

Review

Vitamin D Dietary Intake through Dairy Products to Reduce the Risk of Osteoporosis

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Received: date; Accepted: date; Published: date

Abstract: Background: Vitamin D and calcium are important dietary compounds that affect bone mass, even if other minerals (potassium, zinc, etc.) and other vitamins (A, C and K) are also involved. Vitamin D and other minerals, in fact, play an important role in calcium homeostasis and calcium absorption. Hip fractures incidence is higher in western countries, where calcium is frequently included in human diet, while the occurrence of these fractures is lower in developing countries, where diets are often poor in calcium. This situation is known as the “calcium paradox”, and may be partially explained considering phosphate toxicity, that can induce a disorder of mineral metabolism. It is important to maintain adequate dietary calcium-phosphate balance in order to perform a healthy life, reducing the risk of osteoporotic fracture in older people. Vitamin D can also act as a hormone; vitamin D2 (ergocalciferol) is derived from the UV-B radiation of ergosterol, the vitamin D precursor naturally found in plants, fungi, and invertebrates. Vitamin D3 (cholecalciferol) is originated by sunlight exposure from 7-dehydrocholesterol, a precursor of cholesterol that can also act as a provitamin D3. Dietary intake of vitamin D3 is very important when skin is exposed for short times to ultraviolet B light (UV-B) one of the three kinds of invisible light rays together with UV-A and UV-C. This can be considered the usual situation in northern latitudes and in winter season, or the typical condition for older people and/or for people with very white delicate skin. Actually, the recommended daily intake of dietary vitamin D is strictly correlated with age, ranging from 5 µg for infants, children, teen-agers and adults, including women during pregnancy and lactation, to 15 µg for people over 65 years.

Keywords: vitamin D; calcium; bone mass; osteoporosis; dairy foods; fortified foods

1. Introduction

Vitamins are nutrients characterized by low-molecular weight; these compounds are obtained from external sources in the diet and play a crucial physiological and metabolic role [1]. Vitamins are classified into two categories based on liquid solubility [2], specifically the water-soluble vitamins (B complex and vitamin C) and the fat-soluble vitamins (A, D, E, and K). Most of the vitamins cannot be synthesized by humans; for this reason, they must be provided by food sources or manufactured dietary supplements [3]. Vitamins are bioactive nutrients showing several health-promoting properties which strongly affect human growth and human health [4]. The term Vitamin D was created in 1922, describing a vitamin able to promote calcium deposition [5]. Vitamin D in nature is available as ergocalciferol (vitamin D2) or cholecalciferol (vitamin D3) [6]; vitamin D2 is especially found in plants or plant products, while vitamin D3 is mainly contained in animal products [7].

Provitamin D3 (7-dehydrocholesterol) is converted to previtamin D3 by the action of ultraviolet radiation on the skin, especially the ultraviolet B light of wavelength ranging between 290–315 nm. [8]; previtamin D3 is converted in 25-hydroxy vitamin D3 (25OHD3) which in turn is transformed in 1,25 dihydroxy vitamin D3 [7]. Vitamin D3 synthesis induced by sunlight is strictly correlated with the season during the year, the time of the day, the length of exposure, the skin pigmentation and the latitude; in extreme latitudes such as beyond 35 degrees North or South, vitamin D synthesis is

greatly reduced or does not occur during winter season [9]. Moreover, skin synthesis of vitamin D decreases with advancing age, consequently, the percentage of people with low vitamin D levels is higher in the elderly [10]. Therefore, vitamin D3 dietary intake must increase in the elderly (Table 1), but it is not easy to fulfill this target if the diet is not abundant in vitamin D-rich foods. Fatty fish, fish liver oils and egg yolk represent the most important natural dietary sources of vitamin D [11], but these foods are not frequently eaten by most people [12]. In meat and offals (see Table 2), vitamin D content is normally low [13]. The concentration of vitamin D in meat and liver is strictly correlated to the vitamin D source in the feed [14].

Table 1. Dietary recommendations for vitamin D.

Age	Nutrient intake ($\mu\text{g/day}$)	Nutrient intake (IU/day)
0-3 months	8.5	340
4-6 months	8.5	340
7-9 months	7	280
10-12 months	7	280
1-3 years	7	280
> 65 years	10	400
Pregnancy	10	400
Lactation	10	400

Source: modified from Lanharn-New et al. [15].

Vitamin D, both obtained from food or produced by cutaneous synthesis, undergoes hydroxylation in the liver to 25-hydroxyvitamin D [25(OH)D] which represents the most abundant circulating form [16]. Later in the kidney 25(OH)D is converted into 1,25-dihydroxy vitamin D, which is strictly correlated to the metabolism of calcium and phosphate absorption from the intestine [17], also influencing bone cells [18]. There is also a direct effect of parathyroid hormone on the production of 1,25-dihydroxy vitamin D, with a specific control of the physiologic conditions necessary to link active vitamin D to calcium homeostasis [19].

Table 2. Vitamin D3 and Calcidiol (25(OH)-D-3) content in meat and offal.

Foodstuff	Vitamin D3 ($\mu\text{g/kg}$)	25(OH)-D-3 ($\mu\text{g/kg}$)
Beef steak	< 0.5	0.8
Beef liver	< 0.5	3.4
Beef kidney	1.3	3.0
Pork fillet	1.1	< 0.6
Pork liver	4.0	4.4
Lamb leg steak	0.4	10.4
Chicken leg	3.0	< 2.0
Chicken fillet	2.0	< 2.0

Source: modified from Lanharn-New et al [15].

The best method to determine in human body vitamin D level is represented by the detection of serum concentration of 25(OH)D [20]; the optimal level for either skeletal or extra-skeletal health is not the same for everybody but it is correlated with the specific population tested.

In the human body, ingested vitamin D2 and endogenously produced vitamin D3 are converted to the biologically active form, 1,25-dihydroxyvitamin D [1,25(OH)2D], named calcitriol. In 1969 was detected the nuclear vitamin D receptor (VDR) for 1,25(OH)2D, that till today has been determined in at least 38 human tissues and organs [21]. In fact, VDR has been firstly detected in the bone, kidney and gastrointestinal tract; later has been found in several other tissues, including those in the brain, breast, colon and prostate [21]. The phosphoprotein VDR is involved in different biological functions of calcitriol; because of its widespread distribution, Vitamin D is not considered as just a

calcaemic hormone, and vitamin D deficiency is now implicated in a series of different other diseases [22], such as psoriasis, multiple sclerosis, inflammatory bowel disease, type 1 and type 2 diabetes, hypertension, cardiovascular disease, the metabolic syndrome and various cancers [21].

Severe lack of vitamin D in adults can cause the development of osteomalacia, a disease characterized by the incomplete mineralization of osteoid [23], while in children is responsible for rickets [24]. Traditionally, vitamin D was known as the “antirachitic factor” [22]. Rickets is a condition characterized by a decreased mineralization of bone tissue and growth plates, causing weak bones in infants and children [5]. Severe vitamin D deficiency (25 OHD < 12.5 nmol/L and levels < 25 nmol/L over a long period) in both infants and children results in rickets, a disease where bones are deformed [25].

Chronic lack of vitamin D intake is the cause of secondary hyperparathyroidism, that is responsible for an increased bone turnover, with a consequent progressive bone loss and finally an increased risk of bones fracture [26]. Several clinical trials have been performed in older patients in order to evaluate whether vitamin D supplements can decrease the incidence of fractures [27]. In Table 3 are described results obtained in studies showing a decrease of fracture incidence in patients receiving vitamin D supplementation.[27].

Vitamin D supplementation, with or without calcium, can increase bone mineral density (BMD), decrease bone turnover and decrease fracture incidence [28]. Vitamin D optimal dose may differ between individuals: different genetic polymorphisms, presence of chronic diseases and possible use of other drugs play an important role [29].

Table 3. Fracture risk reduction in patients receiving vitamin D supplementation.

Patients	Vitamin D dose	Obtained 25(OH)D nmol/L	Fracture risk reduction
3270	800 IU/d	71	Hip: - 43%
799	150,000 – 300,000 IU/yr	Not detected	Fractures: - 24%
2686	100,000 IU/4 times per day	74	Non vertebral fractures: - 22%
9605	400 IU/d	47	Non vertebral fractures: - 16%
3195	800 IU/d	75	Fractures: - 13%

Source: modified from Lips & Van Schoof [27].

2. Osteoporosis

Osteoporosis is a progressive disease caused by the deterioration of the bone structure because of the loss of bone mineral density (BMD) [27]; the main effect in people affected by osteoporosis is an increase of the risk of fractures for the rest of the life [30]. Considering the situation of the adult population (over 50 years) in the United States, more than 40 million adults are at high risk of developing osteoporosis because of a low BMD, while the total amount of patients affected by osteoporosis in the States is about 12 million [19]. Evaluating the situation in Europe, specifically in Italy, 3,5 million women and 1 million men are affected by osteoporosis; each year are reported 250,000 fractures due to osteoporosis, specifically 80,000 hips and 70,000 femurs [19]. Unfortunately,

within year, patients with fracture of the proximal femur show a mortality rate of 15-30% [19]. It is interesting to evaluate the occurrence of osteoporosis in african-americans, who are less affected by this disease compared to white population, and in small white women, that is, according to epidemiological available data, the most affected human category [31].

The World Health Organization defined the “calcium paradox” as the high intake of calcium consumed by populations in countries with the highest prevalence of osteoporosis [32]. Dairy cows milk consumed in these countries contains high levels of both calcium and phosphorus, while human milk contains about six times less phosphorus compared to dairy cows milk [33]. The high phosphorus content of cow milk intended for calves along with calcium’s poor bioavailability in humans has the potential to modify the serum calcium–phosphorus balance in humans, triggering parathyroid hormone to release calcium from bone. The high protein content of dairy cows milk can also contribute to negatively affect calcium balance; the final result is that the more dairy consumed in combination with other dietary sources of phosphorus, the higher the risk for osteoporosis [32].

Low BMD characterizes osteoporosis, with a consequent deterioration of the microarchitecture with trabeculae smallness, associated with reduced mineralization and an increase in cortical porosity [34]. BMD declines more quickly during winter compared to summer [35]. Vitamin D supplements (800 IU/day) combined with calcium eliminate the faster fall in BMD during winter [36]. A clinical trial showed a 37% lower risk of osteoporotic fracture in postmenopausal women younger than 65, if they consume vitamin D in a dose of 12.5 µg/day, compared to women consuming less than 3.5 µg/day vitamin D [37]. Furthermore, three other studies demonstrated that the combination of calcium and 20 µg vitamin D together reduced fracture risk in adults older than 65 years [35].

Vitamin D status is related to bone mineral density (BMD), not only in vitamin D deficient subjects, but also in vitamin D insufficient subjects [27]. A global study on vitamin D status and BMD in 7441 postmenopausal women with osteoporosis showed a significant positive relationship between serum 25(OH)D and BMD in the trochanteric area of the hip with a threshold below 50 nmol/L [38].

Patients with osteoporosis are usually treated with bisphosphonates, calcium and vitamin D are added, too [27]. In Italy, 1515 women with postmenopausal osteoporosis treated with bisphosphonates were classified as vitamin D deficient or vitamin D replete; the mean BMD increase per year in the lumbar spine was 0.22% in vitamin D deficient patients versus 2.11% in vitamin D replete patients, and similar differences were observed in the hip [39]. These data confirm that vitamin D and calcium addition in anti-osteoporotic treatment is necessary, unless the patient is vitamin D replete (serum 25(OH)D > 50 nmol/L) and has a dietary calcium intake of 1200 mg/d [28].

The guidelines for the management of postmenopausal osteoporosis describe importance of satisfying vitamin D requirements in order to obtain the best response in BMD, showing recommendations for its supplementation [31], together with calcium [40]. Diet appears to have only a moderate relationship to osteoporosis, but calcium and vitamin D are both important, at least in older populations; diets low in dairy products have been associated with increased risk of osteoporosis [41]. A meta-analysis of nine studies reported lower BMD of the spine and hip in vegans than in those who consume milk [42].

3. Vitamin D and Cancer

More than 8,000 clinical trials have been performed in order to investigate the inverse association among vitamin D, its metabolites, and cancer [43]. Women with higher solar UVB exposure in the Third National Health and Nutrition Examination Survey (NHANES III) showed 50% of the incidence of breast cancer compared to those with lower solar exposure [44]. In another survey, men with a higher solar UVB exposure showed only half the incidence rate of fatal prostate cancer [45]. The ultraviolet-B (UVB)–vitamin D–cancer hypothesis was based on a geographical ecological study of colon cancer mortality rates in the United States with respect to annual sunlight exposure [46].

Meta-analyses report significant inverse correlations between serum 25(OH)D concentration and incidence of all, bladder, breast, colorectal, kidney, and lung cancer [43]. Two studies were reported for colon and rectal cancer. In the first one, an inverse correlation was found between 25(OH)D concentration and incidence of distal colon cancer and rectal cancer; in the second one, colon cancer cases were directly correlated with 25(OH)D concentration, whereas no correlation was found for rectal cancer [47].

Another way to assess vitamin D's role in cancer is to examine the disparity in cancer survival rates between black and white Americans. In the period 2001-2004, black Americans older than 40 y had mean 25(OH)D concentrations between 35 and 43 nmol/L, whereas white Americans had mean concentrations around 63-65 nmol/L [48]. Considering these results, black Americans would have 60% higher cancer mortality rates than white Americans. According to the literature, disparities are evident for 13 cancers: bladder, breast, colon, endometrial, lung, ovarian, pancreatic, prostate, rectal, testicular, and vaginal cancer; Hodgkin's lymphoma; and melanoma [43]. Cancer-specific mortality rates for black Americans averaged about 25% higher than for white Americans.

Several other health benefits are associated with higher 25(OH)D concentrations, including reduced risk of autoimmune diseases [49], diabetes mellitus type 2 [50], adverse pregnancy and birth outcomes [51], respiratory tract infections [52] and Celiac Disease [53]. Recently vitamin D supplementation has been tested also to prevent coronavirus [54]. Thus, raising 25(OH)D concentrations in an effort to reduce cancer risk will yield additional benefits [55]. The optimal 25(OH)D concentration is certainly above 75 nmol/L and more likely 100-150 nmol/L; reaching those concentrations could take 1,000-5,000 IU/d of vitamin D3 or a moderate amount of sensible sun exposure [55]. The only way to ensure reaching the desired concentration is to determine serum 25(OH)D concentration [56].

The UVB-vitamin D-cancer hypothesis has considerable supporting scientific evidence from several study types [55]: geographical ecological, observational, and laboratory studies of mechanisms, as well as many clinical trials. Actually, people can spend more reasonable time in the sun and use vitamin D3 to prevent and treat many cancers [55]. The hypothesis that vitamin D supplementation can reduce the risk of cancer incidence should be further investigated in clinical trials, in order to determine the right doses of vitamin D and serum 25(OH)D concentrations, and eventually the presence of other possible safety issues.

4. Vitamin D in dairy products

Dairy cows breeding started around 5,000 years ago during the late Neolithic and early Bronze Age in northern and central Europe [57]. Domestication of animals for livestock has played a key role in the development of human civilizations. The cow has now become the main dairy animal associated with milk, with the term "milk" being almost synonymous with cow milk in most people's minds; however, milk from a range of other animal species is also consumed [58].

Milk is a complete food providing several nutrients to the consumers, specifically carbohydrates (mainly lactose), proteins, fat, minerals and vitamins, contributing in an average human diet a mean daily intake of 134 kcal, 8 g of proteins and 7.3 g of fat [59]. Water is the most represented compound in all different milks, ranging from water content lower than 50 percent in whale milk to a content close to 90 percent in donkey milk [60]. Knowledge of differences in nutrients in milk from various species facilitates development of products for consumers with specific needs, e.g. substitutes for cow milk for people with cow milk allergy [58], and milks formulated for the rehabilitation of malnourished individuals and other nutritionally vulnerable groups.

Milk is a natural source of calcium and vitamin D; both these nutrients interact in the human body [61]: when the level of ionized calcium in the blood falls, parathyroid hormone is secreted by the parathyroid gland, stimulating the conversion of vitamin D to its active form, calcitriol (1,25-dihydroxyvitamin D) and thus depleting vitamin D status (measured by the amount of the

inactive form). Vitamin D, as calcitriol, influences calcium absorption across the intestine, and inadequate vitamin D status is associated with reduced absorption of calcium from the diet [62].

Dietary intake of vitamin D through milk has been investigated since the 1960s [1]. Some years later, vitamin D content in cow's milk was determined in the range of 0.125–1 g/L [63], while a value of 240 IU per liter was detected in cow's milk for vitamin D activity, 85% of which is water soluble, attributed to vitamin D₃-sulphate [64]. Total amount of Vitamin D content can be described using different units, such as micrograms (µg) or International Units (IU); the most common unit used in Europe to describe vitamin D content is represented by µg, while to convert µg to IU the content in µg must be multiplied by 40 [21].

Human milk contains 24,25-dihydroxycholecalciferol and 1,25-dihydroxy vitamin D₃; vitamin D content in human milk is considered very low [64]. A study performed on 198 children, followed up to 9 years of age, evaluated the effect of maternal vitamin D status during pregnancy on childhood skeletal growth [15]. Results obtained in the children 9 years old fed by mothers who had vitamin D insufficiency (25 OHD levels < 40 nmol/L, 31%, n = 49) or vitamin D deficiency (25 OHD levels < 25 nmol/L, 18%, n = 28) during late pregnancy showed a lower whole body and lumbar spine bone mineral content (BMC). According to the results of this study, vitamin D supplementation is recommended in pregnant women, particularly during the winter months, in order to obtain a long-lasting positive effect on peak bone mass (PBM) attainment and a reduced risk of osteoporotic fracture in elderly age [65].

A recent study [66] determined a very interesting total vitamin D content in donkey milk (23 mg/L, about 920 IU/L), higher compared to the values obtained analyzing milk produced by several mammalian species, including human milk [67]. Even if donkey milk represents a niche product, the importance of this interesting result is correlated to its use in special categories of consumers at risk of nutritional deficiencies, such as children and/or elderly; in these patients, donkey milk could help in avoiding lack of vitamin D [58].

Milk consumption is decreased in the recent years, consequently dietary intake of vitamin D drinking fresh milk has declined [68], while cheese consumption has significantly increased by almost 100% since 1980 [68]. The large growth of human population and the change in food consumption habits created the right condition for producing new fortified foods able to provide the recommended intake of vitamin D in the daily diet. Milk does not provide the dietary requirements of vitamin D (Table 4), while cheese can represent the right food for the recommended dietary intake of this nutrient; in the States the fortification level of vitamin D in cheese is strictly regulated by the U.S. Food and Drug Administration [69].

In patients with osteoporosis, treatment with drugs is surely the best approach for decreasing the risk of other fractures. However, even in these patients the importance of nutrition should not be forgotten, because inadequate intake of Ca, vitamin D and proteins may reduce the efficacy of anti-osteoporotic drugs [69]. A study in which 37 elderly women with vitamin D deficiency received daily a fortified soft plain cheese was performed [69]; the dairy product provided 17–25% of the recommended allowance for vitamin D (10–15 mg), and 25% for both Ca (1200 mg) and proteins (1.0 g/kg body weight). Following the daily consumption of two servings of soft plain cheese during 1 month, the vitamin D supplement caused a small increase in serum 25(OH)D. The findings of this clinical trial demonstrated that fortified soft plain cheese consumed by elderly women with vitamin D deficiency can reduce bone resorption by positively influencing Ca and protein metabolism, as expressed by decreased PTH and increased IGF-I, respectively [69].

Table 4. Natural vitamin D content ($\mu\text{g}/100\text{ g}$) in foods.

Foodstuff	Vitamin D
Whole milk	0.1
Cheese, cheddar	0.3-0.6
Yogurt	0.1
Butter	1.5
Egg yolk	4.9-5.4
Mushrooms, chanterelle	5.3-14.2
Cod liver oil	210-250
Salmon, wild	13.1-24.7
Salmon, farmed	6.0
Herring	5.7-15.4
Cod	Trace-2.6
Sole	Trace-2.8

Source: modified from O’Mahoney et al. [21].

5. Fortified Foods

Vitamin D lack is a public health issue that affects each stage of the lifecycle and crosses sex, economic and educational classifications, causing big human and economic costs [70]. Consumers can improve and maintain vitamin D status through increased consumption of natural or fortified food sources or vitamin D-containing dietary supplements [71]. There are very few foods naturally rich in vitamin D, and most of these are subject to large seasonal variation in vitamin D content [72]. Frequent fish consumption can be a very effective way to maintain desirable circulating levels of 25(OH)D, as observed in elderly Japanese women [73]. Frequent fish eaters were able to maintain desired serum 25(OH)D levels even during the winter. Mushrooms represent another natural source of vitamin D; all edible mushrooms make abundant amounts of ergosterol, which, when irradiated with sunlight or UVB light, is converted to vitamin D2 [74].

An easy and practical method to avoid micronutrients deficiency is represented by fortification of commonly consumed staple foods [75]. The first case of food fortified dated around 4000 years BC, when the Persian physician Melampus enriched wine with iron filings to increase the sailors’ resistance and their sexual activity [76]. Around six thousand years later, in 1833, in France the chemist Boussingault inserted iodine into salt to prevent goiter; later, during 1920s in Denmark vitamin A was put in margarine, while during 1930s in the United States, in order to prevent rickets in children, dairy companies started to add vitamin D to milk [77].

Actually in Canada vitamin D fortification is mandatory for milk and margarine, while the addition of vitamin D to eligible foods in the U.S. is optional, with the exception of fortified milk [78]. Milk is the major fortified staple in the U.S. and Canada milk [72], but the amount of vitamin D added to milk (1 mg/100g fluid milk) is not adequate to produce the desired increase or maintenance of circulating 25(OH)D, while fortification at higher levels (10 mg/50g powdered milk) has been shown to be effective in improving vitamin D status and bone mineralization in older women milk [79].

Milk products are systematically, either mandatorily or voluntarily, fortified with vitamin D only in Finland, Norway, Sweden, Canada, and United States [80]. In Finland, the recommended fortification level of all fluid milks except some organic products is currently 1 $\mu\text{g}/100\text{ g}$, but some products with a concentration of 2 $\mu\text{g}/100\text{ g}$ are available on the market [80]. The fortification is voluntary, but all manufacturers unanimously follow the recommendations. In Norway, only one type of milk is recommended to be fortified with vitamin D at a concentration of 0.4 $\mu\text{g}/100\text{ g}$ [80]. Sweden recently doubled the fortification levels of fluid milks to 1 $\mu\text{g}/100\text{ g}$ and extended the mandatory fortification to cover all fluid milk products with <3% fat [9,10].

In other countries, such as United Kingdom, Ireland, Spain, and Australia, the fortification is not systematic, but there is a varying number of vitamin D-fortified milk products available [81]. In

countries with wide vitamin D fortification policies (Finland, Canada, United States), the total vitamin D intake as well as the contribution of milk to total vitamin D intake is higher compared to the countries without fortification policies, such as Ireland, United Kingdom, Spain, Australia [80]. The consumption of vitamin D-fortified milk was positively associated with 25(OH)D status in almost all studies reviewed by Itkonen et al [80], without an influence of country-specific vitamin D-fortification policies. Even though the consumed amounts of milk varied, the associations between milk and 25(OH)D status were seen also at fairly low consumption levels [82].

Vitamin D fortification of foodstuffs has proven to be a suitable vehicle to increase vitamin D dietary intake, and vitamin D-fortified fluid milk products contribute to both vitamin D intake and 25(OH)D status [83]. However, fortification of fluid milks may not be enough; country-specific staple foods should be chosen as optimal vitamin D carriers based on the results of simulation studies. In many countries without a current fortification policy (see Table 5), the option of systematic vitamin D fortification of food is under consideration, and simulation studies have been performed in recent years [84].

Table 5. Prevalence of Vitamin D deficiency (25-OH-D3 < 50 nmol/L) in Southeast Asia.

Country	Age (yers)	Prevalence (%)
Vietnam	Childbearing age	7
Indonesia	18-40	63
Thailand	15-98	5.7
Malaysia	7-12	72.4
Malaysia	48-53	41 males, 87 females

Source: modified from Yang et al. [84].

The optimal vitamin D status has not been yet determined; the Endocrine Society's Clinical Practice Guidelines established the lower serum threshold for 25(OH)D level as 75 nM or 30 ng/mL [85]. The Institute of Medicine (IOM) determined these thresholds for S-25(OH)D status: < 30 nmol/L is vitamin D deficient, 30–49.9 nmol/L is insufficient, and > 50 nmol/L is sufficient [86].

6. Vitamin D Fortification Strategies

Vitamin D₃, given in the form of cod liver oil, has been used in infant nutrition in northern Europe since the 1700's using a small teaspoon-full daily [87]. This arbitrary dose of cod liver oil was effective, as discovered in studies performed two centuries later [88]; the 375 IU (9 µg) of vitamin D₃ contained in the teaspoon was confirmed as being appropriate for infants [89].

Compared to the adult, vitamin D nutrition in the children has been well characterized [90]. In the 1960s, an expert committee on vitamin D could determine a requirement for vitamin D in adults and recommended one-half the infant dose [87]. Approaches to improve vitamin D status in the population include increasing intake of naturally vitamin D containing food, food fortification, vitamin D supplements, increasing solar UV-B exposure and weight loss [91]. Vitamin D food fortification seems to be the best method of improving vitamin D intake and status in human population in order to meet dietary vitamin D recommendations [92]. Considering the lack of natural vitamin D-rich foods, some countries, particularly populations at high latitudes, have initiated national policies of fortifying certain foods with vitamin D to prevent vitamin D deficiency [93]. Usually these vitamin D-fortified products are low-fat milk, fat spreads, breakfast cereals, and certain baby foods [83]. To better cover different populations with differing food habits, a wider vitamin D fortification of different products instead of concentrating on only a few staple foods has been suggested [83]. In general, food can be enriched with vitamin D by simply adding vitamin D to food (i.e., traditional vitamin D food fortification) or by so called "bioaddition" [94]. Bioaddition of vitamin D, which has also been called "biofortification," refers to various ways of increasing vitamin D content of food without direct exogenous addition of vitamin D. [94]

Milk fortification with vitamin D started in the USA during the 1930s [70]. At the beginning milk was fortified by irradiating milk with vitamin D or, as an alternative, by feeding the cows using irradiated yeast [70]. During the 1940s was developed a new simple and valid method based on vitamin D concentrate direct addition to milk; this practice is still used today. [70].

In the States, several RTE (ready-to-eat) breakfast cereals are fortified with vitamin D, that it is also added in some yogurt and margarines, while in Canada it is not permitted to fortify RTE breakfast cereals; However, in permitted foods, fortification of vitamin D must not exceed 20 IU/100 Calories [95]. The efficacy of vitamin D food fortification to increase vitamin D serum level has been tested [70]. Foods fortified with vitamin D normally contain 100 IU per serving; considering specifically milk fortified with vitamin D, its consumption increased vitamin D intake and was responsible for a significant increase of 25(OH)D levels [21]. An average daily intake of about 11 µg (440 IU/day) using fortified foods (range 120–1000 IU/day) achieved 25(OH)D concentrations to 7.7 ng/mL. This corresponded to a 0.5 ng/mL daily increase in 25(OH)D for each 40 IU (1 µg) ingested [70]. The most common food fortified with vitamin D is milk, contributing 44% of total daily vitamin D intake. Teen-agers males (13 to 18 years) [82] had the highest vitamin D intakes among the age/sex categories, but for all the consumers considered in that study dietary requirement level of 400 IU in order to reach serum levels of 16 ng/mL was not enough [96]. Therefore, considering the vitamin D dietary requirement covered when in serum vitamin D level is ≥ 30 ng/mL, the actual consumers mean intake of vitamin D can be considered low and not adequate compared to the daily nutritional requirements [97]. Higher levels of vitamin D fortification are required in order to increase the number of consumers with serum levels of 25(OH)D ≥ 20 ng/mL [98]. Vitamin D fortification strategies have also been evaluated in consumers living in developing countries, using fresh milk, cheeses and margarine, fortifying foods with both vitamins D and A, but results obtained in these programs have not been clearly discussed [99].

When considering vitamin D food fortification, it is important to evaluate whether or not such a public health intervention is likely to be cost-effective [100]. Usually, micronutrient fortification is considered the most cost-effective public health intervention [101]. With reference to vitamin D food fortification there is only few reports available on its cost-effectiveness. The available studies on this issue reported that systematic vitamin D fortification may indeed be highly cost-effective [102]. Regarding the costs for a typical food fortification programme, the following distribution of costs was determined: 80% recurrent production costs, 8% marketing and education costs, 7% food control and monitoring costs, and 5% other production costs [101]. A study estimated [103]. that the implementation of a vitamin D plus calcium fortification programme in Germany would cost 41 million Euros per year while saving 365 million Euros per year as a result of reduced fracture costs [103].

7. Vitamin D supplementation to prevent osteoporosis

The deposition of bone minerals starts during pregnancy, particularly in the last 90 days; bone mass can increase about 40 times from birth to adulthood, with a peak close to 90% occurring close to the age ranging between 18 and 20 years [15]; in fact, the most critical periods for bone minerals deposition are represented by childhood and adolescence [104]. Bone is a living tissue continuously subjected to cycles of bone formation and bone resorption: poor skeletal integrity causes an increased risk of osteoporotic fractures [105].

Clinical trials showed that mild vitamin D insufficiency can have a negative effect on bone mineral mass in adolescent females [106] and children [107]. The effect of vitamin D supplementation (200 IU/day or 400 IU/day) on bone mineral accretion has been examined in 212 adolescent girls (mean age 11.4 years) who were Ca replete [108]. Results showed that bone mineral augmentation at the femur was 14.3% and 17.2% higher, respectively, in the groups receiving the vitamin D supplementations compared with placebo. Furthermore, vitamin D supplementation significantly reduced bone resorption, as assessed by urinary deoxy-pyridinoline excretion [108]

In postmenopausal women, several studies based on vitamin D and Calcium supplementation have been performed, in order to determine the best nutritional strategies [109]. A pooled analysis,

describing the effect of vitamin D supplementation on fracture reduction, showed that there was a significant reduction in the incidence of hip fractures when doses higher than 792 IU/day were administered [110]. However, there was no significant decrease of the hip fracture risk caused by calcium intake [111].

Circulating 25(OH)D is generally considered the most reliable marker of vitamin D status [70]. The serum content of 25(OH)D necessary to maintain adequate levels of PTH is considered ranging between 30 and 100 nmol/L [112]. Because of this great variability, vitamin D insufficiency within populations can be differently evaluated depending on the threshold used. In a study performed using 8532 postmenopausal, osteoporotic European women, 79.6% were considered to have inadequate level of vitamin D if the serum 25(OH)D threshold was fixed to the value of 80 nmol/L, while when the threshold was reduced to 50 nmol/L the women with severe lack of vitamin D were a smaller amount, 32.1 % [113]. Basing on the results obtained in several clinical trials, actually 80 nmol/L is considered to be an overestimate threshold while 50 nmol/L is believed to be an acceptable threshold [109].

Dose used for vitamin D supplementation must be enough to reach the threshold values of serum 25(OH)D, otherwise any desired target will be obtained. Clinical trials performed with the aim of determining the anti-fracture efficacy of different doses of vitamin D found that 400 IU per day was not enough to achieve a significant effect in reducing fracture rate [114]. Oral daily doses of 700–800 IU or 100,000 IU taken quarterly both showed a positive anti-fracture effect, while an annual intramuscular dose of 300,000 IU did not show valid efficacy [114]. The results obtained in these studies show that the most effective vitamin D supplementation in osteoporotic patients is obtained when administered orally either daily or quarterly; in case of a daily supplementation, the dose should be higher than 700–800 IU/day [114].

However, it is important to consider that, according to several clinical trials performed all over the world, the most effective anti-osteoporotic results have been achieved with combined treatment with calcium and vitamin D supplementation [115]. In women over 65 year of age, risk of osteoporotic fracture can be frequent, particularly if a lack of calcium is associated with vitamin D deficiency [70]. In these cases, calcium and vitamin D supplementation can be useful, administering doses respectively of 1000–1200 mg calcium and 800 IU vitamin D daily [116]. The best recommended strategy is combining vitamin D and calcium into a unique supplement, in order to increase patient healthy status, with a consequent improvement of the treatment's efficacy.

8. Conclusions

Risk of osteoporotic fracture is increased in case of vitamin D deficiency [117]. In fact, biologically active vitamin D enhances calcium intestinal absorption by regulating calcium transport proteins in the small intestine, stimulating osteoclastic maturation and helping bone growth [53].

Vitamin D supplementation is required for many individuals to reach 25(OH)D concentrations above 30 ng/ml. However, vitamin D fortification of basic foods such as dairy and flour products can raise serum 25(OH)D concentrations, reducing risk of osteoporosis. Prevention is absolutely necessary, considering that by 2030, 25% of the human population, will be elderly.

Using appropriate feeding strategies in dairy cows, natural vitamin D content can be increased in dairy products, especially in fresh milk; further studies are necessary in this way, optimizing the total natural vitamin D contents in dairy products, considering that in several countries fortification of food is not always permitted as a common practice.

Conflicts of Interest: “The authors declare no conflict of interest.”

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