Bioavailability of iron, zinc, and other trace minerals from vegetarian diets

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ABSTRACT  Iron and zinc are currently the trace minerals of greatest concern when considering the nutritional value of vegetarian diets. With elimination of meat and increased intake of phytate-containing legumes and whole grains, the absorption of both iron and zinc is lower with vegetarian than with nonvegetarian diets. The health consequences of lower iron and zinc bioavailability are not clear, especially in industrialized countries with abundant, varied food supplies, where nutrition and health research has generally supported recommendations to reduce meat and increase legume and whole-grain consumption. Although it is clear that vegetarians have lower iron stores, adverse health effects from lower iron and zinc absorption have not been demonstrated with varied vegetarian diets in developed countries, and moderately lower iron stores have even been hypothesized to reduce the risk of chronic diseases. Premenopausal women cannot easily achieve recommended iron intakes, as modified for vegetarians, with foods alone; however, the benefit of routine iron supplementation has not been demonstrated. It may be prudent to monitor the hemoglobin of vegetarian children and women of childbearing age. Improved assessment methods are required to determine whether vegetarians are at risk of zinc deficiency. In contrast with iron and zinc, elements such as copper appear to be adequately provided by vegetarian diets. Although the iron and zinc deficiencies commonly associated with plant-based diets in impoverished nations are not associated with vegetarian diets in wealthier countries, these nutrients warrant attention as nutritional assessment methods become more sensitive and plant-based diets receive greater emphasis. 

IRON BIOAVAILABILITY FROM A VEGETARIAN DIET

Eliminating meat from the diet can be accomplished with minimal effect on the total dietary iron content. In Western countries, vegetarian diets can contain as much or more iron than mixed diets containing animal flesh (2–4). For example, Calkins et al (2) reported the iron contents of vegan, lactoovegetarian, and nonvegetarian diets of a control group of non–Seventh-day Adventists, and nonvegetarian diets of Seventh-day Adventists, and nonvegetarian diets of a control group of non–Seventh-day Adventists, as (3 ± SE) 18.0 ± 1.6, 14.2 ± 0.8, 14.4 ± 0.9, and 16.1 ± 1.1 mg Fe/d, respectively, when assessed by a 3-d food record. The total iron content of a diet, however, provides little information about its content of bioavailable iron, which is considerably influenced by the foods in the diet and can vary 10-fold from different meals of similar iron content (5). Although a vegetarian diet is likely to contain iron in amounts equivalent to amounts in a nonvegetarian diet, the iron from a vegetarian diet is likely to be substantially less available for absorption (6) because of differences in the chemical form of iron and the accompanying constituents that enhance or inhibit iron absorption [Figure 1, with data from Hunt et al (7–9)].

The chemical form of iron is an important factor affecting the iron availability of vegetarian diets. Less than 40% of the iron in meat, poultry, and fish (10) is in the heme form, which is more efficiently absorbed than the remaining nonheme iron present in these and all other foods (11–15). Nonvegetarian diets with substantial amounts of red meat supply about 2 mg/d, or 10–12%, of the total iron in the heme form (8); in comparison, diets based on poultry or fish contain less heme iron, roughly in proportion to the decrease in total iron content, and vegetarian diets contain no heme iron. Heme iron is better absorbed (∼15–40%) than non-heme iron (∼1–15%) (11–15). Both forms are absorbed in inverse

INTRODUCTION

The trace elements iron and zinc merit special attention when evaluating the nutritional adequacy of vegetarian (ie, strongly plant-based) diets. Although plant foods tend to be rich sources of trace elements such as copper, manganese, and iron, animal products provide most of the zinc in US diets (1), and meat, poultry, and fish provide some iron in the highly bioavailable heme form. The bioavailability of dietary iron and zinc can be reduced considerably by the phytic acid and possibly other constituents of some plant foods. Because these factors may be especially important in vegetarian diets, this paper will review nutritional concerns about trace element bioavailability from vegetarian diets, with emphasis on iron and zinc.

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2 Presented at the Fourth International Congress on Vegetarian Nutrition, held in Loma Linda, CA, April 8–11, 2002.
3 Names are necessary to report factually on available data; however, the US Department of Agriculture neither guarantees nor warrants the standard of the product, and the use of the name by the US Department of Agriculture implies no approval of the product to the exclusion of others that may also be suitable. The US Department of Agriculture, Agricultural Research Service, Northern Plains Area, is an equal opportunity/affirmative action employer and all agency services are available without discrimination.
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vegetarian (/H17039 the trace element bioavailability of experimental nonvegetarian (/H11623 (5, 17, 18; in inhibit the intestinal solubility and absorption of nonheme iron can substantially alter the dietary components that enhance or whole-grain rather than refined-grain products. Such diet choices consumption of dried beans and legumes, fruit and vegetables, and elimination of meat may be accompanied by increased con-

widely as that of nonvegetarian diets, for many vegetarians, the depends on the enhancing and inhibiting food constituents being consumed concurrently.

Although the composition of vegetarian diets can vary as widely as that of nonvegetarian diets, for many vegetarians, the elimination of meat may be accompanied by increased consumption of dried beans and legumes, fruit and vegetables, and whole-grain rather than refined-grain products. Such diet choices can substantially alter the dietary components that enhance or inhibit the intestinal solubility and absorption of nonheme iron (5, 17, 18; Table 1). Hallberg and Hulthen (5) have reviewed these dietary interactions and proposed an algorithm for estimating dietary iron absorption, and the enhancing or inhibiting effects of other dietary components consumed concurrently. For most vegetarian diets, the enhancing effect of ascorbic acid

logarithmic proportion to body iron stores. However, the result is a greater range of efficiency for nonheme iron absorption, compared with that of heme iron, as iron stores vary from low to high normal values (11–15).

Heme iron can account for nearly half of the iron absorbed by people with moderate iron stores consuming moderate to liberal amounts of red meat (12, 16). In contrast, because of apparent upregulation of nonheme iron absorption, nonheme iron contributes more than heme iron to the total amount of iron absorbed in people with low body iron stores (12). Thus, the generally less well absorbed nonheme iron in vegetarian diets is more responsive than heme iron to differences in body iron status: nonheme iron absorption can be more completely limited by those with high iron stores, while being nearly as well absorbed as heme iron by those with very low iron stores. However, the efficiency of nonheme iron absorption by those with low iron stores depends on the enhancing and inhibiting food constituents being consumed concurrently.

Although the composition of vegetarian diets can vary as widely as that of nonvegetarian diets, for many vegetarians, the elimination of meat may be accompanied by increased consumption of dried beans and legumes, fruit and vegetables, and whole-grain rather than refined-grain products. Such diet choices can substantially alter the dietary components that enhance or inhibit the intestinal solubility and absorption of nonheme iron (5, 17, 18; Table 1). Hallberg and Hulthen (5) have reviewed these dietary interactions and proposed an algorithm for estimating dietary iron absorption, and the enhancing or inhibiting effects of other dietary components consumed concurrently. For most vegetarian diets, the enhancing effect of ascorbic acid (19–21) and possibly of carotenoids (18) on nonheme iron absorption is unlikely to counteract the absence of unidentified enhancers provided by meat, poultry, and fish (22, 23) and the likely increased consumption of inhibitors of iron absorption. Nonheme iron absorption is inhibited by phytic acid (6-phosphoinositol), found in whole grains, legumes, lentils, and nuts; polyphenols, such as tannic and chlorogenic acids, found in tea, coffee, red wines, and a variety of cereals, vegetables, and spices; soy protein (apparently independent of the phytic acid in soy); and eggs (5).

In a controlled crossover study design (8), premenopausal women absorbed 3.5 times more nonheme iron and an estimated 6 times more total iron from a nonvegetarian than a lactoovovegetarian diet, after 4 wk of equilibration to each diet. The diets contained similar amounts of iron: nearly 18 mg/d as calculated from food databases, but only 13 mg by actual analysis. Besides containing no meat, the experimental vegetarian diet contained ~20% more ascorbic acid, and 3 times more fiber and phytic acid from whole grains and legumes. The inverse relationship between iron absorption and body iron stores (serum ferritin) was evident with both diets [Figure 2, using data from Hunt and Roughead (8)] and influenced individual iron absorption as much as the difference in diets. These absorption data are consistent with a reduced intestinal solubility of the nonheme iron from a vegetarian diet but a similar homeostatic control of the mucosal uptake of the soluble fraction. Although the vegetarian diet did not support as great an increase in iron absorption by those with low iron stores (all of the volunteers had normal hemoglobin concentrations), it allowed a more complete exclusion of dietary iron by those with higher serum ferritin concentrations. Serum ferritin concentrations were unaffected by consuming each of these diets for 8 wk (8). Such controlled diet studies (8, 16) suggest that even fairly large differences in dietary iron bioavailability do not change iron status within a few months.

The long-term effects of vegetarian diets on iron status are apparent from cross-sectional surveys. In these studies, meat intake is the
The Dietary Reference Intakes recently proposed for iron (6) suggest that vegetarians need to increase dietary iron by 80% to compensate for an estimated lower iron bioavailability of 10% from a vegetarian diet, as indicated by serum ferritin (Table 2) (25, 29–42). However, the results of such surveys remain consistent with the conclusion over a decade ago that “iron deficiency anemia appears to be no more prevalent among vegetarian women than among nonvegetarian women” (43, page 77), at least in Western countries. Of 3 reports of lower hemoglobin or hematocrit in vegetarians compared with nonvegetarians (Table 2), the relatively high incidence of anemia in Chinese adults was twice as frequent among female vegetarians (consuming a diet rich in soy products) as nonvegetarians (39). However, US college women who consumed a lactoovo-vegetarian diet or mainly fish or poultry, compared with those who consumed mainly red meat as a protein source, had lower serum ferritin, hemoglobin, and hematocrit concentrations and greater iron binding capacities, but the hemoglobin and hematocrit values were in the normal range for all groups (35). Similarly, British vegetarian children with significantly lower hemoglobin concentrations compared with matched omnivores had no greater proportion with hemoglobin values less than normal (40). Thus, although several reports indicate that vegetarians in Western societies have lower iron stores and may have lower hemoglobin concentrations, they do not indicate a greater incidence of iron deficiency anemia.

The Dietary Reference Intakes recently proposed for iron (6) suggest that vegetarians need to increase dietary iron by 80% to compensate for an estimated lower iron bioavailability of 10% from a vegetarian diet, compared with 18% from a mixed Western diet (6). These estimates are based on the maximum rates of absorption attainable by individuals maintaining an adequate level of iron nutrition, with a serum ferritin concentration of 15 mg/L (6). Note that the data in Figure 2 suggest the bioavailability of some vegetarian diets may be even lower. Although vegetarian men can easily meet the iron requirement of 14 mg Fe/d, the corresponding recommendation for vegetarian women is lower (10 mg Fe/d) (43), at least in Western countries (Table 2). Rather than routine iron supplementation for vegetarian women of fertile age, but the long-term benefit of such supplementation has not been tested. Iron supplementation reduces the efficiency of iron absorption from the diet (15), must be continuous to have a long-term influence on serum ferritin of women with low iron stores (15, 46), and may be associated with increased oxidative stress by the unabsorbed iron in the lower bowel (47). At this time, low iron stores without anemia have not been clearly demonstrated to adversely affect function (6, 48). This may change with increasing sensitivity functional assessments, such as recent reports of improvement in adaptation to exercise training with iron supplementation of women with low iron stores (49, 50). Although not a functional criterion for iron status, the susceptibility to toxicity of other elements is also increased with low iron stores (51). However, iron deficiency clearly impairs function only when hemoglobin concentrations are measurably decreased (6, 48), which, as indicated above, is not observed in studies comparing vegetarians with omnivores in Western countries (Table 2). Rather than routine iron supplementation, it may be preferable to individualize supplementation recommendations based on hemoglobin screening of women with risk factors for iron deficiency, as recommended by the US Centers for Disease Control and Prevention (52). The listing of low iron intake as one such risk factor (52) could be extended to women who consume vegetarian diets or who avoid red meat (35). Iron absorption from vegetarian diets can likely be somewhat improved by modifying food preparation techniques, food selection, and food combinations. Such modifications could include the use of iron cookware (53) (especially for cooking acidic foods that solubilize iron from the pan), the consumption of iron-containing foods concurrently with sources of ascorbic acid–containing foods while limiting inhibitory foods such as coffee and tea to between meals, and the selection of lower-phytate foods or the use of preparation methods that reduce phytic acid (54). For example, yeast leavening reduces the phytic acid content of whole-grain foods, 3 of dairy or fortified nondairy, 1.5 of nuts and seeds, and 2.5 of oils. Although it may be possible to plan a vegetarian diet that is rich in iron, most assessments of the dietary intake of female vegetarians in developed countries suggest much lower average iron intakes, in the range of 11–18 mg/d (2, 35, 37, 38, 41, 45).
breads, especially if the calcium content of the recipe is minimized (calcium inhibits the phytate degradation associated with yeast fermentation and baking) (55). Unfortunately, the resulting improvements in absorption are likely to be modest, based on the limited improvement in iron absorption when research volunteers modified their self-selected meals to consume more ascorbate (56). And, as noted above, even the more substantial differences in iron absorption observed with controlled research diets do not modify serum ferritin concentrations within several weeks (8, 16).

Lowering iron stores without increasing the risk of iron deficiency anemia may confer a health advantage when vegetarian diets are chosen from an abundant food supply. An association between high serum ferritin and coronary heart disease (26) has not been confirmed in other similar studies (57). However, an increased risk of heart disease has been observed in heterozygous “carriers” of the mutation associated with the iron storage disorder hemochromatosis (58–60); this relatively common heterozygous mutation occurs in 9.5% of the US white, non-Hispanic population (61). Excess iron has been hypothesized to increase colorectal cancer risk (62), and the greater serum ferritin of meat eaters, compared with lactoovovegetarians with similar body mass (body mass index <23 kg/m²), has been associated with reduced insulin sensitivity (42). In general, an increased health risk associated with high iron stores remains to be confirmed; however, this possibility, without an apparent increased frequency of iron deficiency anemia among vegetarians in Western countries, makes the benefit of supplementation without individualized assessment questionable. The committee setting Dietary Reference Intakes for iron indicated that it is prudent for men and postmenopausal women to avoid iron supplements and highly fortified foods (6); this recommendation would seem to be appropriate whether or not a vegetarian diet is chosen.

ZINC BIOAVAILABILITY FROM A VEGETARIAN DIET

More than half of the zinc in US diets is derived from animal foods, and one quarter of the zinc comes from beef (63). Although vegetarian diets can be planned that have zinc content similar to that of nonvegetarian diets, such planning requires special emphasis on the use of legumes, whole grains, nuts, and seeds.

The bioavailability of zinc from vegetarian diets is also likely to be less than that of nonvegetarian diets. Plant foods rich in zinc—such as legumes, whole grains, nuts, and seeds—are also high in phytic acid, an inhibitor of zinc bioavailability (64). Phytic acid values are not listed in most large tables of food composition (65) but can be estimated from smaller published tables (5, 64). Bioavailability of zinc is enhanced by dietary protein (66), but plant sources of protein are also generally high in phytic acid.

Despite high phytate content that lowers the fraction of zinc absorbed from unrefined foods, the higher zinc content of these foods may make these foods preferable to more refined products lower in zinc. For example, nearly 50% more zinc was absorbed from a serving of whole-wheat bread compared with a serving of white bread (0.22 compared with 0.15 mg, respectively) because the zinc content of the whole-wheat bread more than compensated for less efficient absorption of zinc (16.6% compared with 38.2%, respectively) (66).

A World Health Organization publication (67) categorized lacto- and ovovegetarian and vegan diets with phytate-zinc molar ratios of 5–15 as moderate in zinc bioavailability (30–35% absorption). In comparison, high-zinc-bioavailability (50–55% absorption) diets were described as refined, low in cereal fiber, with a phytate-zinc molar ratio of <5, and with adequate protein principally from animal sources. Low-zinc-bioavailability diets (15% absorption) were listed as high in unrefined cereal grains, with phytate-zinc ratio >15, and the majority of energy supplied by high-phytate foods, with soy products as the main protein source and low amounts of animal protein. High levels of calcium fortification may also reduce zinc bioavailability (67).

In a controlled diet study, the replacement of meat with simple carbohydrates substantially reduced the amount of zinc absorbed (from 3.6 to 2.0 mg/d) by postmenopausal women, in proportion to the reduced zinc content of the diet (from 13 to 6.7 mg/d) (68). Fortification of the low-meat diet with minerals from meat did not increase the amount of zinc absorbed, because the addition of minerals reduced fractional zinc absorption (68).

The description of the new Dietary Reference Intakes for zinc (6) suggested that, because of lower absorption of zinc, those consuming vegetarian diets, especially with phytate-zinc molar ratios >15, may require as much as 50% more zinc than nonvegetarians. This is consistent with the measurement of zinc absorption from experimentally controlled diets: the same lactoovovegetarian diet that reduced nonheme iron absorption by 70% and estimated total iron absorption by nearly 85% (7) reduced zinc absorption by about 35% compared with a nonvegetarian diet (Figure 1) (8). Legumes, whole grains, seeds, and nuts replaced meat, resulting in phytate-zinc molar ratios of 14 and 5 for the lactoovovegetarian and nonvegetarian diets, respectively. After allowing 4 wk for dietary adaptation, women absorbed zinc less efficiently from the vegetarian diet (26% compared with 33%). Because the vegetarian diet contained somewhat less zinc, only two thirds as much zinc was absorbed as from the nonvegetarian diet (2.4 compared with 3.7 mg Zn/d, respectively). By the end of 8 wk, this difference was associated with a 5% reduction in plasma zinc (within the normal range) and a positive elemental zinc balance (7). Positive zinc balance has also been reported with another experimental vegetarian diet (69), but this method is not a sensitive measure of zinc nutriture.

Unfortunately, effective evaluation of vegetarian diets and zinc nutriture has been hindered by the lack of a sensitive clinical measure of marginal zinc status. Plasma zinc is relatively insensitive to several weeks of severe dietary zinc restriction (70, 71). Cross-sectional plasma zinc measurements have not usually differed between vegetarians and nonvegetarians (31, 33, 38, 72, 73), although plasma zinc was inversely related to dietary phytate-zinc molar ratios in Canadian adolescent girls following lactoovovegetarian diets (38). In a year-long study of research subjects who changed to a vegetarian diet, plasma zinc as well as urinary zinc were reduced after 3 mo, with no further reductions after 6 and 12 mo (74), suggestive of a new equilibrium on the vegetarian diet. These longitudinal studies (7, 74) suggest that, as a result of a vegetarian diet, plasma zinc concentrations are reduced within a normal range, and this reduction is detectable after several weeks when compared with measurements from the same individuals on a nonvegetarian diet. Vegetarian diets have also been associated with reduced hair zinc (74), compared with baseline values, an increased 3-h plasma zinc response to an oral zinc load, and reduced zinc concentration in the salivary sediment (75). Assessment of the long-term effects of vegetarian diets will continue to be difficult because there are no generally accepted, sensitive clinical criteria for marginal zinc status.
SHOULD VEGETARIAN DIETS BE SUPPLEMENTED WITH IRON OR ZINC? TO ANSWER THIS QUESTION, MORE INFORMATION IS NEEDED ON THE COMPETITIVE INTERACTION BETWEEN IRON AND ZINC ABSORPTION, WHICH IS MOST APPARENT WHEN SUPPLEMENTAL QUANTITIES ARE ADMINISTERED WITHOUT FOOD (76–78). OTHER NUTRIENTS MUST ALSO BE CONSIDERED. EXTENSIVELY FORTIFYING FOODS WITH CALCIUM MAY REDUCE BOTH IRON AND ZINC ABSORPTION (55, 67, 79). SUPPLEMENTATION WITH BOTH IRON AND ZINC WOULD BE EXPECTED TO REDUCE THE ABSORPTION OF OTHER TRACE ELEMENTS SUCH AS COPPER (6).

OTHER TRACE ELEMENTS

MUCH LESS INFORMATION IS AVAILABLE ABOUT THE BIOAVAILABILITY FROM VEGETARIAN DIETS OF TRACE ELEMENTS besides iron and zinc. Because plant foods are generally good sources of elements such as copper and manganese, vegetarian diets are likely to provide greater amounts of these than nonvegetarian diets. Plasma copper was not significantly different between vegetarians and nonvegetarians in a cross-sectional study (73). In Swedish subjects who followed self-selected vegetarian diets for 12 mo, plasma copper decreased by 22%, together with reductions in hair copper concentrations and urinary copper excretion (74). Plasma copper was slightly but significantly lower after consumption of an experimental lactoovovegetarian than after consumption of a nonvegetarian diet (7), but this slight difference was not significant in a follow-up experiment (9). As assessed by monitoring the fecal excretion of a stable copper isotope (apparent absorption), copper was absorbed less efficiently from the vegetarian diet, but more total copper was absorbed because of the greater copper content of a vegetarian diet, compared with a nonvegetarian diet (Figure 1) (9).

The selenium content of foods varies greatly with the selenium in the soil where the food is grown or the animals are raised. Furthermore, the retention and use of selenium from the diet likely depends on the chemical form of selenium in foods. By analysis, the total selenium content of vegetarian diets was similar or lower than that of nonvegetarian diets in a German study (80); in contrast, dietary selenium (calculated) increased by about 40% when Swedish subjects switched to a vegetarian diet (74). In those healthy Swedish subjects who switched from mixed diets to 12 mo of self-selected vegetarian diets, plasma selenium decreased by 11%, to concentrations between 0.6 and 0.7 mmol/L in 4 of 9 subjects (74). Hair selenium concentrations and urinary and fecal selenium excretion also decreased (74). The decrease in plasma selenium in the Swedish study contrasts with a report of higher plasma selenium measured in vegetarians compared with nonvegetarians in Slovakia (73). We conducted retrospective analyses of balance samples from our controlled diet experiment in North Dakota (7) (JR Hunt and JW Finley, unpublished data, 1999), although this was not designed as a selenium study. Compared with the nonvegetarian diet, the lactoovovegetarian diet had similar selenium content (x ± SD: 113 ± 24 and 107 ± 16 mg/d, respectively) but resulted in lower urinary selenium excretion (x 44 and 65, pooled SD 6 mg/d, P < 0.0001) without affecting apparent absorption or elemental balance of selenium (unfortunately, plasma samples were not available for selenium analyses). Such differences in urinary selenium from diets with similar selenium content suggest that the 2 diets may have contained different chemical forms of selenium that were not similarly retained. More information is needed on the influence of geography (i.e., soil selenium content) on plant and animal foods and the use of specific forms of selenium from the food combinations that characterize vegetarian and nonvegetarian diets.

The year-long Swedish study of subjects changing to self-selected vegetarian diets also noted increases in plasma and hair magnesium and decreases in hair mercury, lead, and cadmium concentrations that tended to revert to baseline concentrations when evaluated 3 y after the diet intervention ended (74). It is difficult to evaluate such findings because the baseline and vegetarian dietary intakes of mercury, lead, and cadmium were not known and because hair measurements have not been validated as indicators of systemic elemental exposure. As these elements may share and compete for some of the same cellular transport and absorption receptors as iron, diets with reduced nonheme iron bioavailability could potentially enhance the retention of these toxic elements (51). Alternatively, inhibitors of nonheme iron bioavailability may also reduce the absorption and retention of toxic elements with similar physicochemical properties.

CONCLUSION

The iron and zinc from vegetarian diets are generally less bioavailable than from nonvegetarian diets because of reduced meat intake as well as the tendency to consume more phytic acid and other plant-based inhibitors of iron and zinc absorption. However, in Western countries with varied and abundant food supplies, it is not clear that this reduced bioavailability has any functional consequences. Although vegetarians tend to have lower iron stores than omnivores, they appear to have no greater incidence of iron deficiency anemia. With current methods, it is not possible to effectively evaluate the influence of vegetarian diets on zinc nutrition because there are no reliable and sensitive criteria to identify marginal zinc nutriture in humans. The effectiveness of trace element supplementation of vegetarian diets has not been demonstrated, and any recommendations for supplementation should consider potential adverse effects, including possible competitive interactions between minerals. Research to further define the functional consequences of low iron stores without anemia and to sensitively detect marginal zinc status is needed to detect and prevent any possible problems associated with lower iron and zinc absorption from vegetarian diets.

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REFERENCES

6. Food and Nutrition Board, Institute of Medicine. Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper,