DEPARTMENT OF NUTRITIONAL SCIENCES

INCREASING MINERAL BIOAVAILABILITY IN A VEGAN DIET

VALERIE SULLIVAN
Spring 2011

A thesis
submitted in partial fulfillment
of the requirements
for a baccalaureate degree
in Nutritional Sciences
with honors in Nutritional Sciences

Reviewed and approved* by the following:

Dorothy Blair
Assistant Professor of Nutrition
Thesis Supervisor

Gary Fosmire
Associate Professor of Nutrition
Honors Adviser

* Signatures are on file in the Schreyer Honors College.
ABSTRACT

A vegan diet is able to supply all nutritionally essential minerals without the use of supplements. However, the bioavailability of certain minerals may be compromised by inhibitors that are present in plants. In particular, the absorption of iron, zinc, and calcium is decreased by plant-derived inhibitors. Iron, zinc, and calcium absorption is inhibited by phytate; zinc and calcium absorption is inhibited by oxalic acid; and iron absorption is inhibited by polyphenols. If these minerals are primarily derived from poorly absorbable plant sources, an apparently “healthy” vegan diet may actually be nutritionally deficient.

Reducing the concentrations of inhibitors and/or opposing their inhibitory effects can increase the bioavailability of these minerals in foods. Several food processing methods have been shown to decrease the concentration of such inhibitors. Presoaking, boiling, and draining foods is useful for reducing concentrations of phytate, oxalic acid, and polyphenols. Germination, fermentation, and bread making are additional methods that reduce phytate concentrations. The effects of inhibitors can be opposed by adding absorption-enhancing compounds to the diet. Ascorbic acid and other organic acids improve iron absorption, and citric acid may improve zinc absorption. These strategies can be used to increase the bioavailability of minerals from plants.

New vegans should be taught to distinguish between sources of iron, zinc, and calcium that are well-absorbed versus those that are poorly absorbed. Presently, nutritional advice for new vegans does not fulfill this need. Therefore, an informative brochure has been developed to teach new vegans about mineral absorption inhibitors and ways to reduce their inhibitory effects and/or concentrations in foods.
# TABLE OF CONTENTS

ABSTRACT.................................................................................................................. i

TABLE OF CONTENTS................................................................................................. ii

LIST OF FIGURES ........................................................................................................ iv

LIST OF TABLES ............................................................................................................ v

ACKNOWLEDGEMENTS ............................................................................................... vi

Chapter 1  Introduction ................................................................................................. 1

Phytate ...................................................................................................................... 2
Oxalate ....................................................................................................................... 5
Polyphenols ................................................................................................................. 7

Chapter 2  Iron ............................................................................................................. 10

A. Decrease Inhibitors .............................................................................................. 12
B. Increase Enhancers ............................................................................................. 26
C. Increase Total Iron Intake ................................................................................... 33

Chapter 3  Zinc ........................................................................................................... 41

A. Decrease Inhibitors .............................................................................................. 42
B. Increase Enhancers ............................................................................................. 48
C. Emerging Research: Polyphenols ....................................................................... 48
D. Increase Total Intake ............................................................................................ 50

Chapter 4  Calcium ................................................................................................... 54

A. Decrease Inhibitors .............................................................................................. 60
B. Enhance Absorption: Vitamin D .......................................................................... 66
C. Increase Total Intake ............................................................................................ 69

Chapter 5  Decreasing Inhibitors ............................................................................. 76

Phytate ...................................................................................................................... 76
Milling ......................................................................................................................... 77
Soaking ....................................................................................................................... 79
Cooking ....................................................................................................................... 83
Chapter 6  Developing an Educational Guide.......................... 100
Chapter 7  Discussion............................................................ 104
References................................................................................. 106
LIST OF FIGURES

Figure 6-1 Brochure interior ................................................................. 102
Figure 6-2 Brochure exterior ................................................................. 103
LIST OF TABLES

Table 2-1 Iron in Selected Grains ................................................................. 34
Table 2-2 Iron in Selected Legumes .............................................................. 35
Table 2-3 Iron in Selected Nuts and Seeds .................................................... 36
Table 2-4 Iron in Selected Fruits ................................................................. 36
Table 2-5 Iron in Selected Vegetables ............................................................ 37
Table 3-1 Zinc in Selected Grains ................................................................. 51
Table 3-2 Zinc in Selected Legumes .............................................................. 51
Table 3-3 Zinc in Selected Nuts and Seeds .................................................... 52
Table 3-4 Zinc in Selected Fruits and Vegetables ......................................... 52
Table 4-1 Calcium in Selected Legumes ....................................................... 71
Table 4-2 Calcium in Selected Nuts and Seeds ............................................. 72
Table 4-3 Calcium in Selected Fruits ............................................................. 72
Table 4-4 Calcium in Selected Vegetables .................................................... 73
Table 5-1 Mineral Losses in Milled Grains .................................................. 78
ACKNOWLEDGEMENTS

I would like to thank Dr. Dorothy Blair, the supervisor of my thesis, for offering steady guidance throughout the entire writing process. Her thoughtful comments and reflective questions motivated me to thoroughly research my topic to understand its complexities from all viewpoints. She has given generously of her time to revise my work and help locate necessary resources. I am grateful for her tireless commitment to this project.

I also thank Dr. Gary Fosmire, my honors advisor and second reader of my thesis. He has been instrumental in the formulation of this thesis by helping me to turn my interest in vegan nutrition into a creative thesis project. I am especially grateful for his patience in awaiting the final draft and taking time from his other responsibilities to read and respond to it. In the midst of a chaotic semester, he has helped me to maintain a sense of calm by offering a listening ear and reassurance.

Finally, I thank my family for encouraging me to persevere and see this thesis through to its completion. They have tolerated my distraction, messiness, and anxiety throughout months of research and writing. I appreciate their enduring love and inexhaustible forgiveness.
Chapter 1

Introduction

Once an obscure term known only by its adherents, “veganism” has recently been popularized by celebrities and the media. For instance, actress Alicia Silverstone’s *The Kind Diet*¹ and author Mark Bittman’s “vegan before six” diet² advocate total and primarily vegan diets, respectively. Books and documentaries, such as Robert Kenner’s *Food, Inc.*,³ have also raised awareness about factory farming systems that supply most American animal foods, thereby increasing the appeal of vegan diets. Vegans, or strict vegetarians, abstain from eating meat, poultry, fish, eggs, and dairy products as well as any animal-derived ingredients like gelatin, casein, and whey. As more people adopt vegan eating patterns, it is important to ensure that they are informed about planning nutritionally adequate diets.

New vegans can research their diet through Internet websites, magazines, and books. These sources generally focus on replacing foods that are eliminated from a vegan diet. Replacing animal-derived foods with alternative nutrient sources is certainly an important component of vegan diet planning. However, the bioavailability of these nutrients is an equally important concern that is often overlooked. Bioavailability refers to a nutrient’s ability to be absorbed. Foods that are apparently rich in a nutrient may not necessarily be reliable sources if their bioavailability is low. Vegans must not only attend to the total nutrient content of their diets but also the bioavailability of these nutrients.

The content and bioavailability of many vitamins is high in a plant-based diet. Vitamins A, C, and E, for instance, are common and well-absorbed from plant foods. Certain minerals are also consumed frequently and absorbed sufficiently to meet or exceed estimated requirements, such as potassium, magnesium, manganese, and copper. In contrast, vegan diets eliminate
common sources of iron, zinc, and calcium and increase foods containing substances that jeopardize their bioavailability. Flesh foods, for instance, are concentrated sources of iron and zinc while dairy supplies zinc and calcium. Plant foods that replace such sources may contain comparable concentrations of these minerals yet yield lower net absorption due to the presence of inhibitors. Phytate, oxalic acid, and polyphenols are abundant inhibitors in vegan dietary staples that compromise the absorption of iron, zinc, and calcium to varying degrees.

**Phytate**

Structurally, phytate is a salt composed of an alcohol, inositol, attached to six phosphate groups, calcium, and magnesium. Phytate is primarily concentrated in plant seeds, where it stores phosphate and minerals for plant growth upon germination. Approximately 50-85% of seeds’ phosphorus exists in the form of phytate. In contrast, relatively little phytate resides in roots and tubers. Humans mostly consume phytate in the diet as grains, legumes, and oil seeds.

The distribution of phytate within seeds varies. In most cereals, it is primarily concentrated in the aleurone layer. The germ contains a smaller fraction of a grain’s phytate but, expressed per unit mass, is also rich in phytate. An exception to this pattern is corn, which contains most of its phytate in the germ. In all cereals, the endosperm does not contain much phytate. In contrast, phytate in legumes, seeds, and nuts is concentrated in organelles called protein bodies within their cotyledons rather than the exterior seed coat or hull.

Variability within the same plant species is also observed. For instance, the phytate content may vary due to genetic differences or the conditions (temperature, hydration, and exposure to pathogens) under which they are grown. In addition, the maturity of a seed affects its phytate content, since phytate accumulates in seeds as they develop. All of these factors contribute to differing phytate content in the plants of the same species.
Though the terms are often used interchangeably, phytate and phytic acid (PA) are chemically distinct compounds. Phytate is a salt whereas the dissociated acid (i.e. phytate without Ca and Mg) is called phytic acid, formally named myo-inositol (1,2,3,4,5,6) hexakisphosphate. Plants contain phytate, not phytic acid; however, their “phytic acid” content can be measured using analytical procedures that extract the compound in an acidic medium.

The molecular weight of phytate varies depending on the types and proportions of minerals that form the salt, but 660 mg/mol is commonly used in calculations to translate phytic acid into approximate phytate concentrations.

The antinutritional properties of phytate are attributable to the phytic acid it contains. Phytic acid is negatively charged and binds di- and trivalent minerals in mildly acidic or neutral conditions. In the nearly neutral human intestine, dietary phytic acid binds minerals and forms poorly soluble complexes that are not absorbed. Of concern to vegan nutrition is the formation of insoluble complexes between phytic acid and the essential minerals iron, zinc, and calcium. Most vegan sources of these minerals are rich in phytate, so the bioavailability of the minerals is reduced. While magnesium can also bind phytate, vegans generally consume sufficient amounts of this mineral to compensate for its lower bioavailability.

Inositol phosphate compounds with fewer than six phosphate groups may also be consumed in the diet. The compounds are called inositol mono-, bis-, tris-, tetrais-, and pentakisphosphates, and they bind one, two, three, four, and five phosphate groups, respectively. Plant seeds naturally contain a small amount of these lower inositol phosphates in addition to inositol hexakisphosphate (InsP₆). They are also formed during seed germination and certain food processing methods that hydrolyze phosphate groups from InsP₆. Enzymes collectively known as “phytases” are responsible for catalyzing this reaction. Phytase activity thereby reduces the amount of InsP₆ in foods and increases the proportion of lower inositol phosphates.
Converting inositol hexakisphosphate to lower inositol phosphates could be nutritionally beneficial for people primarily obtaining minerals from high-phytate foods, such as vegans. With the exception of inositol pentakisphosphate (InsP_5), lower inositol phosphates do not significantly decrease mineral absorption. Inositol tetra- and triphosphates (InsP_4 and InsP_3) apparently only inhibit mineral absorption in the presence of higher inositol phosphates (InsP_5 and InsP_6), while inositol di- and monophosphates (InsP_2 and InsP_1) have no effect. Degradating InsP_5 and InsP_6 to these lower forms can, therefore, improve the bioavailability of minerals from phytate-containing foods.

Phytic acid degradation depends on the activity of the previously mentioned enzymes, phytases. The human digestive tract cannot produce phytases to degrade dietary phytic acid. Instead, humans rely on the activity of endogenous phytases in plants or can introduce exogenous phytases from microbial sources. Plants make phytases during germination to release stored phosphate for energy and minerals for growth. Microbial phytases from bacteria and fungi can also hydrolyze dietary phytate. Plant and microbial phytases function optimally at different temperatures and pH levels. Identifying and providing conditions that favor the activity of these enzymes maximizes phytate degradation.

Vegans derive most of their energy from phytate-rich whole grains, legumes, nuts, and seeds. Due to their high phytate content, minerals in these foods may not be sufficiently absorbed to meet recommendations through portions normally consumed. Educating vegans about the antinutritional effects of phytate and how to reduce it enables them to increase the bioavailability of minerals from these dietary staples.
Oxalate

Oxalate is the general name for salts of oxalic acid. Oxalic acid is primarily found in plants and its concentration varies depending on the species, cultivar, growing conditions, and organ of the plant that is analyzed. For instance, commonly consumed plant species that contain the highest oxalic acid concentrations are spinach, beets, amaranth, and rhubarb. Different cultivars of a single species, like rhubarb, can exhibit three-fold differences in oxalic acid concentrations, even when all other growing conditions are equivalent. Growing conditions independently affect oxalic acid accumulation; increased light exposure and dry environments, for example, both promote higher levels of this compound. Finally, oxalic acid is distributed differently throughout plants. In most species, concentrations are highest in the leaves; intermediate in seeds; and lowest in stems. Of course, exceptions to this pattern do exist, and the concentrations are all relative. In other words, seeds and stems are not necessarily “low” in oxalic acid. They are simply lower in the compound than the leaves.

The role of oxalic acid in plants is uncertain. It may accumulate as a waste product of metabolism, deter pests from consuming the plant, or serve as a buffer by regulating free ion concentrations. The latter role is made possible by the ability of oxalic acid to bind ions, thereby forming salts called “oxalates.” Oxalic acid forms water-soluble salts with sodium and potassium and water-insoluble salts with ferrous iron (Fe^{2+}), calcium, and magnesium. Both water-soluble and –insoluble oxalates are present in plants in variable relative concentrations.

When insoluble oxalates are consumed, they remain intact and cannot be absorbed. Both oxalic acid and the mineral to which it is bound are excreted. Soluble oxalates, however, dissociate during digestion and release free oxalic acid and minerals. Free oxalic acid can be absorbed in the colon and possibly the small intestine and, together with endogenous oxalic acid that forms as a metabolic end product, it is excreted in urine. However, free oxalic acid may
instead form insoluble salts with calcium, magnesium, and iron in the intestinal lumen. These salts, like the insoluble oxalates consumed directly from plants, are not absorbed but rather are excreted. Thus, soluble oxalates can inhibit mineral absorption when consumed at the same meal.

Both insoluble and soluble oxalates are relevant to nutrition in different ways. Minerals bound as insoluble oxalates cannot be absorbed but are included in totals of analyzed nutrient content. Consequently, a substantial proportion of an apparently rich mineral source may not be absorbable. People may thereby overestimate their absorption of such minerals. Soluble oxalates, upon disassociation, can bind minerals in the food from which they are derived as well as other dietary sources. Mineral absorption from the entire meal can be compromised by soluble oxalates in a single food.

Animal flesh may also contain oxalic acid, but it is present in negligible amounts. Fungi contain oxalic acid, as well. Its concentration in commonly consumed fungi, namely edible mushrooms, has not been determined but is believed to be low. Thus, from a human dietary perspective, plants are the main source of oxalic acid. As plant consumption increases, oxalic acid intake increases as well. Vegans, who practically subsist on plants alone, presumably consume more oxalic acid, on average, than omnivores and vegetarians who consume eggs and milk, lactoovovegetarians. If vegans rely on high-oxalate foods for iron and calcium, or consume large amounts of oxalate-rich foods with other sources of these minerals, their absorption may be lower than realized. To ensure that absorption of such minerals is not compromised by oxalates, vegans should be educated about low-oxalate alternative mineral sources and ways to decrease oxalate content in foods.
Polyphenols

Polyphenols are secondary metabolites of plants that contribute to plant pigmentation, resistance against pathogens, and protection from predators. Most polyphenols share a basic three-ringed flavan structure. Hydroxylation and esterification of other groups transforms this simple compound into thousands of related compounds that collectively share the designation as polyphenols. Their complexity varies widely, ranging from the simplest phenolic acids to complex polymers, such as tannins. Though unspecific, the term “polyphenol” is commonly used to simplify discussions about the role of these compounds in human health.

Since polyphenols are plant products, they are ingested by humans from plants and plant-derived foods. Three kinds of polyphenols predominate in the human diet: phenolic acids, flavonoids, and complex polymers of either flavonoids alone or flavonoids and phenolic acids. Fruits and vegetables are generally rich in phenolic acids and flavonoids. Legumes and cereals contain all three groups, while nuts and oil seeds primarily contain tannins and phenolic acids, respectively. Beverages also contribute dietary polyphenols. For instance, phenolic acids are found in coffee; flavonoids are in herb teas and green tea; complex polymers are in cocoa and black tea; and all types are found in wine. Diets containing five daily servings of fruits and vegetables supply an estimated one or more grams of total polyphenols. Vegans almost certainly exceed this modest estimate with higher fruit and vegetable consumption as well as reliance on grains, legumes, nuts, and seeds to replace animal products.

Quantification of polyphenols in the diet is hindered by wide natural variations that occur in plants. The total content and proportion of specific types of polyphenols varies within the same food due to genetic differences, growing conditions (e.g. exposure to light), and level of maturity when harvested. Reactions that occur in food processing methods, such as tea leaf fermentation and roasting of coffee and cocoa beans, also change the polyphenolic composition
of foods and, therefore, complicates estimates of polyphenols in the diet.\textsuperscript{14} Reported polyphenol contents from laboratory assessments cannot accurately be extended to identical species grown in different conditions, harvested at different times, or processed differently. Such measurements are helpful to study nutrient-polyphenol interactions in experiments but they are not reliably extensible beyond the specific experiment in which they were reported.

Generous consumption of polyphenol-rich foods is recommended to promote health. One of the well-researched roles of polyphenols is their antioxidant function. They can neutralize free radicals by donating hydrogen atoms, and they can bind metal ions that would otherwise catalyze lipid peroxidation.\textsuperscript{14} Specifically, polyphenols form complexes with cations including sodium, aluminum, copper, iron, and zinc.\textsuperscript{14} Since oxidative reactions are implicated in carcinogenesis and atherogenesis, high polyphenol intakes may prevent or delay the development of cancer and cardiovascular disease.

However, certain populations should be aware of potential antinutritional roles of polyphenols. Tannins known as proanthocyanidins and ellagitannins, for instance, can decrease dietary protein utilization.\textsuperscript{18} Populations relying on plant protein sources that are high in these polyphenols might unknowingly foster a protein deficiency.\textsuperscript{18} Another antinutritional effect of polyphenols results from their ability to bind metals. Whereas metal chelation serves a salutatory antioxidative function in cells, it inhibits mineral availability in the gut. Polyphenols tend to form stable complexes with minerals within the pH range 5.0 – 8.0, which corresponds to human intestinal pH levels.\textsuperscript{19} Iron and possibly zinc absorption are decreased by polyphenols.\textsuperscript{14} Vegans derive most dietary minerals from plants containing polyphenols, so the bioavailability of such minerals may be compromised. Given that polyphenols are abundant and polyphenol-free mineral sources (e.g. meat) are virtually absent in a vegan diet, vegans should be informed about their inhibitory effects and ways to minimize their interference with mineral absorption.
To prevent deficiencies, nutrition education for vegans should explain how to plan a diet that provides sufficient bioavailable iron, zinc, and calcium. Diet planning guides for new vegans are presently inadequate to educate vegans about bioavailability. Therefore the purpose of this thesis is, first, to elucidate the role of inhibitors in a vegan diet and, second, to develop an educational guide to advise new vegans in planning a diet that supplies adequate absorbable minerals. Specifically, this guide will summarize the effects of common inhibitors in vegan diets, develop strategies to reduce inhibition, and identify well-absorbed sources of iron, zinc, and calcium.
Chapter 2

Iron

The Institute of Medicine established a recommended daily allowance (RDA) of 8 mg iron for men and postmenopausal women and 18 mg for premenopausal women.\textsuperscript{20} The iron RDA assumes that 18\% of consumed iron is bioavailable. This value was chosen to represent an average omnivorous Western diet, in which 90\% of iron is present in its nonheme form with the remaining 10\% as heme iron. Heme iron is absorbed more efficiently (25-40\%) than nonheme iron; thus, it contributes more absorbable iron per milligram iron consumed. In contrast, only 6-8\% of nonheme iron is absorbed. The RDA was calculated using “conservative” fractional absorption estimates of 25\% for heme iron and 16.8\% for nonheme iron. The reason for choosing such a high approximation of nonheme iron absorption is unclear but may be based on the observation that dietary animal flesh enhances nonheme iron absorption.\textsuperscript{21} In the average American diet, nonheme absorption is likely facilitated by meat, poultry, and fish with which it is consumed.

However, a vegan diet lacks heme iron as well as flesh foods (meat, fish, and poultry) that enhance nonheme iron absorption in mixed diets. Estimated absorption must be adjusted to account for the lower bioavailability of nonheme iron in an animal flesh-free diet. The Institute of Medicine accounted for these differences by recalculating iron needs based on a diet entirely derived from nonheme iron that is absorbed with 10\% efficiency.\textsuperscript{20} Consequently, the RDA for vegetarian diets (vegans included) is 1.8 times higher than the regular RDA: 14 mg for men and postmenopausal women, and 33 mg for premenopausal women.\textsuperscript{20} Note that these estimates pertain to vegetarians, in general; these values may underestimate the requirements of vegans, who replace dairy and egg calories with plant foods that contribute more inhibitors. In a diet
based largely on unrefined grains, legumes, nuts, fruits, and vegetables, iron absorption may be as low as 5%.\textsuperscript{20}

As a starting point to improve iron nutrition in vegans, the current status of vegans’ diets should be observed. Few recent studies exist in the literature. Therefore, a generous range of dates were included as the basis for this summary. In general, mean daily iron intakes among vegans were higher than, or comparable to, those of non-vegans: 23 mg in both genders averaged\textsuperscript{22}; 24 mg males and 38 mg in females\textsuperscript{23}; and 18.9 mg for males and 14.8 mg for females.\textsuperscript{24} All three of these studies used British samples. A more recent study collected 4-day weighed food records from 26 vegans in the UK and calculated average iron intakes for supplement users and non-users separately.\textsuperscript{25} Average intakes were similar: 16.7 mg for non-users versus 17.9 mg for users. These results indicate that based on the vegetarian RDA, vegans’ iron intakes appear to be adequate for males and postmenopausal women (RDA 14 mg/d) but insufficient for premenopausal women (RDA 33 mg/d).

More insight into the iron status of women is provided by a German study that evaluated vegan women on the basis of two 9-day food frequency questionnaires and blood samples.\textsuperscript{26} The mean intake of women aged 19-50 was 20.0 mg, and that of older women (age 50+) was 19.6 mg. Hematological values for many women in both age groups were indicative of deficiency. Serum ferritin was less than 12 ng/ml for 40% of young and 12% of older women, and transferrin saturation fell below 16% for 36% of both groups. Only 4% of both groups had diagnosable iron deficiency anemia, but impaired iron status was clearly prevalent in the vegan sample. This study suggests that even when vegans meet the RDA, as was the case of the older presumably postmenopausal women, physiological iron status may be impaired.

One potential explanation for this observation is that iron absorption is inhibited to varying degrees in a vegan diet. The estimated 10% nonheme iron absorption used to set the RDA may be too generous for diets high in unrefined foods and low in fortified products. It may
also result from purposeful avoidance of refined foods – the most commonly fortified foods – by health-conscious individuals. Therefore, the unique composition of a vegan’s diet must be considered when predicting iron absorption and recommending a specific intake value.

Vegans differ in their use or avoidance of processed foods, fortified products, meat substitutes, and supplements. In addition, energy needs and motivation to prepare food vary between individuals. To provide vegans with flexibility in meeting their iron needs, several strategies are presented. The three overarching themes are to decrease inhibitors, increase enhancers, and increase total intake.

A. Decrease Inhibitors

Substances in plant foods that inhibit nonheme iron absorption operate by chelating iron ions. Chelators do not always interfere with iron absorption; in fact, some improve its absorption. Whether a chelator decreases or increases iron absorption depends on its ability to release ions to enterocytes. Those that improve iron absorption do so by protecting iron from forming insoluble complexes while in the intestine and then releasing it to enterocytes at the brush border to be absorbed. In contrast chelators that decrease iron absorption, or “inhibitors,” remain bound to iron and are excreted as such.

Inhibitors do not solely bind iron coexisting in the same food source but rather their effects are extended to all food that enters the gut in an eating episode. Thus, their inclusion in a meal jeopardizes all nonheme iron consumed at that meal. The most powerful and abundant inhibitors in a vegan diet include phytate and polyphenols. Oxalic acid also has the potential to inhibit iron absorption, but its actual effect on iron during meal consumption contradicts expectations. Thus, oxalic acid will be classified as an inhibitor and described in this section,
but advice to limit its consumption with meals containing iron will be deferred until more conclusive evidence is generated.

Phytate

Vegans generally consume significantly more phytate than omnivores. Omnivores are estimated to consume between 400 – 800 mg/d while vegans easily consume two to three times this amount. Phytate is concentrated in some of the most iron-rich foods in a vegan diet, including whole grains, legumes, seeds, and nuts. The inhibitory effect of phytate decreases iron absorption from these foods and foods consumed with them, so vegans may not be absorbing sufficient iron even when intakes are high. Reviewing the effects of phytate on nonheme iron absorption and improvements that occur when it is decreased can inform recommendations to improve iron bioavailability in a vegan diet.

Even at low concentrations, phytate powerfully inhibits nonheme iron absorption. Hurrell et al., for instance, compared iron absorption from liquid semisynthetic formulas (soy isolate protein mixed with cornstarch, corn oil, vanilla extract, and water) containing varying concentrations of phytate to a control formula, in which soy protein was replaced with egg whites. Iron, as FeCl₃, was added in to all formulas to standardize total iron content for absorption comparisons. Native phytate from soy isolate was incrementally decreased to achieve different phytate levels in formulas. As phytate levels were decreased, no significant improvements were observed in iron absorption until just ≤0.3 mg phytate per gram of soy isolate remained. In relation to iron content, the authors hypothesized that less than 10 mg phytate would have to be present in a meal containing 5 mg iron to protect iron absorption. Vegan meals could easily exceed this ratio. A 100-g serving (approximately one-half cup) of boiled red kidney
beans, for example, contains 460 mg phytate with just 2 mg iron. Impaired iron absorption is highly likely in vegan diets, given that phytate is abundant in most iron-rich foods.

Phytate also inhibits absorption of nonheme iron from other foods in the meal with which it is consumed. A study by Rossander-Hulthén et al. illustrated this effect by serving an identical low-phytate breakfast meal alone or with oatmeal, thereby increasing the phytate and iron content of the meal.\(^{31}\) Iron absorption was decreased by 60% (from 12.4% to 5.2%) when the meal contained oatmeal. Even though oatmeal raised the total iron content of the meal by 1.2 mg, the net effect of adding oatmeal was a 40% decrease in milligrams of iron absorbed (from 0.29 mg to 0.18 mg). Presumably, sufficient phytate was present in the oatmeal to bind not only its own iron but also some from the rest of the meal. When judging the bioavailability of iron in foods, it is important to consider inhibitors contributed by foods eaten at the same time.

To decrease the inhibitory effect of phytate, it must be degraded or completely removed from its food source. Degradation of phytate means that phosphate groups are removed from the inositol hexaphosphate molecule. Regarding iron absorption, inositol hexakisphosphate (IP\(_6\)) is the most inhibitory form, followed by inositol pentakisphosphate (IP\(_5\)); lower inositol phosphates (IP\(_4\) and IP\(_3\)) can bind iron but to a lesser degree.\(^{10}\) Thus, degradation must be thorough to significantly improve iron absorption.

During the process of making bread, endogenous phytase enzymes in grains actively degrade phytate to lower inositol phosphates by hydrolyzing phosphate groups. As the content of higher inositol phosphates (InsP\(_6\) and InsP\(_5\)) remaining in prepared bread decreases, iron absorption from the bread generally increases.\(^{32}\) Evidence for this relationship is provided by an experiment in which iron absorption from bread rolls containing varying amounts of inositol hexa- and pentakisphosphates, but fortified to contain approximately equal iron contents (3.7 – 4.0 mg), was compared.\(^{32}\) Specifically, iron absorption from control rolls (white flour, 55% extraction) was compared to absorption from whole grain-based test breads. When phytate,
reported as the sum of inositol hexa- and pentakisphosphates (InsP$_6$ + InsP$_5$), was reduced from 89.4 mg (flour) to 0.25 mg (rolls) per serving of test rolls, absorption of iron was not significantly different than from the control rolls. Approximately 18% of iron was absorbed from both rolls. But as higher concentrations of InsP$_6$ and InsP$_5$ were left in the rolls, absorption progressively decreased; with merely 4.9 mg phytate (InsP$_3$ + InsP$_6$) per serving in the whole grain test rolls, fractional iron absorption was 6.1% whereas 16.2% was absorbed from the control rolls. Evidently, decreasing the concentration of hexa- and pentakisphosphates improved iron absorption. These two inositol phosphates potently inhibit iron absorption, even in small concentrations. Therefore, the greatest improvements in iron absorption will occur when degradation is thorough.

Thorough degradation of phytate (to ≤0.002%, by weight) from cereal porridges significantly increased iron absorption in human subjects, measured by erythrocyte incorporation of labeled isotopes. This thorough degradation was accomplished through the addition of exogenous phytase enzymes. Such a technique is impractical for home food preparation, but it helps to demonstrate the benefits of nearly eliminating phytate from foods in this experiment. The most profound effects were observed for wheat: fractional iron absorption increased from 0.99% to 11.54%. Less drastic improvements, but improvements nonetheless, are observed by decreasing phytate when elimination is impractical. For example, decreasing phytate in oat porridge from 437 μmol to 107 μmol/serving significantly improved iron absorption (from 4.4% to 6.0%) from an otherwise identical meal. Cumulatively, even small improvements in iron absorption from high-phytate foods could help vegans to meet their requirements.

Removal of phytate, by mechanical processing, is another way to increase fractional iron absorption. Phytate is concentrated in the outer layers of grains, so removing this component removes phytate. For example, milling rice improved fractional iron absorption from 5.5% (in brown rice) to 10.3%. However, since iron is concentrated in the same location as phytate,
milling also reduces the total iron content of grain. Thus, the milling may not improve net iron absorption. In the rice comparison, net iron absorption was nearly identical for brown (0.13 mg) and milled (0.14 mg) rice due to the higher total iron content of brown rice.\textsuperscript{35} In the United States, milled grain products are enriched to replace iron that is lost during milling. Enriched products are good sources of absorbable iron because they are low in phytate yet high in iron. However, such foods in other countries are not always enriched and are, therefore, not good sources of iron in spite of their lower phytate concentrations.

The best way to improve net iron absorption from high-phytate foods is to decrease the concentration of the most inhibitory inositol phosphates (InsP$_5$ and InsP$_6$) while preserving iron content. Methods by which this can be achieved will be discussed in Chapter Five: Decreasing Inhibitors.

**Polyphenols**

Polyphenols are a diverse class of compounds with different properties. Various types of polyphenols are present in individual foods; isolating specific types and evaluating their effects on iron absorption facilitates predictions of iron absorption from foods based on their polyphenol composition. Several were tested in a human absorption study comparing the effects of adding equimolar amounts of purified phenolic compounds to radioiron labeled rolls containing 3.8 mg iron per serving.\textsuperscript{36} Gallic acid and chlorogenic acid, two nonflavonoids, both significantly inhibited absorption. Absorption decreased 52\% with the addition of 14.7 mg gallic acid and 33\% with 30.5 mg chlorogenic acid. Gallic acid is found in blueberries, dark cherries, coffee, and green and black tea, and chlorogenic acid is distributed throughout diverse sources including potatoes, sweet potatoes, prunes, coffee, and apples.\textsuperscript{37} For instance, 200 ml of instant coffee contains between 50 – 150 mg chlorogenic acid.\textsuperscript{38} A diet rich in fruit, vegetables, teas, and coffee
provides ample amounts of both of these phenolic compounds and likely decreases iron absorption from meals.

The same study also measured iron absorption as the concentration of another phenolic compound, tannic acid, was increased.\textsuperscript{36} Tannins are polymers of various phenolic acids and simple sugars.\textsuperscript{37} They are found in various foods including grains and legumes, especially those with red-colored seed coats, like red winter wheat.\textsuperscript{37} At the lowest tannic acid concentration, 5 mg, iron absorption was decreased by 20%. Increasing tannic acid inhibited absorption to progressively greater degrees until 50 mg, at which impairment of absorption seemed to plateau around 86%; higher levels of tannic acid (100 mg and 200 mg) did not reduce absorption significantly more than the 50 mg level. A similar study measuring the same outcome also observed a plateau in inhibition as tannic acid was increased beyond 55 mg in a meal containing 3 mg iron.\textsuperscript{39} Iron absorption reached a plateau at ~20% that of the tannic acid-free control meal. Since tea contains about 30 mg tannins per 8-oz. cup\textsuperscript{40}, it is conceivable that the maximum level of inhibition could be achieved by drinking just two cups of hot or iced tea with a meal.

Another way to evaluate iron absorption is by simulating digestion and absorption \textit{in vitro} using cultures of human intestinal Caco-2 cells. Such studies can help researchers to identify mechanisms by which absorption is altered. Kim et al. provided Caco-2 cell cultures with a stock solution containing FeCl\textsubscript{3} alone or with one of two polyphenol extracts, (\textnormal{-\textit{E}})epigallocatechin-3-gallate (EGCG) or grape seed extract.\textsuperscript{41} Whereas EGCG is often consumed in fruits like apples, berries, and plums\textsuperscript{37} and green tea\textsuperscript{41}, grape seed extract is generally taken as a supplement.\textsuperscript{41} It is also added to certain breakfast cereals, namely Kashi Heart to Heart\textsuperscript{®} cereals. Though uncommon in the diet itself, grape seed extract contains a mixture of diverse polyphenols that are consumed in other foods, including gallic acid, catechin, EGCG, epigallocatechin, and proanthocyanidins.\textsuperscript{41} Apples, grapes, plums, and berries are just a few foods that contain these polyphenols.\textsuperscript{37}
When EGCG and grape seed extract were added to the iron-enriched stock solution, both decreased transepithelial iron transport during three-hour absorption period. Relative to the control culture, EGCG reduced transfer by 94±0.1% and grape seed extract reduced it by 90±0.1%. Yet when the cells were analyzed for iron content, both cultures that received polyphenol extracts contained significantly more iron than control cells. Cells accumulated iron in concentrations that were 6.3-fold and 16.2-fold those of control when supplemented with ECGC and grape seed extract, respectively. These results suggest that apical iron uptake is enhanced while basolateral exit is decreased by polyphenols. The authors hypothesized that polyphenols may increase uptake by binding iron in the gut and facilitating absorption through a non-active transport pathway. Exit from enterocytes is likely prohibited by these complexes. Unless these complexes dissolve over time, iron-polyphenol complexes that accumulate in enterocytes will eventually be sloughed off and excreted, thereby decreasing the overall bioavailability of dietary iron.

Observing the effects of isolated polyphenols can reveal the causative agent of iron malabsorption from many foods. However, people do not eat purified polyphenols; their sources in the diet are widespread and may be more or less inhibitory, depending on the other substances coexisting in the food or meal. Several studies have observed how polyphenol-rich foods impact dietary iron absorption.

Certain beverages are particularly concentrated sources of inhibitory phenolic compounds. Teas, coffee, red wine, cocoa, and herbal infusions reduce iron absorption to different degrees. Black tea exerts the greatest influence over absorption. Iron absorption from a meal (hamburger, string beans, and mashed potatoes) containing 3.0 mg nonheme iron decreased from 10.8% (0.32 mg) when consumed with water to 4.1% (0.12 mg) with 250 mL Ceylon breakfast tea, representing a statistically significant reduction of 61%. When served with just a hamburger, 200 mL tea decreased absorption by 64%, or from 3.71% to 1.32%. Both of these
test meals included meat, which has been shown to increase nonheme iron absorption. Thus, the inhibitory effect of tea may have been buffered by the hamburger.

Another study used a simpler “meal” of white (low-phytate) bread; nonheme iron absorption decreased by 79 – 94% when served with 275 mL Assam tea instead of water. Brune et al. also used a bread meal, served with either 150 mL water or tea; absorption fell from 14.4% to 4.5% - a 68% drop. An exact level of inhibition may be impossible to predict because it depends on the composition of the accompanying meal and its nonheme iron content.

Furthermore, the polyphenol content can vary between varieties of tea and the strength to which it is brewed. Consumers should be cautioned, nonetheless, that significant inhibition can occur even when tea is diluted to 5% of its usual concentration. In other words, polyphenols may potently inhibit iron absorption in small doses. Iron availability from meals consumed with black tea will almost certainly be inferior to meals sans tea.

Coffee also decreases iron absorption. When served with the hamburger meal described previously, 250 mL coffee cut absorption by 33%, from 12.9% (0.39 mg) to 8.4% (0.26 mg). Two-hundred milliliters of coffee served with a hamburger, alone, similarly decreased nonheme iron absorption by 39%, from 3.71% to 2.25%. In contrast, a smaller 150 mL serving of coffee decreased absorption from a bread meal by 61%, from 12.0% to 4.8%. Iron absorption was apparently protected by the enhancing effect of meat in the first two studies. When a meal low in enhancers is consumed with coffee, however, inhibition may approximate the higher level, ~60%, predicted by the latter study.

Another beverage consumed with meals, wine, is also a concentrated source of polyphenols. Red wine is a better source of polyphenols than white wine because it contains polyphenols that have been extracted from grape skins. Not surprisingly, relative to white wine or water, red wine consumption significantly reduced iron absorption from a bread test meal. Less than half as much iron was absorbed when consumed with red versus white wine. With 120
ml red wine, only 4-5% of iron from an unenriched low-phytate dinner roll containing 0.54 mg iron was absorbed. Fractional absorption from the same roll consumed with 120 ml white wine was 11.43% and did not differ significantly from fractional absorption of the roll consumed with water (15.89%). Thus, the polyphenols in red wine seemed to be responsible for the inhibition.

In contrast, red wine served with a hamburger meal increased iron absorption relative to water. This discrepancy may be explained by the absorption-enhancing effect of meat in the second study. Another important difference was the serving size of wine: 120 mL in the first study versus 250 mL in the second. Alcohol, alone, has been shown to increase iron absorption. The higher alcohol load provided in the second study may have helped to overcome inhibition caused by the polyphenols. The wine used in the second study was also higher in iron than those tested in the first study: ~1.56 mg versus ~0.62 – 0.83 mg Fe per 100 mL. Any of these factors may have contributed to the conflicting results. Given that a vegan diet lacks the enhancing effect of meat, an appropriately-sized (4 oz) serving of red wine is predicted to inhibit iron absorption, albeit to a lesser degree than either tea or coffee.

Cocoa also decreased iron absorption from a bread meal. The hot cocoa was prepared from 5 g of cocoa mixed with 275 ml water and 10 g sugar, and it was consumed with a white bread enriched to contain 2.1 mg iron per serving. Geometric mean iron absorption from the meal served with cocoa was just 1.61%; mean absorption was significantly higher (28.5%; \( P < 0.001 \)) when water was served in place of cocoa. Expressed differently, addition of cocoa caused a 71% decrease in absorption. Relative to black tea, cocoa was found to be a weaker inhibitor at the concentration served to subjects. Still, cocoa consumption with nonheme iron sources can jeopardize absorption significantly. This study also suggests that iron from cocoa, itself, is poorly available. A single tablespoon of cocoa powder contains 0.75 mg iron, but its polyphenol content probably inhibits most of its absorption.
In addition to the specific beverages described, a wide variety of herbal infusions can also inhibit iron absorption. Peppermint, pennyroyal, vervain, lime flower, and chamomile teas all decreased iron absorption from a radioiron labeled roll. The degree of inhibition was not as severe as black tea. Peppermint tea had the greatest effect, an 84% reduction, followed by pennyroyal (73%), vervain (59%), lime flower (52%), and chamomile (47%).

All of these beverage studies served the drink and test meal concurrently. Thus, the inhibitory compounds were well mixed with foods in the gut and able to bind nonheme iron as it was released. Drinking such beverages before or after meals presumably does not impair absorption as strongly, since the food can be digested and absorbed without encountering the inhibitors. However, no known human study has observed the effect of separating meal and beverage consumption for varying periods of time. Rats consuming black tea with or after an iron dose supported the hypothesis that iron absorption is better preserved when tea consumption is delayed for longer periods of time. Consumed concurrently, tea inhibited absorption by 62%; waiting for 0.5, 1, 2, or 3 hours resulted in 30%, 21%, 10%, and 15% inhibition, respectively. Other researchers have extended these findings to humans, estimating 20% inhibition caused by tea consumed between meals. Whether this is a valid assumption is debatable, but the results in rats are suggestive that tea – and perhaps other inhibitory beverages – can impact absorption even when consumed several hours after a meal.

Beverages are not the only dietary sources of polyphenols. Vegetables differ in the types and concentrations of polyphenols they contain. Gillooly et al. studied a variety of vegetables, each in isolation, to compare iron absorption. The researchers found that absorption was poorest in vegetables with high concentrations of polyphenols: eggplant, spinach, brown lentils, beet greens, and green lentils. They also demonstrated a significant inverse correlation between iron absorption and polyphenol content. A limitation of this study is that such vegetables are
rarely eaten in isolation. The foods with which they are consumed may prevent some of the iron from binding to polyphenols.

In addition to vegetables, many fruits, legumes, nuts, and grains also contain high concentrations of polyphenols that can inhibit iron absorption. Consider, for example, a study comparing iron uptake into Caco-2 cells from black, red kidney, and white great northern beans. The beans were subjected to *in vitro* digestion and “fed” to Caco-2 cells to simulate intestinal absorption, and iron absorption was evaluated by measuring ferritin formation in each cell culture. A significantly greater ferritin concentration accumulated in the culture that received white beans. Whereas only 1.5 – 2.7% of iron was taken up by cells fed colored beans, those fed white beans absorbed 12.1 – 18.8%. This difference was attributed to the higher phenol concentration in colored beans, which contained 4 to 17 times more total phenols than white beans. While *in vitro* studies do not give exact measures of absorption that would occur in actual physiological conditions, they do illustrate the direction and relative magnitude of changes in absorption that occur when inhibitors are added. Based on these results, iron absorption from otherwise comparable foods can be significantly different due to polyphenols.

Completely avoiding polyphenols is not practical because they are widely distributed throughout plant foods that constitute a vegan diet. These foods are valuable sources of nutrients in the diet, and they often contain substances that oppose the inhibitory effect of phenolic compounds. Polyphenol-rich beverages, however, should be avoided at meals. These beverages generally do not contribute enough iron to the meal to compensate for their inhibitory effect on total iron absorption. Habitual consumption of such beverages with meals could eventually impair iron status from a plant-based diet in which iron bioavailability is already low. Vegans should reserve these beverages for consumption between meals.
**Oxalic Acid**

Oxalic acid is capable of forming an insoluble salt with ferrous iron (Fe\(^{2+}\))\(^{12}\) whereas ferric salts (Fe\(^{3+}\)) are highly soluble.\(^{27}\) When foods containing nonheme iron are digested, both forms of iron are released in the stomach and intestine. Ferric iron generally predominates in the stomach, but a portion of the ferric iron is reduced to ferrous iron by ascorbic acid and ferrireductase enzymes on the duodenal brush border.\(^{50}\) Theoretically, ferrous iron in the small intestine may be rendered unavailable for absorption if oxalic acid is consumed in the same meal due to formation of insoluble ferrous oxalate.

The clearest illustration of how oxalic acid impacts absorption is provided by an *in vitro* study that evaluated uptake of ferrous and ferric iron as FeCl\(_3\) and FeSO\(_4\), respectively, in the presence of oxalic acid.\(^{51}\) Uptake of ferrous iron by Caco-2 cells consistently decreased as higher concentrations of oxalic acid were added to the culture. In contrast, ferric iron uptake was increased; absorption was greatest at low concentrations but declined and reached an apparent plateau as the concentration was increased. Even at this lower level, absorption was approximately 3-fold that of cells lacking oxalic acid. While the directionality of modified absorption – i.e. decreased for Fe\(^{2+}\) and increased for Fe\(^{3+}\) – is probably consistent with actual human absorption, the exact level of inhibition or enhancement is difficult to predict from *in vitro* studies. Oxalic acid in foods is generally present as a salt, oxalate, rather than pure acid. Furthermore, oxalate and iron are not consumed together in isolation. Both likely interact with other components of food, which could interrupt the formation of insoluble ferrous oxalate complexes. Human studies are required to demonstrate whether oxalate actually affects iron absorption in realistic meals.

Limited evidence is available to show whether formation of ferrous oxalate significantly inhibits iron absorption in humans. In one study, iron absorption from meals based on high-
oxalate spinach and low-oxalate kale were compared. Each test meal consisted of two low-
phytate wheat bread rolls with 150 g of either pureed spinach or kale. The spinach meal
contained 407 mg soluble oxalates, which can dissociate and bind minerals in the intestine, while
the kale meal contained just 5 mg. Since the spinach meal naturally contained more iron per
serving, ferrous sulfate (1.38 mg) was added to the kale puree so that total iron was equivalent
(2.86 mg/serving) in each meal. Absorption of iron was measured by administering test meals to
thirteen women between the ages of 19 and 38; each woman consumed one test meal (spinach or
kale) at breakfast and the other test meal at lunch for three consecutive days. Since the meals
were labeled with different stable isotopes, $^{57}$Fe and $^{58}$Fe, absorption could be evaluated by
collecting blood samples 14 days after the final meal and measuring erythrocyte incorporation of
the isotopes. Iron absorption from the two types of meals, reported as geometric means, did not
differ significantly; absorption was 8.4% and 11.0% from spinach and kale, respectively ($P =
0.19$). The high oxalic acid content of spinach did not appear to inhibit iron absorption.

A second comparison in the same study lends greater credibility to this conclusion. In
this part of the study, the same subjects consumed spinach at one meal and kale plus 1.26 g
potassium oxalate, a soluble oxalate, at the other meal. This addition raised the total oxalate
content of the kale meal to the level contained in spinach, 1.27 g. Again, iron absorption did not
differ significantly; absorption from spinach and kale plus potassium oxalate were 8.0% and
11.2%, respectively ($P = 0.16$). Furthermore, iron absorption from kale with and without added
oxalate was indirectly compared by normalizing the values for slight differences in spinach iron
absorption between each part of the study. Absorption did not differ significantly (11.5% vs.
10.7%; $P = 0.86$). This reiterates the finding that oxalate does not decrease iron absorption, at
least in the amount present in these test meals.

The authors offered two hypotheses to explain why iron absorption was not impaired by
high-oxalic acid meals. First they suggest that the majority of iron could have been present in the
ferric form following digestion. Since the trivalent iron cation forms soluble salts with oxalic acid, absorption of this type of iron would not have been decreased by foods high in oxalic acid. The soluble salt would have released ferric iron for absorption. Alternatively, iron may have been present as a mix of both cations, Fe$^{2+}$ and Fe$^{3+}$. Oxalic acid probably inhibited absorption of ferrous iron, and it may have enhanced absorption of ferric iron. Recall that in vitro studies have demonstrated increased ferric iron absorption in the presence of oxalic acid. These two effects would have canceled each other out so that no net effect would be observed.

Another study supports the conclusion that oxalic acid does not impair iron absorption. In this study, iron absorption was measured from a test meal consisting of 200 g white rice to which ferrous sulfate was added to achieve a total iron content of 3.4 mg. The meal was labeled with a stable isotope and fed to 15 Indian women along with 100 g of a puree of rhubarb that had been heated gently. Blood samples were collected 14 days later to measure how much of the isotope was absorbed, and absorption was corrected to 40% reference dose absorption. Rhubarb slightly increased iron absorption from the basal meal; the geometric corrected mean absorption from rice alone was 0.025, and it increased to 0.042 when served with rhubarb.

Factors other than oxalic acid may have enhanced absorption when rhubarb was added, thereby overshadowing any inhibitory effect of oxalic acid. It is also possible that iron was primarily present as ferric iron during digestion and, thus, not affected by oxalic acid. In meals where more iron is present as ferrous iron, inhibitory effects of oxalic acid may be realized. However, this hypothesis must be substantiated by evidence. Importantly, iron absorption is thought to be improved when greater proportions of iron are present as the ferrous ion because this ion remains relatively soluble in the alkaline intestinal environment. It is presently unknown whether the enhanced absorption of ferrous iron in the intestine outweighs the depressed absorption resulting from increased formation of insoluble ferrous oxalate in meals that contain iron and oxalic acid. Finally, the oxalic acid in rhubarb may have already formed
insoluble complexes with calcium, given that a relatively high concentration – 145 mg per 100 mg cooked\textsuperscript{45} – is present in rhubarb. If all the oxalic acid was already bound to calcium, it would not have inhibited iron absorption from the rice. The observed increase in iron absorption could then be attributed to other organic acids present in rhubarb, like ascorbic acid.

Presently, insufficient evidence exists to recommend either avoiding or increasing oxalic acid intake with meals containing iron. Under controlled laboratory conditions, ferrous iron does form a relatively insoluble complex with oxalic acid.\textsuperscript{27} During digestion, however, the extent to which such complexes form could be affected by the presence of other minerals that preferentially bind oxalic acid, thereby preventing it from binding ferrous iron, or by other substances in foods that enhance iron absorption. In addition, a substantial portion of dietary nonheme iron may remain in the ferric form until just before absorption. Enzymes attached to the duodenal brush border can reduce ferric iron to ferrous iron in close proximity to where it is subsequently absorbed.\textsuperscript{50} Oxalic acid may, therefore, have little time to bind the iron and prevent absorption. Ascorbic acid, when consumed at the same meal, may facilitate oxalic acid’s inhibitory role by reducing greater amounts of iron than would normally be reduced by intestinal enzymes, alone. Until future studies test this hypothesis, the effect of oxalic acid on iron absorption is unclear.

\textbf{B. Increase Enhancers}

Enhancers of iron absorption may chelate nonheme iron cations in the intestine, just as inhibitors do. The distinguishing feature of this bond that makes an enhancer \textit{increase} iron absorption is that the iron is loosely bound and able to be released to enterocytes cells for absorption. A variety of acids seem to improve iron absorption, especially ascorbic acid.
Ascorbic Acid

The enhancing effect of ascorbic acid, or vitamin C, is well-documented by research. For instance, adding ascorbic acid to a simple rice meal labeled with radioiron significantly improved absorption.\(^5\) At a molar ratio of 2:1 ascorbic acid to iron, 270% more iron was absorbed; at 4:1, 343% more was absorbed.\(^5\) Ascorbic acid may enhance iron absorption by chelating ferric ions (Fe\(^{3+}\)), thereby preventing them from forming insoluble Fe(OH)\(_3\) complexes in the gut. Ascorbic acid does not remain bound to iron but rather releases it to enterocytes to be absorbed. Another mechanism by which ascorbic acid facilitates absorption is by reducing ferric iron to its more absorbable ferrous (Fe\(^{2+}\)) form. Both mechanisms may account for the significant increase in nonheme iron absorption from the diet.

Ascorbic acid can mitigate or even negate the effects of inhibitors in meals. Served alone, rolls containing 25 mg phytate as sodium phytate decreased fractional iron absorption from ~16% (in phytate-free rolls) to ~7%.\(^4\) When served with 50 mg ascorbic acid, absorption from the phytate-containing rolls more than doubled, from 7.1% to 14.2%. With 100 mg, absorption tripled, increasing from 6.5% to 19.5%. Thus, researchers estimated that the inhibition caused by 25 mg phytate could be overcome by approximately 80 mg ascorbic acid.\(^4\) However, unprocessed grains, legumes, nuts, and seeds are substantially higher in phytate than these test rolls. One-hundred grams of cooked lentils, for instance, contains approximately 250 mg while the same amount of cooked oatmeal contains 110 mg.\(^4\) Eighty milligrams of ascorbic acid could be expected to improve iron absorption in these high-phytate foods but not completely restore it to a level that would be observed from a phytate-free food.

MacFarlane et al. tested absorption from a more realistic meal of nuts, bread, and milk.\(^5\) Nuts were found to inhibit iron absorption from bread, presumably due to the combined effects of phytate and polyphenols. Therefore, the study evaluated the potential to restore iron absorption
by adding ascorbic acid to a test meal of white bread (60g), peanuts (50g), and 150 mL whole milk. Adding 25 mg ascorbic acid increased absorption from 3.1% to 5.2%. Adding 50 mg restored absorption to that observed from bread alone (~10%).

In the previous study, the effect of ascorbic acid on inhibition caused by polyphenols could not be distinguished from that caused by phytate because both inhibitors were present in the test meal. To determine whether ascorbic acid specifically improves iron absorption from polyphenol-rich foods, ascorbic acid was added at three levels (25, 100, and 500 mg) to a low-phytate bread meal containing 3 mg iron and 420 mg purified tannic acid. Subjects consumed each bread meal with tannic acid alone and again with ascorbic acid. Ascorbic acid significantly increased iron absorption at all levels; absorption increased 3-fold with 25 mg, 5.5-fold with 100 mg, and 7-fold with 500 mg ascorbic acid. At the highest level (500 mg), iron absorption was restored to that expected from bread without tannic acid.

Such a high intake at a single meal cannot be attained from foods, alone; supplements or purified ascorbic acid would be required to achieve this drastic improvement. Still, even a modest 25 mg ascorbic acid addition restored absorption to approximately half that expected from bread sans tannic acid, and 100 mg restored it to 80%. Evidently, any increase in ascorbic acid will improve nonheme iron absorption when it is depressed by tannic acid. Whether or not similar improvements occur in the presence of other polyphenols cannot be determined from this study. Until future research indicates otherwise, adding ascorbic acid to polyphenol-rich meals is a recommended strategy to reduce inhibition of iron absorption.

Vegans are likely to incorporate ascorbic acid into their diets via foods rather than a purified powder. Many fruits and vegetables are rich sources of this nutrient that are easily incorporated into iron-containing meals. When food sources of ascorbic acid are added to meals, substantial improvements are consistently observed. For example, a hamburger, string bean, and mashed potato meal containing 3.0 mg nonheme iron was served with 250 mL orange juice,
which supplied 110 mg ascorbic acid. Nonheme iron absorption doubled when served with the juice rather than water, from 0.29 mg to 0.63 mg. Neither the phytate nor polyphenol content of this meal were reported, but they were apparently low relative to a typical vegan diet.

The meals used by Hallberg et al. are better approximates of meals that might be consumed by vegans, given that they were meat-free, high in plant foods, and apparently high in phytate and polyphenols. Navy beans, brown rice, bread with margarine, apples, nuts, and yogurt constituted the low ascorbate (7 mg), high phytate (271 mg) meal. Though the meal contained 5.8 mg nonheme iron, only 0.13 mg were absorbed, or 2.31%. Adding 60 mg ascorbic acid in the form of boiled cauliflower (125 g) increased total nonheme iron content to 6.5 mg and enhanced absorption to 0.32 mg, or 5.11%.

Another vegetarian meal used in this study contained kidney beans, tomato sauce, bread and margarine, boiled cauliflower, cottage cheese, canned pineapple, and banana. Relative to the previous meal, this one was higher in ascorbic acid (74 mg) and lower in phytate (82 mg). As expected, baseline absorption was high: 0.98 mg of the total 5.8 mg nonheme iron, or 17.14%. Removing cauliflower (125 g) reduced total nonheme iron to 5.1 mg and ascorbic acid to 14 mg. Consequently, iron absorption dropped to 0.32 mg, or 6.31%.

Whereas dairy foods were included in the previous two meals, absorption from a completely vegan meal was also evaluated in a later study. The meal comprised of corn tortillas, polished rice, and black beans served with or without boiled cauliflower. Alone, the meal contained 4.4 mg nonheme iron, and subjects absorbed 0.18 mg. Adding 125 g boiled cauliflower increased iron content to 5.3 mg and absorption approximately tripled to 0.58 mg.

Together, these studies show that nonheme iron absorption from a meatless meal containing 60 mg ascorbic acid as cauliflower is at least 2.5 times that of the meal without cauliflower. The 125 g boiled portion used in all three absorption study meals is equal to approximately one cup, a portion that could easily be added to meals. Other foods that could
deliver a similar amount of ascorbic acid are one cup of strawberries, one-quarter of a red bell pepper, or eight ounces of orange juice. Though these foods have not been evaluated in controlled test meals, their effects are predicted to be similar.

Importantly, the enhancing effect of ascorbic acid only occurs when it is ingested with nonheme iron-containing foods. Simply increasing ascorbic acid in one’s daily diet may not significantly improve iron absorption. When five days of nutrient intake were averaged, for instance, subjects with daily ascorbic acid intakes of 247mg absorbed just 35% more iron than subjects consuming just 51 mg/d. Instead, the acid must be consumed within the same eating episode so that it binds iron cations in the gut and/or reduces ferric to ferrous iron, thereby potentiating its absorption. Including ascorbic acid with all iron-rich meals is recommended to optimize absorption.

*Other Organic Acids*

Acids other than ascorbic acid are less well-researched for their effects on iron absorption. Notable among the available studies is that of Gillooly et al., which added purified citric, malic, and tartaric acids to plain rice. This straightforward design demonstrated that all of these acids, in one-gram amounts, significantly improved iron absorption. A much smaller dose of citric acid (0.05 g) was also added separately to rice and chickpea meals and increased iron absorption by 62% and 55%, respectively. In contrast, adding one gram of citric acid to a meal of corn tortillas, black beans, and rice apparently reduced iron absorption, from 0.09 mg to 0.03 mg. The researchers could not explain this unexpected result and, in fact, cited other studies that supported the enhancing property of citric acid.

The variability of iron absorption in response to addition of citric acid can be clarified by an *in vitro* study by Salovaara et al. When Caco-2 cells were fed ferrous iron as FeSO₄ along
with citric acid, cellular iron uptake was drastically reduced relative to control cultures lacking the acid. As the concentration of the acid was increased, a gradual increase in absorption was observed but absorption was still just one-quarter that of the control culture at the highest concentration tested, 4 mmol/L. When ferric iron was administered as FeCl$_3$, a fairly steady increase in absorption was observed as the citric acid concentration was increased. At the highest concentration (4 mmol/L), cellular uptake of ferric iron had more than doubled relative to the control. Evidently, the effect of citric acid on iron absorption depends on which form of iron predominates as well as the concentration of acid used. The meals used in the aforementioned human absorption studies varied in composition, iron content, and size. These differences, in turn, probably influenced the form of iron that predominated during digestion and the concentration of citric acid available to interact with iron. Given that most nonheme iron is released as ferric iron during digestion, citric acid is likely to increase iron absorption rather than suppress it. Citric acid does not seem to increase iron absorption as much as ascorbic acid, but even small increases are cumulatively valuable when aiming to improve iron status.

The experiments that tested the effects of organic acids used purified forms of the acids. While citric acid powder can be purchased, the other acids may be difficult to obtain. More likely, people will naturally consume these acids as components of their normal diet. Citric acid, for instance, is concentrated in lemons and limes; malic acid is in apples, pears, bananas, and cherries; and tartaric acid is most commonly encountered in grapes. Evidence to support the iron absorption-enhancing benefits of these acids when consumed from foods is lacking, so recommendations to increase consumption of them (for the sake of iron nutrition) are tentative. More research is needed to determine whether iron absorption is actually enhanced when such acids are consumed from foods that are also rich in inhibitory polyphenols, like berries, cherries, and grapes.
Before presenting a third iron-optimizing strategy, the limitations of the previous two strategies should be noted. First, the effect of inhibitors and enhancers on iron absorption can vary. Factors such as the iron status of subjects, iron load of the test meal, and size and composition of the test meal can influence observed iron absorption. Researchers often account for these differences within their studies, but comparing the results of two or more different studies is complicated by these factors.

In addition, most absorption studies are based on comparisons of single meals. The relevance of such studies to real-world situations has been challenged. Iron absorption can vary widely between single meals, but the range of absorption from the overall diet tends to be more narrow. This is because a varied diet usually provides a mixture of inhibitors and enhancers that can counterbalance the effect of any individual component on iron absorption. Still, the observations of single meal studies remain valuable for generalizing about the characteristics of a diet. Based on these studies, one can predict that a diet characterized by abundant inhibitors and few enhancers may provide less iron than would be estimated by merely summing iron content from food labels.

Finally, measured iron absorption from individual foods cannot simply be added together to predict iron absorption from a meal. Researchers summed the predicted absorbable iron from all components of a meal, accounting for their relative proportions in the meal. When they compared the estimate to measured absorption from the same meal, the values differed significantly. Inhibitors and enhancers from different foods can interact, changing their effects on absorption. Without measuring absorption from all possible combinations of foods, one can only estimate the net iron availability from a composed meal. Several equations have been developed as tools for such estimates. The complexity of the equations limits their use among most people. Simply increasing enhancers and limiting inhibitors in one’s primary iron sources will suffice to improve iron nutrition without tedious calculations. Individuals can be sure that
their diets provide sufficient absorbable iron if they are able to maintain appropriate biochemical markers of iron adequacy.

Thus, it is important to recognize that iron availability is not a static value. It changes within the context of a meal and overall diet. Predictions of iron availability best serve as guidelines for choosing a diet that optimizes iron absorption.

C. Increase Total Iron Intake

The simplest strategy to improve iron nutrition is to increase total iron intake. Even foods with “poor” iron availability or low iron content can provide adequate iron when consumed in sufficiently large amounts. However, intake is restricted by both (1) volume (i.e. stomach capacity) and (2) energy needs. People must, first, be physically able to consume the foods to benefit from their iron content. Second, the foods must provide sufficient iron to meet needs without exceeding a person’s calorie needs. Identifying a variety of iron-rich vegan foods gives vegans flexibility to meet their iron needs with a diet that is tolerable and provides an appropriate amount of calories from foods they enjoy.

Foods may either be naturally high in iron or acquire iron from exogenous sources. Natural sources in a vegan diet can be grouped into five groups: grains, legumes, nuts and seeds, fruits, and vegetables.

Grains

Grains contain most of their iron in the outer layers, the aleurone layer and bran. Therefore, unenriched refined grains (e.g. white rice) are poorer sources than whole grains. Phytate, as mentioned previously, is also concentrated in these external layers. Due to the
presence of this potent inhibitor, the amount of iron absorbed from whole grains is less than the value listed on a nutrition fact label. A compromise between high-phytate, high-iron whole grains and low-phytate, low-iron refined grains is attained by parboiling grains. During parboiling, some of the iron from the outer layers of whole grains migrates into the central endosperm. The grain is subsequently milled, thereby removing most of the phytate while allowing the grain to retain more iron. The iron content of several grains and grain products is listed in Table 2.1.

Table 2-1 Iron in Selected Grains

<table>
<thead>
<tr>
<th></th>
<th>mg /1 cup cooked</th>
<th>Bioavailability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barley, pearled</td>
<td>2.09</td>
<td>-</td>
</tr>
<tr>
<td>Buckwheat Groats (Kasha)</td>
<td>1.34</td>
<td>-</td>
</tr>
<tr>
<td>Bulgur – parboiled, milled wheat</td>
<td>1.75</td>
<td>+</td>
</tr>
<tr>
<td>Millet</td>
<td>1.10</td>
<td>-</td>
</tr>
<tr>
<td>Rice, long grain, brown</td>
<td>0.82</td>
<td>-</td>
</tr>
<tr>
<td>Rice, long grain, white (enriched)</td>
<td>1.90</td>
<td>+</td>
</tr>
<tr>
<td>Rice, long grain, white, parboiled (enriched)</td>
<td>2.86</td>
<td>+</td>
</tr>
</tbody>
</table>

**Legumes**

Legumes, too, are rich sources of both iron and inhibitors. Vegans are cautioned to recognize that iron absorption from these sources is lower than the nutrition fact label indicates. A sample of the richest sources include the legumes and legume-based foods in Table 2.2.
Table 2-2 Iron in Selected Legumes

<table>
<thead>
<tr>
<th></th>
<th>Mature Beans, Boiled Bioavailability</th>
<th>Sprouted Beans Bioavailability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/1 cup</td>
<td>mg/1 cup</td>
</tr>
<tr>
<td>Black Beans</td>
<td>5.27</td>
<td>-</td>
</tr>
<tr>
<td>Blackeye Peas</td>
<td>4.32</td>
<td>-</td>
</tr>
<tr>
<td>Garbanzo Beans</td>
<td>4.74</td>
<td>-</td>
</tr>
<tr>
<td>Kidney Beans</td>
<td>5.20</td>
<td>-</td>
</tr>
<tr>
<td>Lentils</td>
<td>6.59</td>
<td>-</td>
</tr>
<tr>
<td>Mung Beans</td>
<td>2.83</td>
<td>-</td>
</tr>
<tr>
<td>Peas</td>
<td>2.46</td>
<td>-</td>
</tr>
<tr>
<td>Pinto Beans</td>
<td>4.46</td>
<td>-</td>
</tr>
<tr>
<td>Soybeans</td>
<td>8.84</td>
<td>-</td>
</tr>
<tr>
<td>Soy Products</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tempah</td>
<td>2.24</td>
<td>+</td>
</tr>
<tr>
<td>Tofu</td>
<td>1.83</td>
<td>+</td>
</tr>
<tr>
<td>Natto</td>
<td>7.53</td>
<td>+</td>
</tr>
</tbody>
</table>

*Nuts and Seeds*

Nuts and seeds also provide inhibitors along with iron. Like grains and legumes, consuming large quantities of nuts and seeds can compensate for their relatively low iron bioavailability. However, they are more energy-dense than other food groups. Individuals with high energy needs can afford to eat nuts and seeds in sufficiently large quantities to make this group a significant iron source. In contrast, low-calorie diets may limit this group to a single daily serving and, instead, emphasize less energy-dense iron sources. To maximize iron obtained from this group, some of the best iron sources are identified in Table 2.3.
### Table 2-3 Iron in Selected Nuts and Seeds

<table>
<thead>
<tr>
<th>NUTS</th>
<th>mg /1 oz. dry roasted</th>
<th>Bioavailability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Almonds</td>
<td>1.28</td>
<td>-</td>
</tr>
<tr>
<td>Cashews</td>
<td>1.70</td>
<td>-</td>
</tr>
<tr>
<td>Hazelnuts</td>
<td>1.24</td>
<td>-</td>
</tr>
<tr>
<td>Pistachios</td>
<td>1.19</td>
<td>-</td>
</tr>
</tbody>
</table>

| SEEDS           | mg /1 oz. roasted   | |
|-----------------|---------------------||
| Pumpkin seed kernels | 4.24      | - |
| Sesame seeds, whole | 4.18      | - |
| Sunflower seed kernels | 1.93      | - |

### Fruits

Fruits are generally poor sources of iron. The volume required to obtain just one milligram (e.g. four apples; three cups of cherries; or three mangos) illustrates the limitations of relying on fruit for iron, especially in a filling high-fiber vegan diet. Greater iron-to-volume ratios are found in dried fruits; adding a daily serving of dried fruit to the diet supplies a small, but valuable, dose of iron. Additionally, a few fresh fruits are noteworthy for their “high” iron content, relative to most other fruits. These dried and fresh fruits are listed in Table 2.4.

### Table 2-4 Iron in Selected Fruits

<table>
<thead>
<tr>
<th></th>
<th>mg /Serving (as indicated)</th>
<th>Bioavailability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dried apricot halves</td>
<td>0.87 per ¼ c</td>
<td>+</td>
</tr>
<tr>
<td>Dried figs</td>
<td>1.26 per 3 figs</td>
<td>+</td>
</tr>
<tr>
<td>Dried plums (prunes)</td>
<td>1.05 per 5 prunes</td>
<td>+</td>
</tr>
<tr>
<td>Raisins</td>
<td>1.08 per ¼ c</td>
<td>+</td>
</tr>
<tr>
<td>Blackberries</td>
<td>0.82 per 1 c</td>
<td>+</td>
</tr>
<tr>
<td>Raspberries</td>
<td>0.70 per 1 c</td>
<td>+</td>
</tr>
<tr>
<td>Strawberries, whole</td>
<td>0.55 per 1 c</td>
<td>+</td>
</tr>
</tbody>
</table>
Vegetables

Most vegetables are higher in iron than fruits, expressed per volume, and higher than grains, legumes, nuts, and seeds, per kilocalorie. The diverse and abundant selection of vegetables common in a vegan diet may represent a substantial portion of one’s total iron intake. Raw vegetables might be perceived as too filling, or take too long to eat, to consume large amounts. If so, cooking may improve the acceptability of such vegetables without destroying the iron. Table 2.5 identifies some of the best iron sources among raw and cooked vegetables.

Table 2.5 Iron in Selected Vegetables

<table>
<thead>
<tr>
<th></th>
<th>mg /1 cup</th>
<th>Bioavailability</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RAW</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Broccoli, chopped</td>
<td>0.77</td>
<td>+</td>
</tr>
<tr>
<td>Romaine Lettuce, chopped</td>
<td>0.62</td>
<td>+</td>
</tr>
<tr>
<td>Snow Peas</td>
<td>1.31</td>
<td>+</td>
</tr>
<tr>
<td>Spinach, chopped</td>
<td>0.81</td>
<td>+</td>
</tr>
<tr>
<td>Zucchini</td>
<td>0.47</td>
<td>+</td>
</tr>
<tr>
<td><strong>COOKED</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acorn Squash, cubed, baked</td>
<td>1.91</td>
<td>+</td>
</tr>
<tr>
<td>Asparagus, boiled</td>
<td>1.32</td>
<td>+</td>
</tr>
<tr>
<td>Brussels Sprouts, boiled</td>
<td>1.88</td>
<td>+</td>
</tr>
<tr>
<td>Butternut Squash, cubed,</td>
<td>1.23</td>
<td>+</td>
</tr>
<tr>
<td>Green Beans, boiled</td>
<td>1.60</td>
<td>+</td>
</tr>
<tr>
<td>Kale, boiled</td>
<td>1.17</td>
<td>+</td>
</tr>
<tr>
<td>Mustard Greens, boiled</td>
<td>0.98</td>
<td>+</td>
</tr>
<tr>
<td>Pumpkin, canned</td>
<td>3.41</td>
<td>+</td>
</tr>
<tr>
<td>Spinach, boiled</td>
<td>6.43</td>
<td>+</td>
</tr>
<tr>
<td>Swiss Chard, boiled</td>
<td>3.96</td>
<td>+</td>
</tr>
</tbody>
</table>

Cast-Iron

Foods can also acquire iron from the vessels in which they are cooked. By habitually using such vessels, vegans can easily add iron to their meals. Cast-iron and steel cookwares have
been shown to release iron into foods that are cooked in them, and the bioavailability of such iron has been demonstrated in rats\textsuperscript{70}, in vitro\textsuperscript{71}, and in humans.\textsuperscript{72-73} Therefore, preparing foods in cast-iron and steel cookware is an easy and reliable way to enhance iron nutrition in a vegan diet.

Several studies have attempted to quantify the amount acquired, but acquisition varies widely depending on the properties of foods. A comparison of 20 foods cooked in cast-iron versus an unspecified “non-iron utensil” revealed that the iron content of 18 foods increased significantly when cooked in the cast-iron pan.\textsuperscript{74} Iron acquisition was highest for acidic, moist, and long-cooked foods. For example, 100-g applesauce increased from 0.35 mg to 7.38 mg iron, and tomato sauce increased from 0.61 mg to 5.77 mg per 100-g. Less, but still significant, amounts were acquired by less acidic foods like pancakes (0.63 mg to 1.31 mg) and white rice (0.67 mg to 1.97 mg). The only two recipes that did not significantly increase in iron content were liver and onions and cornbread. The researchers could not explain why liver and onions did not gain iron, but they hypothesized that cornbread’s uptake was insignificant because it forms a crust upon contacting the hot pan and it is not stirred. These procedural differences limit the batter’s contact with the pan; only the thin layer contacting the pan can acquire the iron. Thus acidity, moisture, cooking duration, and stirring appear to be positively correlated with iron acquisition from cast-iron cookware.

Another variable suspected of altering iron yield from a cast-iron skillet is frequency of use. To test this variable, applesauce and spaghetti sauce were cooked in two separate new, seasoned cast-iron skillets for 50 trials.\textsuperscript{75} Iron uptake in the first two trials for both recipes was significantly less than the remaining trials. Researchers attributed this to the protective effect of seasoning the pan. With repeated trials, uptake by spaghetti sauce slightly, but significantly, increased. In contrast, applesauce did not differ after the first two trials. Thus, the first few uses of a seasoned cast-iron skillet may contribute less iron to a recipe. Such a small difference may
not matter relative to one’s overall iron needs. Whether the pan is old or new, cooking in cast-iron can easily add iron to one’s usual diet.

In addition to cast-iron, steel cookware also yields iron to foods during cooking. The iron content foods was compared before and after cooking in a steel wok. As with cast-iron, steel yielded more iron to foods that were acidic and cooked for a longer duration. Sweet and sour sauce, for instance, increased from 0.12 mg to 7.10 mg iron per 100-g whereas stir-fried rice only increased from 0.13 mg to 0.39 mg. Yet even the smallest increases were statistically significant, indicating that the steel wok did improve iron content. In contrast, only 7 of 10 foods cooked in a stainless steel saucepan contained significantly more iron than the same food cooked in glass. The foods that did not acquire significant amounts of iron were rice, green beans, and white sauce. Iron in similarly non-acidic foods (e.g. pancakes and scrambled eggs) did increase significantly, so the reason for the three non-significant results is not clear. Nonetheless, the authors of both studies concluded that iron contributed by steel cookware is significant enough consider as a contribution to one’s dietary intake.

*Fortified Foods*

Finally, many foods are enriched or fortified with iron. In the U.S., milled grains are enriched with certain nutrients to replace those that are lost during milling; iron is one of these nutrients. White flour, corn flour, and white rice provide iron without the phytate that accompanies whole grain products. Occasional refined grains, if enriched, can be valuable absorbable iron sources for vegans.

Fortification adds nutrients to foods that are not naturally present or adds them in amounts exceeding natural concentrations. Some of the most common iron-fortified foods are ready-to-eat breakfast cereals. A one-cup serving of Cheerios, for instance, provides 8.10 mg
iron; Corn Flakes contain 8.40 mg per cup; and Grape Nuts contain 16.20 mg per half-cup serving.45

The three strategies recommended for improving iron nutrition – decrease inhibitors, increase enhancers, and increase total iron intake – provide vegans with flexibility in meeting their recommended daily intake. Different strategies may prove more suitable than others for certain situations. Since the best strategy differs by individual preferences and situations, providing vegans with the knowledge to make an informed decision is essential.
Chapter 3

Zinc

Zinc is another trace mineral that a vegan diet may undersupply. The Institute of Medicine recommends a daily intake of 11 mg for males and 8 mg for females over age 19.\textsuperscript{20} This RDA assumes approximately 45\% zinc absorption from the average American diet. Nearly half of American adults’ zinc is supplied by animal products such as beef, poultry, seafood, pork, and dairy.\textsuperscript{78} A vegan diet excludes these rich sources and, instead, derives zinc from plant foods that commonly contain zinc inhibitors. Therefore, absorption is likely less than predicted for the average population. Vegan diets based largely on whole grains and legumes – both high in inhibitors – may require 50\% more zinc than estimated by the RDA.\textsuperscript{20} If the diet is based on such foods, recommended intake might be as high as 16.5 mg for men and 12 mg for women.

Epidemiological studies generally show that vegans and omnivores consume comparable amounts of zinc.\textsuperscript{79} However, vegans may absorb less of this zinc than omnivores because many staples of a vegan diet – whole grains, legumes, nuts, and seeds – contain inhibitors that decrease zinc absorption. The adequacy of vegans’ zinc intakes is difficult to determine, given that requirements vary with diet composition. A largely refined vegan diet that includes foods fortified with zinc is likely to provide sufficient well-absorbed zinc. In contrast, a diet based on unrefined and unfortified foods may provide adequate amounts of zinc but contains inhibitors that decrease its absorption. Net zinc absorption from this diet may not meet an individual’s physiological requirement.

A brief review of studies measuring the zinc intake of vegans shows that total zinc intake in this population almost meets recommendations for U.S. adults. A Canadian vegan sample
assessed by 3-day diet records reported a mean daily intake of 8.5±2.2 mg. A UK sample fared slightly worse with an average intake of just 7.9 mg/d, as measured by 4-day weighted food records. Adequate, or near adequate, intakes were reported in diet histories of Swedish men (10 mg) and women (7.8 mg) as well as 4-day diet records of American men (12.2 mg) and women (7.7 mg). Similarly, Australian male vegans assessed by three 4-d weighted diet records had intakes of 10.9 mg/d. Judged against the U.S. RDI, vegans may just barely meet recommendations. The IOM’s estimated 50% elevated requirement for a primarily unrefined diet, however, is not achieved.

Whereas the adequacy of iron nutrition could be evaluated by hematological values, reliable biochemical measures do not exist for zinc. Furthermore, signs and symptoms of inadequate zinc consumption do not appear until late stages of deficiency and the effects of chronic marginal zinc status are unknown. To avoid subclinical deficiencies and preserve stores, vegans should be conscious of their zinc intakes. Educating vegans about the relative bioavailability of zinc sources in their diets can help them judge whether their intakes are adequate and, if not adequate, adjust accordingly.

A. Decrease Inhibitors

Phytate

Zinc, like iron, is susceptible to irreversible binding by phytate in the intestinal lumen. High-phytate diets threaten adequate zinc absorption. This inhibitory relationship was first noted among Egyptian and Iranian populations in the 1960s. Classic zinc deficiency symptoms developed in people following diets low in flesh foods and based on unleavened whole grain breads. Though the bread contained high levels of zinc, absorption was poor due to its phytate
content. Without a varied diet and alternative sources of absorbable zinc, such as meat, the grain-based diet was deficient in zinc.

Similarly, the primary vegan zinc sources are high-phytate grains, nuts, and legumes. Zinc content of such plant foods is comparable to many animal foods, according to food nutrient reference tables. These tables, however, are inappropriate for estimating actual absorption, which is substantially reduced by phytate. To isolate its inhibitory effect, phytate was added to a test meal as 2.34g sodium phytate. Zinc absorption from this meal was halved, relative to an identical control without sodium phytate. However, one cannot assume that absorption from all phytate-containing zinc sources is halved. Rather, the inhibition caused by phytate is dose-dependent; that is, inhibition increases as phytate increases, assuming zinc content remains unchanged. Total zinc and phytate content must both be considered when estimating bioavailable zinc.

To facilitate estimation of zinc availability, the World Health Organization categorizes foods by their molar ratios of phytate to zinc. Briefly, moles of phytic acid are calculated by dividing milligrams phytate by its molecular weight, 660 mg/mol; moles of zinc are calculated as milligrams zinc divided by zinc’s atomic weight, 65.4 mg/mol. The relationship between this molar ratio and zinc bioavailability was derived from controlled single meal studies. In general, ratios below 5 indicate 45-55% zinc absorption; 5-15 indicates 30-35% absorption; and greater than 15 indicates 10-15% absorption. While these ratios have helped to guide efforts to improve zinc nutrition, they are no longer considered valid. Single meal absorption studies, on which these ratios were based, show the direction and relative response in absorption caused by dietary changes. However, whole diet studies offer better approximations of dietary zinc absorption over longer periods of time.

Improved estimates of dietary zinc absorption in the presence of phytate are provided by a recently developed trivariate mathematical model. The model estimates fractional zinc
absorption at increasing levels of dietary phytate. First, fractional absorption was graphed from 32 isotope studies that reported zinc and phytate intakes and zinc absorption from daily diets in humans. Then, a model was fit to these data points using nonlinear regression analysis. The model can be used to predict the amount of dietary zinc that must be consumed to absorb sufficient zinc to meet estimated physiological needs at various levels of daily phytate intake. In a diet free of phytate, men and women can meet requirements by consuming 8.5 mg and 6.6 mg zinc, respectively. For every 1000 mg phytate, recommended intakes increase by 9.3 mg (men) and 6.5 mg (women). Thus, the zinc intakes predicted to fulfill requirements on a 1000 mg phytate diet increase to 17.8 mg (men) and 13.1 mg (women).

Though not valid to predict overall dietary zinc absorption, single meal studies help to illustrate the dose-dependent inhibition of zinc absorption caused by phytate. One such study compared zinc absorption from whole grains prepared as porridges. Phytate content ranged from 150 μmol (100 mg) in rye to 615 μmol (~406 mg) in oats per serving of porridge, prepared from 60 g raw grain. Absorption was greatest from rye, the cereal with the least amount of phytate; the opposite was true for oats, which contained the most phytate per serving. The other two grains, whole wheat and barley, contained intermediate amounts of phytate: 420 μmol (~277 mg) and 360 μmol (238 mg), respectively. Zinc absorption was greater from barley, and absorption from both grains was intermediate to that from rye or oats. In other words, the order of ranking for phytate content was the inverse of zinc absorption ranking. This study helps to elucidate the effect of phytate on zinc absorption in food. Given that people do not generally consume single foods in isolation, the applicability of its results are limited. More complex analysis is necessary for evaluating absorption from an entire diet.

Compromised zinc status following consumption of high-phytate diets has been demonstrated in several studies. Kim et al. conducted a crossover study to compare zinc absorption in women following two 9-day periods of either a low- or high-phytate diet. Foods
served in the low-phytate diet had been treated with phytase, but they were otherwise identical to the high-phytate diet. Relative absorption was estimated by measuring changes in zinc excretion on each diet. Absorption from the high-phytate diet (molar phytate:zinc ratio 23) was approximately 40-50% less than absorption from the low-phytate diet (molar ratio 10). Decreasing phytate in diets containing equal amounts of zinc clearly improved zinc absorption.

A longer crossover study in women demonstrated similar results by comparing a lactoovovegetarian (LOV) diet to an omnivorous diet with molar phytate:zinc ratios of 14 and 5, respectively, followed for four weeks each. The percentage of zinc absorbed from the LOV diet (26%) was significantly less than that from the omnivorous diet (33%). Likewise, net absorption was superior in the omnivorous diet, which contained slightly more total zinc. It is noteworthy that the lower total zinc content of the LOV diet (9.4 versus 10.9 mg, by analysis) would actually favor improved fractional absorption, but this was not observed. The greater phytate content of the LOV diet was identified as the causative difference in fractional absorption. However, greater zinc absorption from the omnivorous diet cannot solely be attributed to its lower phytate content because meat has been observed to enhance zinc absorption. The relative effects of phytate and meat were not teased apart in this study.

Reporting zinc absorption as a percentage of total zinc is useful for studying the dose-dependent effects of phytate. However, net absorption also depends on total zinc present in a food. If a vegan diet is higher in total zinc content, then net absorption may equate that of an omnivorous diet in spite of its inferior bioavailability. Evidence seems to support this idea. Compared to a very low phytate white bread and cow’s milk meal, percentage zinc absorption was significantly lower from a high-phytate (phytate:zinc molar ratio 20) whole wheat bread and soy milk meal. The meals contained 1.84 mg and 2.74 mg zinc, respectively, and absorption from the white bread/cow’s milk diet exceeded that from wheat bread/soy milk by just 0.08 to 0.10 mg zinc. Relative to dietary requirements, this difference is negligible. Therefore, the high-
phytate diet typical of vegans may still supply adequate zinc if zinc-rich foods are regularly consumed.

Another comparison of zinc absorption supported the same concept. In this study, phytate-rich whole wheat bread was compared to white bread. Expressed as a percentage of total zinc, absorption from white bread is superior to its whole wheat alternative: 38% versus 17%, respectively. Vegans may misinterpret these results by assuming that white bread is a better zinc source. However, whole grains concentrate both phytate and zinc in their aleurone layer and bran, which are removed by milling to make white flour. Whole wheat flour retains more zinc, resulting in greater net absorption from whole wheat bread than white bread: 0.22 mg versus 0.15 mg. Admittedly, the 0.07 mg greater absorption from whole wheat bread may be insignificant relative to daily requirements. An important point to take from this study, nonetheless, is that higher phytate foods may still be valuable zinc sources if they are richer in zinc that their low-phytate alternatives. Simply favoring low-phytate foods does not guarantee better zinc nutrition.

After reviewing the evidence, it is clear that phytate jeopardizes zinc absorption and that decreasing may increase zinc bioavailability. Importantly, the method by phytate is decreased should preserve zinc content in order to significantly improve bioavailability. Methods to achieve such reduction are the topic of Chapter Five: Decreasing Inhibitors.

Oxalic Acid

Zinc, as a divalent cation (Zn$^{2+}$), is capable of forming an insoluble salt with oxalic acid. When oxalic acid is consumed with zinc, zinc absorption may be mildly inhibited.

The degree to which oxalic acid inhibits zinc absorption may depend on the fiber content of the meal with which it is consumed. Evidence for this relationship was reported by Kelsay and Prather in a study evaluating mineral balance in twelve men following each of three controlled
One diet was low in fiber and contained spinach (high oxalic acid) on every other day; another diet was high in fiber and also contained spinach on every other day; and the final diet was high in fiber but provided cauliflower (low oxalic acid) instead of spinach. Each diet was followed for four weeks, and all urine and feces were collected during the third and fourth weeks for analysis of zinc excretion. By subtracting the sum of urinary and fecal zinc excretion from dietary zinc intake, zinc balance was determined.

When zinc balances were compared across the three diets, no significant differences were observed during week three. During week four, however, zinc balance was significantly lower on the second diet (high-fiber with spinach) than on either of the other two diets. Zinc balances on these other two diets did not differ significantly. Mean zinc balance on the high-fiber spinach diet was negative (-1.4 mg/d) while positive balances were observed following the low-fiber spinach diet (2.0 mg/d) and high fiber diet without spinach (0.4 mg/d). Neither oxalic acid nor fiber, alone, impaired zinc balance but together, they promoted net excretion.

A vegan diet is typically high in fiber, and oxalic acid-rich vegetables may be common constituents of the diet, as well. In the high-fiber spinach diet of Kelsay and Prather, the main source of oxalic acid – spinach – was only consumed every other day. A vegan could consume comparable amounts of oxalic acid equally as often from not only spinach but also sweet potatoes, yams, beets, and black tea. It is reasonable to predict that vegans’ zinc balance may be compromised by frequent consumption of oxalic acid in their high-fiber diets. Therefore, avoiding concentrated sources of oxalic acid or using cooking methods to decrease oxalic acid in foods may be advisable to promote optimal zinc nutrition.
B. Increase Enhancers

*Citric Acid*

Limited evidence suggests that citric acid may increase zinc absorption. A 0.05 g dose of citric acid was pressure cooked for 10 minutes with 10 g rice or chickpeas and subsequently the mixture was subjected to *in vitro* digestion.\(^5^8\) Zinc dialyzability, an indicator of absorbability, increased 40% and 31% in rice and chickpeas, respectively. Likewise adding 0.75 g amchur, an ethnic ingredient made from dried green mango powder, increased dialyzability in rice by over 100% and in chickpeas by 22%. This amount, 0.75 g, was chosen to equate the pH-lowering effect of 0.05 g citric acid. Since citric acid is the main acid in amchur, the results further support citric acid’s enhancing effect. A limitation of this study is that *in vitro* dialyzability only demonstrates relative changes in absorption. Exact predictions of absorbable zinc for humans cannot be made. In other words, this study merely demonstrates that zinc absorption increases with the addition of citric acid. The percentage by which absorption increases in humans, however, is unknown.

C. Emerging Research: Polyphenols

Little evidence is available to describe the influence of polyphenols on zinc absorption, and available studies present mixed results. Yet so far, the effects of polyphenols have only been demonstrated *in vitro* using Caco-2 cell cultures to model absorption. For instance, Kim et al. measured cellular uptake and transepithelial absorption of zinc as ZnCl\(_2\) in the presence of (−) epigallocatechin-3-gallate (EGCG), grape seed extract, and green tea extract.\(^9^9\) Only grape seed extract significantly (P < 0.05) decreased zinc absorption; both apical uptake and basolateral exit were reduced. The other two polyphenol sources, ECGC and green tea extract, had no effect on
zinc absorption. The authors hypothesized that the inhibitory effect of grape seed extract results from procyanidin, one of the many polyphenols that comprise the extract. It is possible that procyanidins strongly bind zinc and prevent uptake by enterocytes. Procyanidin is found in many plant foods, including blueberries, apples, and grapes. However, more research is needed to replicate and extend these results to human absorption studies before recommending any dietary changes.

In contrast to the results of Kim et al.\textsuperscript{99}, Sreenivasulu et al. observed increases in zinc absorption in Caco-2 cells when polyphenols were added.\textsuperscript{100} The source of polyphenols included green tea, which was shown to have no effect on zinc absorption when tested by Kim et al.\textsuperscript{99}, as well as red wine and red grape juice. In this study, all three polyphenol sources significantly increased zinc absorption over a three-hour period compared to control cultures. Relative to control, absorption was 25%, 32%, and 47% greater in the presence of red grape juice, green tea, and red wine, respectively; all reported data was significant at $P<0.05$. Furthermore, the increase was significantly greater in red wine than in either red grape juice or green tea. This difference could not be attributed to the alcohol in wine because alcohol, alone, inhibited absorption.\textsuperscript{100} The authors concluded that differences in relative concentrations of specific polyphenols were responsible for the differences in absorption.

An important difference between the studies by Kim et al.\textsuperscript{99} and Sreenivasulu et al.\textsuperscript{100} is the source of zinc used to measure absorption. Whereas Kim et al. provided zinc as ZnCl$_2$,\textsuperscript{99} Sreenivasulu et al. used isotope-labeled polished white rice.\textsuperscript{100} Interactions with the food matrix could have modified the effect of polyphenols on zinc. Since humans consume zinc and polyphenols with food rather than as isolated extracts, the results of Sreenivasulu et al. may be more relevant to real life. However, evidence is certainly lacking at this point in time. Additional \textit{in vitro} studies will likely be conducted in the future to elucidate these conflicting findings before progressing to human absorption studies. Until more conclusive evidence emerges,
recommendations to increase or decrease dietary polyphenols for the sake of zinc absorption are not justified.

D. Increase Total Intake

The most concentrated vegan zinc sources are often high in phytate, the most potent inhibitor of absorption. Yet theoretically, small fractions that are absorbed throughout a day can collectively provide an adequate intake if total zinc intake is high enough. Specifically, Messina et al. advise all vegetarians to exceed the RDA by 50%. A diet conscientiously constructed around zinc-rich foods is essential to allow for inevitable losses and ensure adequate net absorption from a high-phytate diet. Educating vegans about zinc sources and promoting their consumption will improve the adequacy of a vegan diet. Examples of zinc sources in vegan diets can be found in grains, legumes, nuts and seeds, and fruits and vegetables.

Grains

Grains concentrate most of their zinc in their outer layers, which are removed when grains are milled into refined flours. Therefore, whole grains contain more zinc than their refined counterparts. However, since phytate is also concentrated in this region, zinc absorption is relatively poor from whole grains. Whereas parboiling grains facilitates the migration of iron into the central endosperm, the distribution of zinc is not significantly altered by parboiling. Parboiled rice and raw rice, for instance, lose comparable amounts of zinc when they are milled. Table 3.1 lists some grain sources of zinc.
Table 3-1 Zinc in Selected Grains

<table>
<thead>
<tr>
<th></th>
<th>mg /1 cup cooked</th>
<th>Bioavailability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barley, pearled</td>
<td>1.29</td>
<td>-</td>
</tr>
<tr>
<td>Bulgur – parboiled, milled</td>
<td>1.04</td>
<td>+</td>
</tr>
<tr>
<td>Millet</td>
<td>1.58</td>
<td>-</td>
</tr>
<tr>
<td>Oatmeal</td>
<td>1.15</td>
<td>-</td>
</tr>
<tr>
<td>Rice, brown, long grain</td>
<td>1.23</td>
<td>-</td>
</tr>
<tr>
<td>Rice, white, long grain</td>
<td>0.77</td>
<td>-</td>
</tr>
</tbody>
</table>

Legumes

Legumes, like grains, are also rich sources of zinc but contain phytate that inhibits its absorption. Germinated legumes contain smaller concentrations of inhibitory inositol phosphates and, therefore, offer more bioavailable zinc. The zinc contents of several legumes and legume-based products are identified in Table 3.2.

Table 3-2 Zinc in Selected Legumes

<table>
<thead>
<tr>
<th></th>
<th>Mature Beans, Boiled mg /1 cup</th>
<th>Bioavailability</th>
<th>Sprouted Beans mg /1 cup</th>
<th>Bioavailability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black Beans</td>
<td>1.41</td>
<td>-</td>
<td>0.55 (boiled)</td>
<td>+</td>
</tr>
<tr>
<td>Blackeye Peas</td>
<td>2.22</td>
<td>-</td>
<td>1.12 (stir-fried)</td>
<td>+</td>
</tr>
<tr>
<td>Chickpeas</td>
<td>2.51</td>
<td>-</td>
<td>2.00 (stir-fried)</td>
<td>+</td>
</tr>
<tr>
<td>Kidney Beans</td>
<td>1.89</td>
<td>-</td>
<td>0.98 (boiled)</td>
<td>+</td>
</tr>
<tr>
<td>Mung Beans</td>
<td>1.70</td>
<td>-</td>
<td>1.12 (stir-fried)</td>
<td>+</td>
</tr>
<tr>
<td>Lentils</td>
<td>2.51</td>
<td>-</td>
<td>2.00 (stir-fried)</td>
<td>+</td>
</tr>
<tr>
<td>Peas</td>
<td>1.90</td>
<td>-</td>
<td>0.98 (boiled)</td>
<td>+</td>
</tr>
<tr>
<td>Soybeans</td>
<td>1.98</td>
<td>-</td>
<td>0.98 (steamed)</td>
<td>+</td>
</tr>
<tr>
<td>Soy Products</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tempeh</td>
<td>0.95</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tofu, firm</td>
<td>1.27</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Natto</td>
<td>2.65</td>
<td>+</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**Nuts and Seeds**

Nuts and seeds provide large amounts of zinc per serving, as well. Since they also contain phytate, this zinc is poorly absorbed. The zinc content of nuts and seeds is listed in Table 3.3.

Table 3-3 Zinc in Selected Nuts and Seeds

<table>
<thead>
<tr>
<th>NUTS</th>
<th>mg /1 oz. dry roasted</th>
<th>Bioavailability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Almonds</td>
<td>1.00</td>
<td>-</td>
</tr>
<tr>
<td>Cashews</td>
<td>1.59</td>
<td>-</td>
</tr>
<tr>
<td>Peanuts</td>
<td>0.94</td>
<td>-</td>
</tr>
<tr>
<td>Pecans</td>
<td>1.44</td>
<td>-</td>
</tr>
<tr>
<td>Walnuts</td>
<td>0.88</td>
<td>-</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>SEEDS</th>
<th>mg /1 oz. roasted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pumpkin seeds</td>
<td>2.11</td>
</tr>
<tr>
<td>Sesame seeds</td>
<td>2.90</td>
</tr>
<tr>
<td>Sunflower seeds</td>
<td>1.50</td>
</tr>
</tbody>
</table>

**Fruits and Vegetables**

Fruits and vegetables are relatively poor sources of zinc. However, since vegans consume many servings of such foods daily, their cumulative contribution to zinc intake can be significant. This zinc is believed to be well-absorbed unless accompanied by oxalic acid. Some of the best sources among fruits and vegetables are identified in Table 3.4.

Table 3-4 Zinc in Selected Fruits and Vegetables

<table>
<thead>
<tr>
<th>Fruits and Vegetables</th>
<th>mg /Serving (as indicated)</th>
<th>Bioavailability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beet greens, boiled*</td>
<td>0.72 per 1 c.</td>
<td>+</td>
</tr>
<tr>
<td>Blackberries</td>
<td>0.76 per 1 c.</td>
<td>+</td>
</tr>
<tr>
<td>Broccoli, boiled</td>
<td>0.70 per 1 c.</td>
<td>+</td>
</tr>
<tr>
<td>Cremini mushrooms, raw, sliced</td>
<td>0.79 mg per 1 c.</td>
<td>+</td>
</tr>
<tr>
<td>Shiitake mushrooms, sliced, cooked</td>
<td>1.93 mg per 1 c.</td>
<td>+</td>
</tr>
<tr>
<td>Potato, baked</td>
<td>0.73 per 1 potato</td>
<td>+</td>
</tr>
<tr>
<td>Zucchini, boiled</td>
<td>0.70 per 1 c.</td>
<td>+</td>
</tr>
</tbody>
</table>

*Beet greens contain oxalic acid but boiling reduces its concentration, so the bioavailability of zinc is thought to be improved.
Fortified Foods

Even though milled grains lose a substantial portion of their zinc contents, refined grain products are not customarily enriched with zinc. Some manufacturers, however, may choose to fortify their products with zinc. In particular, ready-to-eat breakfast cereals and nutritional yeast are common zinc-fortified foods. For example, Cheerios and Corn Chex each contain 3.75 mg/cup, and Wheaties contain 7.50 mg/cup. Nutritional yeast is prepared by growing yeast on a nutrient-rich medium so that the yeast take up these nutrients and, when consumed, provide a valuable source of absorbable vitamins and minerals. Zinc is one of the mineral contained in this medium, so nutritional yeast contains an impressive 2.1 mg zinc per tablespoon. Vegans can easily increase their zinc intakes by seasoning meals with nutritional yeast.

Zinc absorption is limited by inhibitors in its most concentrated natural sources in a vegan diet. Decreasing these inhibitors, along with increasing enhancers and overall intake, ensures that sufficient zinc can be absorbed to meet physiological needs. By choosing from these strategies, vegans can plan a diet that meets zinc requirements while satisfying individual preferences and lifestyles.
Chapter 4

Calcium

One defining feature of a vegan diet is the exclusion of dairy products. Since dairy foods supply nearly three-quarters of the calcium in an average American diet\textsuperscript{102}, the calcium sufficiency of a dairy-free American diet is questionable. Dairy is certainly not the only calcium source but it is most widely known. Furthermore, the availability of calcium from plants may be limited by inhibitors that co-exist in plant calcium sources or that are consumed along with calcium sources in the overall diet. Assessing the content and availability of calcium in a vegan diet will reveal potential strategies to improve the adequacy of the diet.

Unlike iron and zinc intake recommendations, the Institute of Medicine’s recommendations regarding calcium intake do not consider bioavailability as a factor that influences requirements.\textsuperscript{103} Calcium absorption from most foods is comparable, so calcium load generally affects absorption more than availability. However, the IOM acknowledges that high intakes of phytate and oxalate, two absorption inhibitors, decrease absorption.\textsuperscript{103} Phytate is abundant in a vegan diet, and oxalate intake may also be higher than that of omnivores due to greater intakes of plants that contain the substance. Theoretically, calcium recommendations for the average American diet may not apply equally to a diet that contains higher concentrations of absorption inhibitors, such as a vegan diet.

Specifically, the Institute of Medicine’s calcium recommendations were established based on data from calcium balance studies. Calcium balance measures the difference between calcium intake and excretion. Balance studies generally assume that intake is adequate when it equates excretion; in other words, there is no net loss or gain. In calcium studies, adequate intake was defined as the minimum intake that would enable the body to retain (i.e. not excrete)
sufficient calcium for bone growth and maintenance throughout different stages of life.\textsuperscript{103} Beyond this level of intake, no further retention occurs and excess intake is excreted. This intake was predicted with a non-linear regression model based on available balance study data.\textsuperscript{103} For men and women between the ages of 19-50, recommended intake is 1000 mg/d; beyond 50, recommendations are raised to 1200 mg/d.\textsuperscript{103} The appropriateness of using balance studies to predict optimal calcium retention is questionable, and longitudinal data was unavailable to confirm predictions. Therefore, recommendations are presently designated as adequate intakes (AI) rather than estimated average requirements (EAR).

Since studies that were used to establish the calcium AI did not all report the specific sources of dietary calcium, recommendations could not be tailored to diet composition. Instead, the IOM assumed that absorption from a varied diet will, on average, meet calcium needs for most people when the recommended intakes are consumed. A vegan diet supplies a variety of calcium sources but they are entirely derived from, and consumed with, plants. The average American diet, for which IOM recommendations are established, derives calcium from plants as well as dairy foods. Dairy and plant foods contain different concentrations of calcium as well as different types and amounts of absorption inhibitors. Thus, it is likely that absorption of calcium from a vegan diet differs from calcium absorption in a mixed diet.

Not only does calcium absorption differ between vegans and omnivores but calcium excretion also apparently varies by diet composition. In five Chinese counties, for instance, women’s animal protein intakes were assessed by 3-day weighted food records and related to their urinary calcium excretion.\textsuperscript{104} As animal protein intakes increased, urinary calcium excretion also increased; the inverse was true for plant protein. The relationships were statistically significant and remained so after adjusting for age and calcium intakes. Vegans solely consume plant protein and, based on these results, would be expected to minimize calcium losses.
Hypothetically, if calcium is conserved better when protein is derived from plants, then vegans may require less calcium because they do not have to compensate for as much urinary waste.

The observed positive relationship between animal protein intake and urinary calcium excretion may be explained by the acid-ash hypothesis. According to this hypothesis, a diet that produces a lot of acidic metabolic waste will increase bone resorption to buffer the acidity with calcium, which is subsequently excreted in urine.\textsuperscript{105} In contrast, diets that yield alkaline byproducts protect against bone loss.\textsuperscript{105} Most plant foods are classified as “net base-producing” while animal-derived foods are “net acid-producing,” although grains – a staple of vegan diets – are also acid producers, and most legumes are nearly neutral.\textsuperscript{106} Two meta-analyses confirmed that urinary acid excretion exhibits a significant, positive linear relationship with calcium excretion\textsuperscript{105,107}, and a vegetarian sample did excrete less urinary acid and calcium than omnivores, as predicted.\textsuperscript{108} Theoretically, vegans probably excrete the lowest levels of urinary acid and calcium, especially if they favor fruits and vegetables and moderate grain consumption.

Though plausible, the acid-ash hypothesis is still accumulating evidence. Less calcium excretion does not necessarily guarantee better bone health if other calcium homeostatic mechanisms compensate for changes in excretion. Evidence favoring the hypothesis shows that high intakes of fruits and vegetables do appear to benefit bone density. In the Framingham Osteoporosis Study, for example, the highest bone mineral densities were measured in men and women consuming the greatest amounts of fruits and vegetables.\textsuperscript{109} This may be attributable to greater alkaline ash production, but it may also be attributed to their high magnesium and potassium contents, which are involved in bone metabolism.

The Framingham Osteoporosis Study sample is not representative of vegans, whose bone densities may differ for other reasons. To see if bone health is better among vegans, vegan samples must be compared to omnivorous samples that are otherwise comparable. Chiu et al., for instance, compared vegan and non-vegan postmenopausal Taiwanese women who had adhered to
their diets for at least 15 years.\textsuperscript{110} Calcium intakes, assessed by a 24-hour recall and food frequency questionnaire, did not differ significantly; vegans consumed 364 mg/d while non-vegans consumed 343 mg/d. However, vegans had poorer lumbar spine and femoral neck bone densities. Chiu et al. concluded that following a vegan diet elevated subjects’ fracture risk in spite of similar calcium intakes.\textsuperscript{110} A possible explanatory difference is lower calcium bioavailability from the vegan diet. Other important determinants of bone health that were not assessed – namely, vitamin D status –may have also influenced bone density. Regardless of the underlying cause, the vegan sample’s bone density did not appear to be “protected” by an exclusively plant-derived diet.

Less drastic differences were observed in a meta-analysis of studies comparing bone density in vegans and non-vegans. Ho-Pham et al. collected nine observational studies from diverse samples that examined the bone mineral density (BMD) of adult omnivores, vegetarians, and vegans.\textsuperscript{111} Calcium intakes of lactoovovegetarians (LOVs) and vegans were comparable, but vegans’ mean femoral neck BMD was slightly lower than that of lactoovovegetarians. The ratio of mean BMD in LOVs to omnivores was 0.98, whereas the ratio was 0.94 for vegans to omnivores; the significance of this difference was not reported, presumably because the difference was not significant. Evidently, a strictly plant-based diet did not promote superior bone density relative to diets that included dairy and eggs or even animal flesh, significant sources of acid precursors.

Similarly, a cross-sectional study of Vietnamese women over the age of 50 found comparable bone densities in vegans and omnivores.\textsuperscript{112} The vegans (n=105) and omnivores (n=105) had comparable mean body mass indices (24 kg/m\textsuperscript{2}), weights (53-54 kg), ages (62 years), and activity patterns but vegans’ mean daily calcium intake, assessed by a two-day dietary habit questionnaire, was 375 mg while that of omnivores was significantly higher at 638 mg. Even though vegans had followed their diet for 10 – 72 years, presumably with chronic low
calcium intakes, their average femoral neck, lumbar spine, or whole body bone mineral densities were not significantly different from those of omnivores. The prevalence of osteoporosis, defined by having a femoral neck bone mineral density lower than 2.5 standard deviations below the population average, was not significantly different either. Prevalence was 18% and 15% in vegans and omnivores, respectively. This study suggests that factors other than calcium intake may protect vegans against bone loss, like a better acid-base balance resulting from their high fruit and vegetable intake.

Whereas Chiu et al.\textsuperscript{110} and Ho-Pham et al.\textsuperscript{112} defined bone density as their outcome, Appleby et al. analyzed bone fracture risk in British vegans consuming different amounts of calcium.\textsuperscript{113} Since Britain is a Western country with an ethnically diverse population like the United States, bone fracture data is probably comparable to that which would be observed in Americans. According to data from the EPIC-Oxford study, bone fracture rates were higher in vegans than omnivores and lactoovovegetarians. However, separate analysis of vegans consuming at least 525 mg calcium per day revealed fracture rates comparable to those of non-vegans. Insufficient total calcium intakes, rather than a vegan diet \textit{per se}, seemed to explain higher fracture rates. Perhaps above a minimum necessary amount, such as 525 mg, net calcium absorption is sufficient to promote bone mineralization in a plant-based diet even at an intake that barely exceeds half of the IOM’s AI. It is presumable that calcium retention may be sufficient at this relatively low intake because of reduced urinary calcium excretion secondary to a favorable acid-base balance. At present, however, this conclusion cannot be made because other unreported bone- and fracture-related variables may have changed along with calcium intakes, like physical activity, body mass, and other nutrients like vitamin D.

Currently, available evidence is inconsistent and does not confirm whether vegan diets promote calcium retention at lower intakes. Urinary calcium excretion does increase as dietary acid-precursors increase and is counterbalanced by largely plant-derived base-precursors, but this
may not necessarily translate into superior bone health among people exclusively eating plants. For instance, it is possible that higher calcium retention is offset by reduced absorption from a vegan diet, if the vegan diet derives most calcium from sources rich in inhibitors. Until evidence clarifies the relationship, advising vegans to consume less than the IOM’s adequate intakes for the general population is premature.

Instead of setting a different recommended level of intake, a more cautious approach should be taken regarding vegan calcium recommendations. Specifically, vegans should be encouraged to meet the AI through a diet that favors bioavailable calcium sources. Identification of calcium sources that are naturally low in inhibitors, as well as knowledge of food preparation methods that can decrease inhibitors, will enable vegans to choose a diet rich in absorbable calcium. This protects against the possibility that plant-based diets are lower in bioavailable calcium than mixed diets. If calcium requirements are actually lower in plant-based diets, due to reduced urinary excretion, there is little risk in consuming excess bioavailable calcium. There is presently no established upper level at which toxicity is a concern, but even if one does exist, it is likely that unfortified plant sources would have to be consumed in prohibitively large amounts to exceed it. Thus, advice regarding calcium nutrition to vegans should aim to ensure adequate total intake and maximize bioavailability from food sources.

Since the Institute of Medicine’s calcium recommendations yet so far remain applicable to vegans, the adequacy of vegan populations’ intakes will be evaluated against these standards. One way to evaluate vegans’ calcium intake is to collect food records and sum calcium content using a nutrient database. Total intake can then be compared to omnivores’ average intakes, a standard of adequacy like the IOM’s adequate intake, or both. For instance, seven-day weighted food records of age- and sex-matched British vegans and omnivores were compared by Rana et al. Males’ intakes were not significantly different, but only omnivores’ mean intake would have met the current calcium AI for Americans. Male omnivore consumed 1070 mg/d while vegans
consumed 700 mg/d. Neither vegan nor omnivorous females met the AI, but omnivores’ intakes were significantly greater than vegans’ intakes. Mean female intakes were 855 mg/d for omnivores but only 484 mg/d for vegans. The difference between omnivores’ and vegans’ calcium intakes was approximately 350 mg for both males and females, even though the difference was only statistically significant for females. Such a large deficit – approximately one-third of the AI for adults – demands attention.

Similarly, Lightowler et al. observed low calcium intakes in vegans in the UK. Four-day weighted food records of 26 adults, undifferentiated by sex, showed mean dietary intakes of 502 mg/d. This fulfills just half of the IOM’s adequate intake. Yet mean intakes per individual in this study were highly variable, ranging from 107 to 1239 mg/d. Clearly, a vegan is capable of consuming more than sufficient calcium to meet recommendations from diet alone. A portion of the vegan population, however, may drastically fall short of recommendations. Promoting consumption of calcium-rich vegan food sources could correct this gap between vegan and omnivore calcium intakes.

Collectively, studies report that vegans’ calcium intakes are inadequate relative to the Institute of Medicine’s recommended adequate intake. Since average intakes fall short of this goal, vegans will benefit from strategies that not only increase total calcium intake but also maximize net calcium absorption. Strategies that will increase net calcium absorption include decreasing inhibitors and securing a reliable, adequate source of vitamin D.

A. Decrease Inhibitors

Calcium is a divalent mineral, like iron and zinc, that can be irreversibly bound by substances in the intestine. The degree to which calcium absorption is inhibited depends on the
concentration and affinity of the substance for calcium in the presence of other dietary components.

To contextualize calcium availability from tested foods, it is often compared to a reference food for which calcium availability is known. Milk is commonly the reference standard to which calcium availability from other foods is compared. Approximately 30% of the calcium from cow’s milk is absorbable. Relating fractional absorption from a test food to milk, when consumed at similar calcium loads, helps to illustrate the degree of inhibition caused by substances within the food.

Researchers have used milk comparisons, as well as comparisons of the same food with and without the inhibitor of interest, to identify and characterize calcium inhibitors. The two significant inhibitors identified by such research are oxalates and phytates.

**Oxalic Acid**

Oxalic acid is the most potent inhibitor of calcium absorption from a plant-based diet. Its presence in certain foods means that apparently “good” calcium sources may, in fact, contain little available calcium. Even when calcium and oxalate are not present in the same food, oxalate from other foods consumed at the same meal can bind calcium in the intestine. Examining the potency and distribution of this inhibitor in the diet will inform food choices and preparation methods to reduce oxalate’s interference with calcium absorption.

A striking illustration of oxalate’s potency is provided by spinach. Spinach appears to be a concentrated source of calcium with approximately 250 mg per cup, cooked. Yet its exceptionally high oxalate concentration prevents most of its absorption. Absorption from a spinach dose providing 200 mg total calcium was only 5.1% in thirteen healthy adult subjects with a mean age 28.1±6.4 years. Expressed differently, more than 16 cups of spinach must be
consumed to obtain the amount of absorbable calcium in 1 cup of milk. In portions normally consumed, spinach does not substantially contribute to daily calcium intake because its calcium is excreted with oxalate.

Oxalate has been confirmed as the absorption-reducing agent by comparisons of low- and high-oxalate foods. For instance, Weaver et al. determined calcium absorption from low-oxalate leafy greens to be comparable to that of milk. Absorption from several radioisotope-labeled Chinese greens and milk, consumed at doses providing 200 mg calcium, were compared. Fractional absorption from the two low-oxalate greens, Chinese mustard greens and Chinese cabbage flower leaves, was 1.080 and 1.097 times that of milk, respectively. In contrast absorption from Chinese spinach, which contained over 33 times as much oxalate as the other greens, is drastically reduced. Only 9.3% of its calcium is absorbed, representing a spinach:milk absorption ratio of 0.257. Independent of oxalate, calcium from leafy greens is equally available as the calcium in milk. Availability of calcium in greens that are concentrated with oxalate, however, is substantially depressed.

Inhibition of calcium absorption also occurs at lower oxalate concentrations than that found in spinach. Watercress, for example, contains approximately one-third as much oxalate as spinach. Yet calcium absorption from a watercress soup sample containing 131mg calcium was only 27.4%, whereas subjects absorbed 45.5% of the calcium from milk. In other words, absorption from the watercress soup was just 60% that of milk. Researchers hypothesized that either oxalate or fiber caused reduced absorption. The bioavailability of calcium from fibrous, oxalate-free vegetables such as broccoli, bok choy, and kale meets or even exceeds that of milk. Therefore oxalate, rather than fiber, is likely responsible for decreasing absorption from watercress.

Clearly, calcium bioavailability from foods is largely dependent on oxalate. Bioavailability could, in theory, be predicted based on the relative concentrations of oxalate and
calcium in foods. If calcium exceeds the binding capacity of oxalate in the food, absorption will be relatively good, whereas the opposite predicts poor absorption. This logic guided Heaney et al. to propose that a 1:1 oxalate to calcium ratio represents saturation of oxalate with calcium.\textsuperscript{115} While the ratio can be used as a tool to compare calcium sources, it is merely an estimate. In the study by Heaney et al., for instance, the ratio of oxalate to calcium in spinach was 1.08:1.\textsuperscript{115} This ratio predicts complete inhibition of calcium absorption because sufficient oxalate is present to bind all of the calcium; actual absorption, however, was about 5%. The disparity likely results from other food components that compete for binding with oxalate. Weaver and Heaney list other foods, namely common beans and soybeans, that also exceed predicted absorption; consequently, these authors insist that bioavailability must be measured rather than predicted.\textsuperscript{116} As long its limitations are acknowledged, this ratio is sufficiently informative to assist vegan diet planning.

Vegans should realize that fruits and vegetables that are high in oxalic acid are relatively poor sources of bioavailable calcium. Low-oxalate calcium sources offer more absorbable calcium and should be favored over high-oxalate types. Alternatively, the oxalic acid content of fruits and vegetables can be reduced through cooking. Food preparation methods that can reduce oxalic acid will be discussed in the next chapter, Decreasing Inhibitors.

\textit{Phytate}

Calcium absorption is reduced by phytate, but the inhibition caused by phytate is less severe than that of oxalate. Phytate is less of a threat to calcium absorption than it is to either iron or zinc because phytate binds calcium less securely, allowing some ions to escape and be absorbed.\textsuperscript{116} Still, the high phytate content of a vegan diet may cumulatively cause substantial
calcium losses. The potency of phytate, and its potential impact on a vegan diet, is best exemplified by absorption studies in a variety of foods.

Beans, for instance, are recommended as valuable calcium sources for vegans. However, beans contain phytate that could reduce the availability of their calcium. Weaver et al. fed subjects three bean varieties (pinto, red beans, and white beans), one dephytinized bean (pinto), and milk to compare calcium absorption from servings providing approximately 70 mg of calcium.\textsuperscript{119} The calcium in the beans and milk was labeled with different isotopes so that absorption could be compared between sources. Mean absorption from the three regular beans was 21.9\% and did not significantly differ between varieties, which is not surprising because they contained similar amounts of phytate (1.3 – 1.9\%, by weight). In contrast, pinto beans that had been enzymatically-treated to reduce phytate to just 0.25\% yielded significantly greater (31.8\%) absorbable calcium. Phytate reduction clearly improved calcium absorption but did not restore it to a level comparable to milk, from which 45.1\% of calcium was absorbed. Authors attributed the residual inhibition to beans’ oxalate content.

Another staple in many vegan diets is soybeans and products derived from them. Soybeans contain more calcium than common beans (175 mg versus 50 – 120 mg per one-cup boiled\textsuperscript{45}) but vary widely in phytate content.\textsuperscript{120} If phytate affects calcium absorption, then absorption should differ between high- and low-phytate varieties. Heaney et al. evaluated this difference in absorption by feeding low- (108 mg/portion) and high-phytate (352 mg/portion) soybeans to subjects in 88-g portions, each providing 2.445 – 2.475 mmol of intrinsically-labeled calcium.\textsuperscript{120} Fractional calcium absorption was significantly higher from low-phytate soybeans (41.4\%) than the high-phytate variety (31.0\%). Thus, the inhibitory effect of phytate on calcium was reconfirmed.

While consumers cannot determine whether their soy foods are made from high- or low-phytate varieties, the study’s results are still applicable to other dietary choices. Specifically, it
demonstrates the importance of considering phytate when choosing calcium sources. The 250 mg phytate difference between soybeans translated into a 0.258 mmol difference in calcium absorption in a single half-cup soybean serving. Independently, this difference seems negligible, but even small losses can accumulate throughout a day. If these losses are avoided by favoring low-phytate calcium sources, net absorption from a vegan diet could improve substantially.

Phytate and calcium from separate food sources can also combine and form complexes when consumed together. Consequently, even calcium absorption from low-phytate foods may be compromised when they are consumed with foods rich in phytate. For instance, fractional calcium absorption from extrinsically-labeled milk was reduced from 37.5% (milk alone) to 25.8% when consumed with bran cereal. Both test portions – milk alone and milk with cereal – provided 5 mmol total calcium, with cereal contributing 0.60 mmol in the milk/cereal condition. The most distinctive difference between conditions was phytate content; the bran cereal contained 472-477 mg phytate per serving, while milk is free of phytate. Weaver et al. concluded that phytate from the cereal significantly decreased calcium absorption from the milk. These findings can be extended to vegans who, likewise, consume meals that contain high-phytate foods with low-phytate, calcium-rich foods, such as beans with collard greens.

As demonstrated by the above studies, phytate decreases calcium absorption. However, the degree of inhibition caused by phytate varies and cannot be precisely predicted. In particular, a phytate:calcium molar ratio, analogous to the phytate:zinc molar ratio that is frequently used to predict zinc absorption, was determined to be unreliable for estimating calcium bioavailability. Weaver et al. propose that the inability to predict absorption is due to variable digestive conditions (e.g. temperature, pH, and endogenous calcium secretions) that influence the formation of calcium-phytate complexes. Since phytate’s affinity for zinc is greater than that
For calcium, it is logical that calcium complex formation is likelier than zinc to respond to fluctuations in the digestive environment.

While precise predictions of calcium bioavailability based on phytate content cannot be made, the overall trend is clear: phytate inhibits calcium absorption. Reducing dietary phytate will, therefore, improve calcium absorption. With the high-phytate content common in a vegan diet, this strategy may address a significant contributor to suboptimal calcium status. Methods for decreasing phytate will be discussed in the next chapter, Decreasing Inhibitors.

B. Enhance Absorption: Vitamin D

Unlike typical mineral absorption enhancers, vitamin D does not directly interact with calcium in the intestine to facilitate its uptake. Rather, vitamin D functions in physiological processes that maintain calcium homeostasis. In its active form, calcitriol (1,25-dihydroxycholecalciferol), vitamin D acts on kidneys, bones, and the intestine to keep serum calcium levels within appropriate ranges. The entire homeostatic mechanism is relevant to calcium balance. However, calcitriol’s role in intestinal absorption will be the focus of the present discussion because the bioavailability of calcium ultimately depends on intact absorptive capabilities.

Intestinal absorption of calcium at levels found in unfortified foods primarily occurs by active transport. A transporter known as calbindin D9k binds calcium at the brush border, facilitates uptake into enterocytes, and transports the mineral to the basolateral membrane. Calcium exits the cell at the basolateral membrane through an ATP-dependent Ca\(^{2+}\)-Mg\(^{2+}\) pump. Both of these processes are affected by vitamin D status. Specifically, calcitriol upregulates calbindin D9k synthesis to increase calcium uptake when serum calcium is low. Calcitriol also increases the activity of the Ca\(^{2+}\)-Mg\(^{2+}\) pump so that calcium can be released from
enterocytes into circulation.\textsuperscript{123} Adequate vitamin D is essential to ensure that calcium homeostasis can be maintained by dietary calcium absorption. If insufficient vitamin D levels are maintained, intestinal absorption cannot be increased sufficiently to meet needs. Instead, calcium will be drawn from bones and potentially result in osteomalacia or osteoporosis.\textsuperscript{123}

Dietary strategies to improve calcium absorption, such as decreasing oxalic acid and phytate, will ultimately fail unless intestinal absorption mechanisms are functional. Hence, vitamin D sufficiency is critical to calcium nutrition. From a dietary perspective, vegans are particularly vulnerable to insufficient intake. Naturally occurring vitamin D is primarily found in foods from animals, including liver, beef, eggs, and fish.\textsuperscript{123} Most plant foods contain negligible amounts of the vitamin.

Alternatively, vitamin D requirements can be met entirely from endogenous synthesis if bare skin is exposed to appropriate wavelengths of sunlight. Sebaceous glands in the skin synthesize a vitamin D precursor, 7-dehydrocholesterol, which is converted to previtamin D\textsubscript{3} when exposed to ultraviolet B radiation from the sun.\textsuperscript{123} Previtamin D\textsubscript{3} is isomerized to cholecalciferol, which is converted into 25-hydroxyvitamin D [25(OH)D\textsubscript{3}] at the liver.\textsuperscript{123} When active vitamin D is required by the body, 25(OH)D\textsubscript{3} is converted to active calcitriol at the kidney.\textsuperscript{123} For vegans unwilling to consume supplements or fortified foods, endogenous synthesis is the only way to maintain adequate serum vitamin D.

Approximately 1000 IU vitamin D can be produced with 10 – 15 minutes sun exposure between 10 am and 3 pm during most of the year.\textsuperscript{123} However, the wavelengths of sunlight that reach earth during the winter at certain latitudes are unable to stimulate previtamin D\textsubscript{3} production.\textsuperscript{124} During these months, the earth’s axis is tilted in such a way that sunlight must travel further to reach earth.\textsuperscript{124} As the wavelengths travel through atmosphere, they are scattered and absorbed along the way. Wavelengths that ultimately reach people at higher latitudes are inadequate to induce endogenous synthesis of vitamin D. For example, people living in Boston
(42°N) cannot synthesize previtamin D$_3$ from November to February, and in Edmonton, Canada (52°N), it cannot be synthesized from November through March.$^{124}$ At lower latitude locations, such as Los Angeles (34°N) and Puerto Rico (18°N), previtamin D$_3$ synthesis is still possible during January.$^{124}$ People living further from the equator cannot rely on endogenous vitamin D synthesis during the winter.

Hypothetically, if sufficient previtamin D$_3$ stores are synthesized during the summer, accumulated serum 25-hydroxyvitamin D [25(OH)D$_3$] could meet needs during the winter without exogenous vitamin D sources. This hypothesis was tested by Lamberg-Allardt et al. in Helsinki, Finland, where sunlight is inadequate to induce cutaneous previtamin D$_3$ synthesis from mid-October through early March.$^{125}$ The researchers tracked 25(OH)D$_3$ concentrations in ten vegan subjects throughout the winter and compared them to levels in twelve omnivores; all subjects were white, so skin color did not alter comparisons between groups. Levels in vegans were 43% lower ($P < 0.01$) than those of omnivores and were indicative of vitamin D deficiency ($<25$ nmol/L) in six of ten subjects. Casual sun exposure during the summer was evidently insufficient to accumulate enough vitamin D to last throughout winter without a dietary source. The authors recommended that vegans consume a supplement or foods fortified with vitamin D to maintain normal serum 25(OH)D$_3$ concentrations during the winter.

Consuming a reliable source of vitamin D is also advisable for older adults, who do not synthesize vitamin D as efficiently as young adults. This is attributable to reduced production of 7-dehydrocholesterol; levels of this precursor in skin exhibit an inverse relationship with age.$^{126}$ People with darkly colored skin may benefit from exogenous vitamin D, as well. The melanin that colors their skin absorbs UVB radiation, so less radiation actually reaches 7-dehydrocholesterol (7-DHC) to convert it to previtamin D$_3$. $^{127}$ Chen et al. exposed black and Caucasian human skin samples to sunlight to measure the extent to which 7-DHC was converted to previtamin D$_3$. $^{127}$ The skin samples were excised from subjects, placed in petri dishes, and
exposed to noon sunlight on a cloudless day in June in Boston (42°N) for up to 30 minutes. In Caucasian skin, 2.78±0.09% of 7-DHC was converted to previtamin D₃; just 0.29±0.05% underwent conversion in black skin. In other words, conversion was nearly 10-fold more efficient in Caucasian skin than black skin. The researchers concluded that people with highly pigmented skin are probably unable to synthesize sufficient vitamin D, even when exposed to ideal sunlight wavelengths.

Clearly, vitamin D plays a significant role in calcium absorption from the diet. Maintenance of adequate serum 25(OH)D₃ levels is, therefore, crucial to maximizing calcium bioavailability. For vegans, natural food sources of vitamin D are not widely available. Many vegans living closer to the equator can probably synthesize sufficient vitamin D year-round with regular sun exposure. At higher latitudes, however, endogenous synthesis is inadequate during winter months. Likewise, the elderly and people with darkly-pigmented skin may not be able to meet requirements through cutaneous synthesis at any time of year. Instead, exogenous sources must be consumed to meet needs. Vegans can choose from a variety of fortified foods, such as soy milk and breakfast cereals, or use supplements. Individuals should select a source that fits their budget, is compatible with their lifestyle, and guarantees adequate vitamin D status to ensure that calcium absorption can function optimally.

C. Increase Total Intake

Calcium absorption inhibitors like oxalate and phytate are common in vegan dietary staples. However, many alternative calcium sources are available that are low in inhibitors. Identifying vegan sources of bioavailable calcium can help new vegans to plan a diet that provides adequate absorbable calcium. Consuming these foods regularly can help a vegan to meet recommendations without relying on supplements.
In a modified food guide pyramid for vegetarians and vegans, Venti and Johnston recommended that all vegetarians exceed the RDA by 20% to compensate for reduced availability. Vegans strictly obtaining calcium from unfortified foods are advised to attain this goal because most vegan sources are relatively low in calcium and consumed with inhibitors, either in the food itself or an accompanying food. In contrast, fortified foods can provide larger doses of calcium in portions normally consumed, which means the calcium load is likelier to exceed the binding capacity of inhibitors and permit greater net absorption. If vegans regularly consume fortified food, the regular RDA is likely sufficient.

Calcium is widely distributed throughout the plant kingdom but the bioavailability of this calcium for human nutrition varies depending on the concentration of inhibitors the plants contain. As previously described, phytate and oxalate are the most potent inhibitors of calcium absorption. To guide selection of calcium sources, several foods from each of four food groups – legumes, nuts and seeds, fruits, and vegetables – will be listed and categorized as highly available or poorly available, based on plants’ phytate and oxalate contents. Grains are poor sources of calcium in amounts normally consumed. In general, they contain less than 30 mg calcium per cup of cooked whole grain. Specific grain sources of calcium will not be listed because all are equally low.

**Legumes**

Legumes and legume-based products contain calcium in varying amounts. Some are particularly high in calcium, as shown in Table 4.1, but they also contain phytate. Therefore, the bioavailability of their calcium is low.
Table 4-1 Calcium in Selected Legumes

<table>
<thead>
<tr>
<th></th>
<th>Mature Beans, Boiled</th>
<th>Bioavailability</th>
<th>Sprouted Beans</th>
<th>Bioavailability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg / 1 cup&lt;sup&gt;69&lt;/sup&gt;</td>
<td></td>
<td>mg / 1 cup&lt;sup&gt;69&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Black Turtle</td>
<td>102</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Blackeye Peas</td>
<td>41</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Garbanzo Beans</td>
<td>80</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Great Northern Beans</td>
<td>120</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Kidney Beans</td>
<td>50</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lentils</td>
<td>38</td>
<td>-</td>
<td>18 (stir-fried)</td>
<td>+</td>
</tr>
<tr>
<td>Mung Beans</td>
<td>55</td>
<td>-</td>
<td>16 (stir-fried)</td>
<td>+</td>
</tr>
<tr>
<td>Navy Beans</td>
<td>127</td>
<td>-</td>
<td>20 (boiled)</td>
<td>+</td>
</tr>
<tr>
<td>Peas</td>
<td>43</td>
<td>-</td>
<td>33 (boiled)</td>
<td>+</td>
</tr>
<tr>
<td>Pinto Beans</td>
<td>82</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Soybeans,</td>
<td>175</td>
<td>-</td>
<td>55 (steamed)</td>
<td>+</td>
</tr>
<tr>
<td>Soy Products</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tempeh</td>
<td>92</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tofu*</td>
<td>204</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Natto</td>
<td>190</td>
<td>+</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Precipitated by calcium salt (calcium sulfate)

**Nuts and Seeds**

Nuts and seeds are generally not good calcium sources in amounts typically consumed. Almonds and sesame seeds are notable exceptions to this generalization. Still, nuts and seeds are high in phytate, so the amount of calcium absorbed from these foods is probably low. The calcium contents of almonds and sesame seeds are listed in Table 4.2, along with those of other nuts and seeds for comparison.
Table 4-2 Calcium in Selected Nuts and Seeds

<table>
<thead>
<tr>
<th>NUTS</th>
<th>mg /1 oz. dry roasted&lt;sup&gt;69&lt;/sup&gt;</th>
<th>Bioavailability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Almonds</td>
<td>75</td>
<td>-</td>
</tr>
<tr>
<td>Cashews</td>
<td>13</td>
<td>-</td>
</tr>
<tr>
<td>Peanuts</td>
<td>15</td>
<td>-</td>
</tr>
<tr>
<td>Pecans</td>
<td>20</td>
<td>-</td>
</tr>
<tr>
<td>Pistachios</td>
<td>31</td>
<td>-</td>
</tr>
<tr>
<td>Walnuts</td>
<td>28</td>
<td>-</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>SEEDS</th>
<th>mg /1 oz. toasted&lt;sup&gt;69&lt;/sup&gt;</th>
<th>Bioavailability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pumpkin seed kernels</td>
<td>12</td>
<td>-</td>
</tr>
<tr>
<td>Sesame seeds, whole</td>
<td>280</td>
<td>-</td>
</tr>
<tr>
<td>Sunflower seed kernels</td>
<td>20</td>
<td>-</td>
</tr>
</tbody>
</table>

**Fruits**

Fruits are relatively low in calcium per serving. However, for vegans consuming several servings daily, the cumulative contribution may be significant. Furthermore, the bioavailability of the calcium is likely good. Some examples of fruits containing calcium are listed in Table 4.3.

Table 4-3 Calcium in Selected Fruits

<table>
<thead>
<tr>
<th>Fruits</th>
<th>mg /Serving (as indicated)&lt;sup&gt;69&lt;/sup&gt;</th>
<th>Bioavailability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dried apricot halves</td>
<td>18 per ¼ c</td>
<td>+</td>
</tr>
<tr>
<td>Dried figs</td>
<td>81 per 3 figs</td>
<td>+</td>
</tr>
<tr>
<td>Dried plums (prunes)</td>
<td>20 per 5 prunes</td>
<td>+</td>
</tr>
<tr>
<td>Orange</td>
<td>52 per 1 medium (2 ½” diam)</td>
<td>+</td>
</tr>
<tr>
<td>Raisins</td>
<td>20 per ¼ c</td>
<td>+</td>
</tr>
<tr>
<td>Blackberries</td>
<td>46 per 1 c</td>
<td>+</td>
</tr>
<tr>
<td>Raspberries</td>
<td>27 per 1 c</td>
<td>+</td>
</tr>
</tbody>
</table>

**Vegetables**

Vegetables can also make significant contributions to daily calcium intakes, and their calcium is generally well-absorbed. Those containing high concentrations of oxalic acid,
however, are not dependable sources. The calcium contents of some fresh and cooked vegetables are presented in Table 4.4.

Table 4-4 Calcium in Selected Vegetables

<table>
<thead>
<tr>
<th></th>
<th>mg /Serving (as indicated)</th>
<th>Bioavailability</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RAW</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fennel bulb, sliced</td>
<td>43 per 1 c.</td>
<td>+</td>
</tr>
<tr>
<td>Rhubarb</td>
<td>105 per 1 c.</td>
<td>-</td>
</tr>
<tr>
<td>Romaine Lettuce, chopped</td>
<td>20 per 1 c.</td>
<td>+</td>
</tr>
<tr>
<td>Spinach, chopped</td>
<td>30 per 1 c.</td>
<td>-</td>
</tr>
<tr>
<td>Watercress, chopped</td>
<td>42 per 1 c.</td>
<td>-</td>
</tr>
<tr>
<td><strong>COOKED</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acorn Squash, cubed, baked</td>
<td>90 per 1 c.</td>
<td>+</td>
</tr>
<tr>
<td>Butternut Squash, cubed, baked</td>
<td>84 per 1 c.</td>
<td>+</td>
</tr>
<tr>
<td>Kohlrabi, sliced, boiled</td>
<td>41 per 1 c.</td>
<td>+</td>
</tr>
<tr>
<td>Broccoli, boiled</td>
<td>83 per 1 med. Stalk</td>
<td>+</td>
</tr>
<tr>
<td>Asparagus, boiled</td>
<td>30 per 10 spears</td>
<td>+</td>
</tr>
<tr>
<td>Brussels Sprouts, boiled</td>
<td>56 per 1 c.</td>
<td>+</td>
</tr>
<tr>
<td>Beet greens*, boiled</td>
<td>328 per 1 c.</td>
<td>+</td>
</tr>
<tr>
<td>Collard greens, boiled</td>
<td>226 per 1 c.</td>
<td>+</td>
</tr>
<tr>
<td>Okra, boiled*</td>
<td>54 per 8 (3 inch) pods</td>
<td>+</td>
</tr>
<tr>
<td>Green Beans, boiled</td>
<td>58 per 1 c.</td>
<td>+</td>
</tr>
<tr>
<td>Kale, boiled</td>
<td>94 per 1 c.</td>
<td>+</td>
</tr>
<tr>
<td>Mustard Greens, boiled</td>
<td>104 per 1 c.</td>
<td>+</td>
</tr>
<tr>
<td>Spinach, boiled*</td>
<td>245 per 1 c.</td>
<td>+</td>
</tr>
<tr>
<td>Swiss Chard, boiled*</td>
<td>102 per 1 c.</td>
<td>+</td>
</tr>
</tbody>
</table>

*While these vegetables are naturally high in oxalic acid, boiling them has been shown to decrease this inhibitor; for this reason, their calcium is believed to be bioavailable.

Other Sources

Two additional food sources do not fit into the above food groups yet can be valuable sources of calcium for vegans. These foods are black strap molasses and mineral water.

Blackstrap molasses is a byproduct of the process used to refine cane sugar. It is a densely concentrated source of minerals with a strong flavor, so it is used in small quantities.
Even in small amounts, this product provides a surprising amount of calcium: 172 mg per tablespoon. However, the bioavailability of this calcium has not been studied.

Mineral water contains dissolved minerals that naturally occur in the sources from which they are derived. The amount of calcium in mineral waters can vary depending on the source, but most contain approximately 40 mg per 8-oz serving. A 20-oz bottle of mineral water can easily add 100 mg to one’s daily calcium intake. The calcium in mineral water calcium is believed to be absorbed as well as that from milk.

**Fortified Foods**

Like zinc, a considerable fraction of the calcium in grains is lost during milling, and the losses are not customarily replaced through enrichment of refined grain products. For example, white all-purpose flour contains 19 mg whereas whole wheat flour contains 40 mg calcium per cup. However, replacing such a small loss (~20 mg per cup) would not significantly improve the value of grains as calcium sources. Neither product – refined or whole – is a good source of calcium.

In contrast, many foods are fortified with calcium in amounts that can make appreciable contributions to daily calcium intakes. A variety of foods that are acceptable to vegans are fortified with calcium, including some ready-to-eat breakfast cereals, soymilk, calcium-set tofu, and orange juice. For example, calcium-fortified cereals like Cheerios and Corn Chex provide 100 mg per one-cup serving while Total supplies 1000 mg per cup. Soymilk is often fortified with 200-300 mg calcium per cup, but not all soymilks are fortified. Consumers should read nutrition fact labels to ensure that their soymilk has been fortified, since unfortified soymilks only contain approximately 10 mg calcium per eight-ounce serving. Likewise, tofu can be a valuable source of calcium but only if it has been precipitated by a calcium salt. Firm tofu set with
calcium sulfate, for example, contains 204 mg per half cup. Finally, calcium-fortified orange juice may contain as much as 500 mg per cup.

Another food that may not be immediately recognized as “fortified” is corn tortillas. Traditionally, corn kernels are soaked in lime (calcium hydroxide) to soften the kernels before they are ground into a dough called masa, which is subsequently formed into tortillas. The resultant corn product is significantly higher in calcium than corn itself, which is a poor source of calcium. Three six-inch corn tortillas, a reasonable meal portion, supplies approximately 120 mg calcium. The bioavailability of calcium from tortillas has not been reported. If degermed masa is used to make the tortillas, then calcium is probably well-absorbed because most of corn’s phytate is concentrated in the germ. If not, phytate likely inhibits the absorption of this calcium; the degree to which absorption is inhibited, however, is unknown.

Calcium absorption from vegan meals may be impaired if inhibitors, namely oxalic acid and phytate, are present in high concentrations. By decreasing these inhibitors, vegans can maximize the calcium they absorb from their diets. However, optimal calcium absorption cannot occur unless appropriate serum levels of vitamin D are maintained. Vitamin D, thus, functions as an indirect enhancer of calcium absorption. In addition to decreasing inhibitors and obtaining adequate vitamin D, vegans can increase their total calcium intakes by choosing from a variety of natural and fortified foods. Offering these three strategies to vegans enables them to plan a diet that meets calcium recommendations while minimizing losses.
Chapter 5

Decreasing Inhibitors

As described throughout previous sections, numerous nutritious foods contain mineral absorption inhibitors. Avoiding these foods can be difficult, given that they are widespread in plant-based diets. Such restrictions could also limit the nutritional adequacy of a vegan diet by eliminating valuable sources of vitamins and other minerals. A preferable alternative is to decrease inhibitors in foods, thereby enhancing mineral availability while permitting consumption of these foods. Food processing techniques that decrease inhibitors vary in efficacy and practicality. Several methods for reducing phytate, oxalate, and polyphenols are evaluated below.

Phytate

In order to understand methods of decreasing phytate, it is important to recall some of its properties. First, phytate is heat stable. Exposing phytate to high temperatures will not destroy the compound, at least at temperatures achievable in household cooking. Second, the distribution of phytate varies between foods. Depending on where phytate is localized, mechanical removal from its source may or may not be possible. Third, phytate is a water soluble compound. Theoretically, submerging phytate-containing foods in an aqueous medium will allow simple diffusion to occur without disrupting the structure of phytate.
In addition, phytate is susceptible to enzymatic hydrolysis. Enzymes collectively known as phytases can hydrolyze phytate to lower inositol phosphates by removing phosphate groups from inositol in a stepwise fashion. Since lower inositol phosphates appear to be less inhibitory\textsuperscript{10}, phytate hydrolysis generally improves mineral availability from foods.

The optimal environment for phytase activity varies depending on their source. Wheat phytases, for instance, favor pH 5.0 at 55 °C\textsuperscript{130} while those in rye favor pH 6.0 at 45 °C.\textsuperscript{131} Pea phytases function optimally at 45 °C at a pH of 5.2.\textsuperscript{132} Brown bean phytases are most active at pH 7 at 55 °C.\textsuperscript{133} The rate of phytase-mediated hydrolysis is altered by deviations from ideal environments.

These properties – heat stability, variable distribution, water solubility, and susceptibility to hydrolysis by phytases – determine whether food preparation methods effectively reduce phytate. Methods that may reduce phytate include milling, soaking, cooking, germination, and fermentation. The relative effectiveness of these techniques will be evaluated using studies in a variety of grains and legumes, the richest sources of phytate in a vegan diet. Another method commonly used for grain preparation, bread making, will subsequently be presented because it encompasses some of these other techniques.

**Milling**

Grains generally concentrate phytate in their outer layers and germ and store very little in the endosperm. For instance, the pericarp contains 80% of a rice grain’s phytate; the aleurone layer of wheat contains 87%; and the germ of corn stores 88%.\textsuperscript{6} Therefore, phytate is substantially reduced by milling grains and discarding these fractions. Refined grain products such as low extraction (“white”) flour, degermed corn flour, and white rice primarily consist of grain endosperms and are low in phytate.
While milling effectively removes phytate from grains, it also removes most of their iron, zinc, and calcium because these minerals are concentrated in the same grain fractions that concentrate phytate.\(^6\) Consider nutrient losses in the following grains as they are processed into refined products:

Table 5-1 Mineral Losses in Milled Grains

<table>
<thead>
<tr>
<th></th>
<th>Wheat (100% extraction)</th>
<th>Whole Wheat Flour (100% extraction)</th>
<th>White Flour (66-75% extraction)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Iron</strong> (mg/100g)</td>
<td>3.5</td>
<td>1.0 – 1.3</td>
<td></td>
</tr>
<tr>
<td><strong>Zinc</strong> (mg/100g)</td>
<td>2.9</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td><strong>Calcium</strong> (mg/100g)</td>
<td>440</td>
<td>230 - 250</td>
<td></td>
</tr>
<tr>
<td><strong>Rice</strong> Brown (bran + aleurone layer removed)</td>
<td>0.88</td>
<td>0.42</td>
<td></td>
</tr>
<tr>
<td><strong>Zinc</strong> (mg/100g)</td>
<td>3.3</td>
<td>1.8</td>
<td></td>
</tr>
<tr>
<td><strong>Calcium</strong> (mg/100g)</td>
<td>120</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td><strong>Maize</strong> Whole Dehulled, Degermed, + Milled</td>
<td>2.33</td>
<td>1.08</td>
<td></td>
</tr>
<tr>
<td><strong>Iron</strong> (mg/100g)</td>
<td>2.10</td>
<td>0.44</td>
<td></td>
</tr>
<tr>
<td><strong>Calcium</strong> (mg/100g)</td>
<td>3.08</td>
<td>1.45</td>
<td></td>
</tr>
</tbody>
</table>

Therefore, refined grain products will only improve net mineral availability if they are fortified or enriched. In the United States, iron is the only mineral that is required to be added to refined grain products. Consumers must check to see whether manufacturers choose to fortify their products with zinc and calcium.

In contrast to grains, legumes concentrate approximately 90% of their phytate within their cotyledons.\(^5\) Nuts and oil seeds, like sunflower and sesame seeds, also concentrate phytate in protein bodies; a relatively small portion is contained within their hulls.\(^5\) Consequently, removing the seed coats and hulls of these foods will not significantly reduce phytate. Expressed as a percentage of total mass, phytate will actually *increase* in when these outer layers are removed. Milling is not an effective method to reduce phytate in these foods.
Soaking

Soaking exploits the water soluble property of phytate by permitting passive diffusion of the compound into soaking liquid. By hydrating phytases, soaking may also promote the water-mediated enzymatic hydrolysis of phytate.\(^7\) Determining the degree and mechanisms by which phytate content is decreased during soaking will inform preparation techniques that minimize phytate.

Whole grains are often soaked to moisten the seeds and reduce cooking times. To characterize phytate losses, Egli et al. soaked a diverse selection of grains under conditions that approximate household soaking conditions.\(^{137}\) Specifically, one-hundred gram samples of grains were soaked in 500 mL water for 16 hours at 25 °C. Only the grains were analyzed after soaking, and IP\(_3\) through IP\(_6\) were measured. Phytic acid losses, reported as the sum of IP\(_5\) and IP\(_6\) losses, ranged from no loss in several grains (including oats, sorghum, and wheat) to 29% loss in rice. Since lower inositol phosphates (IP\(_3\) through IP\(_5\)) did not accumulate substantially during soaking, the authors concluded that IP\(_6\) was either hydrolyzed by phytases to the unmeasured lowest inositol phosphates (IP\(_1\) and IP\(_2\)) and myo-inositol phosphate or leached into soaking water.\(^{137}\) Distinguishing between these two possible explanations is important because if grains are cooked in the same soaking water and consumed with this water, inhibitory inositol phosphates that simply diffused from grains may be consumed. If diffusion alone accounts for phytate losses during soaking, soaking water must be drained to get reduce phytate.

The uncertainty of the cause for phytate losses in the study by Egli et al. is partly resolved by Lestienne et al.\(^{138}\) This study analyzed phytate losses, reported as IP\(_6\), from four grains. One-hundred gram samples were individually soaked in 300 mL water for 24 hours at 30 °C and then drained for analysis. Statistically significant phytate losses, reported as the change in IP\(_6\), were observed from millet (28%), maize (21%), and rice (17%) but not from sorghum. When the
soaking water was also analyzed for IP₆, none was detected. This implies that phytases hydrolyzed all of the “lost” phytate. It remains possible that phytate diffused from the grains before hydrolysis occurred. Phytases could have leached into the soaking water along with phytates and hydrolyzed the compound in this location.¹³⁸ The authors could only conclude that hydrolysis by phytases, independently or subsequent to diffusion, explained the disappearance of phytate. Disposal of soaking water is, therefore, not necessary to get rid of IP₆. However, lower inositol phosphates in soaking water were not analyzed by Lestienne et al. If these are present in high concentrations, soaking water should be discarded to minimize mineral absorption inhibition.

Both Egli et al.¹³⁷ and Lestienne et al.¹³⁸ showed that phytate is reduced by soaking whole grains, but the decrease is relatively small. Losses are apparently greater when whole grain flours are soaked. For instance, rice flour (20 mg) soaked in water (80 mL) at 30 °C lost 60% of its summed IP₆ and IP₅ in just one hour and 98% after 12 hours.¹³⁹ Likewise, maize flour soaked in the same ratio of water to flour (4:1) for just one hour at 25 °C lost 97% of these summed inositol phosphates.¹⁴⁰ The authors of both studies hypothesized that diffusion likely accounted for most of the losses because the concentration of IP₃ did not greatly increase relative to the sum of IP₅ and IP₆, as would have occurred if phytate was hydrolyzed. Increasing the surface area of grains increases their exposure to water, so diffusion is probably more rapid and extensive in soaked flours compared to soaked grains.

Unless diffused phytate is subsequently hydrolyzed by phytases, soaking water must be decanted to obtain reduced-phytate flour. Centrifugation was used to thoroughly separate soaked flours from water in the previously mentioned studies¹³⁹-¹⁴⁰ but is impractical for home food preparation. Manually decanted flour-water mixtures are less thoroughly separated. Only about 65% of water was removed when a maize flour-water mixture (1:4 w/v) was manually decanted.¹⁴⁰ Consequently, a larger portion of phytate that had diffused from flour still remained
in the soaking water. After soaking the flour for 1 hour at 25 °C, phytate was reduced by 57% in decanted flour whereas 97% was removed by centrifugation. Thus, soaking reduces phytate in grain flours via diffusion but incomplete removal of the phytate-containing soak water limits the thoroughness of phytate reductions.

The efficacy of soaking to decrease phytate is presumably different in grains and legumes because phytate is concentrated in different parts of the kernel and seed, respectively. Recall that most phytate is close to the kernel surface of grains, in the aleurone layer; in legumes, it is concentrated in internal protein bodies within the cotyledons.

Simply soaking whole legumes in room temperature water does not appear to substantially reduce phytate. When a sample of common dried beans (variety Dore de Kirundo) was soaked in water at a ratio of 1:10 (w/v) for 12 hours at 20˚C, only a small decrease in phytate (7.02%) was observed. The decline was not statistically significant, so authors concluded that soaking was not effective. Likewise, Schlemmer et al. soaked 20-g samples of various legumes in 50 mL water at room temperature for 16 hours without detecting any change in phytate.

In contrast, Greiner and Konietzny observed a slight increase in phytate content after soaking three Brazilian Phaseolus vulgaris (common bean) varieties for 15 hours at room temperature. Sutardi and Buckle also observed an increase after soaking soybeans for 24h at room temperature. Expressed as a percentage of total dry weight, phytate increased from 1.07% to 1.69%. Sutardi and Buckle attributed the increase to reactivation of a phosphoinositol kinase that reportedly occurs in early germination. Greiner and Konietzny instead hypothesized that soaking improved the extractability of phytate during analytical procedures, thereby increasing total measured phytate rather than actually increasing phytate.

Whereas most studies reported no change or minor increases in phytate, two reported decreases in phytate after soaking smaller legumes. Samples of black grams and mung beans were soaked in water in a 1:5 ratio at 37 °C. Phytate decreased 25% and 17%, respectively, after
12 hours of soaking. It is possible that phytate diffuses more easily from smaller legumes, like grams and mung beans, since water may penetrate the phytate-containing cotyledons more extensively. Alternatively, the higher soaking temperature may have stimulated phytase activity so that hydrolysis at least partly accounted for increased losses. At a lower soaking temperature of 24 °C for 12 hours, for instance, Tabekhia and Luh measured a smaller phytate decrease of approximately 13% in soaked mung beans. Collectively, these results suggest that phytate reductions during soaking may partly depend on legume size but are also dependent on soaking conditions.

Beans prepared for household consumption are usually soaked at room temperature for 24h or less. Based on available evidence, limited degradation – if any – is attributable to soaking under these conditions, even in small legumes.

Whereas the cotyledons of whole legumes are sheltered by a seed coat, ground legumes permit extensive exposure to soaking mediums. Greater passive diffusion of phytate would logically be expected from soaked ground legumes. Mung bean flour, for instance, was soaked in 30 °C water in a 1:4 (w/v) ratio. Inositol penta- and hexaphosphate decreased by 10% after 1 hour of soaking and 47% after 6 hours. In the same study, whole mung beans soaked under the same conditions did not decrease in phytate. Legume flours likely permit greater diffusion of phytate by eliminating the seed coat barrier and increasing the exposure of phytate-containing particles to water.

Though difficult to replicate in home food preparation, ideal soaking conditions for phytate degradation have been identified for certain foods. Phytate (IP₆) from pea flour, for instance, was almost completely degraded after soaking for 10 h at 45 C at pH 7.5. Summation of all inositol phosphates with inhibitory potential (ΣIP₃ – IP₆) showed that under these conditions, 66% of all inhibitory inositol phosphates had been degraded. For brown bean flour,
ideal conditions were 55 °C at pH 7.0. After 17 hours, 98% of phytate (IP₆) had been degraded; the two most inhibitory inositol phosphates, IP₅ and IP₆, were decreased to 0.45 umol/g. Lower inositol phosphates, IP₃ and IP₄, were not degraded quickly enough to keep up with the hydrolysis of IP₃ and IP₆, so these byproducts accumulated. These lower forms are relatively benign with regard to their effects on mineral absorption, so accumulation of such products is probably not problematic.

Soaking liquids were not decanted from these flours, so none of the phytate loss was attributable to diffusion. Instead, all reported decreases represent hydrolysis caused by endogenous phytases. Given that hydrolysis was maximized at the identified conditions, it is likely that endogenous phytases are most active at these temperatures and pH levels. An alternative, or contributing, explanation put forth by Fredrikson et al. is that proteins bound to phytate become more soluble, thereby releasing phytate and making it more accessible to phytases.

**Cooking**

Phytate is heat stable, but decreases can occur during cooking due to leaching into cooking water. It is also possible that cooking could temporarily enhance phytate hydrolysis by providing a favorable temperature for phytase activity. Too much heat will ultimately inactivate these enzymes. However, the increased degradation that occurs during the short period of high phytase activity might exceed the level that would occur at consistent lower temperatures. Phytate losses in cooking can be maximized by understanding what factors enhance these two causes.
A wide range of phytate losses were observed in several dried bean varieties boiled in water for 3 hours at a 1:4 (w/v) ratio: red kidney beans, 7.7%; black-eyed peas, 13.3%; pink beans, 16.4%; and mung beans, 36.3%. In these four beans, phytate losses appeared to be greater for smaller legumes. This trend is supported by two similar studies in which samples were boiled for 90 in water at a 1:5 (w/v) ratio. In one, boiling a type of dried common bean (Phaseolus vulgaris, variety Dore de Kirundo) only decreased phytate by 8.73%. In contrast, the second study reported that more than 25% of phytate was lost when smaller mucuna beans were cooked. Boiling various other beans up to 90 minutes in water at a 1:2.5 ratio did not decrease phytate at all.

Presoaked, boiled legumes may yield the greatest phytate reductions. When Dore de Kirundo beans were soaked for 12 hours at 20 °C and then boiled for 1 hour, phytate decreased by 52.82%. Neither soaking nor cooking, alone, were nearly as effective; losses from these individual processes were just 7.02% and 8.73%, respectively.

Three Phaseolus vulgaris varieties from Brazil were presoaked for 15 hours at room temperature and boiled in the same soaking water for 2 hours. As observed by Barampama and Simard, IP₆ decreased significantly after boiling the presoaked beans; losses ranged from 20 – 24%. In contrast, lower inositol phosphates (IP₃ – IP₅) increased. Decreased IP₆ and increased lower inositol phosphates were observed after boiling presoaked beans but not after soaking, alone. Warmer temperatures likely facilitated the conversion of IP₆ to lower inositol phosphates by increasing the activity of endogenous phytases, which generally function optimally at 55 °C in beans. Since IP₅ and IP₆ were only reduced by approximately 7%, the authors concluded that boiling soaked beans would not improve mineral availability.
Mung beans were presoaked at 37 °C for 12 hours, drained, and then boiled in water “until soft”. Phytate decreased by 20%, which just slightly exceeded the losses observed from soaking alone (17%). When these legumes were cooked without presoaking, phytate decreased 15%. Black grams were soaked and cooked according to the same procedure, and phytate decreased 29%. Soaking, alone, caused a 25% decrease; cooking, alone, caused just a 5% decrease.

Hypothetically, refreshing cooking water could potentiate greater phytate losses through diffusion and phytase-mediated hydrolysis. Maintenance of a steep concentration gradient promotes passive diffusion, and removal of hydrolysis products could prevent product inhibition of phytases. Such a procedure was tested by boiling dried mucuna beans (bean to water ratio 1:5, w/v) for a total of 130 minutes, with disposal and replacement of boiling water every 40 minutes. Contrary to expectations, losses were not significantly different from those observed after 90 minutes of uninterrupted boiling. There are no known advantages of this procedure over regular boiling.

**Germination**

Extensive phytate hydrolysis occurs during germination due to heightened activity of phytases. Chickpeas, black grams, and mung beans were germinated for 48 hours and pigeon peas and soybeans were germinated for 72 hours until sprouts measured 1.5 cm in length. Phytate reductions in the decorticated legumes ranged from 37.2% (mung beans) to 65.8% (pigeon peas). The extent of phytate hydrolysis did not directly reflect the duration of the germination period. For example, chickpeas were soaked for 48 hours and phytate decreased 64.1%, whereas soybeans were soaked for 72 hours yet phytate only decreased 38.9%.
Germination conditions were uniform across all five legumes, so it is likely that phytases in different legumes were differentially active in the given environment.

To determine whether longer germination periods cause more extensive phytate reductions, a single variety of legume must be studied over increasing germination durations. Two studies tracked the decreases in phytate after 24, 36, 48, and 60 hours in black grams\textsuperscript{145} and mung beans.\textsuperscript{146} In black grams, the greatest increase in phytate hydrolysis occurred when germination was extended from 24 to 36 hours.\textsuperscript{145} An impressive 32\% of phytate had decreased after 24 hours but with just 12 additional hours, the total decrease reached 48\%. Hydrolysis appeared to taper off beyond 36 hours; at 60 hours, 54\% reduction had been attained. The decline was steadier and smaller for mung beans.\textsuperscript{146} The 27\% reduction achieved after 24 hours of germination only improved to 38\% after 60 hours. While the rate of hydrolysis in black grams peaked relatively early and then declined, no obvious pattern was detectable for mung beans. In an earlier study, when mung beans were germinated for 5 days, phytate decreased for the first three days until approximately 30\% had been hydrolyzed.\textsuperscript{147} No further decreases occurred in the remaining 2 days. Relative to black grams, phytate hydrolysis in mung beans takes longer and ultimately does not decrease much during germination.

Observing a greater diversity of legumes over a longer germination period helps to elucidate phytate degradation during germination. Tabekhia and Luh germinated black-eyed peas, red kidney beans, and pink beans for 24, 48, 72, 96, and 120 hours.\textsuperscript{147} Phytate decreased the most between 24 and 48 hours for pink beans (12.93\% to 28.03\%); 48 and 72 hours for red kidney beans (12.43\% to 33.94\%); and 72 and 96 hours for black-eyed peas (37.28\% to 71.43\%). The thoroughness of degradation attained at 120 hours also differed: red kidney beans, 35.90\%; pink beans, 46.52\%; and black-eyed peas, 77.44\%. Comparing these results demonstrates variable phytase activity in different kinds of legumes.
**Fermentation**

Fermentation in food production is induced by introducing a microorganism into a food. The microorganism metabolizes some of the nutrients and releases an acidic byproduct, often lactic acid. Fermentation can increase the activity phytases that function optimally in an acidic environment. In addition, the added microorganisms may produce phytases.

Fermented legumes are essential ingredients in miso and tempeh. Tempeh is a fermented soybean product prepared by inoculating steamed soybeans with a mold and incubating them in a warm environment. After a 24-36 hour incubation period, tempeh contained 30% less phytate than whole dried soybeans. Steaming the soybeans before inoculation probably inactivates endogenous phytases, so the decrease in phytate was primarily attributed to mold phytases. The activity of these phytases also appears to persist during storage. When the tempeh was stored for 2 weeks at 5 °C, phytate was reduced by 82% relative to dried soybeans. Based on comparisons of phytate, it is predicted that mineral availability from tempeh is superior to that from whole beans.

While tempeh is made from fermented whole beans, other foods like miso may contain fermented bean pastes. A variety of bean pastes were fermented by Chitra et al. to evaluate the efficacy of reducing phytate in beans. Pigeon peas, chickpeas, and soybeans were presoaked in room temperature water for 16 hours, and mung beans and black grams were soaked for 2 hours, before grinding the samples into pastes. The pastes were then inoculated with lactic acid-producing bacteria, incubated for 24 hours at 30 °C, and freeze-dried for analysis. Fermentation reduced phytate more effectively than pressure cooking but not quite as effectively as germination. Percent reductions ranged from 26% in mung beans to 54% in pigeon peas.
Relative to soybeans in the previous study, average phytate reductions reported by Chitra et al. were comparable. About one-third of phytate was hydrolyzed over approximately a day of fermentation. When fermented foods are refrigerated for later consumption, phytases apparently remain active to some degree, as evidenced by additional phytate reductions in 2-week aged refrigerated tempeh. Since purchased fermented foods are typically older than one day, the percentage reductions observed by Chitra et al. are likely to be substantially greater by the time the food is consumed. Therefore, fermenting iron-, zinc-, and calcium-rich legumes that contain a lot of phytate is an effective way to decrease this inhibitor and thereby improve their nutritive value.

Bread Making

Degradation of phytate can occur in even the simplest breads, composed of flour and water. Grains contain endogenous phytases that are activated by water, so phytate hydrolysis begins as soon as flour is moistened. Provision of an environment conducive to phytase activity maximizes phytate hydrolysis. To identify conditions that promote optimal activity, and thus minimize phytate in bread, studies have compared various bread making procedures.

Phytate hydrolysis during bread making has primarily been studied in three types of bread: unleavened, yeast-leavened, and sourdough. The most basic unleavened bread, or “flatbread”, is made from flour and water. In yeast-leavened breads, single-celled fungi ferment some of the dough carbohydrates and produce alcohol and carbon dioxide that flavor and leaven the bread. Sourdough breads are formed from flour and water mixed with a starter. Starters are often pieces of dough from previous batches of sourdough, but they may also be freshly prepared mixtures of flour and water (at minimum) that are allowed to ferment before mixing with
remaining sourdough ingredients. Importantly, starters feed a population of diverse microbes, including bacteria and yeasts, that are purposefully added by bakers or fortuitously occur in the air or on grains used to make flour. Bacterial fermentation produces acids that give sourdough its sour flavor, and yeast fermentation leavens the bread. These three bread making procedures demonstrate factors that affect the activity of endogenous phytases in grain flours and, thereby, reveal ways to maximize phytate hydrolysis.

Simple unleavened dough will decrease in phytate content due solely to the activity of endogenous phytases. Faridi et al., for example, combined whole wheat (100% extraction) with water and salt and allowed the mixture to rest for 3 hours around 30 °C. The resultant 22% decrease in phytate illustrates the potential to use a grain’s endogenous enzymes to reduce phytate in bread. Pita bread, for instance, is made with the same basic ingredients, so phytate losses are probably similar under the same conditions. However, modifying the environment of phytases in dough changes their activity. Identification and provision of conditions that approximate the “ideal” for a particular grain’s phytases promotes higher activity and greater losses of phytate.

Phytate hydrolysis in yeast-leavened breads is assisted by exogenous phytases from the yeast. These additional phytates expedite the reduction of phytate. The efficiency of phytate reduction in yeasted breads was assessed by Leenhardt et al. with a simple dough composed of whole wheat flour, water, salt, and fresh yeast. A second dough was prepared without yeast for comparison. Both doughs were rested at 30 °C for four hours, and samples were collected every half hour to measure phytate. After just 30 minutes, 20% and 13% of phytate had been hydrolyzed in the yeast and yeast-free doughs, respectively. By four hours, phytate levels had stabilized with a total phytate loss of 50% in yeasted dough versus 40% in yeast-free dough. Evidently, phytate hydrolysis was more rapid and thorough in dough containing yeast. The difference was small but significant, which indicates that yeast enhanced phytate degradation.
Since yeast-leavened breads are only slightly lower in phytate than unleavened breads, most of the hydrolysis is probably attributable to endogenous grain phytases.

While the recipe tested by Leenhardt et al. used fresh yeast, breads may also be leavened with dried yeast. The efficiency of these yeasts in reducing phytate differs, as shown by McKenzie-Parnell and Davies. In this study, whole wheat breads were prepared using fresh (12.5g) or active dry (7.5g) yeast. All other ingredients and procedures were identical, yet total phytate degradation was 48% in the bread made with dried yeast whereas only 30% decreased with fresh yeast. The reason for greater phytate hydrolysis in dough leavened with dry yeast is uncertain and not likely due to age, given that the fresh yeast was used within one day of purchase. Though other studies have not confirmed the observation, these results suggest that using dry yeast in place of fresh yeast may slightly improve phytate degradation.

Since yeasts contribute phytases to bread doughs, increasing the amount of yeast added to dough should logically increase phytase hydrolysis. This hypothesis was tested by doubling yeast in otherwise identical doughs. Faridi et al. compared phytate reductions in whole wheat doughs containing 1% or 2% (dry weight) fresh yeast. The percentage by which phytate decreased was only slightly greater when yeast was doubled: 38% versus 35%. Similarly, Harland and Harland compared whole wheat breads made with one or two tablespoons of dried yeast. The doughs fermented for 8 hours, and samples were analyzed every 2 hours. Neither the rate of phytate hydrolysis nor total hydrolysis differed between the doughs. Adding more yeast to bread dough is, therefore, not recommended for improving phytate hydrolysis.

When flours are hydrated, endogenous phytases will begin to hydrolyze phytates. The rate of degradation varies under different fermentation conditions. Altering the temperature and pH, in particular, affect the activity of phytases in the dough. Fretzdorff and Brümmer compared
whole wheat bread doughs prepared in various ways to identify the conditions under which phytate hydrolysis was maximized. Samples of whole wheat dough were fermented for 4 hours in temperatures ranging from 25 to 75 °C. The greatest amount of phytate degradation occurred when fermentation was carried out at 55 °C. Phytases were less active at lower temperatures, and higher temperatures inactivated the enzymes. Warm environments are recommended for allowing breads to rise, but bakers seeking to maximize phytate hydrolysis in wheat breads should avoid exceeding 55 °C.

Phytases are also pH-sensitive and, consequently, are affected by ingredients that make dough too acidic or alkaline. Baking soda, for instance, increases dough alkalinity. The effect of adding baking soda to bread (whole wheat flour, water, and salt) was tested at two levels: as 0.2% or as 0.4% of dry weight. Phytate hydrolysis decreased significantly when baking soda was added. Whereas 22% was hydrolyzed in bread without baking soda, only 10% decreased with baking soda addition at 0.2% dry weight; even less (7%) decreased with 0.4% baking soda. Similarly, excessively acidic dough inhibits phytase activity. Fretzdorff and Brümmer acidified whole wheat dough with different amounts of lactic, citric, or tartaric acids to compare of phytate degradation. When water, alone, was added to the dough, four hours of fermentation at 30 °C produced dough in which the final pH was 6.0 and approximately 30% of phytate was degraded. More acidic dough (pH 4.5) allowed the greatest reductions to occur; phytate was almost completely degraded in the same period. However, further increases in acidity (pH 3.5-3.9) inhibited phytate degradation. Thus, a pH of 4.5 was determined to be the ideal pH for wheat phytase activity.

While Fretzdorff and Brümmer clearly showed an effect of pH on phytate hydrolysis, their utilization of several different acids provoked questions regarding the importance of the acid source. A simple procedure used by Türk et al. resolved this uncertainty by comparing phytate
Mildly acidified dough is created by bacterial fermentation during preparation of sourdough bread. Leenhardt et al., for example, prepared whole wheat sourdough bread by mixing the flour, water, and salt with a starter containing *Lactobacillus brevis*. The starter was incubated at 30 °C for 24 hours before mixing with the rest of the dough ingredients, so lactic acid had already accumulated in the starter and thereby created dough with an initial pH of 5.6. Since the pH was closer to the ideal wheat phytase pH (~4.5), phytate hydrolysis proceeded more quickly and thoroughly in sourdough compared to yeasted or yeast-free whole wheat dough lacking an acid source. After 30 minutes of fermentation at 30 °C, 36% of phytate had been degraded in sourdough bread; only 20% and 13% had been degraded in yeasted and yeast-free bread, respectively. By the end of the four-hour proofing period, more than 65% had been degraded in sourdough, compared to approximately 50% in yeasted and almost 40% in yeast-free dough. Thus bacterial fermentation proved to be even more effective than yeast for decreasing phytate.

Just as yeasts contributed phytases to yeast-leavened breads, it is probable that phytate hydrolysis in sourdough breads may be assisted by bacterial phytase. To determine whether phytase activity in bacteria was partly responsible for enhanced phytate reductions in sourdough bread, Leenhardt et al. compared sourdough to a dough that contained identical amounts of whole...
wheat flour, water, and salt but lacked bacteria. Lactic acid was added to this dough to equate the final concentration present in fermented sourdough, at which the pH was 5.25. Neither the rate of degradation nor final degradation achieved were significantly different from those observed in true sourdough bread. The same results were achieved independently of bacteria. Therefore the acid byproduct of bacteria, rather than bacteria per se, is responsible for enhancing phytate hydrolysis in sourdough breads.

Evidence clearly shows that adding yeast and adding bacteria independently promote phytate hydrolysis when dough is proofed. However, their effects are not additive, as shown by Lopez et al. The researchers added yeast to a sourdough bread recipe and observed phytate hydrolysis over a 5-hour fermentation period at 30 °C. Phytate losses in regular sourdough and yeasted sourdough breads were not significantly different.

The rate and thoroughness of phytate degradation in any of the bread making procedures described is affected by the consistency of the flour. Coarsely ground flours, sometimes called “meals”, are less vulnerable to phytate degradation than finer flours. This was demonstrated through experiments by Fretzdorff and Brümmer, in which doughs based on whole wheat flour or coarse meal were compared. A simple dough composed of whole wheat flour or meal and water was rested for four hours at 30 °C, and phytate was measured to determine losses. Whereas approximately 30% of phytate was degraded in flour-based dough, almost no degradation occurred in the meal-based dough. Similarly, when equal amounts of lactic acid were added to both doughs to facilitate hydrolysis, phytate reductions were greater in the flour-based dough. After four hours, nearly all of the phytic acid had been degraded in the flour dough while 40-50% still remained in the meal dough. These differences are probably explained by the distribution of phytate and phytases in grains. Both phytate and the endogenous enzymes that degrade them are localized in aleurone cells. Finely grinding grains produces tiny fractions of this layer, thereby
increasing their exposure to water. Since hydration activates endogenous phytases, greater exposure to water facilitates the activation of phytases. Coarser meals, in contrast, contain large fragments of grain; hydration of phytases in these fragments is delayed until water seeps into them.

Breads made solely from coarse meal are uncommon, but a portion of flour is sometimes substituted with meal for texture. If maximum phytate degradation is desired, dough should be entirely made from flour. Whole grain flours and meals contain equivalent nutrients, but the nutrient availability of breads made from them is theoretically greater when flour is exclusively used. Hydrated flour permits more extensive phytate hydrolysis than meals, so a greater proportion of its minerals remain unbound and absorbable when consumed.

Phytate hydrolysis during bread making has yet so far been described in whole wheat-based dough. Wheat flour predominates in American breads but other grains may be combined with wheat flour or used exclusively. Importantly, phytases in other grains are not equivalent to wheat phytases; “ideal” conditions for phytase activity, therefore, can vary between grains. Both rye and wheat phytases, for instance, function optimally within a pH range of 4.3 – 4.6 at 55 ºC.155 Rye phytase, however, appears to exhibit continued activity at higher temperatures. Whereas wheat phytase activity was substantially lower at 65 ºC, rye phytase was still highly active.155 In contrast to wheat and rye phytases, oat phytase is most active at 37 – 40 ºC.158 Oat phytases, however, are denatured during steam-treatment of oats, a standard processing technique that delays rancidity.
Oxalic Acid

Heat does not degrade oxalate, so dry cooking methods like baking or roasting will not reduce the oxalate content of foods. However, heating oxalate-containing foods in an aqueous medium can facilitate reductions attributable to leaching. The water soluble portion of oxalate is most easily extracted by boiling vegetables in excess water. Since oxalate is not degraded in the process, the cooking liquid will contain any “lost” oxalate and should be discarded if reduced oxalate intake is desired.

The degree of oxalate reduction caused by boiling varies between vegetables and depends on cooking duration. For instance, spinach and Swiss chard are among the most concentrated commonly consumed sources of oxalate. Boiling these leaves for 12 minutes led to soluble oxalate reductions of 87% in spinach and 84% in red Swiss chard. Delicate spinach and Swiss chard leaves are not usually cooked for such a long duration. A more reasonable boiling time, 2 minutes, was still highly effective, reducing soluble oxalate by 66% in spinach; with 6 minutes of boiling, 53% was lost from Swiss chard. This suggests that in leafy vegetables, diffusion of oxalate occurs readily. Prolonged boiling times may not substantially enhance oxalate reduction in such foods.

Firmer vegetables are usually boiled for longer periods of time to soften their texture. Oxalate losses were, therefore, analyzed after cooking them for realistic durations. For example, rhubarb stalks lost 61% of soluble oxalates when they were boiled for 15 minutes. Similarly, Savage et al. reported a loss of 72% after just 10 minutes of boiling. The reason for the difference between these results is not immediately evident but may be a consequence of different measurement techniques or unique characteristics of the particular rhubarb plants that were studied in each experiment. Even though the exact measured values are different, both studies
indicate that a substantial proportion of soluble oxalate can be drawn out of firm vegetables by boiling them.

Interestingly, insoluble oxalates can also decrease slightly during boiling. Nearly 37% of insoluble oxalate decreased in Swiss chard leaves after 6 minutes of boiling, while just 3% was lost from rhubarb after 15 minutes and none was lost from spinach boiled for 2 minutes. Chai and Liebman also observed insoluble oxalate losses in Swiss chard (36%) and rhubarb (5%) but not spinach. When cooking water from spinach was also analyzed, the lack of insoluble oxalate losses was confirmed; all oxalate was present in the soluble form. Apparently, insoluble oxalates in certain vegetables, like spinach and rhubarb resist leaching during boiling. However, since oxalic acid is already bound to a mineral in this form, it cannot bind dietary calcium from other sources in the diet.

In contrast to boiling, steaming eliminates relatively little oxalate. Steamed spinach lost half as much soluble oxalate as spinach boiled for an equivalent 12-minute duration; losses were 42% and 87%, respectively. Red Swiss chard lost 14% when steamed for 12 minutes whereas 84% was lost during boiling. Insoluble oxalate did not decrease in either of these vegetables when they were steamed. All oxalate was recovered from the steaming water, suggesting oxalate leached into water droplets on the steaming vegetables. Not surprisingly, vegetables that were submerged in boiling water leached more oxalate than those that were merely coated with water droplets. Boiling is clearly a more effective cooking method than steaming for reducing oxalate from vegetables.

While boiling is preferable for reducing oxalate, it also reduces water-soluble vitamins. The extent of losses was illustrated by Pingle and Ramasastri, who boiled amaranth leaves in excess water for 15 minutes. Substantial amounts of riboflavin (87%), folic acid (86%), and ascorbic acid (94%) were lost due to leaching into cooking water. Calcium, in contrast, was
preserved. Since vegan diets typically contain ample sources of the vitamins most vulnerable to leaching, boiling high-oxalate vegetables will not likely compromise the nutritional adequacy of the overall vegan diet. Furthermore, by reducing oxalate and preserving calcium, boiling can improve vegans’ net calcium absorption. For vegans consuming a varied diet rich in fruits and vegetables, the beneficial preservation of calcium absorption is likely outweighs concerns about reduced water-soluble vitamins. Thus, boiling is recommended for cooking high-oxalate foods in a vegan diet.

**Polyphenols**

The inhibitory effects of polyphenols on iron absorption are well documented. Logically, reducing the polyphenol content of foods should improve the bioavailability of dietary iron. Research has not evaluated this hypothesis yet so far. Perhaps interest in such a topic is lacking because polyphenols are more commonly regarded as desirable components of the diet. In a vegan diet, polyphenols are abundantly supplied; decreasing their concentrations in certain foods could potentially improve iron absorption while offering polyphenols from the rest of the diet.

One of the best targets for reducing polyphenol concentrations is beans. Several studies have measured losses of total phenolic compounds, reported as gallic acid equivalents, in beans after soaking and boiling under typical household cooking conditions. Xu and Chang, for example, soaked pinto beans and black beans in room temperature tap water at a 1:3 weight/volume ratio for 2.3 hours and 4 hours, respectively.\(^{162}\) The soaked pinto and black beans were subsequently boiled for 90 and 80 minutes, respectively. Soaking and cooking times were individualized to the beans to obtain desirable hydration (50%) after soaking and texture (beans yield to tactile pressure) after cooking. The beans were then analyzed for total phenolic contents
and compared, on a dry weight basis, to their raw forms. Losses were comparable in both beans, with a 63% reduction observed in pinto beans and 61% observed in black beans. These losses were statistically significant ($p < 0.05$) and illustrated substantial losses that can occur with cooking.

Another study by the same authors compared decreases in total phenolic content in yellow and black soybeans. The yellow and black soybeans were soaked in room temperature tap water at a 1:3 weight/volume ratio for 16 hours and 2.5 hours, respectively, to achieve hydration ratios of 100% and 50%. (The lower ratio was preferred for black soybeans for the sake of conserving polyphenols, since the goal of the study was to evaluate losses under normal cooking conditions rather than to maximize polyphenols losses.) Subsequently, both beans were boiled for 120 minutes. Even though yellow soybeans were soaked longer than black soybeans, losses were greater in the latter beans: 27.4% versus 62.4%. Yellow soybeans naturally contain fewer phenolic compounds than black soybeans (2.15 vs. 6.96 mg gallic acid equivalents/g), and the types they contain are different. This fact is obvious from the strikingly color differences in their seed coats. The darkly colored soaking water from black soybeans evidenced leaching of soluble phenolic compounds from their skins. The phenolic compounds responsible for their color, anthocyanins, are evidently prone to leaching into surrounding water and contribute to the overall losses measured in soaked, boiled black soybeans. Even with these losses, the total phenolic content of yellow soybeans was still lower than that of black soybeans after processing: 1.56 mg versus 2.62 mg GAE/g. Thus, choosing lighter colored beans is a simpler way to consume fewer phenolic compounds, regardless of the cooking method used.

It is important to reiterate that the effect of reducing polyphenol concentrations on iron absorption has not been evaluated. Therefore, this strategy is only recommended for foods that are not compromised by such treatment. Beans are customarily presoaked and boiled, so this
polyphenol-reducing strategy is easily applied without changing usual preparation methods. Similar decreases might also be achieved by soaking and boiling grains, which are also customarily prepared in this way. However, this technique is not recommended for softer fruits and vegetables, which would develop an unpleasant texture with prolonged cooking.

Various food preparation methods present opportunities to decrease inhibitors in foods. Decreasing concentrations of inhibitors should improve mineral absorption from these foods as well as foods with which they are consumed. Informing new vegans about these techniques enables them to consume foods with high concentrations of inhibitors while preserving mineral absorption. Vegans can thereby select a nutritious diet that supplies adequate absorbable iron, zinc, and calcium without completely eliminating foods that are high in phytate, oxalic acid, or polyphenols.
Chapter 6

Developing an Educational Guide

In previous sections, factors affecting iron, zinc, and calcium absorption have been described along with strategies to their bioavailability. This valuable information needs to be translated into a form that is easily comprehensible so that people can employ these strategies. To convey the information to the public, an educational guide has been developed with the following goals in mind:

*Use simple, non-scientific language.*

The target audience of this guide is new vegans who prepare foods for themselves. The range of ages included in this group is potentially wide, spanning from teenagers to aged adults. The educational background of such an audience is also diverse. To communicate effectively with an audience that varies widely in age and educational background, scientific terms should be avoided unless they are accompanied by a simple definition.

*Translate recommendations into actual foods and food preparation methods.*

Providing examples of foods that contain inhibitors and enhancers, and ranking the bioavailability of their minerals, can equip vegans with the knowledge to plan a diet that suits their individual preferences while meeting nutritional recommendations. Food preparation methods, likewise, should be described in relation to the specific foods for which they are recommended.
Present information in a useable, visually-appealing format.

A guide that is too detailed or visually dense with words may deter people from reading the information. Including white space and presenting information as bullet points, rather than paragraphs, can make the guide less overwhelming. Pictures and charts also increase its aesthetic appeal and usability, respectively.

Designing the guide to meet these goals ensures that new vegans are able to understand and use the strategies that improve mineral bioavailability.
Plants contain all the minerals our bodies need to keep us healthy. A vegan diet can supply these minerals in the amounts we need without requiring supplements. However, most Americans rely on animal products to help meet recommended mineral intakes. In particular, iron, zinc, and calcium are largely derived from animal sources like meats, seafood, and dairy products. As a new vegan, it is important to find alternatives to replace animal sources.

Finding good sources of iron, zinc, and calcium is not as simple as comparing nutrition facts labels. Foods that appear to be high in these nutrients may not actually be good sources if their minerals cannot be absorbed. Substances called "inhibitors" can attach to minerals in the foods you eat and prevent your body from absorbing them. If foods contain high concentrations of inhibitors or are eaten with other foods that contain them, their minerals will not be absorbed well.

By using strategies that reduce the effect or concentration of inhibitors, like adding enhancers or decreasing inhibitors, these foods can become valuable mineral sources.

This guide identifies sources of minerals, their inhibitors and enhancers, and food preparation methods to help you absorb more iron, zinc, and calcium from your vegan diet.

### Food Preparation Methods

The amount of inhibitors in some foods can be decreased during the processes which they are prepared. Getting rid of some inhibitors helps your body absorb more minerals.

- **Soak, drain, and boil dried beans.**
  Soaking beans overnight (12-24 hours) before boiling them not only reduces their cooking time but also decreases inhibitors. The inhibitors move into the soaking and cooking water, so it is important to get rid of this water.

- **Roll and drain high oxalic acid vegetables.**
  When these foods are boiled, oxalic acid moves into the cooking water. By draining the water, you will decrease the amount you eat and increase calcium absorption.

  **NOTE:** Boiled vegetables also lose water-soluble vitamins, like vitamin C and riboflavin (B2). Ensure that you are consuming enough of these vitamins by choosing a variety of fruits and vegetables prepared in different ways (e.g., raw, steamed, stir-fried, etc.).

- **Eat fermented beans and grains.**
  Fermentation decreases the amount of phytate in foods so that more iron, zinc, and calcium can be absorbed. Fermented bean and grain foods, like miso and tempeh, are easily found in many well-stocked supermarkets.

- **Germinate beans, lentils, and grains.**
  Like fermentation, germination decreases phytate so that iron, zinc, and calcium absorption is improved. You can germinate your own seeds or buy them as fresh sprouts or sprouted products like breads, tortillas, flours.

- **Make bread from whole grain flours.**
  Whole grain flours are high in phytate, but the amount can be decreased when it is processed into bread. Phytate is decreased the most when bread dough is allowed to rest for long periods of time at warm temperatures. Traditional sourdough breads also lose more phytate than regular yeast-leavened breads.

  Most breads in grocery stores — even “artisan” breads — are leavened for relatively short periods of time. For substantial phytate losses to occur, the bread should be leavened for at least 12 hours. Ask your local bakers how they make their breads, and buy those that are leavened for the longest periods of time. If you make your own bread, allow dough to rest overnight or throughout the day in a warm place in your kitchen.

### Inhibitors and Enhancers Table

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Sources</th>
<th>Minerals Affected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phytate</td>
<td>Whole grains, beans and lentils, nuts and seeds</td>
<td>Iron, zinc, calcium</td>
</tr>
<tr>
<td>Oxalic Acid</td>
<td>Spinach, rhubarb, sweet potatoes and yams, beets, chocolate, black tea, coffee</td>
<td>Iron* zinc* calcium</td>
</tr>
<tr>
<td>Polyphenols (plant pigments naturally found in fruits and vegetables)</td>
<td>Almost all fruits and vegetables, tea, coffee, wine, whole grains, beans and lentils, nuts and seeds</td>
<td>Iron</td>
</tr>
</tbody>
</table>

*TIP: To absorb more minerals, avoid drinking tea or coffee within an hour of meals. Reserve these beverages for between meals.

For example, you can add absorption enhancers to meals. Enhancers have the opposite effect of inhibitors. That is, they increase mineral absorption. Enhancers include:

- Enhancers that increase iron and zinc absorption:

<table>
<thead>
<tr>
<th>Enhancer</th>
<th>Sources</th>
<th>Mineral Affected</th>
<th>Suggested Combinations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin C (ascorbic acid)</td>
<td>Broccoli, cauliflower, bell peppers, strawberries, oranges</td>
<td>Iron</td>
<td>Black bean tacos + diced red bell pepper</td>
</tr>
<tr>
<td>Citric Acid</td>
<td>Lemons, limes, grapefruit, oranges</td>
<td>Iron</td>
<td>Oatmeal + orange juice</td>
</tr>
<tr>
<td>Other Organic Acids (malic, tartaric)</td>
<td>Apples, grapes, tomatoes, pineapple</td>
<td>Iron</td>
<td>White beans + tomato sauce</td>
</tr>
</tbody>
</table>
The Basics

The Institute of Medicine establishes nutrient intake recommendations in the United States. Current recommendations for vegetarians are listed below:

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ages 19-50</td>
<td>14 mg</td>
<td>33 mg</td>
</tr>
<tr>
<td>Ages 50+</td>
<td>14 mg</td>
<td>14 mg</td>
</tr>
<tr>
<td>Zinc</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ages 19+</td>
<td>11 mg</td>
<td>8 mg</td>
</tr>
<tr>
<td>Ages 50+</td>
<td>1000 mg</td>
<td>1000 mg</td>
</tr>
<tr>
<td>Calcium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ages 19-50</td>
<td>1200 mg</td>
<td>1200 mg</td>
</tr>
</tbody>
</table>
| Choose from a variety of foods to supply these recommendations. An example:

<table>
<thead>
<tr>
<th>Grains</th>
<th>Serving</th>
<th>Iron (mg)</th>
<th>Zinc (mg)</th>
<th>Calcium (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barley, pearled*</td>
<td>1 cup (cooked)</td>
<td>2.1</td>
<td>1.3</td>
<td>15</td>
</tr>
<tr>
<td>Brown Rice *</td>
<td>1 cup (cooked)</td>
<td>1.0</td>
<td>1.2</td>
<td>20</td>
</tr>
<tr>
<td>Bulgar Wheat</td>
<td>1 cup (cooked)</td>
<td>1.8</td>
<td>1.0</td>
<td>20</td>
</tr>
<tr>
<td>Beans/Lentils</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brown Lentils*</td>
<td>1/4 cup (cooked)</td>
<td>8.3</td>
<td>1.0</td>
<td>15</td>
</tr>
<tr>
<td>Kidney Beans*</td>
<td>1/4 cup</td>
<td>2.6</td>
<td>1.0</td>
<td>25</td>
</tr>
<tr>
<td>Navy Beans*</td>
<td>1/4 cup (cooked)</td>
<td>2.3</td>
<td>1.0</td>
<td>65</td>
</tr>
<tr>
<td>Nuts &amp; Seeds</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Almonds*</td>
<td>1 oz.</td>
<td>1.3</td>
<td>1.0</td>
<td>75</td>
</tr>
<tr>
<td>Cashews*</td>
<td>1 oz</td>
<td>1.7</td>
<td>1.6</td>
<td>15</td>
</tr>
<tr>
<td>Pumpkin Seeds*</td>
<td>1 oz.</td>
<td>4.2</td>
<td>2.1</td>
<td>10</td>
</tr>
<tr>
<td>Fruits</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dried Apricots</td>
<td>1/4 cup</td>
<td>0.9</td>
<td>0.1</td>
<td>20</td>
</tr>
<tr>
<td>Dried Figs</td>
<td>3 figs</td>
<td>0.3</td>
<td>0.3</td>
<td>80</td>
</tr>
<tr>
<td>Dried Peaches</td>
<td>5 prunes</td>
<td>1.1</td>
<td>0.2</td>
<td>20</td>
</tr>
<tr>
<td>Vegetables</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kale</td>
<td>1 cup (cooked)</td>
<td>1.2</td>
<td>0.3</td>
<td>95</td>
</tr>
<tr>
<td>Butternut Squash</td>
<td>1 cup (cooked)</td>
<td>1.2</td>
<td>0.3</td>
<td>84</td>
</tr>
<tr>
<td>Green Beans</td>
<td>1 cup (cooked)</td>
<td>1.6</td>
<td>0.3</td>
<td>55</td>
</tr>
<tr>
<td>Soy Foods</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tempeh</td>
<td>3 oz</td>
<td>2.3</td>
<td>1.0</td>
<td>95</td>
</tr>
</tbody>
</table>

*Iron and zinc from these foods are not absorbed well; consume with enhancers or prepare in ways that reduce inhibitors, as described. Source: Data from USDA National Nutrient Database for Standard Reference, Release 28, 2016.

Foods to Try

A few foods are uncommon in most American diets yet contain impressive amounts of absorbable minerals. Consider trying these:

- Nutritional Yeast: 3.2 mg Iron per 1.5 T
- Mineral Water: ~45 mg Calcium per cup
- Blackstrap Molasses: 170 mg Calcium, 3.5 mg Iron per 1T

Begin Today!

It's simple to plan a vegan diet that maximizes iron, zinc, and calcium absorption. Start by identifying a variety of mineral-rich foods to keep on hand for meals and snacks. Then, pair them with enhancers to improve your absorption of these minerals. You can also prepare foods in ways that decrease the inhibitors they contain or buy foods prepared by such methods.

Here are some foods and recipes to get you started:

Make It...

- **Bread**
  Whole wheat bread that is fermented for 12+ hours and requires minimal effort and no kneading!
- **Sprouts**
  How to safely sprout a variety of grains, beans, and lentils
  [http://www.primal-swede.org/sprouting.htm](http://www.primal-swede.org/sprouting.htm)

Or Buy It...

- **Sprouted Products**
  Flours made from germinated grains
  [http://www.organiclate.com/sprout.htm](http://www.organiclate.com/sprout.htm)
  Breads made from sprouted grains and lentils
  Tofu made from sprouted soybeans
- **Fermented Foods**
  Tempeh and miso are available in most supermarkets and online

Learn More

Find more recipes and advice for following a vegan diet by visiting the American Dietetic Association (ADA) Vegetarian Nutrition dietetic practice group website:
[http://vegetariannutrition.net/](http://vegetariannutrition.net/)

Mind Your Minerals:

Iron, Zinc, & Calcium in a Vegan Diet

**Figure 6-2 Brochure exterior**
The standard American diet is based on animal products, such as meat and dairy, and refined grain products. While such a diet is linked to increasing rates of obesity, type 2 diabetes, and heart disease, it typically provides adequate iron, zinc, and calcium to meet recommended levels of intake established by the Institute of Medicine. Meats and seafood are rich sources of bioavailable iron and zinc, as are refined grain products enriched or fortified with these minerals. Calcium supplied by dairy products is not more bioavailable than most plant sources, except for those containing oxalic acid. However, calcium is more densely concentrated in dairy than in plants, so net absorption per serving is generally higher from dairy products. A standard American diet including animal products and enriched refined grain products is unlikely to be deficient in such minerals unless caloric intake is not severely restricted.

Vegan diets, in contrast, eliminate all animal products and replace them with plant protein sources, like beans, nuts, and seeds. Vegetable, fruit, and whole grain consumption is also higher among most vegans than omnivores. Mineral absorption inhibitors like phytate, oxalic acid, and polyphenols are present in much higher concentrations in these foods than in animal products or refined grains. Consequently vegans may absorb a smaller proportion of iron and zinc, and potentially calcium, from their diets than omnivores. To compensate for reduced absorption, higher total intakes from foods are recommended so that net absorption is sufficient to meet physiological requirements. Alternatively, or in conjunction with increased total intake, vegans can decrease inhibitors and consume more absorption enhancers at meals rich in these minerals. As evidenced by the described research studies, these strategies are feasible and effective.
When Americans transition from a standard American diet to a vegan diet, it is important to educate them about potential nutritional deficiencies and how to avoid them. Most advice for new vegans merely focuses on replacing eliminated animal products with plant substitutes without considering differences in the bioavailability of nutrients from these sources. Therefore, a guide has been developed to address both total intakes and bioavailability of three minerals – iron, zinc, and calcium – that are susceptible to inhibitors in a vegan diet.

The guide explains how to increase intake and absorption of iron, zinc, and calcium from a vegan diet. It is visually attractive and written using simple, non-scientific language to extend its usability to a wide range of ages and educational backgrounds. By presenting a variety of food sources and cooking methods, the guide enables vegans to customize their diet to fit individual preferences and lifestyles.


49. Laparra JM, Glahn RP, Miller DD. Bioaccessibility of phenols in common beans (*Phaseolus vulgaris* L.) and iron (Fe) availability to Caco-2 cells. *J Agric Food Chem.* 2008;56:10999-11005.


139. Perlas LA, Gibson RS. Use of soaking to enhance the bioavailability of iron and zinc from rice-based complementary foods used in the Philippines. *J Sci Food Agric.* 2002;82;1115-1121.
113


VITA

Valerie Sullivan

Education

8/2007-Present: The Pennsylvania State University, University Park, PA
  Major: Nutritional Sciences
  Minor: Psychology
  Honors: Nutritional Sciences

Work Experience and Professional Membership

9/2010-Present: Member of the American Dietetic Association

10/2010-Present: Volunteer, Nutrition and Food Services
Mount Nittany Medical Center, State College, PA
  • Visit patients to assess appetite, food tolerance, and unexplained weight changes
  • Record observations and enter notes into computerized patient records
  • Call discharged patients to survey patient satisfaction regarding food service

1/2010-12/2010: Undergraduate Research Assistant
The Center for Childhood Obesity Research
The Pennsylvania State University, University Park, PA
  • Recruited subjects and obtained informed consent for child eating behavior research
  • Conducted tasting trials with children, following research protocol
  • Weighed and recorded food consumption data

1/2010-12/2010: Teaching Assistant
The Pennsylvania State University, State College, PA
  • Assisted lecture component of two Nutrition department courses, Food Preparation (NUTR 120) and Nutrition Assessment (NUTR 358)
  • Led review sessions to answer students’ questions and reiterate key concepts
  • Evaluated exams, class activities, and projects with professor
The Pennsylvania State University, Abington, PA
• Assisted one Fall and two Spring semester courses, including an introductory Nutrition class and a multicultural communication class
• Communicated weekly with students via Webmail to engage in class content

Dance Dynamics, Huntingdon Valley, PA
• Identified and corrected mistakes to cultivate age-appropriate dance technique
• Communicated student needs with teacher
• Facilitated progress toward spring performance

Summers 2008-2009: Summer Day Camp Counselor
Carson-Simpson Farm Christian Center, Hatboro, PA
• Supervised approximately 15 preschool and kindergarten-aged children
• Delegated appropriate responsibilities to teenage assistants and evaluated performance

Awards/Honors
2011: College of Health and Human Development Alumni Recognition for Student Excellence Award
2010-2011: Evan Pugh Scholar Award
2009: The President Sparks Award
2008: The Freshman Award
2007: The United States Congressional Award Gold Medal