effect in alleviating iron-deficiency anemia [6, 7, 25].

Rice

Even though iron fortification of rice has been successfully carried out and practiced by few companies in Nigeria, majority of the bagged rice products largely consumed, are not iron fortified [6, 7, 13, 25].

Beans

Beans is one of the commonest foods in Nigeria with very high iron content. Its high protein content and easy availability make it widely known and consumed both by the rich and the poor. The different species with their iron content (in mg) are listed below [5, 7, 21].

Butter and Margarine

Butter and Margarine brands in Nigeria have been observed to be iron unfortified. Currently, a lot of butter and margarine brands, manufactured in Nigeria are now being fortified.

Amala

Amala is a thick brown paste or porridge, made from yam or plantain, which had been peeled, cleaned, dried, and then blended into flour. It is widely consumed in Nigeria, primarily by the "Yorubas." Iron fortification of this food has not commenced, probably because it is largely processed locally.

Cocoa Powder and Chocolate

Chocolate is now showing more and more health benefits each day. The pure cocoa powder without any cocoa fat, milk, or sugar provides the most iron with 36 mg in a 100 g serving, or 200 % of the RDA [3, 8, 29].

Packaged and Unpackaged Snacks

There has been an influx of different snacks into the Nigerian market, both, packaged and unpackaged. These come in different names, colors, texture, taste, and so on. But none of these has been observed to be fortified with iron, even though the raw material "flour" may have been fortified. However, majority of the road-side snacks in Nigeria are not iron-fortified.

Pumpkin Seeds

Pumpkin seeds contain about 15 mg (83 % RDA) of iron/100 g serving. It is highly seen and consumed in the villages, where it is mainly preserved for the next farming season. It can be consumed cooked or roasted. The dried seeds have been observed to contain more iron than roasted.

Sweets and Gums

Iron fortification of our sweets and chewing gums can be suggested to be a strong tool in reducing iron deficiency anemia, since they are widely and casually consumed by many, especially, children. But it has been observed that most of the common sweets and chewing gums available in Nigeria are iron unfortified [5, 19, 25, 30, 31].

Sun Dried Tomatoes

Sun Dried Tomatoes are delicious in a sandwich or as an ingredient in pasta sauce. 100 g (about 2 cups) will provide 9.1 mg or 51 % of the RDA. When further processed into industrial products, such as tin tomatoes and canned pasta sauces, they leave these products iron unfortified.

Fruit Juice and Soft Drinks

Iron fortification of fruit juice and soft drinks seems not to have taken effect in Nigeria. This is observed in some manufactured fruit juice and soft drinks in Nigeria.

Dried Herbs

These are mainly used as spices and for medicinal purposes. These herbs are packed with nutrients and iron is no exception. Dried Thyme contains the most with 124 mg/100 g serving or 687 % of the RDA.

Liver

Liver is a vitamin rich food, and it is packed with iron. In the early 1900s, liver was prescribed as a cure for anemia, and as a supplement for pregnant ladies. It can be taken boiled or preserved by drying. Duck liver (Foie Gras) provides the most iron with 30.5 mg (170 % RDA)/100 g serving, or 13.4 mg (75 % RDA)/liver, chicken liver (72 % RDA/100 g), and beef liver (36 % RDA). It is available in every part of the country [5, 7, 9, 18, 30, 31].

Clams, Oysters, and Mussels

Shellfish can be eaten raw, baked, steamed, fried, or made into pottage. Clams provide the most iron with 28 mg (155 % RDA)/100 g serving or about 27 mg (150 % RDA) in 10 small clams. Oysters provide 12 mg (67 % RDA)/100 g serving or 5 mg (28 % RDA) in six medium sized oysters. Mussels provide 6.72 mg (37 % RDA)/100 g, or 5.7 mg (32 % RDA) in a 3 oz serving. Even though present in every part of the country, they are mainly consumed in the riverine areas such as South–South Zone of Nigeria (Calabar, Port Harcourt, etc.).

Others

Tables 33.1 and 33.2 show some Nigerian Foods rich in iron, of which, when taken as a single diet or highly contained in a cooked or processed food, makes iron fortification unnecessary [8, 11, 21, 24, 30].

Table 33.1 Iron levels of Nigerian bean varieties

Beans	Iron content (mg)
Green beans, cooked	0.60
Black eye beans	2.6
Navy beans	2.5
Pinto beans	2.2
Lima beans	2.2
Kidney beans Rajmah	1.5
Soybeans	4.4

Table 33.2 Iron levels in some Nigerian foods

Foods	Iron content (mg)
Sea vegetables	18.1–42.0
Sweet potatoes, canned	1.7
Potato, baked with skin	1.7
Watermelon, 1/8 medium	0.5
Peas, cooked	0.65
Tomato juice	0.6
Chickpeas (200 g)	6.2
Tofu	6.6
Soy milk	0.9
Dried apricot, 5	1.6
Sesame	1.2
Sunflower seeds	1.2
Cashew nuts	1.0
Bran flakes, 1 cup	11.0
Oatmeal, 1 packet	6.3
Pasta, 1 cup, cooked	1.7
Semolina, 1/2 cup, cooked	5.5
Wheat germ, 2 tablespoon	1.2
Whole wheat bread, 1 slice	0.9
White bread, 1 slice	0.7
Lamb beef, cooked	2.2
Turkey, white meat	1.6
Turkey meat, cooked	4.8
Tuna in oil	1.2
Sardines	4.0
Shrimp, cooked, 4 large	0.7
Pork, loin, broiled	1.05
Egg, 1	0.7

Conclusions

The role, prospects, and challenges of iron fortification of Nigerian foods are highlighted. The current nutrition programs and the need for nutritional education on food fortification of staple foods in Nigeria were emphasized.

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Chapter 34
Food Fortification: What More Is There to Know?

Rajkumar Rajendram, Roshanna Rajendram, Vinood B. Patel, and Victor R. Preedy

Key Points

- Diet quality scores are related to health outcomes. Thus diet quality is as important as quantity; a fact which is overlooked when food security is measured.
- Micronutrient malnutrition affects billions of people worldwide and is associated with significant morbidity and mortality.
- Food fortification is one potential solution to micronutrient malnutrition.
- This chapter lists the most up-to-date resources on the regulatory bodies, journals, books, professional bodies, and websites that are relevant to an evidence-based approach to food fortification.

Keywords Food • Food fortification • Diet quality • Nutrition • Evidence • Resources • Books • Journals • Regulatory bodies • Professional societies

Introduction

The availability of or access to sufficient calories (i.e., "food security") is increasing worldwide [1]. However food secure individuals and populations with access to sufficient calories may still lack essential nutrients that are important for human health. So despite "food security" nutritional inadequacies in poor quality diets ("hidden hunger") increase the risk of both short- and long-term morbidity and

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V.R. Preedy et al. (eds.), *Handbook of Food Fortification and Health: From Concepts to Public Health Applications Volume 2*, Nutrition and Health, DOI 10.1007/978-1-4614-7110-3_34,

mortality. Diet quality is as important as quantity; a fact which is overlooked when food security is measured.

Hidden hunger from micronutrient malnutrition is a gargantuan public health issue. Worldwide it affects billions of people, in both developing and developed countries. Vitamins and minerals are required in many physiological processes, including the maintenance of pH, osmotic pressure and electrical stability of cell membranes. Deficiencies of these vitamins or minerals cause serious health problems. For example, over two billion people are at risk of iron deficiency worldwide [2]. Adverse effects on the health of these individuals range from exhaustion, to impaired cognition and increased susceptibility to infection [3]. Besides significantly reducing quality of life, the complications of micronutrient malnutrition can cause significant mortality; 0.8 million deaths each year are related to iron deficiency [4]. The high morbidity and mortality of micronutrient malnutrition has important economic sequelae, including the loss of individual and government income and financial stability.

Potential solutions to micronutrient malnutrition include promotion of dietary change (requiring education, advice and incentives), dietary supplementation, and fortification of food. Educational interventions are, in theory, the ideal solution. However, changing dietary habits on the population level is challenging [2], and may have poor efficacy, at least in the short term [5]. Dietary supplementation is a rapid and cost-effective solution for individuals at risk of deficiency that also limits overdose in those with adequate dietary intake. However, supplements may have adverse side effects and compliance may be poor. Food fortification is another potential solution. This involves enrichment of food with nutrients to greater concentrations than those naturally present [6]. Food fortification has increased recently, as it has been recognized that fortification is a public health intervention for nutritional deficiencies that has a wider and more sustained impact than supplementation. Although not without limitations, food fortification is an important intervention to treat micronutrient malnutrition that should be used in combination with promotion of dietary change and dietary supplements. Examples, of the definitions, measurement and applications of food fortification can be found in this book and also via the recommended resources in the tables below.

Tables 34.1, 34.2, 34.3, 34.4, and 34.5 list the most up-to-date information on the regulatory bodies (Table 34.1), journals (Table 34.2), books (Table 34.3), professional bodies (Table 34.4) and websites (Table 34.5) that are relevant to an evidence-based approach to food fortification.

Table 34.1 Regulatory bodies

Agência Nacional de Vigilância Sanitária	www.anvisa.gov.br
Bundesministerium für Ernährung, Landwirtschaft und Verbraucherschutz (German Federal Ministry for Nutrition)	www.bmelv.de
Bundesinstitut für Risikobewertung (German Federal Institute for Risk Assessment)	www.bfr.bund.de
Canadian Food Inspection Agency	www.inspection.gc.ca/english/fssa/labeti/guide/toce.shtml
Codex Alimentarius	www.codexalimentarius.net/
EU-lex	eur-lex.europa.eu/
European Commission, Food Safety	ec.europa.eu/food/food/labellingnutrition/vitamins/index_en.htm
European Food Safety Authority	www.efsa.europa.eu/
Food Chemicals Codex	www.usp.org/fcc/
Food Standards Australia New Zealand (FSANZ)	www.foodstandards.gov.au/
Governo do Brasil/Ministério da Saúde/Política Nacional de Alimentação e Nutrição	nutricao.saude.gov.br/
Governo do Brasil/Agência Nacional de Vigilância Sanitária	www.anvisa.gov.br/
Government of Canada: Food and Drugs Act and Regulations	laws-lois.justice.gc.ca/eng/acts/F-27/
Health Canada	www.hc-sc.gc.ca/fn-an/index-eng.php
International council for control of iodine deficiency disorders (ICCIDD)	www.iccidd.org/
Micronutrients Initiatives	www.micronutrient.org

(continued)

Table 34.1 (continued)

Natural Health Products Directorate	www.hc-sc.gc.ca/dhp-mps/prodnatur/index-eng.php
Regulation (EC) No 1925/2006 on the addition of vitamins and minerals and of certain other substances to foods	ec.europa.eu/food/food/labellingnutrition/vitamins/index_en.htm
Regulation (EC) No 1924/2006 with regard to the list of nutrition claims	eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:037:0016:0018:EN:PDF
Subcommittee on Poultry Nutrition, Committee on Animal Nutrition, Board on Agriculture, National Research Council	agriculture.house.gov/singlepages.aspx?NewsID=32&LSBID=44
Thailand's Food and Drug Administration	www.thaifda.com
United Nations Children's Fund (UNICEF)	www.unicef.org/nutrition/index_iodine.html
United States Department of Agriculture (USDA)	www.cnpp.usda.gov/dietaryguidelines.htm
US Food and Drug Administration	www.fda.gov/
World Bank	www.worldbank.com
World Health Organization	www.who.int/nutrition/topics/micronutrients/en/

This table lists the regulatory bodies involved with food fortification

Table 34.2 Journals

American Journal of Clinical Nutrition	www.ajcn.org
The American Journal of Nutrition	www.ajcn.org/
Archivos Latinoamericanos de Nutricion	www.alanrevista.org
Asia Pacific Journal of Clinical Nutrition	apjcn-apcns@umail.hinet.net
British Journal of Nutrition	journals.cambridge.org/action/displayJournal?jid=BJN
Eastern Mediterranean Health Journal	www.emro.who.int/emhj.htm
Environmental Law & Management	www4.dr-rathfoundation.org/NHC/studien_pdf/new/European_regulation_of_food_supplements_etc_-_ELM_-_2007-19_-_Schwitters_et_al.pdf
European Journal of Clinical Nutrition	www.nature.com/ejcn/index.html
European Poultry Science	www.ulmer.de/2972.html
European Thyroid Journals	content.karger.com/ProdukteDB/produkte.asp?Aktion=JournalHome&ProduktNr=255331
Food and Nutrition Bulletin	www.foodandnutritionbulletin.org/fnbhome.php
Food and Nutrition Research	www.foodandnutritionresearch.net
Food Chemistry	www.journals.elsevier.com/food-chemistry/
International Journal of Behavioral Nutrition and Physical Activity	www.ijbnpa.org
International Journal of Paediatric Obesity	informahealthcare.com/loi/jpo
International journal of vitamins and nutrition research	http://www.verlag-hanshuber.com/zeitschriften/journal.php?abbrev=VIT
Journal of the Academy of Nutrition and Dietetics	www.adajournal.org
Journal of Adolescent Health	jahonline.org
Journal of Agricultural and Food Chemistry	pubs.acs.org/journal/jafcau
Journal of the American Dietetic Association	www.ADAJournal.org
Journal of Clinical Nutrition	www.nutrition.org
Journal of Internal Medicine	www.jim.se
Journal of functional foods	www.journals.elsevier.com/journal-of-functional-foods/
Journal of food science	www.ift.org
Journal of Nutrition	jn.nutrition.org
Journal of Nutrition Education and Behavior	www.jneb.org
Journal of public policy and marketing	www.marketingpower.com/AboutAMA/Pages/AMA%20Publications/AMA%20Journals/Journal%20of%20Public%20Policy%20Marketing/JournalofPublicPolicyMarketing.aspx
Journal of Thyroid Research	www.hindawi.com/journals/jtr/
Meat Science	www.journals.elsevier.com/meat-science/

(continued)

Table 34.2 (continued)

Nutrition	www.elsevier.com/wps/find/journaldescription.cws_home/525614/description#description
Nutritional Neuroscience	www.maney.co.uk/index.php/journals/nns
Nutrition Research	www.nrjournal.com
Public Health Nutrition	journals.cambridge.org/action/displayJournal?jid=PHN
Sight and Life Magazine	www.sightandlife.org

This table lists the journals publishing original research and review articles related to food fortification

Table 34.3 Books

Allen L, de Benoist B, Dary O, Hurrell R. Guidelines on food fortification with micronutrients. World Health Organization and Food and Agriculture Organization of the United Nations, 2006 Geneva, Switzerland

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Chen C, He W, Zhenying F. Ten Year Tracking Nutrition Status in China: 1990–2000. People's Medical Publishing House, 2004, Beijing, China

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Feldman D, Pike WJ, Adams JS. Vitamin D 3rd Ed. Elsevier, 2011, San Diego, California

Garrow JS, James WPT. Human nutrition and dietetics 10th ed, Churchill Livingstone, 2000, London, UK

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Immerseel FV, Nys Y, Bain M. Improving the safety and quality of eggs and egg products: Egg safety and nutritional quality. Woodhead Publishing Limited, 2011, Sawston, Cambridge, UK

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Kirchhoff E. Food composition and nutrition tables 7th ed., German Research Centre for Food Chemistry. CRC Press, 2008, Boca Raton, USA

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Sim JS, Nakai S. Egg Uses and Processing Technologies. CABI Publishing, 1994, Wallingford, UK

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World Health Organization, Food and Agriculture Organization. Vitamin and mineral requirements. Second edition. World Health Organization, 2004, Geneva-Switzerland

Weaver CM and Heaney RP. Calcium in Human Health. Humana Press Inc., 2006 USA

World Bank. Enriching Lives. World Bank, 2000, Washington DC, USA

This table lists some important books on food fortification

Table 34.4 Professional societies

The Academy of Nutrition and Dietetics	www.eatright.org
American Heart Association	www.heart.org/HEARTORG/
American Society for Nutrition	www.nutrition.org/
American Society for Nutritional Sciences	www.faseb.org/asns
Associação Brasileira de Nutrologia	www.abran.org.br
Canadian Nutrition Society	www.cns-scn.ca
Deutsche Gesellschaft für Ernährung (German Nutrition Society)	www.dge.de
Dieticians of Canada	www.dieticians.ca
Flour Fortification Initiative	www.sph.emory.edu/wheatflour/
FoodDrinkEurope	www.fooddrinkeurope.eu/
Fortifying Africa's Future (FORTAF)	www.fortaf.org/partners.htm
Global Alliance for Improved Nutrition (GAIN)	www.gainhealth.org
International Council for Control of Iodine Deficiency Disorders	ICCIDD.org
International Margarine Association of the Countries of Europe (IMACE)	www.imace.org/
The International Society for Nutraceuticals and Functional Foods	isnff.org/
International Society for the Study of Fatty Acids and Lipids	www.issfal.org
Institute of Food Technologists	www.ift.org
Institute of Medicine (IOM), Food and Nutrition Information Centre USDA	www.iom.edu/Activities/Nutrition/SummaryDRIs/~/media/Files/Activity%20Files/Nutrition/DRIs/New%20Material/5DRI%20Values%20SummaryTables%2014.pdf
Joint FAO/WHO Expert Committee on Food Additives (JECFA)	www.fao.org/ag/agn/agns//jecfa_index_en.asp
Micronutrient Forum	www.micronutrientforum.org/
Micronutrients Initiative	www.micronutrient.org/
Sociedade Brasileira de Pediatria	www.sbp.com.br
Sociedad Española de Nutrición Comunitaria (Spanish Society of Community Nutrition)	www.nutricioncomunitaria.org/
Sharing United States Technology to Aid in the Improvement of Nutrition (SUSTAIN)	www.sustaintech.org/
World's Poultry Science Association (WPSA)	www.wpsa.com/

This table lists the professional societies involved with food fortification

Table 34.5 Relevant internet resources (i.e., those devoted to food fortification)

A2Z Project (USAID)	www.a2zproject.org/~a2zorg/
American Egg Board	www.aeb.org/
BASF, Food fortification	www.food-fortification.com/Home.aspx
British Nutrition Foundation	www.nutrition.org.uk/
Codex Alimentarius—International Food Standards	www.codexalimentarius.org
Department of Health and Senior Services, NJ, USA	www.nj.gov/health/fhs/prenatal/folic.shtml
European Commission, Scientific Committee on Food, Opinion of the Scientific Committee on Food on the Tolerable Upper Intake Level of Vitamin D	ec.europa.eu/food/fs/sc/scf/out157_en.pdf
European Food Safety Authority (EFSA), Calcium and Vitamin D and Bone Strength	www.efsa.europa.eu/en/efsajournal/pub/828.htm
European Recommendations Aligned, FP6 EC Funded Project (EURRECA)	www.eurreca.org/everyone
Flour Fortification Initiative	www.sph.emory.edu/wheatflour/index.php
Food and Agriculture Organization (FAO)	www.fao.org
Global Alliance for Improved Nutrition	www.gainhealth.org
Golden Rice Humanitarian Board	www.goldenrice.org/index.html
Harvest Plus	www.harvestplus.org
Helen Keller International	www.hki.org

(continued)

Table 34.5 (continued)

International Osteoporosis Foundation	www.iofbonehealth.org
Institute of Medicine	www.iom.edu/Activities/Nutrition/SummaryDRIs/DRI-Tables.aspx
Institute of Medicine, Food and Nutrition Board, 2010, Dietary Reference Intakes for Calcium and Vitamin D	www.iom.edu/Reports/2010/Dietary-Reference-Intakes-for-Calcium-and-Vitamin-D.aspx
International council for Control of Iodine Deficiency Disorders (ICCIDD) Newsletter	www.iccidd.org/pages/idd-newsletter.php
International Food Information Council Foundation	www.foodinsight.org/
MERCK	magazine.merck.de/en/Life_and_Assistance/iodine_deficiency_screening/Thyromobil1.html?wt.srch=1
Micronutrient Initiative	www.micronutrient.org
National Health and Medical Research Council, 2006, Nutrient Reference Values for Australia and New Zealand	www.nhmrc.gov.au/guidelines/publications/n35-n36-n37
Nutrition, National Agricultural Library, USDA	www.nutrition.gov/nal_display/index.php?info_center=11&tax_level=1
Office of Dietary Supplements—National Institutes of Health	ods.od.nih.gov/factsheets/folate/
OPTIFORD (Vitamin D Fortification)	www.optiford.org/
Osteodiet	osteodiet.ucc.ie
Project Healthy Children	www.projecthealthychildren.org
Public Health Agency of Canada	www.phac-aspc.gc.ca/fa-af/
Scientific Advisory Committee on Nutrition (SACN), Vitamin D	www.sacn.gov.uk/meetings/working_groups/vitamin/index.html
Scientific Committee on Food (SCF)	ec.europa.eu/food/fs/sc/scf/index_en.html
Sundar Serendipity Foundation (suppliers of the multiple micronutrient fortified salt and multiple micronutrient food fortificant to combat multiple micronutrient deficiencies)	www.sundarserendipityfoundation.org
Sweet potato Knowledge Portal	sweetpotatoknowledge.org
United Nations Children's Fund (UNICEF)	www.unicef.org
United States Agency for International Development	www.usaid.gov/our_work/humanitarian_assistance/ffp/crg/sec2.htm
United States Department of Agriculture	www.usda.gov/wps/portal/usda/usdahome
USDA National Nutrient Database for Standard Reference Release 24	ndb.nal.usda.gov/ndb/foods/list
Vitamin D Council	www.vitamindcouncil.org/
World Bank	www.worldank.com
World Food Program	foodquality.wfp.org/FoodSpecifications/tabid/56/Default.aspx
World Health Organization	www.who.int/nutrition/topics/vad/en/
World Health Organization (WHO) Reduction of Micronutrient Malnutrition	www.who.int/nutrition/publications/micronutrients/en/

This table lists some internet resources on food fortification

Acknowledgements We would like to thank the following authors for contributing to the development of this resource. D Alasfoor, OM S Amancio, NK Bhullar, S Bhushan, D Blancquaert, L Bui, MS Calvo, F Capanema, C Cerqueira, V Chavasit, L Corvaglia, H De Steur, MT Díaz Díaz-Chirón, M Dunn, M Fenech, J Fontecha, Fustier P, I S Hussein, J Jastrebova, M Lawrence, J Low, Y Manios, D Mackerras, LH da S Miglioranza, V Mocanu, LM Neufeld, P Pathomrungsiyounggul, J Sacco, MJ Salgueiro, E Scarlett, N Shapira, I Sioen, D Van Der Straeten, L Timotijevic, M Venkatesh-Mannar, M VinodKumar, C von Schacky, SJ Whiting, S Zimmerman.

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V.R. Preedy et al. (eds.), *Handbook of Food Fortification and Health: From Concepts to Public Health Applications Volume 2*, Nutrition and Health, DOI 10.1007/978-1-4614-7110-3,