Chapter

Nutritional Considerations of Vitamin D Deficiency and Strategies of Food Fortification

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Abstract

Vitamins and minerals are crucial for human health. Any deficiency can lead to major diseases; however, the most prevalent one is the vitamin D deficiency. Due to its high risk in the Middle East and Lebanon, besides its major effects, solutions to decrease this deficiency are taken nowadays. Vitamin D food fortification is the most popular solution taken now. Liposomes showed highest efficiency in vitamin D fortification. However, a study must be done in order to deduce the amounts needed in the targeted population. Therefore, before fortification starts, FDA regulations must be reviewed. Several foods succeeded in fortification with vitamin D and increasing its levels such as milk and cheddar cheese. Stability and flavors showed good results over fortification, while according to the odor, water sources showed more aroma depth than oil sources. The AOAC methods for vitamin D amount in fortified foods must be applied. Dietary 25(OH)D3 was 7.14-fold more effective at raising serum 25(OH)D than dietary vitamin D3.

Keywords: vitamin D, food fortification, health, FDA, Middle East and Lebanon

1. Introduction

Human body is a complex matrix that requires various components for its healthy functioning. Vitamins and minerals are crucial elements that are often obtained from one's diet; these are referred to as micronutrients. Most of the latter are not produced by the human body and an unbalanced diet could inevitably lead to various nutrient deficiencies that could pose dramatic health risk for the human body that if severe enough could lead to death. Nutrient deficiency could be a direct effect of the changing eating patterns, but mostly due to poverty and lack of access to proper food especially in low- and middle-income societies. For this reason, studies and research were directed toward fortifying consumed food with vitamins that are most likely to be deficient and their deficiency could pose tremendous health problems. One of the most popular nutritional deficiencies in Lebanon and the Middle East is vitamin D deficiency. Due to the high frequency of vitamin D deficiency in Middle East and Lebanon, it is essential to find a product that is widely consumed among the population and provides a good source of vitamin D regarding its stability. Dairy products are one of the most famous consumed foods in our society, which is why it was chosen to be fortified with vitamin D.

2. Nutrition and vitamins

2.1 Factors contributing to individual wellness

Healthy lifestyle improves individual strength, prevents certain illnesses, and maintains an advisable weight. Wellness incorporates physical, emotional, and spiritual well-being (**Figure 1**). The concept of wellness is not an end point in a person's life but rather an ongoing lifestyle. Diet and exercise are so linked to each other that they can be considered opposite sides of a coin. An individual's nourishment is impacted by how much energy is used to perform daily exercises; meanwhile, physical activity directly affects the utilization of supplements in food. Consuming a nutritious diet allows one to progressively perform strenuous exercises for longer durations and prompts an overall better feeling. On the other hand, an imbalanced food intake could lead to inactivity or, in extreme cases, cause severe medical issues [1].

2.2 Essential nutrients for health

Our daily consumed foods include bread, rice, dairy products, fruits, vegetables, and meats. Each of them is composed of various nutrients, which are each characterized by its unique chemical composition. Each has its own function; however, they work together to complete body's functions. Food nutrients are carbohydrates, proteins, fats, vitamins and minerals, and water. Fiber is likewise a fundamental part of our eating routine [2].

Macronutrients (carbohydrates, lipids, and proteins) are those that the body requires in huge quantities to give energy and maintain growth, whereas micronutrients including vitamins and minerals are those nutrients required in lesser quantities to help normal health and body functions [1].

Figure 1.
Factors contributing to individual wellness [1].

2.2.1 Macronutrients

Macronutrients (carbohydrates, lipids, and proteins) are energy-providing nutrients [1].

- Carbohydrates: consist of the three main constituents: carbon, hydrogen, and oxygen. They are made of 3 groups: monosaccharides, disaccharides, and polysaccharides. Monosaccharides are single unit carbs such as glucose. Disaccharides are composed of two sugars connected together. An example of this class is lactose, which is known as milk sugar. Polysaccharides are those formed by more than two sugars and are generally more complex chains such as starch and dextrin [2].

- Fats: is an essential part of our body. Fat serves a vital role in protecting the cells and tissues of the essential organs in our body such as brain and heart. Food fats are composed of solid fats, fluid oils, fat-soluble vitamins, and cholesterol. Fats contain oxygen less than carbohydrates. Hence, 1 g of lipids gives the body more energy than carbohydrates (9 Cal/g of fat compared to 4 Cal/g of carbohydrates) [2].

- Proteins: are huge natural compounds. Proteins, similar to starches and fats, contain carbon, hydrogen, and oxygen. Likewise, proteins contain around 16% nitrogen, which differentiates them from starches and fats. Proteins are used mainly for growth and development purposes [2].

2.2.2 Micronutrients

Nutrients required in little quantities to have healthy and normal body functions are "micronutrients" [1].

- Minerals: are inorganic elements that are not broken down by digestion, absorption, or heat. Minerals support with the regulation of body functions and are classified into major and trace minerals [1].

Minerals are needed for body-building; enhancing bones, teeth, and structural parts of soft tissues; muscle contraction; clotting of blood; and nerve stimuli [2].

- Vitamins: are organic compounds that assist in controlling physiological processes [1]. They engage in various activities throughout the entire body and help in the release of energy from the macronutrients [3].

2.2.3 Water

Water represents around 60 percent of the body weight. It is necessary for the use of food materials in the body and for disposal of food excess later on [2].

2.3 Vitamins

Vitamins (natural carbon-containing composites) adjust many of the body's processes [1]. They are available in small amounts; however, they are essential for physiological functions as growth and development. Vitamins can work as antioxidants, cofactors in metabolic oxidation-reduction responses, and hormones [4].

2.3.1 Fat-soluble vitamins

Vitamins A, D, E, and K are fat-soluble. They remain inside the fatty parts of foods and are absorbed along with dietary fat. Fat-soluble vitamins have good storage in the body since they are kept in the adipose tissues. Fat-soluble vitamin toxicity symptoms include hair, skin, bones, eye injuries, and anorexia nervosa [1].

2.3.2 Water-soluble vitamins

Fat-soluble vitamins include the B vitamin and ascorbic acid (vitamin C). They are distributed in many foods. Water-soluble vitamins are absorbed easily via the enteral tract straight into the blood and then into the cells. Water-soluble vitamins are not stored in large amounts, except vitamin B12. Thus, they should be eaten daily [1].

2.4 Vitamin deficiencies

Vitamin deficiency is the condition of a long-term lack of a certain vitamin. Most common and serious vitamin deficiencies are as follows [1]:

- Vitamin A, which leads to vision defect, impaired growth, and immunity
- Vitamin D, which leads to rickets, osteomalacia, or osteoporosis
- Vitamin K, which leads to coagulopathy and bone health impacts
- Vitamin B12, which leads to pernicious anemia, nerve damage, memory loss, and dementia
- Vitamin C, which mainly leads to Scurvy disease, fractures, and depression

3. Vitamin D

Vitamin D, known as the sunshine vitamin, is a real hormone delivered from body's sterols by the photolytic activity of UV light on the skin [4]. It is a fat-soluble seco-steroid that may be either created within the skin from its precursor (7-dehydrocholesterol) by exposure to sunlight or offered from diet [5, 6]. Vitamin D with calcium, magnesium, and phosphorus plays a crucial role to support bones and teeth health [4].

3.1 Vitamin D sources

Vitamin D can be attained from foods as vitamin D3 (cholecalciferol) or as nutrient D2 (ergocalciferol). D3 is acquired from animal sources, while D2 is available in parasites and mushrooms irradiated with UVB [7]. Vitamin D approximate content in various foods is shown in **Table 1** [8].

Since most foods contain low measurements of vitamin D (**Table 1**), fortifying common foods is becoming a trend and common practice nowadays. In addition, nutritional supplements are a reliable solution for compensation of the vitamin deficiency [4].

Food	Approximate vitamin D content (µg/100 g)
Fortified	
Milk	0.8–1.3
Margarine	8–10
Nonfortified	
Butter	0.3–2
Milk	<1
Cheese	< 1
Liver	0.5–4
Fish	5–40

Table 1.
Major food sources of vitamin D [8].

3.2 Vitamin D needs and normal levels

The Food and Nutrition Board has set an adequate intake (AI) for vitamin D due to the inability to set a more precise RDA level because of the variability in sun exposure among individuals [9]. Recommendations for vitamin D instructed an AI for vitamin D of at least 500 IU/day (12.5 µg) and more than 1000 IU/day (25 µg) for those not exposed to enough sunlight [8].

International units are used to quantify vitamin D. 1 IU is equal to 25 metric weight unit of cholecalciferol, and 1 g of cholecalciferol is equal to 40 IU [7].

3.3 Metabolism and regulation

Metabolism of vitamin D occurs through different stages that includes hydroxylation. Through these stages, vitamin ingested is transformed into its active form (**Figure 2**) [4].

Vitamin D synthesis starts when 7-dehydrocholesterol (cholesterol precursor found on skin) is visible to sunlight and then transformed to previtamin D [9, 10]. 7-Dehydrocholesterol is an intermediate precursor for vitamin D, it is found throughout the epidermis and dermis, and thus has the most elevated limit with respect to cholecalciferol synthesis [11].

The first step occurs when 7-dehydrocholesterol absorbs the UVB photons to convert them into previtamin D3. Then, photoisomerization occurs in order to covert previtamin D3 into vitamin D3 (cholecalciferol). However, it is not a problem since during delayed sun exposure previtamin D3 is converted into its inactive forms (lumisterol and tachysterol) [12].

Large portion of vitamin D reaches liver from lipoproteins or vitamin D–binding protein and is then transformed by hydroxylation to yield 25-OH-D3 [4]. 25-OH-D3 is the major circulating type of vitamin D used by clinicians to evaluate vitamin D level, though it is latent and must be transformed to its active form (1α,25-dihydroxyvitamin D) in the kidneys [13].

When calcium decreases in the body, parathyroid hormone is released leading to calcitriol synthesis increase (vitamin D active form: 1α,25(OH)2D) (**Figure 3**). Because of the hypocalcemia (declined blood calcium), PTH is released from parathyroid gland resulting in an increase in the hydroxylation of 25(OH)D3 to calcitriol. Then, calcitriol plays its role with PTH or by itself on its target tissues leading to an increase in serum calcium levels. Kidneys, bones, and intestines are the primary target tissues [8]:

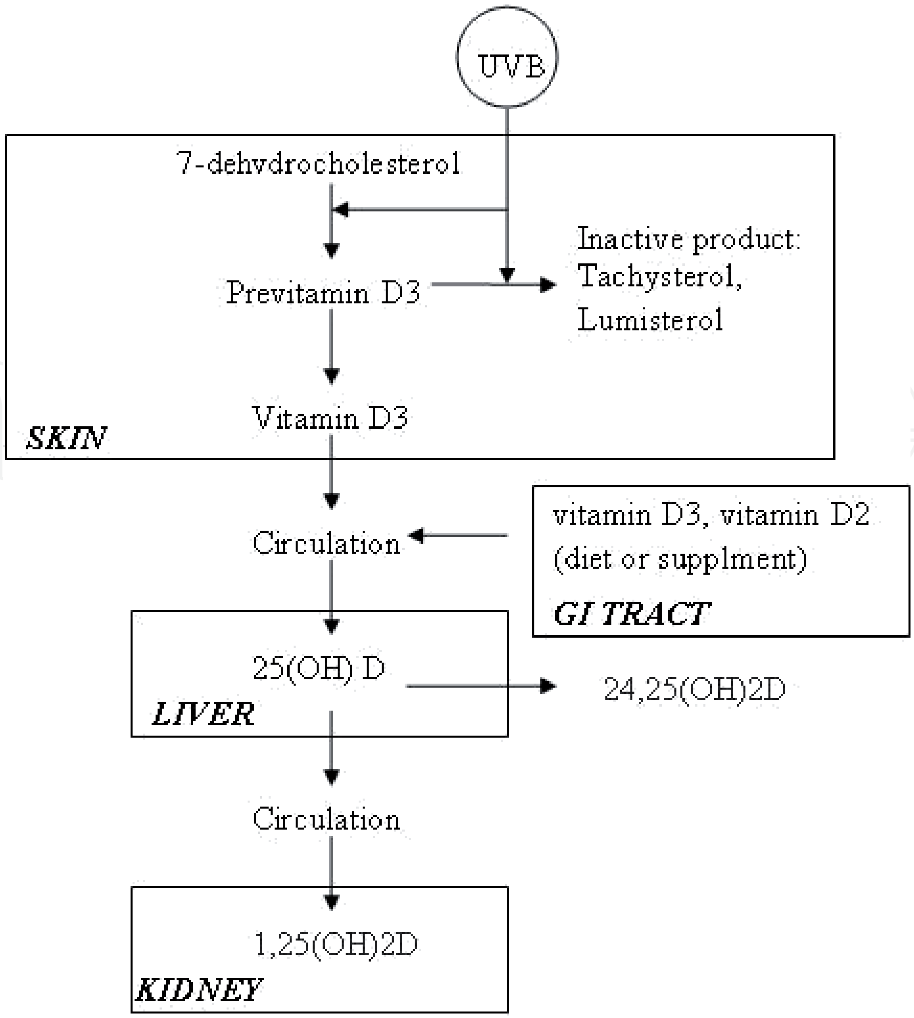

Figure 2.
Sources, sites, and processing of vitamin D metabolites [10].

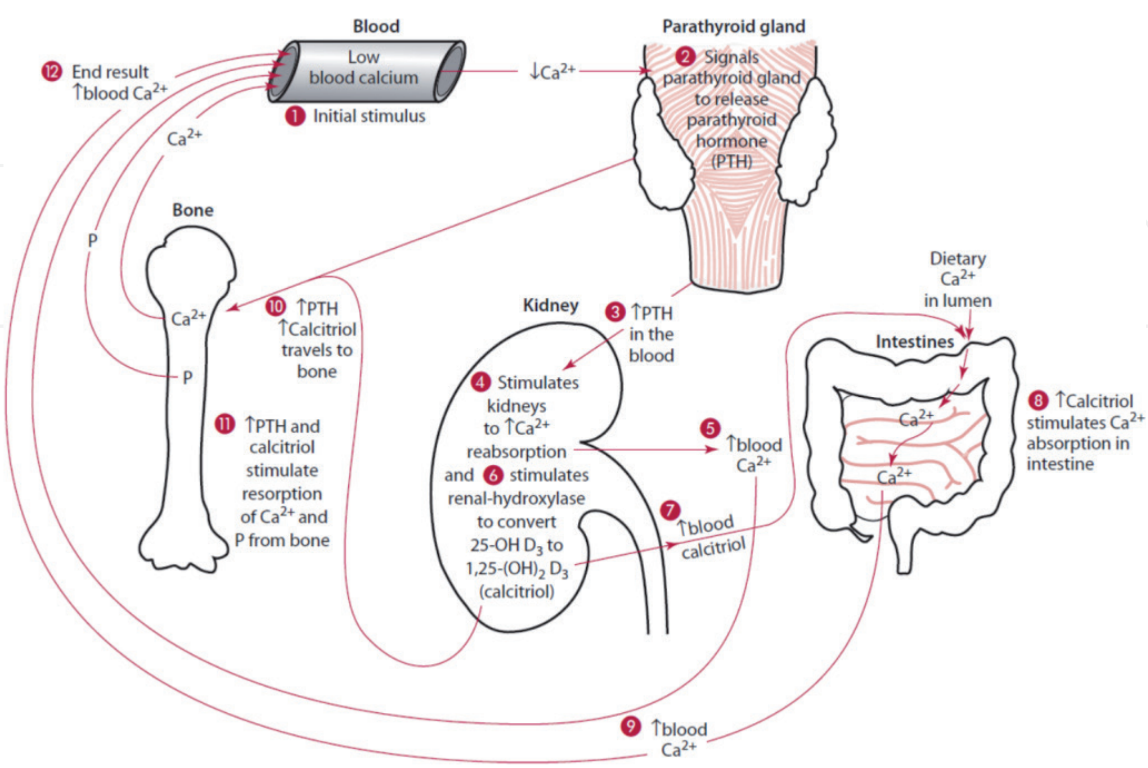

Figure 3.
Calcitriol, 1,25 (OH)2 D3, synthesis and actions with parathyroid hormone (PTH) [8].

3.3.1 Intestine

Increasing calcium and phosphorus absorption is the major role of calcitriol in the intestines. It works by the participation of calcitriol with cell membrane receptors to improve calcium absorption [8].

3.3.2 Kidney

Calcitriol with the help of PTH increases calcium reabsorption in the distal renal tubule into the blood. Phosphorus released by the kidney is boosted and can result in lower serum phosphorus levels [8].

3.3.3 Bone

Regarding the bone, PTH plays its role with calcitriol for reabsorption increase of calcium and phosphorus from bone to reach normal blood calcium level. Osteoclasts catalyze calcium and phosphorus from bone. The net impact of this is to raise blood calcium and phosphorus levels [8].

3.4 Factors affecting vitamin D production

Vitamin D skin synthesis is negatively influenced by factors such as the following [6]:

- Season: winter season decreases the quantity of ultraviolet light reaching the skin, while summer season increases it.
- Skin pigmentation: dark pigments interfere with UV light entering the needed skin layer.
- Clothing: covering the skin leads to inadequate sunlight skin exposure.

3.5 Clinical manifestation of vitamin D levels

Calcidiol (25(OH)D) is the vitamin D metabolite that is estimated to identify a patient's vitamin D status [14]:

- Vitamin D deficiency: 25(OH)D between 21 and 29 ng/mL
- Vitamin D lack: 25(OH)D < 20 ng/mL
- Normal vitamin D status: 25(OH)D > 30 ng/mL
- Vitamin D overproduction: 25(OH)D > 40–60 ng/mL
- Vitamin D toxicity: 25(OH)D > 150 ng/mL

However, females are at 3 times higher risk of having vitamin D deficiency compared to males. There is no relationship between the age and vitamin D levels in males, but in females, those having an age between 30 and 40 are at higher risk for deficiency. But, children (10–20 years) are at highest risk for deficiency [15].

3.6 Vitamin D transport

3.6.1 Transfer from chylomicrons to plasma

Practically, all consumed vitamin D is held in a nonesterified structure, which is believed to be associated with the outside of chylomicrons (lipoprotein particles). Vitamin D that is not moved in the plasma is taken up with chylomicron remainders by the liver, where it is then transferred to the same binding protein and discharged to the plasma [4].

3.6.2 Vitamin D–binding protein

Vitamin D is transferred in the plasma to a great extent in association with protein, as other sterols, which is the vitamin D–binding protein (DBP) [4].

3.7 Vitamin D storage

Vitamin D storage in the liver is minimal, in contrary to other fat-soluble vitamins. Vitamin D levels do not go above 25 nmol per kg in the liver. Plasma calcidiol (25-hydroxyvitamin D) is the storage form of vitamin D, which has a half-life of 3 weeks [11]. Long-standing admission of vitamin D inside the physiological range induces storage in fat tissues and most likely in other tissues of clinical importance [16].

3.8 Vitamin D toxicity

Vitamin D toxicity is incredibly uncommon; however, it can occur by unplanned or purposeful ingestion of unreasonably high portions. Portions of more than 50,000 IU every day raise levels of 25-hydroxyvitamin D to more than 150 ng for each milliliter (374 nmol/L) as well as hypercalcemia and hyperphosphatemia. Portions of 10,000 IU of vitamin D3 every day for as long as 5 months do not cause toxicity [13]. Excessive exposure to sunlight represents no danger of toxicity through overproduction of endogenous cholecalciferol [8].

When calcium plasma concentrations reach 2.75–4.5 mmol/L, vitamin D toxicity causes several symptoms such as nausea, appetite loss, cramps, and diarrhea, and in more severe cases, it causes hypercalcemia. When plasma calcium levels exceed 3.75 mmol/L, hypertensive encephalopathy occurs due to the contraction of smooth muscles. Hypercalcemia and expanded vitamin D levels lead to soft tissue calcification (kidneys, heart, and lungs) [11].

4. Vitamin D deficiency

The most popular and worldwide deficiency currently is vitamin D deficiency [17]. It is a worry for public health and has several acute and chronic impacts. It results from wrong lifestyle starting in predominance obesity and inadequate sun exposure [18].

4.1 Vitamin deficiency

Living with a lifestyle that is inadequate in any food group will result in a vitamin deficiency. A vitamin deficiency results in different diseases and disorders. The clinical signs and symptoms are the final stage in hypovitaminosis [4].

Stages of vitamin deficiency are as follows:

1. Marginal deficiency
   - Stage I—depletion of vitamin stores
   - Stage II—cellular metabolic changes
2. Observable deficiency
   - Stage III—clinical defects
   - Stage IV—morphological changes

During marginal deficiency, there is only depletion of vitamin stores and its effect on cells. This depletion cannot be recognized without chemical or biochemical testing, which shows the stores' concentrations. However, in observational deficiency, the signs and symptoms appear and morphological changes take place [4].

4.2 Vitamin deficiency causes and effects

Vitamin D deficiency is caused by the following [13]:

1. Decreased synthesis in the skin: due to creams, aging, and skin pigment, the vitamin D3 synthesis will be reduced.
2. Decreased bioavailability: obesity and malabsorption diminish the availability of vitamin D3.
3. Breastfeeding: decreased amount of vitamin D in human milk can lead to vitamin D deficiency when the child is exclusively breastfed.
4. 25-hydroxyvitamin D diminished synthesis: vitamin D malabsorption caused by liver failure results in 25-hydroxyvitamin D diminished synthesis.
5. 1,25 dihydroxy vitamin D diminished synthesis: decreased phosphorus excretion and decreased serum levels of 1,25-dihydroxyvitamin D are caused by chronic kidney diseases.

4.3 Manifestation of vitamin D deficiency

Vitamin D deficiency's first stages include rickets and osteomalacia, whereas the final and long-term stages include osteoporosis in which there are chronic changes [19].

4.3.1 Rickets

Vitamin D deficiency in children leads to rickets. Rickets is classified by bone mineralization loss [8]. This occurs due to deficiency in both vitamin D and calcium [7]. Consequently, the bones twist due to their inability to hold the body, stand, or walk [3].

4.3.2 Osteomalacia

As rickets occur in children, osteomalacia occurs in adults [4]. Osteomalacia is the result of the demineralization of bones [7]. In the case of osteomalacia, nonmineralized bones are much more than mineralized bones. Consequently, the patient will have muscular weakness, bone tenderness, and pain and will be at higher risk of fractures [4].

The decreased ability to produce vitamin D3 in elderly due to the decrease in 7-dehydrocholesterol in the skin results in osteomalacia also. Therefore, vitamin D is essential for preventing and treating osteomalacia in elderly [7].

4.3.3 Osteoporosis

Osteoporosis is the result of inability to synthesize or get enough vitamin D from sunlight and food. This results in bone calcium loss and thus fractures [3]. It is due to multiple causes as aging, weakened vitamin D digestion, and decreased estrogen levels. It is widely seen in elderly, postmenopausal women, and those taking steroids [4].

4.4 Vitamin D deficiency prevention steps

Vitamin D deficiency can in fact be prevented by different methods. These can include the following [13]:

- Expose body to sun or ultraviolet radiation for the needed time.
- Ingestion of 400–100 IU/day for kids and 800–1000 IU for grown-ups.
- Control serum phosphate in case of kidney disease.
- Sustaining maintenance level.

4.5 Vitamin D deficiency in Middle East and Lebanon

International Osteoporosis Foundation (IOF) study showed that almost all countries have 25(OH)D3 levels beneath 30 ng/ml, while South Asia and Middle East have levels beneath 10 ng/ml [19].

The most widely seen medical issue in Middle East and North Africa is vitamin D deficiency. This results in diseases and disorders and most widely osteomalacia. Results showed 50–90% 25(OH)D3 deficiency, and these patients have levels below 20 or 30 nmol/ml [15].

Results showed higher rates of vitamin D deficiency in Lebanon especially Beirut regions as 57.86% were deficient using a cut-off of 20 ng/ml [15].

Females are 3 times more prone to have hypovitaminosis D. An investigation on Lebanese grown-up population has demonstrated that hidden ladies had just about 3 times more incidence of extreme hypovitaminosis D than nonhidden subjects [15].

Results verified that females are at more risk to have vitamin D deficiency. Besides, hidden ladies are 3 times more at risk than nonhidden ladies [15].

5. Vitamin D fortification

5.1 Food fortification basic principles

Fortification is the process of supplementing food with needed nutrients for their health benefits and in order to prevent diseases as defined by the Codex General Principles [20].

Fortification levels differ from one nation to another according to different factors. These factors include the dimension of enrichment, the bioavailability of the fortificants, and the range of fortified consumed foods. Fortification can be achieved by adding a single nutrient (such as the addition of iodine to the salt), or by adding a blend of nutrients [20].

The general medical advantages of fortification include the following [20]:

- Prevention of micronutrient deficiency
- Correction of a micronutrient deficiency
- Achieving the health benefits of a nutrient (for example, there is some proof to recommend that a diet full in selected antioxidants may aid in preventing cancer and different disorders.)

5.2 Vitamin D fortification history

In order to prevent or defend rickets, humans usually used one teaspoon of cod liver oil daily (1 teaspoon has 10 μg = 400 IU of vitamin D). Vitamin D food fortification started in the 1940s in the US and other nations such as Britain. In the beginning, vitamin D was supplemented to milk and then to other foods and beverages. However, in the 1950s, events of hypercalcemia appeared, and an adjustment was done for fortifying foods with vitamin D. As a result of that, few side effects of hypercalcemia had been seen in newborn children in the former German Democratic Republic, where babies were enriched with discontinuous portions of 15 mg (600,000 IU) of vitamin D as a push to defend rickets. In these newborns, serum 25(OH)D levels increased to a few hundred nmol/L [17].

5.3 Vitamin D3 fortification forms

Various techniques for fortification of foods with vitamin D were accomplished. In a study done for the evaluation of fortifying cheddar cheese with vitamin D methods, vitamin D3 was supplemented by: addition of a water-soluble emulsion, crystalline liposoluble vitamin D, or water-soluble vitamin D multilamellar liposomes [21]. Results showed better recovery of vitamin D3 in liposomes than that in homogenized cream and water-dissolved emulsions. Similar results were shown in commercial water and fat-soluble types of vitamin D3 in delivering an evenly dispersed concentration of vitamin D3 in prepared cheddar [22].

Vitamin D3 was fortified into a cheddar-like matrix, yogurt, or dessert in either a crystalline or an emulsified structure. The emulsified structure was more stable in cheddar over a three-month storage period at 4°C, with roughly 6% of the crystalline vitamin D3 lost under these conditions, while the two types of vitamin D3 were stable in yogurt and dessert with storage for the normal shelf life of the items [23].

5.4 Data required for determination of vitamin D fortification levels

Vitamin D fortification levels determination requires the following [24]:

1. Survey about the quality of the diet and amount of ingested nutrient in a target population
2. Average sun exposure adequacy in accomplishing vitamin D adequate levels
3. The recent vitamin D levels in the country

When the fortification program is achieved, supervising and assessments should continue to get better data and higher effectiveness [24].

5.5 Factors affecting vitamin D food fortification

Vitamin D food fortification is affected by factors such as availability of fortificants and suitable vehicles for the fortificant [24]:

1. Availability of mechanical experience in producing D2 and D3.

2. Having a good capacity for raising 25-hydroxyvitamin D blood concentration.

3. Having different formulas that suit all foods: the fat-rich items and fat-poor foods. For example, a formula of dry preserved vitamin D is found and has benefits as it contains antioxidant that defends the strength of vitamin D for extended time, yet within the appearance of minerals.

4. Having the proper vehicle for fortification:

    a. Widely consumed food must be used as fortification vehicle, financially available, and consumed all through the year.

    b. The fortification vehicle must ensure an even scattering by economical technologies.

    c. Vitamin D bioavailability in fortified items, for example, milk, milk powder, and cheddar.

    d. Vitamin D fortification vehicle must have negligible olfactory impact.

5.6 FDA regulations

FDA authorizes the use of nutrients as supplements and additives after enough scientific safety reviews. The additive must be used under the law to stay in the safe side. Several studies were done to set a law for the use of vitamin D and its safety [25].

Vitamin D is available in different structures. The two main structures are vitamins D2 and D3. Vitamin D is certified and commonly identified as safe (GRAS) to be used in food, with the following exact conditions [25].

FDA authorized adding vitamin D to infant formulas, calcium-fortified foods and beverages, breakfast cereals, certain cheese and cheese products, soy beverages, and milk and milk products [25] (**Table 2**).

Category of food	Maximum levels in food (as served)
Breakfast cereals	350 IU/100 g
Grain products and pasta	90 IU/100 g
Milk	42 IU/100 g
Milk products	89 IU/100 g

Table 2.
Maxim levels of vitamin D in foods [25].

The IOM (Institute of Medicine) specified the maximum allowed daily intake as UL of a supplement that represents safety of conflicting impacts when the supplement is ingested over delayed time [25].

5.7 Foods fortified with vitamin D

The USA began vitamin D milk fortification in 1930s. In the USA, vitamin D food fortification is optional. They focus on food vehicle and the practical and dimensional use in order to avoid fortification above what is recommended by the FDA. The addition of vitamin D commonly in conjunction with calcium is usually to several foods including orange juice, breakfast grains, rice, and soy milk [24].

Vitamin D widely used concentration is 100 IU per serving. The consumption of foods that have been fortified with vitamin D significantly increased 25(OH)D blood levels. The average personal intake of almost 11 μg/day (440 IU/day) from fortified foods (range 120–1000 IU/day) raised 25(OH)D levels by 7.7 ng/mL. Said relation correlates to a 0.5 ng/mL rise in 25(OH)D for every 40 IU (1 μg) ingested/day calories [24].

5.7.1 Milk

A couple of studies have researched the long impact of additional vitamin D3 on the initial vitamin D load of milk. Hollis in his article "Vitamin D and Its Metabolites in Human and Bovine Milk" demonstrated a 10-fold rise of vitamin D3 consumption from 100 to 1000 μg/d and contributed a 7·5-fold rise in vitamin D3 levels of the milk and a 2-fold rise in 25(OH)D3 [26].

5.7.2 Eggs

Two frequently used strategies to fortify eggs with vitamin D are: increasing sunlight exposure and supplementing bird diet with vitamin D. It is proved that eggs that were exposed to light have higher vitamin D3 content. Browning and Cowieson verified the effectiveness of diet enrichment with vitamin in increasing both vitamin D3 and 25(OH)D3. However, Browning, Cowieson, and Duffy verified that addition of 25(OH)D3 had positive impact on 25(OH)D3 level of the egg yolk. Thus, within a diet, 25(OH)D3 could be consumed straightforwardly by hens with no exchange to vitamin D3 within the circulation [26].

5.7.3 Cheddar cheese

It is verified that vitamin D is stable during pasteurization, manufacturing, and maturing. It is evenly distributed in the cheddar and does not influence the flavor. The positive part is that vitamin D has the same effect in supplements and in cheddar matrix. The disadvantage in fortifying cheddar with vitamin D is the loss in the wheying off step. However, it is solved by decreasing the whey load before manufacturing and the use of ultrafiltration (UF) [27]. Ganesan proved vitamin D stability over a 9-month maturity period while using typical types of vitamins. The use of UF to cheddar milk results in little yet considerable reductions in vitamin D losses in whey [28].

5.7.4 Bread

Bread is a widely consumed food item; it is prepared from flour and water by baking, as well as nongrain material such as nuts, fats, raisins, and nutrients to improve the nutritional quality of the meal eaten. Vitamin D can be added during different stages (fermentation, baking, and storage). It is verified that 60 min is the reasonable time for dough fermentation, since when raising the fermentation time, oxidation of vitamin occurs [5].

5.8 Stability, odors, and off-flavors

In the USA, milk is commonly enriched with vitamin D with an amount of 400 IU per quart. Vitamin D fortification occurs before pasteurization step, and the liquid milk shelf life is usually 1 year at room temperature and in dark [29].

5.8.1 Stability

The stability of vitamin D throughout production and storage in dairy and milk products is verified, as well as its stability in high temperature short time handling and storage without light and acid [29].

Vitamin D loses its stability in some conditions. It decreases in acidic media as it transforms into its inactive form isotachysterol, or by heating to a temperature above 150°C in the presence of air [27]. However, it is stable at temperatures below zero, at 4–8°C, at 25°C, and during cooking at 200°C [24].

In the study of elevating vitamin D3 under two different conditions, high temperature processed decreased fat milks (increased temperature/decreased time, pasteurized at 73°C for 15 s, and ultra-heat treated, purified at 138°C for 2 s) and reduced fat yogurt (purified at a temperature of 85°C for a half hour), it is verified that the amount of vitamin D does not decrease during preparation [30]. In addition to that, it was stable during storage and has no effect on sensory qualities [23].

Banville found that vitamin D3 fortification in cheddar in the liposome form decreased after 3–5 months of maturing and lost its stability [21]. It is shown also that vitamin D3 has stability in nonfat foods such as in squeezed orange (storage for 30 days at 4°C) [31]. Both vitamin D3 and vitamin D2 showed their stability in fortified squeezed orange [32].

5.8.2 Flavor

Even at maximal level of fortification (1200 IU), there were no flavor differences in vitamin D–fortified milk. This shows that increasing the concentration of vitamin D is better to improve the nutritional quality and increase vitamin D amount ingested and do not influence the liquid milk flavors [29].

5.8.3 Odors

Odors were affected by vitamin D fortification, as they give rancid oil and painty odor. However, it is shown that the aroma depth is way more in water source than that in oil source. In oil sources, the aroma depth is lesser due to the oil matrices found that defend oxidation. After vitamin D oxidation occurs, aldehydes are found in the product [29].

5.9 Measurement of vitamins D2 and D3 in foods

The Association of Official Analytical Chemists (AOAC) International, which set up authority and "legally defensible analytic" strategies in the United States, has approved the accompanying chemical techniques for the examination of vitamin D in foods [23]:

Method 980.26: vitamin D in multivitamin preparations

Method 981.17: vitamin D in fortified milk and milk powder

Method 982.29: vitamin D in mixed feeds, premixes, and pet foods
Method 992.26: vitamin D3 in ready-to-feed milk-based infant formula
Method 995.05: vitamin D in infant formulas and enteral products (for tube feeding)
Method 2002.05: cholecalciferol (vitamin D3) in selected foods (fortified milk, infant formula, gruel, margarine, and cooking and fish oil)

The AOAC strategies recorded above are alike on a basic level. Every strategy includes four key advances [23]:

1. A digestion step to break down the food matrix (alkaline saponification)
2. An extraction step for the separation of vitamin D from the food matrix
3. A clean-up step, to isolate vitamin D from other food components
4. Quantitative detection by HPLC with UV

However, these strategies are time-, work-, and expert-consuming. Besides that, they are allowed only for restricted items (generally dairy), and not suitable for nonfat items in certain countries. The AOAC techniques target vitamin D3, and not vitamin D2, that might be available either normally or as a fortificant [23].

Instrumental techniques for the analysis of vitamin D in foods incorporate [23]:

- partition by high-pressure liquid chromatography (HPLC),
- detection by ultraviolet absorption (UV), and
- diode array detection (DAD) or mass spectrometry.

The accompanying approach was created, approved, and used to survey vitamin D3 levels in Danish pork and dairy items [33, 34]:

1. Homogenization and addition of vitamin D2 internal standard
2. Digestion by alkali saponification for 45 min in a boiling water bath
3. Extraction with petroleum ether: diethyl ether
4. Clean-up with silica solid-phase extraction columns
5. Purification with semipreparative HPLC (amino + silica columns, normal phase)
6. Detection by reverse-phase HPLC with DAD

5.10 Vitamin D fortification efficacy

It is verified that the oral use of 25(OH)D3 is efficient in rising plasma 25(OH) D levels [26]. The common level of vitamin intake range is between 10 and 20 μg/d. Cashman in his study gave adults (mean age of 57 years) 20 μg vitamin D3 or 20 μg 25(OH)D3 for those having with serum 25(OH)D of 28·9 nmol/l in winter. The results showed after 2.5 months treatment an increase in both vitamin D3 and 25(OH)D3. Jetter in his study provided fit postmenopausal ladies with 20 μg 25(OH)D3 or 20 μg vitamin D3 for about 4 months over the winter. The results showed better effectiveness of 25(OH)D3 than that of dietary vitamin D3 [26].

Results showed better effectiveness for 25(OH)D3 in absorption and increasing the plasma levels. In addition to that, 25(OH)D3 is better for human health as Bischoff Ferrari et al. proved a decline in systolic blood pressure and enhancements in a few markers of immunity in healthy postmenopausal ladies. Also, 25(OH)D3 supplementation corrected the excess bone turnover, improved plasma lipid level rise in HDL cholesterol, and diminished LDL-cholesterol in osteopenic and dyslipidemic postmenopausal ladies [26].

6. Conclusion

After referring to various studies considering fortifying foods and dairy products with vitamin D, it is proven that dairy product vitamin D fortification is an effective strategy to diminish the prevalence and incidence of vitamin D deficiency among populations. This is because this method is cost-effective regarding its positive effect on public health and its impacts with minimal sensorial changes including flavor and order. Dairy products have been shown to be a suitable food type to be fortified with vitamin D. Milk and many cheese products, and yogurt are fortified with vitamin D and have shown to be stable and an effective source of vitamin D, and this is why we can consider other types of dairy products to be fortified with vitamin D like Labneh, in which further research will be done in order to test for stability of vitamin D and the most appropriate form to be used in it. According to the appropriate form of vitamin D used in dairy products, liposomes of vitamin D are effective for cheddar cheese fortification. This method proves the effectiveness and liposomes of food fortification that has been followed for years, a strategy that tremendously leads to decreasing the burden of nutrition deficiency among populations especially in developing countries. In fact, public awareness stays a must in promoting education about vitamin D deficiency, its symptoms, risk factors, and causes.

Conflict of interest

The authors declare no conflict of interest.

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