



POSITION PAPER

Position paper on vegetarian diets from the working group of the Italian Society of Human Nutrition



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Abstract *Background:* Interest in vegetarian diets is growing in Italy and elsewhere, as government agencies and health/nutrition organizations are emphasizing that regular consumption of plant foods may provide health benefits and help prevent certain diseases.

Methods and results: We conducted a Pubmed search, up to September, 2015, for studies on key nutrients (proteins, vitamin B12, iron, zinc, calcium, vitamin D, and n-3 fatty acids) in vegetarian diets. From 295 eligible publications the following emerged: Vegetarians should be encouraged to supplement their diets with a reliable source of vitamin B12 (vitamin-fortified foods or supplements). Since the plant protein digestibility is lower than that of animal proteins it may be appropriate for vegetarians to consume more proteins than recommended for the general population. Vegetarians should also be encouraged to habitually consume good sources of calcium, iron and zinc – particularly vegetables that are low in oxalate and phytate (e.g. Brassicaceae), nuts and seeds, and calcium-rich mineral water. Calcium, iron, and zinc bioavailability can be improved by soaking, germination, and sour-dough leavening that lower the phytate content of pulses and cereals. Vegetarians can ensure good n-3 fatty acid status by habitually consuming good sources of α-linolenic acid (walnuts, flaxseeds, chia seeds, and their oils) and limiting linoleic acid intake (corn and sunflower oils).

Conclusions: Well-planned vegetarian diets that include a wide variety of plant foods, and a reliable source of vitamin B12, provide adequate nutrient intake. Government agencies and health/nutrition organizations should provide more educational resources to help Italians consume nutritionally adequate vegetarian diets.

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In response to increasing interest in eating vegetarian in Italy, in 2012 the Italian Society of Human Nutrition (SINU)

set up a working group to assess the peer-reviewed literature on vegetarian diets so as to distil scientifically sound advice for the Italian public on how best to maximize the benefits and minimize the risks associated with the different types of vegetarian diet. The present position

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paper summarizes the evidence pertaining to the availability of proteins, vitamin B12, iron, zinc, calcium, vitamin D, and n-3 fatty acids, in vegetarian diets and the nutritional status of these factors in vegetarians of all ages and lifestyles. These factors are key for vegetarians since they may not always be present in adequate amounts in some types of vegetarian diet. Based on the evidence, the paper makes recommendations intended for those who wish to follow a vegetarian diet.

Varieties of vegetarian diet

A vegetarian diet excludes consumption of all types of meat (pork, beef, mutton, lamb, poultry, game), meat products (sausages, salami, pâté, etc.), fish (including sushi), and molluscs and crustaceans, etc. Dairy products, eggs, and honey may be included, so that there are two main types of vegetarian diet:

- (a) *Lacto-ovo-vegetarianism (LOV)*. This excludes meat but includes dairy products, eggs, and honey, together with a wide variety of plant foods. Sub-categories are lacto-vegetarianism (LV) which excludes eggs, and ovo-vegetarianism (OV) which excludes dairy products. And
- (b) *Veganism (VEG)*. This excludes meat, dairy products, eggs, and honey, but includes a wide variety of plant foods.

The nutritional profiles of LOV and VEG diets vary widely in relation to the types, quantities, and extent of processing of the plant foods consumed; for LOV, the variation is likely to be greatest, since animal products are also consumed.

However some people adhere to other plant-based diets that limit the foods consumed, and these must be clearly distinguished from LOV and VEG diets. They include:

Raw food diet: consisting exclusively of vegetables, including sprouted cereals and pulses, fresh and dried fruits, and seeds, as well as milk and eggs, all of which are mainly eaten raw.

Fruit diet: consisting exclusively of fresh and dried fruits, seeds, and some vegetables.

Macrobiotic diet: the strictly vegetarian version of this diet consists of cereals, pulses, vegetables, seaweed, and soy products; while dairy products, eggs, and some vegetables are avoided. Fish is consumed by some who adhere to a macrobiotic diet.

The publications reviewed in this paper mainly concern LOV and VEG diets as eaten in western and Asian countries. Consequently the recommendations mainly pertain to these diets, which are generally defined as “vegetarian”.

The nutritional adequacy of raw food, fruit and macrobiotic diets has been assessed by very few studies. What evidence is available on these diets is summarized. The claimed health benefits of these diets are not supported by the available evidence, and in many cases these diets may be nutritionally inadequate.

Methods

PubMed was searched for studies published up to September 2015. We used keywords (words or MeSH terms) within search strings incorporating various terms for vegetarian diet in combination with words relating to age, bioavailability, and nutritional status, in combination with the nutrients of interest (protein, vitamin B12, calcium, iron, zinc, and n-3 fatty acids). We confined ourselves to PubMed as preliminary searches on EMBASE revealed no additional studies. We also searched the reference lists of retrieved studies. We identified 815 publications: 150 on protein, 149 on vitamin B12, 291 on calcium and vitamin D, 59 on iron, 69 on zinc and 92 on n-3.

Review team members screened retrieved titles and abstracts and selected articles that seemed pertinent excluding those not in English or not concerned with humans. The full papers were read independently by two team members to select potentially eligible articles. Review team members then used a checklist (different for each study type: systematic review/meta-analysis, randomized controlled trial, cohort or case–control, or cross-sectional study) to arrive at an assessment of the scientific merit and relevance of each paper value. A total of 295 articles were considered eligible following this assessment.

Protein

Sources and bioavailability

There are concerns that a plant-based diet may not contain protein of adequate quality. Protein quality is determined by digestibility and amino acid content [1]. Purified or concentrated vegetable proteins (e.g. soy protein, gluten) have high digestibility (>95%) – similar to that of animal proteins. For some intact vegetable products, such as whole cereals and pulses, protein digestibility is lower (around 80–90%). Most other vegetable proteins have lower digestibility (50–80%) because of the presence of plant cell walls and anti-nutritional factors. Food processing and heat treatment also influence protein digestibility.

Foods of vegetable origin may contain high levels of antinutritional factors, which may be naturally-occurring (e.g. digestive enzyme inhibitors, tannins, phytate, glucosinolates, isothiocyanates), formed during processing (e.g. D-amino acids, lysinoalanine), or due to genetic modification (e.g. lectins) [2]. Pulses, cereals, potatoes, and tomatoes in particular contain inhibitors of digestive proteolytic enzymes [3]. Soybeans are the most concentrated source of trypsin inhibitors, whereas peas and processed soybean products contain considerably lower levels [2]. Because they are usually proteins, enzyme inhibitors can be inactivated by heat treatment, including extrusion [2], or removed by other processing procedures [3]. Tannins (water-soluble polyphenols) present, for example, in some peas and beans, can complex with proteins reducing digestibility [2]. Phytate, as acid in seeds, grains and nuts, or salts in other plant tissues, can reduce carboxypeptidase and aminopeptidase activity by

chelating cofactors, or by interacting with the enzyme or its substrate [2]. Germination of seeds and grains produces enzymes that reduce polyphenol and phytate levels in the sprouts to thereby improve protein digestibility. Fermentation can also render the proteins of pulses and cereals more digestible [4–6].

Nutritional status of vegetarians at different ages

Consistent data indicate that the protein needs of vegetarians are easily met when the diet includes a variety of plant foods, and calorie intake is adequate [4–6]. A 2003 meta-analysis of nitrogen balance studies found that protein requirements (mg N/kg/day) in healthy adults were not influenced by the source (animal, vegetable, mixed) provided that vegetarians consumed either soy protein or a variety of other vegetable proteins [7]. However, while soy protein can meet protein needs as efficiently as animal protein, proteins from other plant sources (mainly pulses and cereals) are less well digested. Furthermore, when lysine tends to be the limiting essential amino acid – as in diets based mainly on cereals (especially wheat) – small quantities of other vegetable proteins, such as those from pulses or oily seeds, are required to obtain sufficient lysine and other essential amino acids.

Pregnancy and breastfeeding

Inadequate maternal protein intake during pregnancy reduces infant birthweight [8]. The few studies that investigated birthweight in infants born of vegetarian women [9–11] indicate that average birthweights of infants born to mothers on VEG or LOV diets do not differ significantly from the average of infants born to omnivorous (OMN) mothers [10,11]. By contrast, the birthweight of infants born to mothers on a macrobiotic diet was significantly lower than expected, and was attributed to lower maternal weight gain during gestation [10]. The milk of vegetarian mothers is nutritionally adequate, and infants breastfed by well-nourished vegetarian women grow normally [12,13]. However milk from women on a macrobiotic diet has a significantly lower protein content than milk from OMN [14].

Although infants of vegetarian mothers grow normally during the first six months [15,16], their growth rate is at the lower end of normal – interpreted as due to a propensity of vegetarian mothers to breastfeed for longer [17,18].

Studies on infants fed soy-isolate milk formula, irrespective of whether methionine supplemented, indicate no significant differences in growth compared to infants fed conventional cow milk formula [19]; furthermore, blood markers of protein metabolism are similar [19,20]. In a 2001 study [21] no differences were found in average height or weight between young adults fed soy-based formula and those fed cow milk for several months during childhood.

Preschool children (6 months to 3 years)

From the limited data available, it would seem that children who follow a LOV diet have similar growth to OMN children [12]. The growth of non-macrobiotic VEG preschool children is also in the normal range [16,17,22],

although they seem to have an initially smaller stature and tend to be leaner than OMN children [16,17]. By contrast, preschool children on a macrobiotic diet were reported to have significantly lower growth than those on other vegetarian diets [23,24].

Children (4–10 years)

LOV children have similar growth to OMN children [25–27]. Non-macrobiotic VEG children tend to grow at standard rates [25,28], while macrobiotic children grow more slowly [27]. The average protein intake of vegetarian children meets recommendations [22,29–31], although they consume less protein than OMN children [18,22,29,31].

One study found that serum albumin levels were above the normal range in both vegetarian and OMN children [22]. Since plant proteins are less digestible and contain fewer essential amino acids than animal proteins, it may be advisable for VEG children to consume more protein. Messina and Mangels [32] suggested that protein intake should be increased by 30–35% in VEG children under 2 years, and by 20–30% in 2–6-year-olds [32].

Adolescents (11–18 years)

The available studies indicate that the growth of LOV children and adolescents is comparable to that of their OMN peers [8,25,33,34]. However adolescents on a macrobiotic diet have lower growth than reference [25,30,34–36]. As regards protein intake, this was lower in VEG [37] and LOV [33,38] than OMN in some studies, while in others protein intake was adequate [8,30,34,39].

Like younger VEG children, VEG adolescents may require more protein than their LOV or OMN counterparts because of the lower digestibility and poorer amino acid composition of plant proteins. Thus, Messina and Mangels [32] suggested that active VEG teenagers should obtain 7–10% of their calories from protein, and sedentary teenagers should obtain 10–13% of calories from protein.

Adults

Several studies have examined the adequacy of protein intake by adult vegetarians [40–49]. Protein intake in VEG and LOV adults is generally lower than in OMN, but meets requirements. Serum albumin was normal in one study on groups of vegetarians, indicating normal protein nutritional status [41]. Kniskern and Johnston [45] examined food intake over 4 consecutive days in a convenience sample of young adult vegetarian women, finding that animal protein accounted for only 21% of dietary protein, which is below the dietary reference intake (DRI) of animal protein (45–50% of total) considered adequate. The authors suggested that protein DRI for such women should be increased from 0.8 to 1.0 g/kg bodyweight/day to account for the reduced bioavailability of plant proteins.

Elderly

Few studies on the nutritional status of vegetarian elderly are available. Protein intake was lower in vegetarian than

OMN women [50,51], but higher than recommended. In vegetarian men, protein intake was lower than in OMN men, although sufficient to meet requirements [50]. Two studies [52,53] that compared protein intake in elderly Chinese vegetarian and OMN females found that energy from protein was lower in vegetarians and did not always meet the DRI; however, serum markers of protein nutritional status do not seem to differ between vegetarian and OMN elderly [50,52,54].

Recommendations

Since the digestibility and essential amino acid content of plant proteins is lower than that of animal proteins, it may be appropriate for vegetarians to consume more protein than recommended for the general population. This increase can be easily achieved, even in the elderly, pregnant/breast-feeding women, and children, by consumption of a wide variety of plant foods.

Vitamin B12

Sources and bioavailability

Vitamin B12 is reliably present in foods of animal origin but only in small amounts. Some algae contain vitamin B12, however, bioavailability varies with algal species and can be very low [55]. Furthermore some algae contain considerable quantities of inactive vitamin B12 analogues, that can interfere with the absorption of active forms of B12 [55]. Until foods like tempeh are consistently shown to improve vitamin B12 status, they should not be relied upon as a source of vitamin B12.

Vitamin B12 requires intrinsic factor for absorption. Under physiological conditions intrinsic factor-dependent absorption is saturated with 1.5–2.5 µg of the vitamin per meal. Beyond this amount, the bioavailability of B12 decreases markedly.

Unlike the food-bound form, which must be released from its binding proteins, crystalline forms of vitamin B12 from supplements and fortified foods are in the free form, and can combine directly with haptocorrin for protection and subsequently with intrinsic factor for absorption [56]. Vitamin B12 in supplements is usually highly effective in correcting vitamin B12 deficiency [57–59].

The bioavailability of vitamin B12 in a LOV diet depends on the quantities and types of animal foods (dairy products, eggs) consumed, as well as on the consumption of fortified foods (e.g. breakfast cereals) and supplements. For VEG the only reliable sources of vitamin B12 are fortified foods and supplements.

Nutritional status of vegetarians at different ages

Vitamin B12 deficiency develops slowly, as the liver stores sufficient quantities to last several years. If storage is limited or requirements are high (e.g. in infants breastfed by VEG mothers not taking supplements), clinical symptoms may develop earlier. Folate intake is high in

vegetarians, so the typical haematological alterations of vitamin B12 deficiency may not appear; however high folate cannot prevent the deleterious effects of B12 deficiency on the nervous system. Since plasma B12 includes variable amounts of the metabolically inactive form complexed with circulating haptocorrin [60], B12 status is optimally assessed in vegetarians using the markers homocysteine, holotranscobalamin II and methylmalonic acid [61]. Increased methylmalonic acid levels may be present in persons with serum vitamin B12 in the commonly accepted normal range (>156 pmol/L) but not exceeding 360 pmol/L (488 pg/mL) [62], so a reference limit of 360 pmol/L for serum vitamin B12 has been proposed [63] and should be adopted if holotranscobalamin II levels are not available.

Pregnancy and breastfeeding

One study found that vitamin B12 levels in a sample of pregnant LOV women were significantly lower than those of OMN controls: high plasma homocysteine plus low serum vitamin B12 was present in 25% of the LOV women in at least one trimester [64].

Preschool children (6 months to 3 years)

In a review of about a hundred case reports of B12-deficient children from various countries it was found that two thirds were born to vegetarian mothers, and a quarter were born to mothers with pernicious anaemia [65]. Vitamin B12 status seems to have been evaluated in only two cohorts of macrobiotic children. In a Dutch cohort, macrobiotic children had significantly lower “true” cobalamin levels than controls [23,66]. In a Norwegian cohort 85.4% had high serum levels (>0.43 mmol/L) of methylmalonic acid [67].

Children and adolescents (4–18 years)

All available studies indicate low B12 status in macrobiotic persons of this age group [68–70]. By contrast, most available studies on non-macrobiotic vegetarians (LOV + VEG, LOV) indicate that B12 (and when evaluated, homocysteine) levels are within normal range [30,31,71–73]. A small study on 6 LOV Asians who had migrated to Auckland found that half had asymptomatic B12 deficiency [74].

Adults and elderly

Numerous studies on vitamin B12 status in adult vegetarians have been conducted worldwide; many included elderly people, thus results of studies which included both adults and elderly are presented together.

A number of studies on vegetarians (LOV, LV, LOV + VEG) found that mean B12 plasma levels were in the normal range or did not differ from those of OMN [62,75–77].

Twenty-three studies specifically on LOV found compromised vitamin B12 status – as low serum B12 [43,52,78–94], high serum methylmalonic acid [62,77,80,82–84], high homocysteine [43,78,80,82–84,86–89,91,94], or low holotranscobalamin II [80,82–84]. One study [95] reported normal homocysteine levels in vegetarians, which were

however higher than in OMN. Another study [76] found that serum B12 levels in vegetarians were not significantly lower than those in OMN. Finally, a study that examined serum B12, methylmalonic acid, homocysteine and holotranscobalamin II found that LOV had lower B12 status than OMN, but differences were not significant in the LOV subgroup taking B12 supplements [83].

Sixteen studies on VEG found compromised B12 status as low serum B12 [77,78,81,83,85,88,91,96–100], high serum methylmalonic acid [62,77,80,82–84,99], high homocysteine [76,77,80,82–84,88,91,100] and low holotranscobalamin II [80,82–84,99]. Two of these studies included VEG on vitamin B12 supplements [78,83]. Another study that included VEG supplement users found that mean serum B12 and methylmalonic acid levels did not differ between VEG and OMN, nevertheless 10 of the 25 recruited VEG had vitamin B12 deficit as indicated by macrocytosis, circulating vitamin B12 concentrations < 150 pmol/L, or serum methylmalonic acid > 376 nmol/L [101]. In a prospective study on 20 adult OMN followed for 5 years while on a VEG diet, B12 levels were reduced only in those who did not take supplements [102].

The only available study on macrobiotic adults (who occasionally consumed animal products) reported that 51% had low serum B12, and 30% had high urinary methylmalonic acid [69].

Two studies evaluated B12 in VEG who mainly [103] or entirely [104] ate uncooked food. High urinary methylmalonic acid (≥ 4.0 $\mu\text{g}/\text{mg}$ creatinine) was present in 47% [103] and serum low B12 (<200 pmol/L) was present in 57% [104]. A study on raw foodists found increased homocysteine levels due to B12 deficiency in all participants (mixed, LOV and VEG), but LOV and VEG had lower serum B12 and higher total plasma homocysteine than mixed raw foodists [105].

A meta-analysis of 17 studies that compared homocysteine and B12 levels in vegetarians (3230 LOV/LV/VEG) with those in OMN [106] found that VEG had the highest mean homocysteine values, and lowest mean B12 levels, while levels in LOV were intermediate between those of VEG and OMN. In only two of these studies were mean plasma levels of homocysteine and B12 similar to those in OMN [73,101].

Recommendations

The vitamin B12 status of vegetarians should be monitored regularly. All vegetarians should be encouraged to include a reliable source of vitamin B12 in their diet (vitamin-fortified foods or supplement). Persons taking B12 tablets should be encouraged to chew them slowly or allow them to dissolve under the tongue to optimise absorption. For children, droplet formulations are suitable. In view of data [107,108] indicating that B12 absorption is often less than 50% [60], the European Food Safety Authority recommends that vitamin B12 absorption should be assumed to be 40% [109] when formulating recommended daily intakes, which should thus be 4 $\mu\text{g}/\text{day}$ or greater. We therefore propose that, for preserving normal B12 levels in

vegetarians, intake should adhere to the recommendations in Table 1. If B12 deficiency is discovered, supplementation with crystalline cobalamin should begin immediately at doses above 4 $\mu\text{g}/\text{day}$.

Calcium

Sources and bioavailability

Several plant foods, particularly leafy vegetables pulses, and nuts, contain good quantities of calcium, however the bioavailability of this mineral is inversely proportional to the amounts of oxalate and phytate in the diet [110,111] which are abundant in spinach, Swiss chard, and beet leaves. Dietary fibre seems not to impair calcium absorption, since in one study more calcium was absorbed from kale than cow milk [112].

Regardless of solubility, the calcium from calcium salts used to fortify foods is absorbed with similar efficiency to the calcium in cow milk [113] except that the absorption from calcium citrate malate is slightly higher [111]. The tricalcium phosphate used to fortify soy milk is absorbed with only about 75% of the efficiency of cow milk calcium [114]. The calcium from the calcium chloride and calcium sulphate used to produce tofu has similar bioavailability to the calcium from milk [115]. The bioavailability of calcium from mineral water is similar to or better than that from milk [116]. Calcium absorption from water is improved when water is consumed with food [117].

Because sodium and calcium share proximal renal tubule transport systems, high sodium intake promotes calcium excretion [111].

Nutritional status of vegetarians at different ages

Calcium status has been assessed in vegetarians by various methods: dietary calcium intake, serum calcium, ionized serum calcium, bone mineral density (BMD), and bone mineral content (BMC). In physiological conditions,

Table 1 Recommended dietary supplement values for preserving normal B12 levels in persons becoming vegetarians.

Age	LARN ^a (PRI) ^c ($\mu\text{g}/\text{day}$)	EFSA ^b (AI) ^d ($\mu\text{g}/\text{day}$)	Daily multi-dose	Daily single-dose ($\mu\text{g}/\text{day}$)
6–12 months	0.7	1.5	1 $\mu\text{g} \times 2$	5
1–3 years	0.9	1.5	1 $\mu\text{g} \times 2$	5
4–6 years	1.1	1.5	2 $\mu\text{g} \times 2$	25
7–10 years	1.6	2.5	2 $\mu\text{g} \times 2$	25
11–14 years	2.2	3.5	2 $\mu\text{g} \times 3$	50
15–64 years	2.4	4.0	2 $\mu\text{g} \times 3$	50
65+ years	2.4	4.0	2 $\mu\text{g} \times 3$	50
Pregnancy	2.6	4.5	2 $\mu\text{g} \times 3$	50
Breastfeeding	2.8	5.0	2 $\mu\text{g} \times 3$	50

^a LARN is an Italian acronym meaning Reference Levels of Nutrient and Energy Intake for the Italian Population.

^b European Food Safety Authority.

^c Population reference intake.

^d Adequate intake.

serum calcium is maintained within narrow limits (2.25–2.60 mmol/L total serum calcium or 1.1–1.4 mmol/L ionized form) irrespective of calcium intake, with mobilization from bone if necessary. BMD and BMC are sensitive to changes in calcium intake over the long-term (>1 year) [118] and are not now used to assess calcium status.

Pregnancy and breastfeeding

A study [119,120] on breastfeeding women found that calcium intake was lower and 1,25-dihydroxy vitamin D blood levels significantly higher in macrobiotic women than OMN controls, while blood parathyroid hormone levels were similar. The higher 1,25-dihydroxy vitamin D levels suggest a hormonal response, in macrobiotic women, to low dietary calcium and lactation, that may increase the efficiency of calcium absorption [119,120].

Preschool children (6 months to 3 years)

Only one study on this age group seems to be available: it followed a cohort of macrobiotic children from birth [121,122]. At 10–20 months calcium intake and vitamin D in blood were significantly lower in the macrobiotic infants than OMN controls. At the same time (examination in August–November) subclinical or clinical rickets was present in 17% and 28%, respectively, of macrobiotic infants compared to 0% in controls. At follow-up of a subsample of 25 macrobiotic infants in March–April, physical symptoms of rickets were present in 55% of macrobiotic infants [121,122].

Children and adolescents (4–18 years)

Of several studies [22,29–31,123,124] on non-macrobiotic vegetarians, only one, on Chinese vegetarian children [30], found that calcium intake and BMD were similar to those in OMN children. The other studies found that vegetarian children had lower calcium intake than OMN [22,31]. In a large cohort of macrobiotic adolescents evaluated at various times from 1985 [125], calcium intake and BMC/BMD, but not 1,25-dihydroxyvitamin D, were significantly lower than in OMN controls [68,125]. The lower BMC and BMD were unrelated to calcium intake [125]. In a later examination of the same cohort, most of whom had switched to a vegetarian or even OMN diet, low BMD and BMC were associated with B12 deficiency (low serum vitamin B12 or high methylmalonic acid) [68].

Adults

Numerous studies on calcium have been conducted on adult vegetarians worldwide. Most [22,47,126–133] found that calcium intake in vegetarians (LOV, LV, LOV + VEG) did not differ from that in OMN. However in two studies [134,135], calcium intake was higher in vegetarians, and in another [136] calcium intake was lower, but did not correlate with BMD. No significant difference in risk of fracture between vegetarians and OMN was reported after 5.2 years of follow up in one study [127]. Other studies have reported no difference in BMD between vegetarians and OMN [89,130,132,133]. However, a study on Taiwanese vegetarian women found that long-term VEG were at

increased risk of lumbar spine fracture and femoral neck osteopenia compared to other long-term vegetarians. These differences were attributed to lower protein intake in VEG; calcium intake did not differ [136].

A prospective year-long study on pre-menopausal women found that vegetarian women tended to have lower (but stable) BMD than non-vegetarians over the year [137,138]; furthermore BMD at recruitment correlated significantly with vitamin B12 intake [137,138]. A study on 122 Germans (35 OMN, 23 VEG and 54 LV/LOV) and 73 Indian immigrants to Oman (54 OMN, 19 LV/LOV) [84] found increased bone turnover (as measured by alkaline phosphatase, osteocalcin, pro-collagen type I N-terminal peptide and C-terminal telopeptides of collagen I) in vegetarians (LOV + VEG). Multiple regression analysis showed a significant association between increased bone turnover and vitamin B12 status that was independent of vitamin D status [84]. Finally, in a study on Indian and Iranian post-menopausal women, a pure vegetarian diet was a risk factor for osteoporosis among Indian women only, but this was not significant after adjustment for weight and height [139]. From the studies reviewed above we conclude that in adult vegetarians, at-risk bone status correlates with long duration vegetarianism, low protein intake [136], and low vitamin B12 status [84,89,138].

All studies [126–129,136,140–145] found lower calcium intake in VEG than controls. A study that compared meat eaters, fish eaters, vegetarians and VEG found a higher fracture rate in VEG apparently related to markedly lower mean calcium intake [127]. A study on VEG Buddhist nuns found lower calcium intake, but no differences in BMD, fracture incidence, or frequency of osteoporosis compared to non-VEG [140,141]. Another study [130] found no difference in BMD (measured in lumbar spine and femoral neck) between VEG, LOV and OMN.

A study that compared 17 macrobiotic adults with VEG and LOV, found significantly lower calcium intake in the macrobiotic women than the other vegetarian groups [47]. A study on 18 raw foodists in comparison with those eating typical American diets [146] found that the raw foodists had significantly lower BMC and BMD than controls, with no differences in blood markers of bone turnover (C-telopeptide of type I collagen and bone-specific alkaline phosphatase).

Elderly

Elderly persons were included with adults in most of the studies reported above. We identified 2 studies conducted specifically on elderly women [53,147]. The first [53] on elderly Chinese female VEG, LV and OMN found that among the women vegetarian for over 30 years (36 VEG; 40 LV) mean calcium intake was significantly lower than in OMN, and was also significantly lower in VEG than LV. BMD at the femoral neck, but not at the spine, was lower in vegetarians than OMN. The second [147] was a prospective study on elderly white women (49 LOV, 140 OMN). The rate of loss of bone density over the five-year period, at each measurement site, was independent of calcium intake and was similar in both groups.

Intervention studies

Changes in nutritional status were evaluated during intervention studies with plant-based diets in healthy persons (Complete Health Improvement Project) [148] or low-fat VEG diets (about 10% of total energy) in diabetic [149] and in prostate cancer patients [150]. During the intervention period, reduced calcium and vitamin D intake [148] and lower vitamin D blood levels were observed [148]. A short-term study evaluated calcium balance in women who received a VEG diet during the first 10 days, and a LV diet during the following 10 days: calcium balance remained positive regardless of intake. This finding indicates that the lower calcium intake of the VEG diet was compensated for by reduced calcium excretion in faeces. The two diets were not associated with differences in calcium balance, apparent absorption, or bone calcium resorption (assessed by a urine marker) [151].

Recommendations

Vegetarians should be urged to make sure they adopt a diet that ensures their calcium intake is in line with Italian recommendations [60]. VEG especially should be urged to regularly consume foods that are good sources of calcium (vegetables low in oxalate and phytate, soy products, calcium-rich mineral water, and various nuts and seeds).

Iron

Sources and bioavailability

The bioavailability of iron in LOV, VEG and also OMN diets varies markedly. The main source of dietary iron in Italian OMN is cereals and cereal products (31.3%), followed by meat and meat products (16.9%), fresh and processed vegetables (13.5%), fruit (7.3%), pulses (3.2%), and potatoes and other tubers (3%) [152]. Thus Italian OMN obtain close to 60% of their iron from plant sources.

The typical Italian LOV and VEG diet may contain as much or more iron than an OMN diet, however iron bioavailability is lower [153,154] with only 5–12% absorbed, compared to 14–18% from OMN diets [155]. Non-haem iron provides 100% the iron of VEG and LOV diets, but only 85–90% of iron in OMN diets [155]. Much of the iron in soybeans is bound to ferritin and 22–34% of this is absorbed – a bioavailability comparable to that of haem iron (15–35%) [156–159].

Ascorbic acid, which chelates and reduces Fe^{3+} , is the most important facilitator of non-haem iron absorption. Thus the bioavailability of iron in a vegetarian diet can be enhanced by consuming ascorbic acid (citrus fruits, strawberries, kiwi, etc.) during a meal containing iron [160]. Other organic acids in fruits and vegetables (citric, malic, lactic and tartaric acids), as well as carotenes and retinol, also enhance non-haem iron absorption [161–163].

Soaking pulses and cereal grains activates endogenous phytases that reduce the number of phosphates bound to inositol hexaphosphate (phytate) progressively weakening

its ability to sequester iron. Use of the sour-dough method to leaven dough also activates phytases in the flour, again reducing the ability of phytate to sequester iron.

Limited data indicate that the absorption of non-haem iron can increase over the long term in response low iron bioavailability [160] which might explain why the prevalence of iron deficiency is similar in LOV, VEG and OMN [153].

Nutritional status of vegetarians at different ages

The main tests on plasma or serum used to investigate iron nutritional status [60] are haemoglobin (to detect anaemia), transferrin saturation (measure of circulating iron), soluble transferrin receptor (more stable marker of iron levels in inflammation) and ferritin (indicator of iron storage). Since ferritin is also an inflammation marker, reactive protein C should also be determined.

Breastfeeding women and preschool children (6 months to 3 years)

The milk of LOV and VEG women is similar in composition to milk from non-vegetarian women [12] and is not deficient in minerals or vitamins when the maternal LOV/VEG diet is well-balanced [164]. When the children of vegetarian mothers are weaned their iron status should be monitored, and iron-rich foods should be eaten together with food containing ascorbic acid or other fruit acids so as to improve iron absorption [164].

The incidence of iron deficiency anaemia during weaning is not higher in LOV/VEG children than OMN children, and serum ferritin levels (and growth) are usually within normal ranges in LOV/VEG children [158].

Children (4–10 years)

LOV and VEG children have lower iron intakes than OMN children but their serum iron levels are within the normal range [72] and do not differ significantly from those of OMN children [161]. Pre-school and school-age VEG have adequate iron intake and anaemia has not been documented [17,165]. However, the Institute of Medicine [166], suggests that LOV/VEG children should consume 1.8 times more iron than OMN children, to ensure their nutritional needs are met. Macrobiotic children also often have low iron status [161].

Adolescents (11–18 years)

The development of LOV and VEG teenagers is similar to that of non-vegetarian teenagers [12]. Slovak LOV and LV children aged 11–14 years had lower serum iron and haemoglobin than OMN but levels were within the normal range [167]. However to meet iron needs in this period of rapid growth, LOV/VEG teenagers should consider iron supplementation [168]. In a Swedish study [123] the iron intake of adolescent (16–20 years) VEG (at least 6 months) of both sexes was compared with that of OMN. Iron intake in VEG and OMN males was similar. VEG females consumed more iron than OMN females and the population reference intake (PRI); in all cases iron intake was within the recommended range. However serum iron

markers were lower than normal in VEG and OMN females but normal in VEG and OMN males, indicating that menstrual blood loss was responsible for the lower iron levels and that diet had no influence.

Adults

Even after many years on a LOV or VEG diet, serum iron levels in adults do not usually differ significantly from those in OMN [161]. Mean iron intake in LOV/VEG men can in fact be higher than in OMN men, and also higher than the PRI [169]. Nevertheless serum ferritin and haemoglobin are significantly lower in LOV and VEG males than OMN controls [101,134,135,170]. LOV/VEG women also have a similar iron intake to OMN controls [171] and after many years on a vegetarian diet their iron status is adequate [134,171,172]. Nevertheless, the risk of iron anaemia is reported to be about 40% in pre-menopausal women after one year on a VEG diet [100,101]. Haddad et al. [101] found that pre-menopausal VEG and OMN women had similar risks of developing iron anaemia. Studies on young females [170,173] also show that iron deficiency anaemia is present at similar levels in LOV and VEG (at least two years) and OMN. Thus menstrual iron loss rather than diet appears as the main cause of iron deficiency anaemia. In postmenopausal women, high blood ferritin has been found to be a risk factor for cardiovascular diseases [174], so LOV and VEG diets may be protective against these conditions.

Nevertheless iron and vitamin B12 status can both be compromised in adult LOV, LV, and occasional meat eaters; in such cases macrocytosis due to B12 deficiency can be masked by low iron status [77].

Elderly

A study that investigated the adequacy of LOV and VEG diets in elderly persons [134] found that mean daily mineral intake, iron included, and iron serum markers were within normal ranges [134] and did not differ significantly from OMN controls. In older men (59–78 years) undergoing 12 weeks of resistance training designed to maintain muscle mass, serum iron remained within normal limits throughout the training period, irrespective of whether they ate a beef-containing or vegetarian diet [175]. These findings suggest that a vegetarian diet is suitable for elderly persons [134,175].

Recommendations

Vegetarians should be advised increase iron intake above the PRI suggested for OMN, by eating a variety of iron-rich plant foods. Iron bioavailability can be increased by:

1. Eating ascorbic acid-rich foods together with iron-rich foods.
2. Using food preparation methods such as grinding, soaking and germination, and using the sour-dough method to leaven bread (or buy sour-dough bread). These processes lower the phytic acid content of cereals and legumes and thus reduce iron sequestration.

3. Using iron-fortified foods (e.g. breakfast cereals).

Iron supplementation is only recommended if iron status has been shown to be low by appropriate blood tests.

Zinc

Sources and bioavailability

According to the US Department of Agriculture (reported in Hunt [176]) over half the zinc in OMN diets (56%) comes from animal origin foods. Similarly, a 2013 Italian food survey [152] reported that 54.9% of the dietary zinc of Italians comes from animal products (24.8% meat and meat products, 21% milk and milk products, 6.9% fish, seafood and their products, 2.2% eggs), and 40.7% from plant foods (21.5% cereals and cereal products, 9.8% vegetables, 5.5% potatoes and other tubers, 2.8% fruits, 1.1% pulses), with sweet products, water and non-alcoholic beverages providing minimal amounts.

Good zinc sources for VEG and LOV are whole grains, cereals, pulses, nuts and seeds [177]. However, these foods are also rich in phytate which is a strong zinc chelating agent that severely limits intestinal absorption. Oxalate and some dietary fibres also decrease zinc absorption [178,179].

Zinc absorption from VEG/LOV diets is 15–26%, while that from typical OMN diets is 33–35% [178,180]. The consumption of small quantities of animal proteins considerably enhances zinc absorption [181], perhaps due to the release of amino acids during digestion which keep zinc in solution (prevent its chelation) [179]. Sulphur amino acids, cysteine-containing peptides, hydroxy acids (present in fruits) and other organic acids present in fermented food may all increase zinc absorption [181,182]. As with iron, procedures that activate the endogenous phytases present in cereals and pulses, like milling, sprouting, soaking, and sour-dough leavening, increase the bioavailability of zinc [153,183].

Nutritional status of vegetarians at different ages

Because of zinc's protein biochemical roles, various signs of zinc deficiency may manifest that also depend on the severity of the deficiency; for the same reason it is difficult to identify reliable biomarkers of zinc status [184,185]. Markers considered useful for assessing zinc nutritional status are plasma levels [186], serum levels [187] and urinary excretion [186].

Breastfeeding women and preschool children (6 months to 3 years)

If breast-feeding LOV/VEG mothers have adequate zinc intake, the zinc nutritional status of their infants does not differ from that of breast-fed infants of OMN mothers [164]. When breast-feeding is not possible or insufficient [188], adequate zinc intake for LOV infants can be provided by modified cow milk formula, and soy or rice preparations [20,189]. Pre-term birth, low birthweight, and certain

diseases may indicate need for infant iron and zinc supplementation [190]. LOV and OMN women had similar zinc intake during pregnancy [191] although intake was below the PRI. Plasma zinc levels were lower in these women than in non-pregnant women, suggesting that zinc nutritional status is influenced more by pregnancy than by LOV/OMN diet [191]. Nevertheless those on a VEG diet have lower zinc intake [192] and lower zinc plasma levels [101] than those on a LOV diet.

Children (4–10 years)

The zinc intake of pre-school LOV [31,178] or VEG [193] children is similar to that of age-matched OMN. Nevertheless low zinc serum levels have been found in children on low meat/high calcium diets, who also show deficient growth and loss of taste [194,195].

Adolescents (11–18 years)

Teenagers have high zinc requirements, and those on LOV and VEG diets may have inadequate zinc status [196,197]. Thus, Canadian adolescent girls were found to have a mean zinc intake of 7 mg/day – lower than the PRI of 9 mg/day [166]. Serious zinc deficiency in children and teenagers can interfere with bone growth, and sexual and behavioural development [198].

Adults

Mean daily zinc intakes of a population of Canadian 7th Day Adventists on a LOV diet [171] were similar to those of OMN controls and higher than the PRI (9 mg/day); mean zinc serum was within the normal range. In Flemish adults of both sexes on LOV diet for at least a year, mean daily zinc intake was higher than in OMN controls [134]. These findings suggest that people on a long-term LOV or VEG diet have adequate zinc nutritional status and that they adapt to low zinc bioavailability better over the long term than the short term [178]. The adaptation may occur by increasing absorption [178].

The zinc intake of middle-aged Japanese men on LOV or semi-LOV diets was lower than that advised by the Japanese National Health and Nutrition Survey although within the Japanese PRI range [135]. Brazilian (Sao Paulo) men and women who had been vegetarians for at least 5 years had low zinc levels in erythrocytes [199]. In a small group of Swedish men and women who changed from an OMN to an LV diet and pursued it for 12 months, zinc intake did not change but plasma levels had lowered at 3 months, although they remained in the normal range [200]. No further zinc plasma decrease occurred in the following 6–12 months, and excretion, in urine and faeces decreased [200].

Elderly

A LOV diet was compared with an OMN diet in institutionalized elderly persons [50]. Mean daily zinc intake was similar and within the normal range in both groups. However serum zinc was lower than reference in both groups, suggesting that the PRI for zinc in elderly people might be set too low. Since elderly LOV and OMN have

similar nutritional status for zinc and similar health, it can be concluded that a balanced LOV diet is suitable for the elderly [12].

Recommendations

Vegetarians should be encouraged to consume more dietary zinc than the PRI suggested for OMN, especially when the dietary phytate:zinc ratio is high.

Zinc absorption can be improved by adopting food preparation methods (soaking, germination fermentation, sour-dough leavening of bread) that reduce phytate levels in zinc rich foods.

Zinc-fortified foods (e.g. breakfast cereals) can also be used.

Zinc-rich foods should be eaten together with foods that contain organic acids such as fruit, and vegetables of Brassicaceae family.

N-3 fatty acids

Sources and bioavailability

The only n-3 fatty acid present in useful amounts in plant foods is α -linolenic acid (ALA, 18:3 n-3). Its main sources are flaxseeds, hemp seeds, chia seeds, walnuts and their oils, and some seaweeds [201]. For eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3), vegetable sources are extremely limited, the main one being certain seaweeds [201–203].

ALA is an essential fatty acid, while EPA and DHA can be synthesized from ALA. However ALA elongation to EPA and DHA is limited, and influenced by diet: high dietary linoleic acid [204]; inadequate intakes of energy, protein, pyridoxine, biotin, calcium, copper, magnesium, and zinc [205,206]; and excessive intakes of trans fatty acids [201] and alcohol, can all impair EPA and DHA synthesis [207].

Nutritional status of vegetarians at different ages

Pregnancy and breastfeeding

Infants born to vegetarian mothers had low DHA in plasma and umbilical artery phospholipids, but birthweight and head circumference were normal [208]. Breast milk from vegetarian mothers was reported as higher in ALA but lower in DHA than milk from OMN mothers [209]. Vegetarian mothers had lower dietary intakes of EPA, docosapentaenoic acid (DPA, 22:5 n-3), and DHA, higher dietary n-6:n-3 ratio, and higher erythrocyte DPA than OMN mothers [210].

Children (4–10 years)

A study on 20 children reared on a VEG diet found that the dietary n-6:n-3 ratio was very high at 44:1, and that ALA provided 0.2% of the total energy intake [211].

Adolescents (11–18 years)

In a study that compared plasma fatty acid profile in vegetarian (VEG, LOV, and pescovegetarian) and OMN children, all vegetarians had higher ALA than OMN children. VEG had highest ALA levels and highest n-6:n-3 ratio, but lowest EPA, DHA and n-3. Pescovegetarians had highest EPA and DHA and lowest n-6:n-3 ratio [212].

Adults and elderly

Numerous studies on the n-3 fatty acid status of adult vegetarians are available. Findings of studies that did not distinguish VEG from LOV [208,213–227] and studies that did distinguish VEG from LOV [228–233] are reported.

Findings on ALA intake and blood levels are contrasting: some studies [213,219,224,229,230,232,233] found higher levels in vegetarians than OMN; others [215,217,221,222,231] found lower levels; others again found no difference [208,225].

In all studies [208,213,215,217,219,221,222,225,229–233] except one [224], EPA and DHA intake and status were lower in vegetarians. DPA intake was also lower in vegetarians [229,231], while findings on DPA status varied: lower in vegetarians [218,221,230,232], higher in vegetarians [217,229,233], no difference [225].

Vegetarians had lower n-3 intake than OMN who ate a lot of meat, but higher n-3 intake than OMN who ate a moderate amount of meat [231]; total n-3 blood levels were always lower in vegetarians than OMN [217,218, 221,222,225,229,230]. Long chain n-3 intake [231] and status [229] were lower in vegetarians than OMN. The n-6:n-3 ratio of ingested food was higher in vegetarians in two studies [229,231] but lower in another [213]. The n-6:n-3 ratio in blood was always higher in vegetarians [215,217,218,222,229,230].

In one study dietary intake and status of EPA, DHA, DPA, total n-3, long-chain n-3, and n-6:n-3 ratio were all lower in vegetarians than OMN who usually eat fish, but higher than in OMN who do not eat fish [233].

Results on ALA intake and status in VEG are mixed. Some studies reported higher levels in VEG than other dietary groups [229,230,233–235]; others [231,233] reported lower levels; and others again reported no differences [33,229,232,234].

Most studies found higher EPA, DHA, and DPA intake and status in VEG than other dietary groups [229–234]. However, one study [235] found higher EPA and DPA, but lower DHA in VEG, than OMN.

Most studies reported lower total n-3 and long chain n-3 intake and status in VEG than other dietary groups [230,232,233]. However one study [229] reported higher total n-3 intake in VEG and another [233] reported that VEG women had higher total n-3 plasma levels. VEG had also a higher n-6:n-3 ratio in blood [229,230].

Recommendations

Vegetarians can improve their n-3 nutritional status by regularly consuming good sources of ALA (e.g. walnuts, flaxseeds, chia seeds and their oils) and limiting intake of

sources of linoleic acid (e.g. corn and sunflower oils). To enhance the conversion of ALA to EPA and DHA, vegetarians should be advised to ensure their diet contains sufficient proteins, pyridoxine, biotin, calcium, copper, magnesium, and zinc (i.e. to eat a varied diet). They should also be advised to reduce intake of n-6 fatty acids and trans fatty acids, by limiting consumption of processed and deep-fried foods, and alcohol.

Pregnant and breastfeeding women and children < 2 years, with increased requirement for long chain n-3 fatty acids, and those who convert ALA poorly to long chain n-3 (the elderly, people with diabetes or other chronic diseases) should be advised to consume an algae-based supplement of known nutrient content.

Table 2 summarizes dietary recommendations for each of the nutrients considered.

Athletes

The available evidence indicates that a vegetarian diet has neither a beneficial nor a detrimental effect on physical fitness [196], aerobic endurance during running [236,237], lung function, aerobic and anaerobic performance, arm and leg circumferences, hand grip, back strength, and haemoglobin and total serum protein [238]. Varied and well-planned vegetarian diets are compatible with successful athletic performance [196].

However, no long-term studies have assessed the protein intake of vegetarian and OMN athletes in relation to performance. One review [239] concluded (not based on the findings of specific studies) that the protein intake of a LOV diet was adequate for athletes, but that a VEG diet could contain insufficient energy and protein for an athlete with high energy expenditure. Other position papers and reviews [240–242] made recommendations for vegetarian athletes based on the protein requirements of OMN athletes corrected for the reduced digestibility of plant proteins. Thus, the daily protein requirement for athletes who

Table 2 Recommendations for those who wish to follow a vegetarian diet.

- Since the digestibility of plant proteins is lower than that of animal proteins, it may be appropriate for vegetarians to consume more proteins than recommended for the general population
- Vegetarians should be encouraged to supplement their diets with a reliable source of vitamin B12 (vitamin-fortified foods or supplements)
- Vegetarians should be encouraged to regularly consume foods that are good sources of calcium
- Vegetarians should be encouraged to increase their iron intake above the population reference intake suggested for omnivores, by eating a variety of iron-rich plant foods that are low in phytate and oxalate
- Vegetarians should be encouraged to consume more dietary zinc than the population reference intake suggested for omnivores, especially when the dietary phytate:zinc ratio is high
- Vegetarians can improve their n-3 nutritional status by regularly consuming good sources of alpha-linolenic acid and limiting intake of sources of linoleic acid

practice aerobic sports was considered to be 1.2–1.4 g/kg bodyweight/day, while for strength sports 1.6–1.7 g/kg bodyweight/day was suggested; vegetarian athletes were advised to increase their protein intake by 10% to 1.3–1.8 g/kg bodyweight/day for aerobic and strength sports, respectively.

Despite the lower bioavailability of some trace elements, including zinc, in vegetarian diets most studies have found no difference in trace element status between athletes and non-athletes [196]. However, since body glycogen stores are optimized by consumption of plant carbohydrate, the high phytate content of the diet may reduce iron and zinc bioavailability [196,243]. Lower ferritin levels have been found in semi-vegetarian female runners [237] and LOV athletes of both sexes [244,245] compared to OMN controls, but this did not significantly affect their endurance during long distance running.

It has been suggested [246] that zinc supplementation can be advantageous in athletes because urinary zinc excretion may increase with intense training [247]. To avoid zinc supplementation, LOV and VEG athletes can increase zinc intake by consuming high zinc foods (beans, whole grains, nuts, pumpkin seeds and hemp seeds) [246]. Although all these foods contain phytate, they provide sufficient bioavailable zinc.

Conclusions

Interest in and appreciation of vegetarian diets are growing in Italy and elsewhere. The evidence reviewed in this paper makes it clear that well-planned vegetarian diets that include a wide variety of plant foods, and a reliable source of vitamin B12, provide adequate nutrient intake. For Italians, a healthy and nutritionally adequate vegetarian diet can be obtained by choosing from among the large variety of plant foods traditionally consumed in Italy (cereals, pulses, vegetables, fruits, seeds, nuts, olive oil). Consuming foods from other cultures (e.g. soy products) and processed foods (e.g. seitan, wheat meat, extruded soy) is a matter of personal choice: such foods are not necessary to ensure the nutritional adequacy of a vegetarian diet. We urge government agencies and health and nutrition organizations to provide more educational resources to help Italians consume a nutritionally adequate vegetarian diet.

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