

Review

Exploring Vitamin D Synthesis and Function in Cardiovascular Health: A Narrative Review

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Abstract: Vitamin D plays a crucial role in the human body, influencing a wide range of physiological processes from bone health to immune function. The complex biochemical pathways involved in the synthesis, metabolism, and action of Vitamin D are explored, emphasizing its importance in nutrition and food technology. This review also investigates the regulatory mechanisms that control Vitamin D metabolism and its systemic effects on calcium homeostasis, cell proliferation, differentiation, and immune modulation. The role of Vitamin D3 in regulating blood pressure and atherosclerosis in the onset of cardiovascular disorders is discussed. Given the importance of Vitamin D in food science and technology, the regulatory mechanisms that control Vitamin D metabolism and its systemic effects on calcium homeostasis are also investigated, integrating innovative approaches and advanced technologies to improve human health through nutrition. Additionally, the review assesses the influence of food processing on Vitamin D levels and discusses cutting-edge technologies as innovative strategies to mitigate Vitamin D loss during food processing. This comprehensive exploration aims to improve our understanding of the biochemical pathways of Vitamin D and its relevance to food science, contributing to the development of new strategies for food fortification and the promotion of optimal health through diet.

Keywords: Vitamin D; food fortification; nutritional biochemistry; Vitamin D metabolism; clinical nutrition



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1. Introduction

The importance of Vitamin D for human health is widely recognized. It plays a crucial role in a number of physiological processes such as bone health (by promoting calcium absorption in the gut), immune function (by modulating the immune response), and the regulation of calcium metabolism, which is crucial for bone formation and maintenance. Vitamin D deficiency can lead to a range of health problems, including osteoporosis, increased risk of infections, and potentially chronic diseases [1]. Recent research has indeed highlighted a close correlation between Vitamin D deficiency and the onset of cardiovascular disorders [2].

This review aims to explore the complex biochemical pathways involved in the synthesis, metabolism, and action of Vitamin D within the human body, with a particular interest in the prevention of cardiovascular disorders through innovative dietary and technological strategies. The implications of food fortification and food processing on Vitamin D availability and efficacy will also be discussed, integrating advanced approaches and cutting-edge technologies to improve human health through nutrition.

2. Vitamin D Metabolism, Sources and Supplementation

Research on Vitamin D metabolism has focused on calcium homeostasis and bone metabolism [3]. Subsequently, when 25-hydroxyVitamin D (25OHD) and then 1,25-hydroxyVitamin D [1,25(OH)2D] were identified, the research was also extended to other

sectors including infections, immunity, cancer, and cardiovascular diseases. Vitamin D plays a role in regulating the immune system. It works by controlling the activity of suppressor T lymphocytes, cytokine synthesis, and the cell death process [4]. In particular, Vitamin D also stimulates the absorption of phosphate in the intestine and prevents its excretion through the kidneys [5]. Therefore, Vitamin D represents a molecule with a large pleiotropic functional profile.

The term Vitamin D refers to a significant group of fat-soluble substances with the main chain made up of cholesterol rings. 25(OH)D (calcifediol or calcidiol) is the main circulating compound, while 1,25(OH)₂D (calcitriol) represents the active compound and interacts with the Vitamin D/hormone D receptor (VDR) [6]. 1,25(OH)₂D binds to the VDR to initiate or inhibit gene transcription, causing the heterodimerization of the VDR and the retinoid X receptor [7]. When the heterodimer translocates into the nucleus, the complex binds to the Vitamin D consensus sequences, and gene transcription is modulated. At the circulatory level, Vitamin D (2 and 3) attaches to Vitamin D binding protein (DBP) and is then accumulated in adipose tissue [8]. Subsequently, metabolization into 25(OH)D occurs in the liver by hydroxylase (cytochrome P450(CYP)2R1 and CYP27A1). This process can also occur in other tissues in an autocrine and/or paracrine manner [9]. Hydroxylation then occurs in the renal tubule to produce the active molecule 1,25(OH)₂D. During this process, cubilin and megalin, two proteins that allow the DBP-25(OH)D complex to enter through the cellular receptors of the renal tubule [10], intervene. A decrease in cubilin and megalin determines a greater urinary loss of 25(OH)D. Furthermore, the cells of the renal tubule present two hydroxylases belonging to the cytochrome P450 family, i.e., 1-alpha-hydroxylase (CYP27B1) and 24-alpha-hydroxylase (CYP24A1), which, by hydroxylating 25(OH)D, produce the active form of Vitamin D (1,25(OH)₂D-calcitriol) or the inactive metabolite 24,25(OH)₂D [10].

The main production site of Vitamin D is the skin [6]. Vitamin D₃ (cholecalciferol) is produced in the skin when exposed to UVB rays with a wavelength of 290–315 nm. These radiations convert 7-dehydrocholesterol (7-DHC) into pre-Vitamin D, which in turn is converted into Vitamin D₃ in a thermosensitive but non-catalytic process, which involves the formation of three double bonds [11]. Both UVB intensity and skin pigment levels influence the rate of D₃ formation. Vitamin D skin production will therefore be influenced by different factors such as latitude, seasonality, clothing, pollution and the use of UV filters [12,13]. In summer, 15 min of full-body sunlight at midday is equivalent to 250 µg of cholecalciferol [14]. However, many factors influence the effectiveness of this combination (Figure 1).

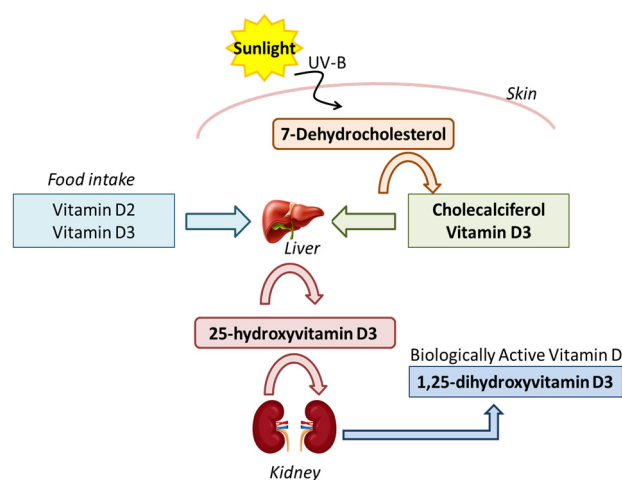


Figure 1. Pathway of Vitamin Production. The vitamin D production pathway involves the conversion of 7-dehydrocholesterol in the skin to vitamin D₃ via UV-B. Vitamin D₃ (and D₂ from food) is hydroxylated in the liver to 25-hydroxyvitamin D₃ and then in the kidneys to 1,25-dihydroxyvitamin D₃, the biologically active form.

Compared to synthesis at the skin level, the intake of Vitamin D from foods is lower. The main food source of Vitamin D is foods of animal origin (cholecalciferol), but mushrooms (ergocalciferol) are also an important resource [15]. Vitamin D is a fat-soluble molecule, and therefore for its absorption it requires bile salts, micelles, and chylomicrons for its transport. Oily fish, eggs, and liver also have a high content of Vitamin D. Over the years, many populations have included foods fortified with Vitamin D (ergocalciferol or cholecalciferol) in their diet, mainly milk (cow or vegetable), butter, margarine, and breakfast cereals. Therefore, for those who suffer from Vitamin D deficiency due to insufficient sun exposure or if skin synthesis is reduced (e.g., in the elderly), a supplement should be prescribed [16]. To date, Vitamin D supplementation is considered an effective treatment to produce better health outcomes. Many clinical studies regarding Vitamin D supplementation show beneficial effects on health, but some of them have not shown any positive effects on different types of pathologies [4]. These differences also depend on the methods and times of administration. In fact, many researchers underline the importance of a very long follow-up especially for chronic diseases; the method of administration is also important: some authors prefer intermittent bolus administration and others daily administration (Vitamin D oral intermittent treatment (DO IT) study, a randomized clinical trial with individual loading regimen). Recent data shows that intermittent bolus administration (which is the highest) should be avoided as it can cause harmful health effects [17]. Finally, the doses of administration also play a decisive role: high doses (i.e., ~1000–2000 IU/day) should be used compared to too low doses (i.e., ~400–800 IU/day) in order to observe significant results. Despite these considerations, Vitamin D supplementation has been shown to reduce the risk of lung disease, cancer, type 2 diabetes, and autoimmune diseases [18–21]. Additionally, individuals with cardiovascular disease may benefit from Vitamin D supplementation, a finding that warrants further investigation [22,23].

3. Methodology

In this comprehensive narrative review, we aimed to collect and summarize the literature concerning the effects of Vitamin D on cardiovascular diseases and the implications of different fortification and processing methods. To achieve this goal, we performed an extensive literature search using Pubmed databases (<https://pubmed.ncbi.nlm.nih.gov>; accessed 1 April 2024). The literature review explored using the search string: targeting publications from January 2008 to December 2024. The search strategy was meticulously crafted to include a wide range of keywords and phrases such as ‘Vitamin D fortification’, ‘Vitamin D metabolism’, ‘Vitamin D and cardiovascular health’, ‘impact of food processing on Vitamin D levels’, and ‘mitigation strategies for Vitamin D content in processed foods’. We also included studies that specifically discussed Vitamin D biochemical pathways and its role in human health, expanding our search to include terms such as ‘Vitamin D biochemical pathways’, ‘Vitamin D in clinical nutrition’, and ‘Vitamin D in food science’. Each article was selected based on its relevance to the central themes of the review, the quality of the evidence presented, and the clarity of the results reported. Once the articles were retrieved, we used a structured approach to data extraction. Key information was catalogued, including study design, sample size, main outcomes, and conclusions regarding the impact of Vitamin D on health and food processing techniques. These data were then critically analyzed to assess the consistency of findings across studies and to evaluate the strength of evidence supporting the role of Vitamin D in improving health and reducing disease. Our analysis aimed not only to summarize the results but also to identify gaps in the current research landscape. The review process was designed iteratively, allowing for the inclusion of new studies that met our inclusion criteria as they became available. The final data synthesis provides a detailed overview of the current understanding of the role of Vitamin D and the efficacy of various fortification strategies, offering valuable insights into potential areas for future research.

4. Vitamin D Deficiency and Cardiovascular Risk

Vitamin D deficiency is associated with many health consequences, including skeletal problems (i.e., rickets, fractures, osteomalacia, osteopenia) and non-skeletal problems [24,25]. Non-skeletal complications include heart disease and complications such as systolic and diastolic dysfunction, abdominal aortic aneurysm, and hypertension [26–31]. Vitamin D deficiency is associated with several cardiovascular risk factors. By increasing the synthesis of renin and angiotensin II, Vitamin D deficiency can induce the production of reactive oxygen species (ROS). Furthermore, being implicated in the processes of calcification and proliferation of smooth muscles, Vitamin D levels directly influence cardiovascular health [32]. A study from the Intermountain Integrated Health System prospective database of 41,504 patients showed an association between Vitamin D deficiency and an increased risk of peripheral vascular disease (PVD), heart failure and coronary heart disease/myocardial infarction, and stroke [33]. Furthermore, following the Hill criteria, low serum 25(OH)D levels have been associated with the risk of cardiovascular disease [34]. Results from a meta-analysis involving 65,994 individuals also show a linear and inverse relationship between Vitamin D levels and the risk of cardiovascular disease [35]. Recent studies demonstrate that Vitamin D can regulate the expression of specific metalloproteinases and their inhibitors, which promote the development of heart failure [36]. Finally, growing evidence reveals the anti-inflammatory activity of Vitamin D in inhibiting the activation of tumor necrosis factor-alpha (TNF- α), nuclear factor kappa B (NF- κ B), and interleukin-10, all known important contributors of cardiovascular disease [37].

4.1. Vitamin D Deficiency and Coronary Artery Disease

The relationship between Vitamin D deficiency and coronary heart disease (CAD) has been demonstrated in various studies. The risk of coronary heart disease seems to be associated with Vitamin D deficiency. The main indicator of this possible relationship is the presence of VDR in the myocardium, vascular endothelial cells, and fibroblasts. In detail, epidemiological studies highlight that Vitamin D deficiency, causing the activation of the renin–angiotensin–aldosterone system (RAAS), causes an increase in CAD by increasing anti-inflammatory and decreasing proinflammatory mediators [38]. Preliminary studies show a relationship between Vitamin D deficiency and cases of acute myocardial infarction (AMI) in the short and long term [38]. The study included 18,225 male patients aged between 40 and 75 years. Over 10 years, results showed an association between low Vitamin D levels and an increased risk of AMI [27]. In particular, men deficient (≤ 15 ng/mL) in 25(OH)D were at increased risk of AMI compared to those considered to be sufficient (≥ 30 ng/mL) in 25(OH)D. It has also been shown that hospitalized patients with AMI had a high incidence of Vitamin D deficiency. Lee et al. found low levels of Vitamin D in 96% of 239 patients with acute coronary syndrome (ACS) [39]. Dziedzic et al. found low levels of Vitamin D in patients who had episodes of myocardial infarction during their lifetime [40]. Increased risks of ischemic heart disease, myocardial infarction, and premature death were also associated with low Vitamin D levels during a large prospective study that included 10,170 individuals [41]. Furthermore, a meta-analysis of 18 studies found an increased risk of ischemic heart disease and premature death in subjects with low Vitamin D levels [41].

Despite these observations, there is still no certain protocol to support the benefit of Vitamin D supplementation in the treatment/prevention of coronary heart disease. This is partly due to the few ongoing or completed clinical studies examining the effects of Vitamin D on patients with AMI or ACS.

4.2. Vitamin D Deficiency and Hypertension

Hypertension is the most common cardiovascular disease in adults (29%), and it is estimated that in 2025 there will be approximately 1.6 billion cases of hypertension in the global population [42]. As mentioned previously, Vitamin D deficiency is assumed to increase blood pressure via the renin–angiotensin system.

An increase in renin expression levels and plasma angiotensin II production are observed in mice lacking the VDR receptor. This condition leads to hypertension, cardiac hypertrophy, and increased water intake. Administering 1,25(OH)₂D₃ suppresses renin expression in VDR-null mice [43]. Vitamin D regulates the proliferation of endothelial and vascular smooth muscle cells and VDR is present in these cells [44]. Endothelial dysfunction plays an important role in cardiovascular diseases as well as hypertension. Vitamin D deficiency affecting endothelial cells can induce hypertension [45]. In endothelium-specific VDR knockout mice, aortic relaxation is impaired by acetylcholine, as are increased sensitivity to the hypertensive effects of angiotensin II and the increased expression of myocardial genes sensitive to hypertrophy induced by angiotensin II infusion [46]. These observations indicate that the endothelial VDR may have an important role in blood pressure control.

Similar results highlight an increase in the production of superoxide anion, angiotensin II, systolic blood pressure, and atrial natriuretic peptide production in rats with Vitamin D deficiency. The same authors also identify 51 genes involved in the regulation of oxidative stress and hypertrophy myocardial [47]. Another study shows how a finger rich in Vitamin D is able to reverse the increase in systolic and diastolic blood pressure, high plasma renin-angiotensin activity, and reduced urinary sodium excretion in Vitamin D-deficient mice after 6 weeks [48]. It therefore seems that hypertension and an acceleration of atherosclerosis are linked to Vitamin D deficiency. A study of 3316 patients showed consistent increases in plasma renin concentrations and angiotensin 2 with a parallel decrease in 25(OH)D and 1,25(OH)₂D [49]. Obese hypertensive individuals also present a significant increase in the activity of the renin-angiotensin system together with a decrease in 25(OH)D levels [50]. Vaidya et al. demonstrate that genetic variability in the Fok1 gene encoding VDR, in association with 25(OH)D levels, is related to hypertension, suggesting that the Vitamin D-VDR complex could be a regulator of renin in humans [51].

4.3. Vitamin D Deficiency and Acute Myocardial Infarction

Referring back to the previous paragraph, regarding coronary heart disease, the association between Vitamin D and acute myocardial infarction (AMI) deserves further investigation. Indeed, many studies present conflicting results. Some authors demonstrate an increase in Vitamin D deficiency in patients who have suffered an AMI both in Italy and in the United States [39,52]. Another study carried out on 314 subjects hospitalized for AMI highlights that 85% of patients had 25(OH)D deficiency regardless of sex or age, but it is associated with some risk factors such as diabetes mellitus and calcium deficiency [53]. This trend has also been confirmed in other prospective studies which state a high incidence of Vitamin D deficiency in patients admitted to hospitals for AMI [54,55]. Further data have been provided on the association between low Vitamin D levels and AMI. Ng et al. in a large study show the link between Vitamin D deficiency and AMI or acute coronary syndrome in 1259 hospitalized patients [56]. As mentioned above there are also conflicting results regarding the association between Vitamin D deficiency and AMI. Ford et al. show how VDR KO mice subjected to experimental AMI had normal cardiac function compared to wild type mice [57]. These results highlight the importance of the contribution of Vitamin D to AMI and other cardiac complications such as thrombosis, arrhythmias, and heart failure, which should still be studied in depth.

4.4. Vitamin D Deficiency and Heart Failure

Many studies also correlate myocardial infarction with Vitamin D deficiency. In particular, a significant increase in biomarkers linked to heart failure, apoptosis, and inflammation was observed in VDR KO mice. The administration of paricalcitol (PC), an activated Vitamin D analog, promotes cardioprotection through the activation of anti-apoptotic and anti-inflammatory mechanisms [58]. In fact, it has been shown that Vitamin D deficiency correlates with an increase in inflammatory cytokines (TNF- α , IL-6, and IL-1 β), which decrease when Vitamin D is supplemented. Alongside this role it seems that Vitamin D also has an anti-fibrotic and anti-hypertrophic role at the cardiac level.

In fact, mesenchymal multipotent cells (MMCs) exposed to 5'-azacytidine to induce a fibrotic phenotype, and then treated with active Vitamin D (1,25D), presented a decreased expression of fibrotic genes [59]. Epidemiological studies also confirm the importance of Vitamin D in subjects suffering from heart failure. Increased mortality in patients with heart failure (due to left ventricular dysfunction) appears to be associated with low levels of 25-hydroxyVitamin D [60]. A recent study published in 2023 shows how Vitamin D can be used today as a prognostic biomarker in heart failure. In particular, the authors demonstrate that in 70 patients with left ventricular heart failure there was also a significant drop in 25(OH)D levels [61]. All these results clarify the importance of Vitamin D in correct cardiovascular function and underline the need to study new types of Vitamin D supplementation and dietary models in relation to heart failure. Table 1 summarizes the possible role of Vitamin D for various cardiovascular disease (CVD) risk factors.

Table 1. The role of Vitamin D3 on CVD risk factors.

CVD Risk Factor	Role of Vitamin D3	Refs.
Blood pressure regulation	Regulation of the renin–angiotensin–aldosterone system (RAAS), which controls vasoconstriction and fluid volume. A Vitamin D deficiency leads to an overexpression of renin and thus the activation of the RAAS.	[62]
Inflammation	Low Vitamin D levels have been linked to elevated levels of inflammatory biomarkers, such as C-reactive protein. Chronic inflammation is a known risk factor for the development of atherosclerosis and other cardiovascular disorders.	[63]
Endothelial function	Vitamin D plays a role in regulating the function of endothelial cells. A Vitamin D deficiency can lead to endothelial dysfunction, a precursor to plaque formation and atherosclerosis.	[64]
Glucose metabolism	Vitamin D is involved in the regulation of glucose metabolism and insulin sensitivity. Vitamin D deficiency may contribute to insulin resistance, a risk factor for type 2 diabetes and cardiovascular disease.	[65]
Arterial stiffness and left ventricular hypertrophy	Vitamin D may influence arterial stiffness and the development of left ventricular hypertrophy, both of which contribute to CVD risk.	[66]
Atherosclerosis	Vitamin D reduces foam cell formation and cholesterol uptake by macrophages, and it facilitates the transport of HDL (high-density lipoprotein) cholesterol. Lower levels of serum 25-hydroxyVitamin D have been linked with metabolic syndrome and its components, particularly affecting HDL cholesterol levels.	[67]

Table 1 summarizes the impact of Vitamin D3 on CVD risk factors, from blood pressure regulation to atherosclerosis. It shows how Vitamin D3 deficiency affects key areas like the renin–angiotensin system, inflammation, endothelial function, glucose metabolism, arterial stiffness, and cholesterol handling, linking low levels of Vitamin D3 to increased CVD risk. Each point is supported by references, illustrating Vitamin D3's critical role in cardiovascular health.

4.5. Vitamin D Supplementation and CVD

Recent investigations into the potential benefits of Vitamin D supplementation in the prevention of CVD have led to conclusions that question the efficacy of such interventions in a large population. Vitamin D supplementation did not significantly reduce the incidence of CVD events [68]. This finding is supported by Virtanen et al. [69], who demonstrated that Vitamin D3 supplementation did not reduce the incidence of major CVD events among older adults, potentially due to the sufficiency of baseline Vitamin D levels in participants. The efficacy of high-dose Vitamin D (200,000 IU, followed a month later by monthly doses of 100,000 IU, or placebo for a median of 3.3 years) supplementation in preventing CVD was questioned in a study by Scragg et al. [70] that revealed no preventive benefit, suggesting the need for further research into the effects of different dosing regimens. Complementing this, Neale et al. [71] reported that the monthly administration of Vitamin D3 (60,000 IU per month) did not reduce all-cause mortality in unselected elderly subjects. A randomized controlled trial [72] showed that high-dose Vitamin D supplementation

did not improve markers of blood glucose, inflammation, neurohormonal activation, or lipids. Similarly, Desouza [73] observed a small favorable effect on atherosclerotic CVD risk score in individuals with prediabetes not selected for Vitamin D insufficiency, but no decrease in major adverse cardiovascular events. Further studies [74,75] found no reduction in major CVD events or improvement in post-stroke outcomes with Vitamin D or omega-3 fatty acid supplementation. Gepner et al. [76] and Pilz et al. [77] also reported no significant improvements in cardiovascular risk factors, with the latter study noting an increase in triglycerides with Vitamin D supplementation in hypertensive patients with low 25-hydroxyVitamin D levels. Finally, Wood et al. [78] concluded that improving Vitamin D status through dietary supplementation is unlikely to reduce conventional CVD risk factors, highlighting the complexity of Vitamin D's role in cardiovascular health and disease prevention. In summary, although Vitamin D supplementation has been widely promoted for its potential health benefits, evidence from RCTs suggests that it does not significantly reduce the incidence of major cardiovascular events or cancer or improve certain cardiovascular risk factors in the general population.

5. Impact of Dietary Vitamin D Fortification on Blood Vitamin D Levels and Cardiovascular Outcomes

Fortification strategies, while jurisdictional in nature, demonstrate variable impacts on cardiovascular health across different states. Dairy products, bread, and breakfast cereals are the foods most commonly fortified with Vitamin D [79]. In countries where there is no mandatory fortification policy, data show that the fortification of foods with Vitamin D could counteract Vitamin D deficiency [80]. In Finland, for example, data modeling studies led to a policy of mandatory Vitamin D food fortification that increased 25(OH)D concentrations in the population by 18 nmol/L [81]. In vitro studies suggest that altering the lipid composition of fortified foods increases the absorption of Vitamin D3 [78]. Proposed fortification strategies may not be as effective because the matrix and composition of foods may alter Vitamin D absorption and bioavailability [82]. Supplements and fortified foods serve as additional sources of Vitamin D. Both Vitamin D2 and D3 can be synthetically produced for supplementation and fortification purposes [83]. The debate on the bioequivalence of Vitamin D2 and D3 in fortification and supplementation suggests that D3 may be superior, especially when administered in the form of supplements or injections. Despite numerous researches that have investigated Vitamin D2 and D3 additionally, there is a lack of research directly comparing the effect of Vitamin D2 and D3 from biofortified foods on 25(OH)D concentrations. The fortification of dairy products with Vitamin D is considered cost-effective and bioavailable, so much so that some countries have mandated fortification [84]. Studies show a significant increase in 25(OH)D levels following the consumption of fortified dairy products, although most research has focused on female participants [85]. The efficacy of fortified foods for males and various dietary preferences remains important [86]. In addition, non-dairy fortification strategies, including bars and juices, meet the needs of those who avoid dairy products. These studies emphasize the importance of selecting appropriate foods for fortification to accommodate different dietary habits and ensure widespread improvement in Vitamin D status [87,88].

Although both D2 and D3 can be effective in increasing Vitamin D levels when added to foods, D3 generally shows superior bioavailability and efficacy in improving Vitamin D status [89]. The choice of foods to be fortified is critical in maximizing the benefits of Vitamin D fortification policies, particularly in meeting the nutritional needs of different population groups [90]. Fortifying bread with 11.3 µg of Vitamin D/100 g could result in a daily Vitamin D intake of 23.7 µg, raising the serum 25(OH)D concentration in the German population from 45 nmol/L to 75 nmol/L during winter as demonstrated by Brawn et al. [80]. Moreover, a 12-month study involving nursing home residents who consumed bread fortified with 125 µg (5000 IU) Vitamin D3 daily observed an increase in serum 25(OH)D levels from 28.5 nmol/L to 125.6 nmol/L [91].

While these strategies have successfully elevated Vitamin D levels, the direct correlation between these increases, and a significant reduction in major cardiovascular events is not clearly established. Despite effectively increasing serum vitamin D levels, supplementation did not yield clear cardiovascular benefits in several studies, suggesting that the role of vitamin D in cardiovascular health might be more complex and not solely dependent on improving Vitamin D status. This underscores the potential limitations of fortification in addressing the multifactorial nature of cardiovascular diseases [75]. For instance, in Finland, despite the rise in 25(OH)D levels due to mandatory fortification, the expected decline in cardiovascular incidents has not been conclusively observed, suggesting that other factors like dietary patterns, genetic predispositions, and lifestyle choices play significant roles [81]. Studies often show that micronutrient enhancements improve markers of general health but fail to demonstrate efficacy in preventing complex conditions such as heart disease, which involve multifactorial etiologies beyond simple nutritional deficiencies. This highlights the need for integrated health strategies that go beyond nutritional fortification, incorporating public health initiatives aimed at overall lifestyle and environmental changes to effectively combat cardiovascular diseases [92]. Table 2 synthesizes *in vivo* studies on the impact of Vitamin D-fortified foods, drawn from systematic reviews and meta-analysis of randomized trials. Based on the variability of results observed in current studies, policy recommendations should consider more localized fortification strategies that take into account regional dietary patterns, sunlight exposure, and genetic factors that may influence Vitamin D metabolism. Adapting policies to regional health profiles could improve the effectiveness of fortification in preventing cardiovascular disease and other complex health conditions [93]. Continued research is essential to better understand the differential impacts of Vitamin D2 and D3, as well as the optimal levels of fortification needed to influence public health outcomes positively. This should include long-term studies that not only assess biochemical markers but also directly correlate these with reductions in disease incidence, particularly in diverse population groups [94].

Table 2. *In vivo* studies on the impact of Vitamin D-fortified food.

Year	Outcome Measured	Summary	Foods Included	Main Findings	Refs.
2008	Improvement in Vitamin D status.	Discusses Vitamin D's health benefits and the effectiveness of fortified foods in increasing Vitamin D levels, and calls for stronger data on food fortification.	Fortified milk, cheese, dairy-based products, orange juice, bread.	Food fortification with Vitamin D effectively improves Vitamin D status in adults.	[91]
2012	Rise in circulating Vitamin D levels.	Examines Vitamin D food fortification's success in raising 25(OH)D levels in adults and stresses the need for high-quality research and reporting.	Milk, yogurt, butter, margarine, cheese, orange juice, bread, breakfast cereals.	Dose-dependent increase in 25(OH)D levels observed with Vitamin D-fortified foods in adults.	[85]
2019	Improvements in Vitamin D status and metabolic health markers.	Reports Vitamin D-fortified yogurt's potential in improving health outcomes across various populations.	Yogurt, milk, butter, margarine, cheeses, orange juice, bread, breakfast cereals.	Fortified yogurt raises serum 25OHD and improves metabolic health parameters.	[95]

Table 2. Cont.

Year	Outcome Measured	Summary	Foods Included	Main Findings	Refs.
2020	Improvement in Vitamin D concentrations.	Discusses Vitamin D deficiency in children, the efficacy of fortification in improving Vitamin D status, and its impact on cognitive function.	Milk, cereal, juice, bread, yogurt, cheese.	Fortification raises 25(OH)D levels and reduces Vitamin D deficiency in children, also improving cognitive function.	[96]
2020	Reductions in waist circumference and ratio.	Meta-analysis on Vitamin D-fortified foods' impact on anthropometric indices, showing benefits on waist measurements but not on weight or BMI.	Vitamin D-fortified foods or calcium plus Vitamin D fortified products.	Vitamin D fortified foods reduce waist circumference and ratio, with variable effects on weight based on intervention duration.	[97]
2021	Significant increase in 25(OH)D concentrations.	Addresses Vitamin D deficiency as a global issue, showcasing the success of fortification strategies in enhancing Vitamin D levels in populations.	Milk, milk powder, milk-based drinks, yogurt, cheese, fruit juice, biscuits, snack bars, crisp breads, lavash bread, eggs, compote, custard, bread with UVB-irradiated yeast.	Both Vitamin D fortification methods enhance 25(OH)D levels, with cholecalciferol being more effective than ergocalciferol.	[98]
2022	Change in bone resorption markers.	Highlights Vitamin D's role in bone health, benefits of fortified foods on bone biomarkers, and positive effects on Vitamin D and IGF-1 levels.	Dairy products, orange juice, bread, biscuit, snack bars.	Fortification decreases bone resorption markers and improves Vitamin D and IGF-1 levels, with limited effects on bone mass.	[99]
2022	Increase in total 25(OH)D levels.	Explores improving Vitamin D status via fortified products, noting the influence of age, BMI, and baseline levels, with implications for policy.	Dairy products.	Vitamin D intake from fortified foods raises 25(OH)D levels, influenced by age, BMI, and initial levels.	[100]
2023	Effects on serum retinol and Vitamin D levels.	Reviews benefits and risks of fortifying oils with vitamins A and D, aiming to guide policy and food fortification program implementation.	Edible vegetable oils and fats.	Vitamin A and D fortified oils show minimal impact on serum levels, suggesting possible insufficient dosing.	[101]
2024	Increase in serum 25(OH)D levels.	Analyzes Vitamin D fortification programs in Europe, underlining the importance of choosing suitable food vehicles for effective outcomes.	Bread/savory biscuits, orange juice, UV-mushrooms, cheese, yogurt, fluid milk, powdered milk, eggs, edible oils, breakfast cereal.	Significant increases in 25(OH)D levels from both animal and plant-based fortified foods, with dairy showing good bioavailability.	[102]

Table 2 provides a comprehensive overview of in vivo studies focused on the impact of Vitamin D-fortified foods, as highlighted in systematic reviews and meta-analyses of randomized trials searched on PubMed with the criteria: Vitamin D Fortified Foods. Spanning from 2008 to 2024, the table documents the effectiveness of food fortification strategies in improving Vitamin D status and related health outcomes across different populations. It summarizes findings from studies involving a variety of fortified foods, including dairy products, orange juice, bread, and more. Key outcomes include improvement in Vitamin D levels, metabolic health markers, cognitive function in children, waist circumference, bone resorption markers, and serum 25(OH)D levels.

6. The Impact of Processing on Vitamin D Levels in Foods and Mitigation Strategies

6.1. Impact of Processing on Vitamin D Levels

The effectiveness of dietary Vitamin D fortification in improving blood Vitamin D levels and cardiovascular outcomes is intricately linked to how Vitamin D is processed and maintained in fortified foods. Fortification strategies are crucial for enhancing Vitamin D levels in the population, especially in regions with limited sunlight exposure. However, the actual benefits of such fortification on cardiovascular health are still under debate, as studies show variable impacts on cardiovascular outcomes. This is compounded by the fact that the processing of Vitamin D-fortified foods can significantly affect the nutrient's stability and bioavailability. Without proper handling during processing, the potential cardiovascular benefits of Vitamin D fortification might not be fully realized, underscoring the need for optimized processing methods to maintain the efficacy of fortified foods in boosting Vitamin D status and potentially improving cardiovascular health. Both Vitamin D3 and D2 are susceptible to degradation from heat, light, oxidation, and other processing factors, which may compromise its stability and bioavailability in food products. Food scientists must account for these considerations during food processing, storage, and distribution to minimize nutrient loss and ensure product quality and safety. Moreover, innovative processing technologies and packaging materials are continuously explored to enhance the stability and retention of Vitamin D in processed foods. Figure 2 illustrates the impact of various processing methods on Vitamin D3 levels in foods and highlights the key mitigation strategies employed to counteract these effects.

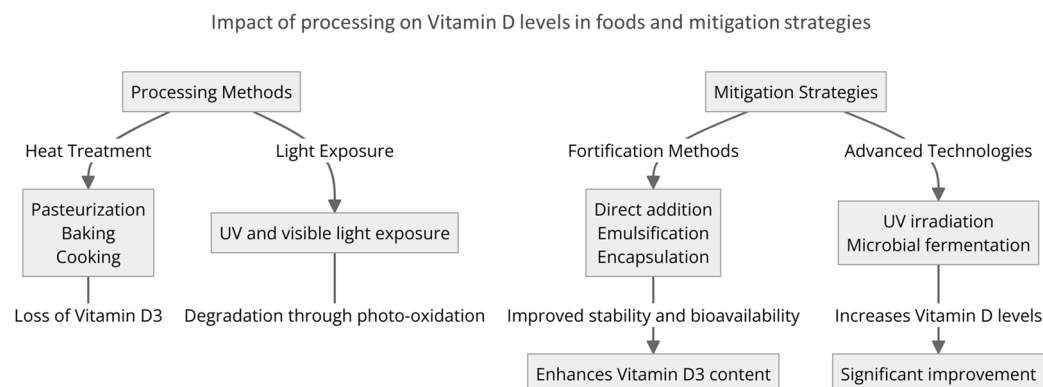


Figure 2. The impact of various processing methods on Vitamin D3 levels in foods and fortification approaches, including direct addition, emulsification, encapsulation, UV irradiation, and microbial fermentation, which are crucial in enhancing the stability and bioavailability of Vitamin D3, thus improving overall nutritional quality.

6.1.1. Pasteurization

Several studies have demonstrated that food processing, especially at high temperatures, alters the Vitamin D content of foods. Pasteurization, a common heat treatment used to extend the shelf life of dairy products, has been shown to cause a significant reduction in Vitamin D levels. Gomes et al. investigated the effects of pasteurization on Vitamin D compounds in donor breast milk. They found significant reductions in Vitamin D2 and D3 concentration, with losses ranging from 10% to 20% after pasteurization. This study highlights the sensitivity of Vitamin D compounds to the pasteurization process [103]. Similarly, another study by Capozzo et al. observed a significant reduction in Vitamin D (56%) following high-temperature, short-time (HTST) pasteurization and ultra-high-temperature (UHT) processing [104]. As reported by Tsai et al. (2017), Vitamin D3 has poor thermal stability, which results in a significant reduction ($p \leq 0.05$) of about 50% in Vitamin D3 in pasteurized milk compared with raw milk [105]. The heat sensitivity of Vitamin D is a key factor contributing to its degradation during pasteurization. Moreover, Vitamin D is susceptible to thermal degradation, especially at elevated temperatures and

prolonged heating times. Research by Jakobsen et al. [106] elucidated the kinetics of Vitamin D degradation during heat treatment, showing that the degradation rate increases with rising temperatures. Jakobsen et al. have highlighted that the stability of Vitamin D in food matrices such as oil and mushrooms significantly decreases when subjected to higher cooking temperatures. Their research found that lower temperatures tend to preserve Vitamin D levels better than high temperatures, which can significantly accelerate the degradation process.

6.1.2. Baking and Cooking

Processing methods such as baking, cooking, frying, and water boiling induce a significant reduction in Vitamin D, although the extent of this loss varies depending on the type of food being cooked and the specific methods of heat application. Jakobsen et al. report that during heat treatment in an oven for 40 min at typical cooking temperatures, eggs and margarine retained about 39–45% of their Vitamin D content while frying preserved 82–84% of the vitamin, whereas boiling eggs achieved a retention rate of 86–88%. In the case of baked bread, rye bread retained 69% of its Vitamin D₃, which was less than the 85% retention observed in wheat bread. Vitamin D₂ retention was, instead, somewhat better, at 73% in rye bread and 89% in wheat bread [107]. Another study by Hill et al. [108] demonstrated that both storage and cooking practices influence Vitamin D metabolites, with certain cooking methods showing true retention rates for Vitamin D₃ ranging from 50–152%. The findings suggest that 25-hydroxyVitamin D₃-enriched eggs can serve as a potent dietary source of Vitamin D, with their total Vitamin D activity being significantly higher in comparison to non-enriched eggs depending on the storage and cooking method used.

6.1.3. Light Exposure

Vitamin D is sensitive to UV and visible light, leading to degradation through photo-oxidation. Foods exposed to light during processing or storage, especially those in transparent packaging, are at risk of losing their Vitamin D content. Saffert et al. have demonstrated the effects of package light transmittance on the vitamin content of milk, showing that transparent packaging led to more pronounced losses in Vitamin D₃ compared to pigmented or less transparent packaging. This aligns with the general observation that light exposure can degrade sensitive vitamins like Vitamin D₃ [109]. Furthermore, Vitamin D₃-enriched bread exposed to light for 2 h showed a reduction of 15–20% in Vitamin D content, underscoring the sensitivity of both forms of Vitamin D to light during post-baking storage [110]. In detail, D₂ and D₃ vitamins exhibit different susceptibilities to oxidative degradation, with Vitamin D₃ generally considered more stable, due to its structure, under similar conditions [110]. The impact of processing on Vitamin D underscores the importance of considering strategies to mitigate nutrient losses in processed foods.

6.2. Mitigation Strategies to Improve Vitamin D Content in Processed Foods

Recent advancements in the field of nutrition and food technology have provided innovative solutions to improve the bioavailability and stability of Vitamin D in processed foods, addressing the challenge of Vitamin D deficiency on a global scale. Stability during processing, storage, and in vitro bioaccessibility are a key point to address for setting the best technological strategies aiming to enhance or maintain nutritional value in terms of Vitamin D in processed foods.

6.2.1. Fortification

The most widely used approach to restore or enhance the Vitamin D content of processed products is fortification. Vitamin D fortification, particularly in dairy products, cereals, juices, and margarine, has been instrumental in enhancing population-wide intake, especially in regions with limited sunlight exposure. In many countries, including the UK, the exposure of the skin to UVB radiation or supplementation is the primary means of obtaining Vitamin D. According to the latest UK National Diet and Nutrition Survey

data, collected in 2012/13 and 2013/14, mean dietary intakes of Vitamin D were reported as 3.1 $\mu\text{g}/\text{d}$ for adult men and 2.5 $\mu\text{g}/\text{d}$ for adult women. However, lower dietary intakes ranging from 1.25–1.6 $\mu\text{g}/\text{d}$ have been observed among South Asian women in the UK [111].

To achieve sustainable fortification, direct addition, emulsification, or encapsulation are widely employed. Direct addition is predominantly used for enriching milk and dairy products [112]. Typically, Vitamin D is dissolved in a food-safe organic solvent, such as ethanol and butter oil, before being evenly distributed through homogenization within the food matrix. However, challenges arise with Vitamin D's stability within aqueous food matrices and its tendency to adhere to packaging materials, like polypacks or tetrapacks, which can lead to degradation. In the emulsification technique, an oil phase containing Vitamin D is dispersed into fine droplets within water, and these droplets are then incorporated into target foods like cheese, milk, and bread. Achieving a stable emulsion faces hurdles such as the homogenization of Vitamin D in the food matrix and the limited availability of food-grade emulsifiers. Manufacturers may utilize fortification techniques to add Vitamin D to dairy products post-pasteurization, offsetting the losses incurred during processing. Adding Vitamin D at stages of processing that expose it to the least amount of heat, light, and oxygen can help in retaining its levels. However, fortification levels may vary by region and regulations but are typically designed to provide a significant portion of the recommended daily intake of Vitamin D per serving. To reduce the loss of Vitamin D upon food processing, complementary approaches include the optimization of processing as the pasteurization parameters, such as temperature and duration, to minimize Vitamin D degradation while ensuring food safety. Modifying processing conditions, such as lowering temperatures and reducing exposure to oxygen and light during manufacturing, can help preserve Vitamin D content.

6.2.2. UV Irradiation and Biofortification

Besides post-processing fortification, alternative fortification methods can be explored to enhance the Vitamin D content of processed foods. Among them, the ultraviolet (UV) irradiation of dairy products post-pasteurization can stimulate the synthesis of Vitamin D, effectively increasing its levels without compromising food safety. The EU Novel Food Regulation oversees the approval and safety evaluation of UV-treated foods, considering them as novel foods. EFSA, under EC regulation no. 258/97, endorsed UV radiation for post-pasteurization milk processing to enhance shelf life and Vitamin D levels [113]. The European Commission specifies allowed UV wavelengths for treating different foods to increase Vitamin D. Ultraviolet (UV) irradiation, predominantly UVB and more recently UVC radiation, stimulates the synthesis of Vitamin D in certain foods, particularly those containing precursor molecules such as ergosterol or proVitamin D₂. After irradiation with UVB light (290–315 nm), 7-dehydrocholesterol undergoes a photoisomerization reaction, followed by a thermal isomerization reaction, resulting in the formation of Vitamin D₃. In this context, the European Commission (EC) allowed specific wavelength ranges for different foods (200–800 nm for mushrooms, 240–315 nm for bread, 200–310 nm for milk, not specified for baker's yeast) [112]. Within the realm of dairy products, UV irradiation is strategically employed post-pasteurization to bolster their Vitamin D content. By subjecting pasteurized dairy items to UV irradiation, manufacturers can catalyze the conversion of precursor molecules inherent in the product, such as ergosterol in milk, into active Vitamin D₂ (ergocalciferol) [112,114]. Studies have shown that Vitamin D₂ levels in fungi can be significantly enhanced by exposing them to UVB light [114]. As reported by Lavelli et al., UV irradiation significantly increased Vitamin D₂ content in mushrooms, reaching up to 57 $\mu\text{g}/\text{g}$ dry weight in *A. bisporus*, and up to 37 $\mu\text{g}/\text{g}$ dry weight in *P. ostreatus* [115]. Additionally, UV irradiation circumvents the need for heat or chemical additives, thereby preserving the sensory and nutritional integrity of the food product, rendering it an appealing option for Vitamin D enhancement without compromising food safety or consumer acceptance.

6.2.3. Microbial Fermentation

Among the innovative approaches to fortifying foods with Vitamin D, microbial fermentation represents a novel strategy. Certain microorganisms such as *Amycolata* sp., *Rhodococcus erythropolis* can convert Vitamin D3 to 1 α ,25-dihydroxyVitamin D3 via 25-hydroxyVitamin D3 during fermentation processes [116]. By selecting appropriate strains and optimizing fermentation conditions, manufacturers can enhance the Vitamin D content of fermented foods such as yogurt and cheese, offering consumers an additional source of this essential nutrient. Recently, EFSA evaluated the safety of Vitamin D-enriched UV-treated baker's yeast, *Saccharomyces cerevisiae*, as a novel food ingredient, by revealing that the UV-treated baker's yeast is capable of converting ergosterol into Vitamin D2, significantly enhancing the Vitamin D2 content of food products without compromising safety [117]. *Amycolatopsis autotrophica* has been shown to convert Vitamin D3 to its more potent forms, 25-hydroxyVitamin D3, and 1 α ,25-dihydroxyVitamin D3, via microbial hydroxylation. The process was further optimized by using cyclodextrin to increase the hydroxylation efficiency significantly [118]. Since Vitamin D3 has inadequate absorption compared to its corresponding hydroxylated derivatives, an efficient biotransformation of Vitamin D3 to 25-hydroxyVitamin D3 by a newly isolated *Bacillus cereus* strain was proposed by Tang et al., 2020 [119]. Moreover, Sasaki et al. reported the bioconversion of D3 derivatives for the first time by screening approximately 300 *Streptomyces* strains. Among them, *S. sclerotialis* FERM BP-1370 and *S. roseosporus* FERM BP-1574 were found to be able to introduce hydroxy group at C-1 α position of calcifediol and C-25 position of 1 α (OH)VD3, respectively. Microbial fermentation presents a viable strategy for enhancing the levels of Vitamin D3, with specific microbial species offering promising routes for the bioconversion and improved bioavailability of this essential vitamin. This approach has implications not only for dietary supplements but also for the fortification of foods, potentially contributing to better health outcomes by addressing Vitamin D3 deficiency.

6.2.4. Encapsulation

In the field of food technology, fortifying foods with Vitamin D presents numerous challenges, such as ensuring compatibility with various food matrices, achieving even dispersion and uniformity, maintaining stability, and delivering bioavailable doses capable of addressing Vitamin D deficiencies. These obstacles have catalyzed the development of innovative fortification methods across different food products. The increasing consumer interest in functional foods has also propelled the creation and application of novel nanomaterials tailored for food fortification, with numerous nanomaterials proving to be effective vehicles for Vitamin D enhancement. Furthermore, cutting-edge processing methods, including microencapsulation and nanoemulsions, are being explored to encapsulate Vitamin D efficiently, thereby protecting it from thermal degradation during food processing. This technique facilitates controlled release, boosts the physicochemical stability and bioavailability of Vitamin D, and enhances its dispersibility and uniformity within complex food matrices. Microencapsulation involves the encapsulation of active ingredients, such as vitamins, within microscopic particles or capsules made of various materials, including proteins, carbohydrates, lipids, and polymers. The encapsulation process creates a protective barrier around the active ingredient, preventing its degradation or interaction with external factors such as heat, light, moisture, and oxygen. Abbasi et al. (2014) investigated the stability of Vitamin D3 encapsulated in nanoparticles of whey protein isolate against degradation parameters such as UV light or oxygen [120]. This study highlights the potential of encapsulation techniques in protecting Vitamin D3 from environmental factors. In addition, Diarrassouba et al. (2014) explored the increased stability and protease resistance of the β -lactoglobulin/Vitamin D3 complex, demonstrating that this complexation can significantly protect Vitamin D3 from the damaging effects of UV light [121]. Similar results has been achieved by Loewen, Chan, and Li-Chan (2018), who aimed to optimize Vitamin D3 loading in re-assembled casein micelles and assess the effect of loading on the stability of Vitamin D3 during storage with light exposure [122]. When

a composite gel made from whey protein isolate and lotus root amylopectin was used as an encapsulation method, Liu et al. (2020) markedly highlighted an enhancement in the bioavailability of Vitamin D3 offering substantial protection against degradation from UV light exposure [123]. Moreover, microcapsules can be designed to release the encapsulated Vitamin D slowly over time, ensuring sustained availability and absorption in the body. Encapsulating Vitamin D in microcapsules can enhance its solubility in aqueous solutions, facilitating its incorporation into dairy products without affecting their texture or sensory properties. Moreover, recent advances suggest that nanotechnology can offer substantial stability and uniformity by protecting many vitamins against a wide range of conditions and substances that could otherwise affect their integrity during food processing [124]. The nanoencapsulation of Vitamin D also offers protection against degradation during processing and storage, ensuring the retention of its nutritional benefits in the final product. As an example, David and Livney used potato proteins as a natural material to protect and deliver Vitamin D3 in beverages [125]. Nanoemulsions can serve as efficient carriers for encapsulating the vitamin within the dispersed oil phase. The nanoemulsion droplets (typically less than 100 nanometers), act as protective compartments, preventing the direct exposure of Vitamin D to heat and other deleterious factors during processing and providing a protective shell that shields it from thermal degradation during pasteurization [126]. For example, research by Gupta et al. demonstrated that Vitamin D3 encapsulated in nanoemulsions showed a 50% improvement in bioavailability compared to non-encapsulated forms. This improvement is attributed to the enhanced solubility and protection against degradation mechanisms provided by the nanoemulsion [127].

Furthermore, the protective capability of nanoemulsions against oxidative degradation is remarkable. A study by Silva et al. [128] found that Vitamin D3 stability increased by up to 70% when encapsulated in nanoemulsions compared to its stability in conventional emulsions under similar conditions. Enhancing the stability of Vitamin D3 is vital for prolonging the shelf life of fortified processed foods, guaranteeing its effectiveness from the moment of production through to the point of consumption. In addition, Lin et al. (2016) focused on the enhancement of Vitamin D3's physicochemical stability and *in vitro* bioaccessibility using corn protein hydrolysate as a novel nano-vehicle, showcasing innovative approaches to maintaining Vitamin D3's stability against UV light [129].

Controlled release is another significant benefit offered by nanoemulsion technology. According to Jaiswal et al. [130], nanoemulsions can be engineered to release encapsulated nutrients in response to specific triggers, such as pH changes or enzymatic action in the gastrointestinal tract. This targeted release mechanism can lead to a more efficient absorption and utilization of Vitamin D3, potentially enhancing its health benefits. Moreover, the development of ovalbumin-pectin nanocomplexes for Vitamin D3 encapsulation showed enhanced storage stability and sustained release in simulated gastrointestinal digestion. The encapsulation efficiency reached up to 96.37%, with a significant improvement in storage stability and a controlled release profile in simulated intestinal fluid, demonstrating that protein-polysaccharide complexes can effectively encapsulate Vitamin D3 for improved bioavailability [131]. Among the innovative strategies for improving Vitamin D3 stability, the nanostructured Lipid Carriers (NLCs) has been shown to provide a controlled release mechanism in simulated gastrointestinal fluids, increasing oral bioavailability and providing a promising strategy for delivering lipophilic vitamins like Vitamin D3. Park et al. [132] examined the release profile of Vitamin D3 from NLCs in simulated gastrointestinal fluids, representing the stomach and intestinal environments. The results showed that NLCs could remain stable and protect Vitamin D3 in simulated stomach fluid, releasing more than 90% of the encapsulated Vitamin D3 upon reaching simulated intestinal fluid. Additionally, Rabelo et al., 2018 focused on chitosan-coated NLCs for Vitamin D loading and studied their physical stability, indicating that coating NLCs with chitosan could effectively stabilize Vitamin D3, preventing its degradation in simulated gastric fluids [133].

Despite these promising outcomes, challenges remain. Concerns about the long-term stability of nanoemulsions and potential impacts on the sensory attributes of fortified foods

need to be addressed. Furthermore, the safety and regulatory aspects of nanotechnology in food products warrant careful consideration. For instance, comprehensive toxicological assessments are essential to ensure consumer safety. The European Food Safety Authority (EFSA) and the Food and Drug Administration (FDA) are actively working on establishing guidelines for the use of nanotechnology in food products [134,135]. In conclusion, the incorporation of nanoemulsion technology in food fortification strategies presents a promising approach to improving the stability and bioavailability of Vitamin D3 in processed foods. However, overcoming the technological and regulatory challenges will be crucial for the successful application of this technology. Ongoing research and innovation in this field are essential to unlock the full potential of nanoemulsions for Vitamin D3 fortification and to contribute significantly to addressing nutritional deficiencies.

7. Conclusions

The main findings of this review emphasize the crucial role of Vitamin D not only in calcium metabolism and bone health but also in blood pressure regulation, inflammation, and endothelial function, all of which are relevant to cardiovascular health. Research has shown that Vitamin D deficiency is associated with an increased risk of cardiovascular disease, suggesting the importance of food fortification and the use of supplements as effective strategies to improve Vitamin D status in the general population. However, current evidence indicates that Vitamin D fortification or supplementation neither significantly reduces the incidence of major cardiovascular events nor improves certain cardiovascular risk factors in the general population. These findings raise questions about the efficacy of Vitamin D fortification strategies in the prevention of cardiovascular disease, suggesting, furthermore, the need for further research to explore the effects of different dosing regimens and the use of Vitamin D in specific population groups with proven deficiency. There is a clear need for further research to optimize intervention strategies, especially for individuals with specific nutritional needs or at risk of Vitamin D deficiency. Moreover, the stability, bioaccessibility, and bioavailability of vitamin-D in the food system are important parameters to pay attention to. Innovative mitigation strategies such as UV irradiation, microbial fermentation, and advanced encapsulation techniques like nanoemulsions have been developed to counteract its losses. Employing these strategies effectively requires a careful consideration of processing conditions and food matrices to optimize Vitamin D retention and functionality. As research progresses, these methods are likely to evolve further, offering more efficient and widely applicable solutions to maintain and enhance Vitamin D levels in fortified foods.

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