

The Role of Vitamin D in Hormonal Balance

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Vitamin D3 is associated with multiple functions, including bone mineralization, regulation of cell growth, and immune and neuromuscular functions. The current article focuses on the role of vitamin D in hormonal balance and how it influences the development of many health issues, including infertility.



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Synthesis of vitamin D in humans

Vitamin D was first identified as a vitamin in the 20th century and is now recognized as a prohormone.¹ There are different types of vitamin D, including fat-soluble secosteroids, i.e., vitamin D1-D5. In humans, vitamin D2 (ergocalciferol) and vitamin D3 (cholecalciferol) are most important for biological functions.² Vitamin D is mostly synthesized in the skin in the presence of sunlight, while some, particularly vitamin D2 and D3, are taken from food sources.

When human skin is exposed to sunlight, 7-dehydrocholesterol is transformed into D3; subsequently, it is converted to 25-hydroxyvitamin D (25(OH)D, also known as calcidiol or calcifediol, in the liver.³ The active form of vitamin D3, which is also known as calcitriol, 1 α ,25-dihydroxy vitamin D3, or 1,25-dihydroxycholecalciferol, is

synthesized in the kidney in the presence of the enzyme 1α -hydroxylase. Vitamin D levels are determined by estimation of serum $25(\text{OH})\text{D}$.

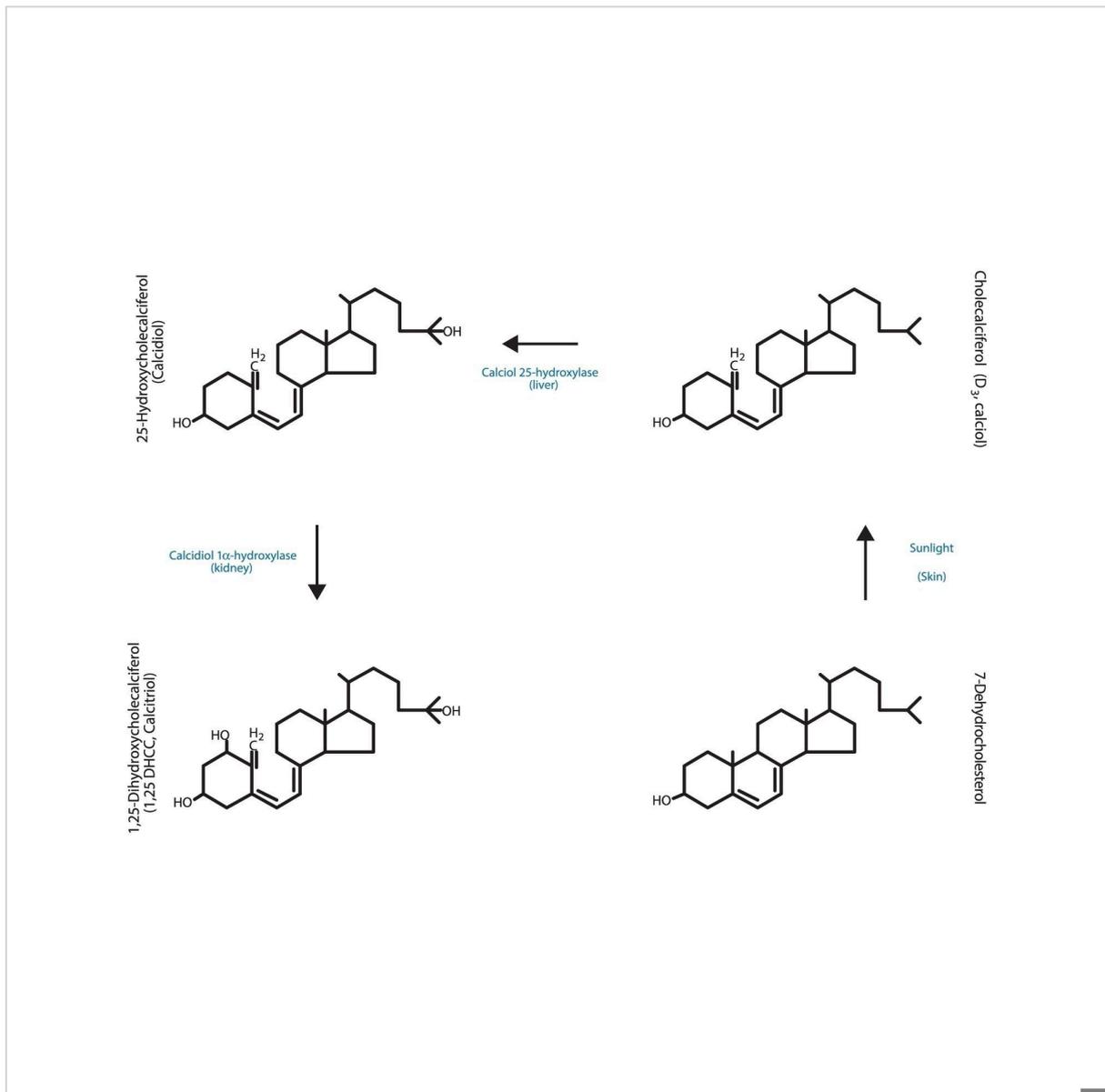


Diagram showing biosynthesis of active form of vitamin D - Calcitriol - via the kidneys and the liver - schematic molecular structure chemical illustration. Image Credit: Bass stock/Shutterstock.com

Vitamin D regulates calcium-phosphate metabolism and enhances calcium levels via multiple pathways.² For instance, it is associated with the stimulation of calcium absorption in the intestine, its reabsorption in the renal tubules, and activation of the RANK/RANKL (receptor activator for nuclear factor κ B/its ligand) pathway in osteoclasts.⁵

Vitamin D and thyroid hormone

Thyroid glands synthesize hormones that regulate the functioning of physiological systems. The hypothalamus-pituitary-thyroid (HPT) axis regulates thyroid hormone synthesis through a feedback mechanism.⁶ When thyroid hormone levels decrease beyond a threshold point, the hypothalamus synthesizes thyrotropin-releasing hormone (TRH). Subsequently, TRH triggers thyrocytes that enhance thyroid hormone synthesis.

Vitamin D and parathyroid hormone (PTH) are involved in the regulation of calcium and bone formation. Vitamin D elevates calcium absorption from the small intestine, inhibits PTH, and decreases calcium excretion in the kidneys. This ultimately increases calcium concentration in the blood and promotes bone formation, growth, and maintenance.⁷

Autoimmune thyroid diseases, such as Hashimoto's thyroiditis (hypothyroidism) and Graves' disease (hyperthyroidism), are characterized by thyroid lymphocytic infiltration.⁸ A meta-analysis observed a significant decrease in 25(OH)D levels in patients with Hashimoto's thyroiditis. Similarly, another study revealed vitamin D deficiency is associated with the onset of Graves' disease in older adults. Animal models of Graves' disease and thyroiditis revealed the efficacy of vitamin D supplementation in alleviating these conditions.⁹

Vitamin D modulates the activity of many immune cells and is involved in immune cell regulation. Mechanistically, vitamin D receptor (VDR) and 1 α -hydroxylase are expressed in immune cells, i.e., T and B lymphocytes, monocytes, dendritic cells, and neutrophils, which entails the production of calcitriol. Vitamin D enhances the production of anti-inflammatory cytokines (e.g., IL-4, IL-5, and IL-10) and inhibits proinflammatory cytokines (e.g., IL-6, IL-8, IL-9, IL-12, IFN- γ , and TNF- α) production. Multiple *in vitro* and *in vivo* studies have shown the positive effect of vitamin D in thyroid cancer treatment.¹⁰

Vitamin D and male hormones

25(OH)D levels regulate multiple enzymes involved in the production of steroid hormones, such as adrenal steroid hormones, sex hormones, and sex hormone signaling. Vitamin D metabolizes enzymes in the human testis and ejaculatory tract and is also associated with spermatozoa maturity.



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Experimental findings have highlighted the possible role of vitamin D in male fertility through the synthesis of male reproductive hormones.¹¹ A Mendelian Randomization analysis revealed the causal relationship between vitamin D deficiency and testosterone levels. This study linked a genetic decrease in 25(OH)D levels with lower testosterone levels. Another cross-sectional study supported this finding and highlighted a positive correlation between 25(OH)D levels and total/bioavailable testosterone levels. Men with lower serum 25(OH)D levels, particularly below 25 nmol/L, had a lower testosterone level.

The Connection Between Vitamin D and Mental Health

These observations have drawn significant attention from the scientific community, which is seeking to understand better the potential impact of this vitamin on male reproductive function. Although more research is needed to understand how age, genetic variations, seasonal variations, and measurement timing influence testosterone levels, a recent study demonstrated that vitamin D supplementation enhances testosterone levels. Future research must also focus on the therapeutic potential of vitamin D in alleviating problems linked with male fertility.

Vitamin D and female hormones

Vitamin D influences estrogen and progesterone levels that impact menstrual regularity, menopause, fertility, and pregnancy.¹² Animal-based experiments revealed that 25(OH)D deficiency reduced fertility rates, compromised mating behavior, and impaired neonatal growth.

Decreased maternal vitamin D levels have been associated with poor maternal and fetal outcomes, including gestational diabetes mellitus, low birth weight, preeclampsia, and small for gestational age. Many women experience polycystic ovary syndrome (PCOS), a heterogeneous endocrine disorder that causes female infertility and is associated with hyperandrogenism. Multiple studies have observed serum vitamin D deficiency in patients with PCOS. Mechanistically, vitamin D deficiency influences insulin secretion and insulin resistance, which could induce PCOS.¹³

Research has established a link between vitamin D and reproductive hormone biosynthesis.¹⁴ An inverse correlation between vitamin D levels and androstenedione and anti-Müllerian hormone, as well as follicle-stimulating hormone (FSH)/luteinizing hormone (LH), has also been found. One recent study recommended women with PCOS consume 400 IU of vitamin D supplementation and 1000 mg of calcium daily for three months to improve fertility and other PCOS symptoms.

The Health Benefits of Vitamin D3 and Vitamin K2 for Women

Vitamin D3, chemically known as cholecalciferol, is an essential fat-soluble vitamin that has taken center stage in recent health discussions. For women, maintaining optimal levels of this vitamin is crucial for a myriad of physiological processes. The interplay of Vitamin D3 and Vitamin K2 presents a fascinating case of how two nutrients can synergistically influence women's health.

Here's why:

1. Bone Health and Osteoporosis Prevention:

One of the most celebrated benefits of vitamin D3 lies in its role in calcium metabolism. Vitamin D3 aids the intestines in absorbing calcium, which is vital for bone mineralization. For women, particularly post-menopausal women, there's a heightened risk of osteoporosis. Adequate levels of vitamin D3 can help mitigate this risk, ensuring bones remain dense and robust.

2. Mood Regulation and Depression Prevention:

Studies have suggested a relationship between vitamin D3 deficiency and mood disorders, including depression. While the mechanisms aren't fully understood, it's believed that vitamin D3 might play a role in the synthesis of serotonin, a neurotransmitter associated with mood regulation.

3. Immune System Support:

Vitamin D3 has been found to modulate both innate and adaptive immune responses. In simple terms, it helps keep the immune system balanced, which is crucial for fighting off infections and warding off illnesses.

4. Heart Health:

Vitamin D3 may play a protective role in heart health, especially hypertension, atherosclerosis, and chronic inflammation — all significant risk factors for heart disease in women.

5. Support in Pregnancy:

Adequate vitamin D3 levels are crucial during pregnancy. A deficiency might be linked to preeclampsia, gestational diabetes, and even bacterial vaginosis. Ensuring optimal levels can support both maternal and fetal health.

6. Alzheimer's Prevention and Cognitive Health:

Emerging research indicates a potential link between vitamin D3 and cognitive health. Here's why:

Neuroprotection: Vitamin D3 possesses neuroprotective properties, possibly reducing the risk of neurodegenerative diseases.

Amyloid Plaque Reduction: A hallmark of Alzheimer's is the buildup of amyloid plaques in the brain. Some studies suggest that vitamin D3 might help in reducing the formation of these plaques.

Inflammatory Response Modulation: Chronic inflammation is believed to play a role in Alzheimer's progression. Vitamin D3 can modulate the body's inflammatory response, potentially offering protection against inflammation-driven cognitive decline.

Recommended Dosages for Women:

The recommended dosage of vitamin D3 varies based on several factors such as age, skin type, geographical location, and dietary habits. Here's a general guideline: Adults 800 IU - 5,000 IU per day.

However, many experts believe that these recommended dosages are conservative and that higher doses might be required for optimal health, especially in regions with limited sunlight.

In Conclusion:

Vitamin D3 is undoubtedly a vital component of women's health. From maintaining bone density to supporting mood and immunity, its benefits are manifold. Women, especially those living in areas with limited sun exposure or those with specific dietary restrictions, should consider their vitamin D3 levels and consult with Complete Healthcare for Women for personalized advice.

Remember, while supplements can be beneficial, natural sunlight remains one of the best sources of vitamin D3. Aim for 10-15 minutes of direct sunlight exposure several times a week to harness its benefits.



How vitamin D can help with menopause

Everything you need to know about vitamin D and menopause, including the causes and signs of vitamin D deficiency, the best types, how much to take and the symptoms it can help with.

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Vitamin D is essential for good health during and after menopause. But lower levels of oestrogen at this time make your body less efficient at producing it and many menopausal women have a vitamin D deficiency.

Taking a vitamin D supplement can prevent this and may also help with a range of other menopause symptoms, such as weak bones or osteoporosis, vaginal dryness and low mood.

In this article, we'll cover everything you need to know about vitamin D and menopause, including:

- why your body needs vitamin D
- the causes and signs of vitamin D deficiency
- the best types of vitamin D
- how it can help during menopause
- how much you should take
- whether you can use it alongside HRT

Vitamin D3 is among the nutrients and herbal extracts in **Better Night**, an expert formulated supplement designed to help with anxiety, low mood and sleep during menopause.

To find out more, visit **The Better Menopause**. And get 10% off your first order with the discount code WELCOME10.

What is vitamin D and why does your body need it?

Vitamin D, also known as calciferol, is essential for your overall health and wellbeing. It's produced by your liver and kidneys when your skin is exposed to sunlight. Vitamin D is not naturally present in many foods, although you can get it from some oily fish like salmon and from mushrooms.

Vitamin D is involved in a range of key functions around your body. It maintains bones and muscles – including by helping you to absorb calcium – supports your immune system, reduces inflammation and keeps the nerves that carry messages from your brain healthy.

Scientists believe vitamin D may also impact how your body **metabolises oestrogen**. Changing levels of oestrogen are responsible for many of the most common **symptoms of menopause**.

Because it can be hard to get enough vitamin D from diet and sunshine alone, the **NHS** suggests taking a daily vitamin D supplement.

Menopause and vitamin D deficiency

During perimenopause – the years leading up to menopause – your levels of reproductive hormones like oestrogen begin to change. After menopause, when your periods have stopped, oestrogen settles at its lowest point.

Reduced levels of oestrogen make your skin and kidneys less efficient at producing **certain forms** of vitamin D, and your gut doesn't absorb it as well. This may partly explain why vitamin D deficiency is **very common** among menopausal women.

After menopause, women with low vitamin D are significantly more likely to have problems with their bone health, such as osteoporosis and fractures.

As we'll discuss later, getting enough vitamin D can reduce your risk of osteoporosis and help with a number of other menopause symptoms, including vaginal health issues and low mood.

What are the signs of low vitamin D?

For adults with low vitamin D, symptoms can sometimes be quite general and hard to pin down, **such as**:

- tiredness / fatigue
- aches and pains
- **muscle weakness**
- feeling generally unwell

People with very severe vitamin D deficiency can develop **osteomalacia**, a softening of the bones. Symptoms include a throbbing pain caused by touch, in areas like the chest, lower back, thighs, hips or pelvis, and increased weakness that could lead to trouble climbing stairs or getting up from a seated position.

Measurements of vitamin D deficiency

If your doctor thinks you could have a vitamin D deficiency, they will give you a **blood test**.

The form of vitamin D that circulates in your blood is known as **25-hydroxyvitamin D** (25(OH)D) or calcitriol. The levels of calcitriol in your bloodstream are usually

measured in one of two ways: in nanograms per millilitre (ng/mL) or in nanomoles per litre (nmol/L). A nmol/L is **approximately 2.5 times** a ng/mL.

There's no definitive agreement on what levels constitute vitamin D deficiency, and **lots of debate** around the subject.

The Endocrine Society has suggested that anything below 20 ng/mL of calcitriol should be considered vitamin D deficiency.

However, the Institute of Medicine (now the National Academy of Medicine) says the right level will differ from person to person. It argues that 16 ng/mL is likely to be healthy for around half the population, with 12 ng/mL still a sufficient level for many.

Your doctor will consider both the results of your blood test and your symptoms when deciding whether you have a deficiency.

What's the best type of vitamin D for menopause?

When it comes to food and dietary supplements, vitamin D comes in **two main forms**, vitamin D2, also known as ergocalciferol, and vitamin D3, known as cholecalciferol.

Studies have shown that in single doses vitamin D3 is better than vitamin D2 at raising your levels of calcitriol, the form of vitamin D that circulates in your bloodstream. Vitamin D3 may also keep your levels **higher for longer**. However, if you take a daily supplement on a regular basis, there may be less difference between the effects of vitamin D2 and D3.

Although in food vitamin D2 comes from plant sources and vitamin D3 comes from animal sources, you can get vegetarian versions of both vitamin D2 and D3 supplements.

Vitamin D3 is one of the ingredients in **Better Night**, an expert blend of nutrients and herbal extracts designed to improve anxiety, mood and sleep during menopause.

To find out more, visit **The Better Menopause**.

How vitamin D could help with menopause symptoms

There's evidence that a higher intake of vitamin D could help to tackle some of these common menopause symptoms.

Reduced bone density

Decreased oestrogen levels due to menopause mean you're less efficient at absorbing calcium from your gut. This can lead to reduced bone density and eventually **osteoporosis**, where bones become weak and more likely to fracture.

One of the key functions of vitamin D is helping your body absorb calcium, so getting enough can keep bones strong and reduce the risk of osteoporosis.

One large-scale, **long-term study** involving postmenopausal women found that those with a higher intake of vitamin D had a significantly lower risk of hip fractures

due to osteoporosis. Interestingly, the study also showed that intake of calcium itself had no effect on this risk.

Meanwhile, a **review** of clinical trials found that postmenopausal women who took a regular vitamin D supplement increased their bone density and reduced their risk of fracture.

Vaginal dryness

Another symptom of low oestrogen during perimenopause and after menopause is **vaginal atrophy**, a thinning of the walls of your vagina that leads to vaginal dryness. This can in turn cause soreness and itching, pain or discomfort during sex and an increased **risk of infection**.

Vitamin D is involved in **regulating the growth** of the different types of cells inside your vagina, including those that produce moisture and maintain a protective barrier.

One **study** involving postmenopausal women found that taking high-dose vitamin D supplements significantly improved several signs of vaginal atrophy after just 6 weeks.

Mood symptoms

Mood issues including **low mood** and depression are common symptoms of perimenopause and menopause.

Areas of your brain involved in processing emotions contain vitamin D receptors. Vitamin D is also integral to how your immune system **responds to stress**, as well

as helping to regulate the mood hormone dopamine. People diagnosed with depression tend to have low levels of vitamin D.

In a **study** involving postmenopausal women, those with the highest intakes of vitamin D were less likely to have depressive symptoms than those with the lowest. They also had a 20% reduced risk of developing symptoms after 3 years.

Urinary tract infections

Low oestrogen during menopause can make the protective linings of your bladder and urinary tract more permeable and prone to bacterial infection.

Studies using infected tissues from postmenopausal women have shown that vitamin D increases the production of proteins that strengthen these linings.

However, more research is needed to demonstrate how these findings might translate to symptoms in women.

How much daily vitamin D should you take during menopause?

Vitamin D is usually measured by weight in micrograms – abbreviated to either mcg or µg – or as IUs (International Units).

IUs are based on how much of a substance it takes to produce a particular biological effect.

The NHS says a daily 10 mcg/µg (400 IU) vitamin D supplement is enough for the average person and recommends not taking more than 100 mcg/µg (4,000 IU) per day.

When it comes to treating vitamin D deficiency in menopausal women, experts have looked at the results of numerous studies to find the optimum dose.

Researchers writing in the journal **Menopause Review** concluded that daily doses of over 2,000 IU were needed to raise levels to 20 ng/mL in the course of a year.

How you take vitamin D is also relevant. **Vitamin D is fat soluble**, meaning it dissolves in fat rather than water. Although you can take vitamin D supplements on an empty stomach, you'll absorb it more efficiently if you take it with food that contains some fat.

Can you take too much vitamin D?

Taking too much vitamin D in the form of supplements could cause a build-up of calcium in your body. Too much calcium is called hypercalcaemia and can actually weaken your bones, as well as damaging organs like your kidneys and heart.

According to the **NHS**, a regular daily dose of more than 100 mcg/µg or 4,000 IU could be harmful.

While you can't get too much vitamin D from sunlight, it's important to protect your skin with sunblock or clothing to reduce the risk of damage to your skin, and skin cancer.

Can you take vitamin D with HRT?

Hormone replacement therapy (HRT) replenishes reproductive hormones like oestrogen and progesterone lost during menopause. It's an effective treatment for a range of common perimenopause and menopause symptoms.

If you're on HRT, you can still take a vitamin D supplement. According to the **Royal Osteoporosis Society**, your doctor may prescribe them at the same time. The **NHS** says everyone should consider taking a vitamin D supplement, especially during the autumn and winter.

Summary

Vitamin D is an essential nutrient for strong bones, immune function and a healthy nervous system.

Lower levels of oestrogen during perimenopause and after menopause can make your body less efficient at producing and absorbing vitamin D and many menopausal women have a vitamin D deficiency.

Signs of vitamin D deficiency can include tiredness, aches and pain and feeling weak and unwell. A severe deficiency can lead to osteomalacia, a softening of the bones.

The main forms of vitamin D used by your body are vitamins D2 and D3. Vitamin D3 can increase and maintain the levels in your blood for longer.

Increasing your vitamin D intake could help with menopause symptoms including weak bones and osteoporosis, vaginal dryness and pain, and low mood and depression. There's also some initial evidence that it could protect against urinary tract infections.

The NHS recommends that most people take a vitamin D supplement, especially during the darker autumn and winter months. Studies suggest that daily doses of over 50 mcg/ μ g (2,000 IU) are the most effective for tackling vitamin D deficiency in menopausal women.

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Review Article

The Synergistic Interplay between Vitamins D and K for Bone and Cardiovascular Health: A Narrative Review

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Vitamins D and K are both fat-soluble vitamins and play a central role in calcium metabolism. Vitamin D promotes the production of vitamin K-dependent proteins, which require vitamin K for carboxylation in order to function properly. The purpose of this review is to summarize available evidence of the synergistic interplay between vitamins D and K on bone and cardiovascular health. Animal and human studies suggest that optimal concentrations of both vitamin D and vitamin K are beneficial for bone and cardiovascular health as supported by genetic, molecular, cellular, and human studies. Most clinical trials studied vitamin D and K supplementation with bone health in postmenopausal women. Few intervention trials studied vitamin D and K supplementation with cardiovascular-related outcomes. These limited studies indicate that joint supplementation might be beneficial for cardiovascular health. Current evidence supports the notion that joint supplementation of vitamins D and K might be more effective than the consumption of either alone for bone and cardiovascular health. As more is discovered about the powerful combination of vitamins D and K, it gives a renewed reason to eat a healthy diet including a variety of foods such as vegetables and fermented dairy for bone and cardiovascular health.

1. Introduction

Worldwide, a large group of people is prescribed to a supplemental regime of both vitamin D and calcium. In Europe, depending on a country and sex, between 1 and 66% of the adult population use vitamin D supplements [1, 2]. Over the last decade, large vitamin D supplementation is promoted to restore 25-hydroxyvitamin D (25(OH)D) concentrations and is considered to be safe with doses up to 4000 international units (IU) per day [3]. However, little is known about potential long-term high-dose vitamin D supplementation [2, 4].

Vitamin D is a fat-soluble vitamin that can be ingested by foods such as fatty fish, dairy products, and eggs, but is mainly synthesized by the human skin when exposed to sunlight. In the liver, vitamin D is hydroxylated to 25(OH)D, the main circulating vitamin D metabolite that is measured to assess and classify vitamin D status. Circulating 25(OH)D is further metabolized by the kidney for full biological activity into its most active form 1,25-dihydroxyvitamin D (1,25(OH)D) also known as calcitriol. Calcitriol is also produced endogenously by extrarenal production through peripheral 1- α -hydroxylase and has positive effects on immune function and anticancer activity [5–7]. Vitamin D

plays a main role in regulating calcium metabolism by increasing intestinal calcium absorption [8]. Ample evidence recommends vitamin D supplementation for the prevention of falls and fractures [9, 10]; however, evidence suggests calcium precipitation in the vasculature and other potential side effects [4, 11–14].

Vitamin K is another fat-soluble vitamin that exists in two forms of vitamin K: vitamin K₁ (phylloquinone, mainly found in green leafy vegetables) and vitamin K₂ (menaquinone, mainly found in fermented dairy and produced by lactic acid bacteria in the intestine) [15]. Vitamin K stores are limited, but they can be recycled to a certain extent [16]. Vitamin K₁ is principally transported to the liver, regulating the production of coagulation factors, while vitamin K₂ is transported to extrahepatic tissues, such as bone and the vascular wall, regulating the activity of matrix Gla protein (MGP) and osteocalcin (bone Gla protein)—the main vitamin K-dependent proteins. They require vitamin K for carboxylation in order to function properly. When circulating concentrations of vitamin K are insufficient, a greater proportion of MGP and osteocalcin remain uncarboxylated, which is associated with unfavorable outcomes such as cardiovascular disease, lower BMD, and osteoporosis [17]. The current recommendation for vitamin K₁ intake is 70 µg/day for all adults defined by an adequate intake [18]. This amount is solely based on maintaining coagulation function and might not be enough for optimal carboxylation of other vitamin K-dependent proteins, which require higher amounts of vitamin K [19].

The role of vitamin K in cardiovascular health has mainly been studied in isolation [20]; however, a growing body of evidence suggests a synergistic effect of vitamin K combined with vitamin D [21–26]. Vitamin D promotes the production of vitamin K-dependent proteins, as shown in rats by Karl et al. already in 1985 [27]. These findings cannot be explained by our current understanding of the biochemical role of vitamin K, but suggest that vitamin D may influence vitamin K-dependent proteins [28].

The purpose of this narrative review is to summarize available evidence in the field of the synergistic interplay between vitamins D and K on bone and cardiovascular health. The primary focus is on the general population and includes observational studies that investigated both vitamin D and vitamin K status with outcome measures and supplementation studies that administered both vitamins D and K.

2. Interaction of Vitamins D and K for Bone Health

2.1. Experimental Studies. In experimental models, the exploration of the interaction between vitamins D and K on bone health is ongoing for decades and a fair amount of literature is available. Recent understanding suggests that vitamin D enhances vitamin K-dependent bone protein concentrations and induces bone formation in vitro [29–31] with stimulation of osteoblast-specific gene expression [32]. Osteoblast-specific expression of osteocalcin is controlled at the transcriptional level by 1,25(OH)₂D through the 1,25(OH)₂D-responsive element within the promoter of the

osteocalcin gene [32]. The underlying mechanism of mineralization induced by vitamin K in the presence of 1,25(OH)₂D was different from vitamin K alone [33]. In rats, 1,25(OH)₂D receptor binding can undergo gamma-carboxylation in the presence of vitamin K. This means that 1,25(OH)₂D receptor carboxylation can potentially modify the intrinsic biochemical properties of the nuclear receptors and modulates its binding to DNA [34].

The effect of 1,25(OH)₂D and warfarin—a vitamin K antagonist—on the vitamin K cycle was studied in cultured osteoblasts [26]. Epoxide reductase, one of the key enzymes in the vitamin K cycle, was strongly inhibited by warfarin, whereas it was not affected by 1,25(OH)₂D, meaning that the vitamin K metabolic cycle functions normally in human osteoblasts.

Human osteoblast cell cultures indicate that glycoxidation interferes with the maturation of osteoblasts; however, this process may be counterbalanced by adding vitamins D and K, which reverses the detrimental glycoxidation on several bone markers [35]. Therefore, the addition of vitamins D and K may induce important biochemical changes in bone, which may exert therapeutic effects on bone metabolic diseases such as osteoporosis [36].

2.2. Animal Models. A growing body of evidence is also documenting the interaction between vitamins D and K in animal models. The effect of vitamin K of bone mineralization is enhanced by plasma 25(OH)₂D concentration. Vitamin K was administered to prevent osteoporosis in ovariectomized rats, but bone loss was only prevented in rats fed with a diet containing vitamin D or vitamin D supplementation [37, 38]. These findings suggest that combined treatment with vitamins D and K is more effective than vitamin K alone particularly in the early phase of estrogen deficiency after menopause.

Vitamin K and vitamin D supplementation on calcium balance was investigated in young rats fed with a normal or low calcium diet, plus vitamin K and/or vitamin D [39]. Vitamin K supplementation promoted the reduction in urinary calcium excretion and stimulated intestinal calcium absorption in rats on a normal calcium diet. Vitamin D supplementation stimulated intestinal calcium absorption with prevention of the abnormal elevation of serum PTH concentrations, prevented hypocalcemia in rats fed with a low calcium diet, and stimulated intestinal calcium absorption in rats fed with a normal calcium diet. The stimulation of intestinal calcium absorption was associated with increased 1,25(OH)₂D concentrations. An additive effect of vitamin K and vitamin D on intestinal calcium absorption was only found in rats fed with a normal calcium diet. This study shows the differential effects of vitamin K and vitamin D supplementation on calcium balance in young rats fed with a normal or low calcium diet.

2.3. Observational Evidence. Human evidence for the role of 1,25(OH)₂D in stimulating vitamin K-dependent proteins is scarce. In hemodialysis patients, vitamin D analog users had much higher concentrations of bone Gla protein (BGP) than nonusers indicating that vitamin D administration

may play a role in stimulating vitamin K-dependent protein activity [40]. More research on the stimulating role of vitamin D on vitamin K-dependent proteins is urgently needed to study the underlying mechanisms.

Some observational studies support the hypothesis that optimal concentrations of both vitamins D and K support bone mineralization and lower fracture risk. In a cross-sectional study among Japanese older men, lower 25(OH)D and vitamin K₁ concentrations were concomitantly associated with BMD, indicating a nonestrogen-dependent pathway in men [41]. In a case-control study of 184 Norwegian older adults, the combination of low vitamin K₁ and low 25(OH)D was synergistically associated with hip fractures: odds ratio 7.6 (95% CI 2.3, 26.7) [42]. In the NOREPOS study, another Norwegian population study, similar results were observed among 1318 older adults [43]. During 8.2-year follow-up, the combination of both low vitamin D and K₁ concentrations was associated with a greater hip fracture risk, hazard ratio 1.41 (95% CI 1.09, 1.82), compared to the high vitamin D and vitamin K category. No increased risk was observed in the groups low in 1 vitamin only. These results indicate that the combination of low concentrations of vitamin K₁ and 25(OH)D is associated with increased risk of hip fractures.

2.4. Human Intervention Studies. A small study among 15 healthy women indicated that 3 weeks of supplementation of 20 ml extra virgin olive oil enriched with vitamins D, K, and B₆ resulted in lower concentrations of uncarboxylated osteocalcin [44]. This means that a vitaminized oil can influence vitamin K-dependent proteins within multiple weeks.

An increasing amount of randomized controlled trials have demonstrated the combined effects of vitamins D and K on postmenopausal osteoporosis mostly pursued in Japan with a study duration between 8 weeks and 3 years (Table 1). A randomized trial with 4 arms (diet, menaquinone-4, cholecalciferol, and menaquinone-4 + cholecalciferol) showed that only the vitamin K plus vitamin D arm increased BMD [45]. Similar results were found in another trial with postmenopausal women with osteoporosis ≥ 5 years after menopause [46]. After 2 years of follow-up, the longitudinal changes in BMD were significant compared with those in the calcium lactate-, vitamin D-, and vitamin K-only groups ($P < 0.001$). A modest synergistic effect of vitamins D and K was found after 2 years in healthy older women from nutritionally relevant intakes of vitamin K₁ together with supplements of calcium plus vitamin D₃ on bone mineral concentration compared to either vitamin D or K alone or placebo [47]. The complementary effect of vitamin K₁ (1 mg/day) and a mineral + vitamin D supplement (8 μ g/day) was most effective in reducing bone loss at the femoral neck after 3 years among postmenopausal women versus vitamin D alone or placebo [48]. The addition of vitamin K to vitamin D and calcium supplements compared to vitamin D and calcium alone in postmenopausal Korean women increased BMD and reduced uncarboxylated osteocalcin concentrations after 6 months compared to vitamin D and calcium alone [49]. In postmenopausal women, 1 year of oral

supplementation with extra virgin olive oil enriched with vitamins D₃, K₁, and B₆ or extra virgin olive oil reduced uncarboxylated osteocalcin concentrations and increased the T-score of BMD [50]. These findings indicate that combined administration of vitamin D and vitamin K appears to be useful in increasing BMD in postmenopausal women. It should be noted that these studies found beneficial effects at some but not all BMD sites measured. Furthermore, treatment with vitamins D and K with calcium increased BMD in older female patients with Alzheimer's disease and led to the prevention of nonvertebral fracture odds ratio: 7.5 (95% CI 5.6, 10.1); however, no placebo capsules were administered, hampering the interpretation of the results [51].

Not all studies observed synergistic effects of vitamin D and K supplementation. A small study among adults with Crohn's disease in Ireland showed generally no effect of combined vitamin D and K supplementation versus placebo on bone mass after 1 year, except a modest increase in bone mass of the total radius [52]. Among healthy women, 1 year of vitamin D and calcium + vitamin K supplementation either by phylloquinone or menaquinone-4 supplementation had no effect on BMD compared to calcium and vitamin D alone [53]. This study does not support a combined role for vitamin D + K supplementation in osteoporosis prevention; however, the relatively short study duration and the inclusion of healthy women could explain the null finding. It is however questionable if BMD can be improved in 12 months since changes in BMD usually require at least 1 year of follow-up time.

Among healthy older men and women, no difference was observed between multivitamin and calcium and vitamin D compared with the addition of vitamin K on BMD after 3 years [19]. An additive effect was noticeable for decreased percentage of uncarboxylated osteocalcin, which indicates an improved vitamin K status in the treatment group.

The ECKO trial among postmenopausal women with osteopenia showed no beneficial effect of vitamin D and calcium + vitamin K supplementation versus vitamin D and calcium alone after 2 years of follow-up in vitamin D-sufficient women [54]. However, the risk of fractures—a clinically more meaningful endpoint—was lower in the vitamin D and calcium + vitamin K groups: hazard ratio 0.41 (95% CI 0.1, 1.18) at 2 years and 0.45 (95% CI 0.20, 0.98) after 4 years of follow-up. This result on fracture risk indicates that bone quality rather than quantity is more important as not all trials showed synergistic effects of vitamin D and K supplementation on bone mineral density.

The protective effect of vitamin D with K on prednisolone-induced loss of BMD in patients with chronic glomerulonephritis after 8 weeks of treatment was similar in the vitamin D-only group [55], meaning that the addition of vitamin K had no synergistic effect. The elevation in serum calcium concentrations in the vitamin D group was, however, attenuated in the vitamin D + K group.

Taken together the evidence for combined vitamin D and K supplementation, the majority of the studies found beneficial effects for BMD among postmenopausal women.

TABLE 1: Summary of clinical trials of combined vitamin D and K supplementation on bone health.

Author, year	Country	Participants	Treatment	Study duration	Outcome	Results for the highest versus the lowest quartiles
Iwamoto et al., 2000 [46]	Japan	N = 92 osteoporotic women \geq 5 years after menopause, mean age 64 years	(i) Calcium (calcium lactate, 2 g/day) (ii) Vitamin D ₃ 0.75 μ g/day (iii) Vitamin K ₂ 45 mg/day (iv) Vitamin D ₃ plus vitamin K ₂	2 years	Bone mineral density % change	Combined vitamins D and K increased BMD
Ushiroyama et al., 2002 [45]	Japan	N = 126 postmenopausal women with osteopenia and osteoporosis, mean age 53 years	(i) Diet (ii) Vitamin K ₂ 45 mg/day MK-4 (iii) 1- α hydroxycholecalciferol 1 μ g/day (iv) Vitamin K ₂ + 1- α hydroxycholecalciferol	2 years	Bone mineral density % change	K + D group increased BMD % change at 2 years $P < 0.001$
Braam et al., 2003 [48]	Netherlands	N = 155 postmenopausal women between 50 and 60 years	(i) Placebo (ii) Mineral + vitamin D (8 μ g/day) (iii) Mineral + vitamin D + vitamin K ₁ 1 mg	3 years	Bone loss	Mineral + vitamin D + vitamin K showed reduced bone loss of the femoral neck
Yonemura et al., 2004 [55]	Japan	N = 60 patients with chronic glomerulonephritis, mean age 32 years, 53% female	(i) Control (ii) Vitamin D (alfacalcidol 0.5 mg) (iii) Vitamin K ₂ MK-4 45 mg/d (iv) Vitamins D plus K	8 weeks	Bone mineral density	The preventive effect in groups K and D + K was similar to D
Sato et al., 2005 [51]	Japan	N = 200 older women with Alzheimer's disease, mean age 78 years	(i) Placebo (ii) 45 mg menatetrenone, 1000 IU ergocalciferol, and 600 mg calcium	2 years	Bone mineral density and fractures	BMD increased in vitamin D + K group Odds ratio nonvertebral fractures 7.5 (95% CI 5.6, 10.1)
Bolton-Smith et al., 2007 [47]	UK	N = 244 healthy women, mean age 68 years	(i) Placebo (ii) 200 mg/d vitamin K ₁ (iii) 400 IU vitamin D ₃ + 1000 mg calcium (iv) Vitamins K ₁ and D ₃ plus calcium	2 years	Bone mineral content	Combined vitamin K with vitamin D plus calcium associated with an increase in bone mineral content at the ultradistal radius
Booth et al., 2008 [19]	US	N = 401 healthy men and women, mean age 69, 59% female	(i) Multivitamin + 10 μ g vitamin D and 600 mg calcium (ii) Multivitamin + vitamin D + calcium + 500 μ g vitamin K ₁	3 years	Bone mineral density	No differences in change in BMD Vitamin D + K group lower uncarboxylated osteocalcin concentrations
Cheung et al., 2008 [54]	Canada	N = 440 postmenopausal women with osteopenia, mean age 59 years	(i) 1500 mg calcium + 800 IU vitamin D (ii) 5 mg of vitamin K ₁ + calcium and vitamin D	2-4 years	Bone mineral density	No effect on BMD
Binkley et al., 2009 [53]	US	N = 381 postmenopausal women, mean age 62 years	(i) Calcium 315 mg + vitamin D ₃ 200 IU (ii) Phylloquinone 1 mg + calcium and vitamin D ₃ (iii) MK-4 (45 mg day) + calcium and vitamin D ₃	1 year	Bone mineral density	No effect on BMD
Je et al., 2011 [49]	South Korea	N = 78 Korean postmenopausal women, mean age 68 years	(i) Vitamin D 400 IU + calcium (630 mg) (ii) Vitamin D + calcium +45 mg of vitamin K ₂	6 months	Bone mineral density	BMD increased significantly in the vitamin D + K group

TABLE 1: Continued.

Author, year	Country	Participants	Treatment	Study duration	Outcome	Results for the highest versus the lowest quartiles
O'Connor et al., 2014 [52]	Ireland	N = 46 adults with Crohn's disease, mean age 45 years	(i) Placebo (ii) Phylloquinone 1 mg, vitamin D 10 µg, and calcium 500 mg/d	1 year	Bone mineral density	Small effect on BMD of the total radius for vitamin D + K group
Mazzanti et al., 2015 [50]	Italy	60 healthy postmenopausal women, mean age 55 years	(i) Extra virgin olive oil (ii) Extra virgin olive oil enriched with vitamins D ₃ , K ₁ , and B ₆	1 year	Bone mineral density	Vitaminized oil D, K, and B ₆ increased the T-score of BMD

BMD: bone mineral density; MK-4: menaquinone-4.

3. Interaction between Vitamins D and K for Cardiovascular Health

Besides bone health, also, the interaction between vitamins D and K with regard to cardiovascular health receives growing research interest. MGP—the vascular marker of vitamin K status—needs γ -glutamate carboxylation to inhibit vascular calcification [56]. In an experimental rat model, warfarin was administered to induce vitamin K deficiency and caused arterial calcification [57], which was accelerated when given toxic doses of vitamin D and resulted in premature death.

The Czech MONICA study cross-sectionally observed that subjects in the highest quartile of dephosphorylated-uncarboxylated MGP (dp-ucMGP) plus the lowest quartile of 25(OH)D concentrations had the highest pulse wave velocity in middle-aged healthy adults [58]. Further, potential interaction between vitamin K status and polymorphisms of the vitamin D receptors was investigated. Pulse wave velocity was higher with the number of G-allele polymorphisms and highest in the top quartile of dp-ucMGP for the GG vitamin D receptor genotype.

A Dutch prospective cohort indicates that the combination of low vitamin D < 50 nmol/L and low K status ≥ 323 nmol/L dp-ucMGP was associated with increased systolic and diastolic blood pressures and incident hypertension after 6 years of follow-up [59]. Up to now, no study investigated the combination of optimal vitamin D and K status in relation to coronary artery calcification and cardiovascular events after long-term follow-up. This would give valuable insight if vitamins D and K are involved in developing cardiovascular disease.

So far, two human intervention studies in healthy populations have investigated the combined effect of vitamins D and K on vascular function and calcification (Table 2) [60, 61]. In postmenopausal women, after 3 years of supplementation (1000 μ g/d vitamin K₁ + 320 IU vitamin D), the vitamin D + K group maintained vessel wall characteristics of the carotid artery, whereas the control group and the vitamin D-only group significantly worsened over 3 years of follow-up [60]. However, vitamin K status was not measured as a marker of compliance to investigate what would have occurred following supplementation. Further, in a 3-year, double-blind, randomized controlled trial in older men and women free of clinical CVD, daily supplemental vitamin K in amounts achievable by high dietary intake of green, leafy vegetables (500 μ g/day) combined with 600 mg calcium carbonate and 10 μ g (400 IU) vitamin D did not result in lower coronary artery calcium progression as assessed by computerized tomography compared to the calcium + vitamin D group. In a subgroup analysis of participants who were $\geq 85\%$ adherent to supplementation, there was less coronary artery calcium progression in the vitamin K + calcium and vitamin D groups than in the calcium and vitamin D group alone [61]; however, MGP carboxylation status was not determined. These data are hypothesis generating, and further studies are warranted to clarify the mechanism.

Among overweight type 2 diabetic patients with coronary heart disease, cosupplementation for 12 weeks of vitamins D

(10 μ g) and K (180 μ g) and calcium (1000 mg) had beneficial effects on maximum levels of left carotid intima-media thickness and insulin metabolism markers [62]; however, no effect on right intima-media thickness was found and the results could be a chance finding. Unfortunately, circulating markers of vitamin K concentrations and vitamin K-dependent proteins were not taken into account to get a better mechanistic understanding.

Two trials studied the effect of vitamin D versus vitamin D + K in nondialyzed CKD patients on vascular calcification and cardiovascular risk factors for 9 months [63, 64]. In 42 CKD patients, the increase in carotid intima-media thickness (IMT) was significantly lower in the K (90 μ g menaquinone-7) + D (10 μ g vitamin D) group compared with the D-only group after 9 months [63]. Another small trial ($n = 38$) from the same research group did not show differences between the D versus D + K groups on cardiovascular risk markers [64]. These few studies show some potential for the combined effect of vitamins D + K versus D alone on subclinical CVD risk markers. It should be noted that very few clinical studies have been conducted in this field and that vitamin D + K supplements have been often combined with different micronutrients making it difficult to solely pinpoint the effect of vitamin D + K. These limited studies indicate that joint supplementation might benefit cardiovascular health.

4. Vitamins D and K with Glucose Metabolism and Inflammation

Another pathway that might affect CVD risk is via disturbances in glucose metabolism. Among Iranian vitamin D-deficient women with polycystic ovary syndrome—a dysmetabolic disorder—cosupplementation of calcium (1000 mg) and vitamins D (400 IU) and K (180 μ g) for 8 weeks improved markers of insulin metabolism and lipid concentrations compared to placebo [65]. The joint supplementation of vitamins D and K might improve insulin metabolism through an effect on upregulation of the insulin receptor genes, the regulation of insulin secretion from the pancreatic beta-cell, the enhancement of β -cell proliferation, and suppression of parathyroid hormone [66–69].

Further, another feature in which both vitamins D and K overlap is on inflammation, which is strongly related to the development of CVD and osteoporosis [70]. In the same Iranian clinical trial among vitamin D-deficient women with polycystic ovary syndrome, the joint supplementation of calcium with vitamins D and K had beneficial effects on endocrine and oxidative stress markers, however no effect on inflammatory markers [71].

5. Effects of Long-Term Vitamin D Supplementation

A large group of people uses both vitamin D and calcium for the prevention of falls and fractures. Given the fact that 25(OH)D is converted to 1,25(OH)D, vitamin D supplementation stimulates the production of 1,25(OH)D [72]. This means that long-term vitamin D supplementation could promote the production of large amounts of vitamin K-

TABLE 2: Summary of clinical trials of combined vitamin D and K supplementation on cardiovascular health and disease.

Author, year	Country	Participants	Treatment	Study duration	Outcome	Results for the highest versus the lowest quartiles
Braam et al., 2004 [60]	Netherlands	N = 181 postmenopausal women, mean age 55, 100% female	(i) Placebo (ii) Minerals + 8 µg vitamin D (iii) Minerals + 8 µg vitamin D + 1 mg vitamin K ₁	3 years	Vessel wall characteristics	MDK group unchanged, placebo and minerals + vitamin D decreased elastic properties
Shea et al., 2009 [61]	US	N = 388 healthy men and postmenopausal women, mean age 66 y, 60% female	(i) Multivitamin + 10 µg vitamin D and 600 mg calcium (ii) Multivitamin + vitamin D + calcium + 500 µg vitamin K ₁	3 years	Coronary artery calcification	No difference between vitamin K ₁ group and control group
Asemi et al., 2016 [62]	Iran	N = 66 overweight diabetic patients with coronary heart disease, mean age 65 y, 47% female	(i) Placebo (ii) Vitamin D (10 µg), K (180 µg), and calcium (1000 mg)	12 weeks	Carotid IMT	Lower left carotid intima-media thickness and improved insulin metabolism markers
Kurnatowska et al., 2015 [63], 2016 [64]	Poland	N = 42 nondialyzed CKD patient stages, mean age 60 y, 3-5, 45% female	<i>Chronic kidney disease patients</i> (i) 10 µg cholecalciferol (ii) 10 µg cholecalciferol + 90 µg MK-7	270 days	Carotid IMT	Reduced progression IMT, reduced dp-ucMGP and osteocalcin

IMT: intima-media thickness; dp-ucMGP: dephosphorylated-uncarboxylated matrix Gla protein; MK-7: menaquinone-7.

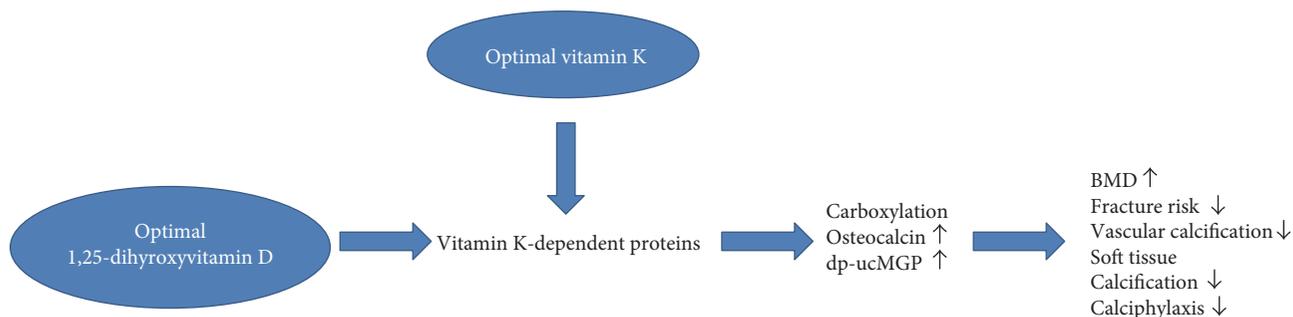


FIGURE 1: Simplified overview of potential synergy between vitamins D and K and bone and cardiovascular health. dp-ucMGP: dephosphorylated-uncarboxylated matrix Gla protein; BMD: bone mineral density. Genetic, molecular, cellular, and human evidence support that optimal concentrations of both vitamin D and vitamin K are beneficial for bone and cardiovascular health. Vitamin K is needed for the carboxylation of vitamin K-dependent proteins such as osteocalcin and matrix Gla protein, while vitamin D promotes the production of vitamin K-dependent protein concentrations. These vitamin K-dependent proteins are needed for extrahepatic organs such as the bone and the vascular system. This will result in bone mineralization and will inhibit soft tissue calcification, which will ultimately lead to lower risks of fractures and coronary heart disease.

dependent proteins, which remain inactive because there is not enough vitamin K to carboxylate (Figure 1). We propose a new hypothesis that if vitamin D concentrations are constantly high, there might not be enough vitamin K for activation of vitamin K-dependent proteins. Consequently, excess vitamin D diminishes the ability of vitamin K-dependent proteins to function properly, to stimulate bone mineralization, and to inhibit soft tissue calcification.

Further, increasing vitamin D intake through dietary or supplemental source increases intestinal calcium absorption, particularly when combined with calcium supplementation, and promotes hypercalcemia [73]. In this context, a human trial was performed in older women who received either 1200 mg calcium or 1200 mg calcium and 800 IU vitamin D per day over a 12-week period [74]. At the end of the 12 weeks, neither group observed a change in calcium concentrations, meaning that calcium was either excreted or stored somewhere. Increased calcium intake by itself may not be problematic as long as there is a steady state between optimal vitamin D and vitamin K concentrations. The disbalance between vitamin D and vitamin K promotes an environment in which excess calcium will be deposited into our vascular tissue instead of bone. The migration of calcification into the vascular tissue is described by the double burden of atherosclerosis and osteoporosis [75–77]. Additionally, as vitamin D increases calcium absorption, it might also promote hypercalcemia as seen in the Women's Health Initiative, which found a 24% higher risk of myocardial infarction in individuals taking calcium and vitamin D supplements and a greater risk for urinary tract stone occurrence: hazard ratio 1.17 (95% CI 1.02, 1.34) [11, 13, 14]. One prospective study found that higher 1,25(OH)D concentrations were strongly associated with the incidence of hypertension, while 25(OH)D was inversely associated with hypertension risk [78]. Higher 1,25(OH)D was associated with lower urinary calcium excretion, which could mean that the calcium meant for bone is stored somewhere else. Unfortunately, vitamin K status was not measured which would have given valuable insight into the association between vitamins D and K with calcium excretion.

6. Calciphylaxis and Vitamin K Antagonist Use

Calciphylaxis is a syndrome of calcification of the blood vessels, coagulopathy, and skin necrosis. It is seen mostly in patients with end-stage kidney disease, but can occur in the absence of kidney failure. Vitamin K antagonist use may contribute to its development [79]. The syndrome may cause a substantial morbidity and mortality. However, it should be acknowledged that the term calciphylaxis refers to a heterogeneous disorder that is characterized by soft tissue and vascular necrosis and has a clinical presentation from mild to severe. The underlying causes of calciphylaxis are not well understood; however, reported risk factors include female sex, obesity, elevated calcium*phosphate product, warfarin use, and vitamin D derivatives, for example, calcitriol, calcium-based binders, or systemic steroids, low blood albumin concentrations, and type 2 diabetes [80]. A recent study among patients with hemodialysis with calciphylaxis versus hemodialysis showed that cases had higher plasma uncarboxylated MGP concentrations than controls, which suggest a role of MGP in the pathophysiology of calciphylaxis. The fraction of total MGP that was carboxylated was also lower in cases than in controls. Vitamin K deficiency-mediated reduction in relative carboxylated MGP concentration may play a role in the pathogenesis of calciphylaxis [81]. This could be further mediated by the combined use of vitamin D derivatives and warfarin. Further, another study indicated that vitamin K antagonist use predisposes to the development of calciphylaxis in end-stage renal disease [82]. More evidence on the combined role of vitamin K antagonist use and vitamin D on bone and cardiovascular health is urgently needed.

7. Vitamin D and K Supplementation

Based on the current body of evidence, there is not enough evidence to recommend combined vitamin D and K supplementation for the prevention and treatment of osteoporosis. Most trials studied low-dose vitamin D in isolation (400–800 IU daily), which demonstrated only

modest or null effects on BMD and fracture prevention in mostly ≥ 65 years postmenopausal women [6–8]. Large clinical trials of moderate–high dose (≥ 800 IU daily) vitamin D supplementation (cholecalciferol) are currently in progress.

The most widely used vitamin K form for supplementation is vitamin K₂ and more specifically menaquinone-4 and menaquinone-7. Menaquinone-4 is more used in trials with bone outcomes, while menaquinone-7 is more in trials with cardiovascular outcomes with dosages between 90–360 μg . Menaquinone-7 has a higher bioavailability and may be of particular importance for extrahepatic tissue [83]. No cut-off value for vitamin K status nor vitamin K supplementation is available yet. Future studies are needed to determine whether vitamin D combined with vitamin K rich foods or vitamin K supplementation could improve bone and cardiovascular health.

8. Recommendations for Future Research

The recommendations for future research are as follows:

- (i) Evaluate the role of vitamin D administration in vitamin K-dependent proteins in human populations
- (ii) Question the possible long-term consequences of high-dose vitamin D supplementation
- (iii) Assess the combined role of vitamin K antagonist use and vitamin D in bone and cardiovascular health
- (iv) Investigate the joint supplementation of vitamins D and K on hard clinical endpoints

9. Conclusion

Taken together, animal and human studies suggest that optimal concentrations of both vitamin D and vitamin K are beneficial for bone and cardiovascular health as supported by genetic, molecular, cellular, and some human studies. However, vitamin D and calcium supplementation along with vitamin K deficiency might also induce long-term soft tissue calcification and CVD, particularly in vitamin K antagonist users and other high-risk populations. At this moment, we should be careful about supplementing high-dose vitamin D, unless indicated differently. More clinical data about the potential interplay between vitamin D and vitamin K metabolism is urgently needed before broader treatment recommendations can be given.

The consumption of a well-balanced diet is key for population-based primary prevention of chronic diseases. As more is discovered about the powerful combination of vitamins D and K, it gives a renewed reason to eat a healthy diet including a variety of foods such as vegetables and fermented dairy for bone and cardiovascular health.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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Review

The Impact of Vitamin D on Skin Aging

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Abstract: The active metabolites of vitamin D₃ (D₃) and lumisterol (L₃) exert a variety of antiaging and photoprotective effects on the skin. These are achieved through immunomodulation and include anti-inflammatory actions, regulation of keratinocytes proliferation, and differentiation programs to build the epidermal barrier necessary for maintaining skin homeostasis. In addition, they induce antioxidative responses, inhibit DNA damage and induce DNA repair mechanisms to attenuate premature skin aging and cancerogenesis. The mechanism of action would involve interaction with multiple nuclear receptors including VDR, AhR, LXR, reverse agonism on ROR α and $-\gamma$, and nongenomic actions through 1,25D₃-MARRS receptor and interaction with the nongenomic binding site of the VDR. Therefore, active forms of vitamin D₃ including its canonical (1,25(OH)₂D₃) and noncanonical (CYP11A1-intitiated) D₃ derivatives as well as L₃ derivatives are promising agents for the prevention, attenuation, or treatment of premature skin aging. They could be administrated orally and/or topically. Other forms of parenteral application of vitamin D₃ precursor should be considered to avoid its predominant metabolism to 25(OH)D₃ that is not recognized by CYP11A1 enzyme. The efficacy of topically applied vitamin D₃ and L₃ derivatives needs further clinical evaluation in future trials.

Keywords: skin aging; photoaging; skin immune responses; vitamin D; vitamin D metabolites; photoprotection



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1. Introduction

Skin, like any other organs, undergoes progressive decline in its physiological, morphological, and functional features during aging [1–4]. The phenomenon of aging is natural and genetically predisposed. The functions of the skin are crucial for the homeostasis and survival. As the largest organ in the human body, the skin, together with the hypodermis (subcutaneous fat), is both the source and the target for several hormones and neuromediators [5–17], making it an independent peripheral endocrine organ [5,18]. The skin has also the capacity of producing the prohormone vitamin D and transforming it to active metabolites [19–25], which can exert several different effects on the main skin cells (keratinocytes and fibroblasts) [20,25–29] and immune cells [4,28,30,31] via the activation of the nuclear vitamin D receptor (VDR) [29,32–35]. Vitamin D plays a pivotal role in skin homeostasis contributing to its barrier function [20,29,36–38]. Moreover, as an essential part of a functioning immune system, active forms of vitamin D modulate the cutaneous immunity [8,30,39].

The gold standard of analyzing a vitamin D status is by measuring its major circulating metabolite, 25-hydroxyvitamin D₃ (25(OH)D₃), via high-performance liquid chromatography (HPLC) or liquid chromatography tandem–mass spectrometry (LC-MS/MS) [40–42].

Recently, a novel sensitive and specific LC-MS/MS method of the simultaneous measurement of 13 circulating metabolites of vitamin D₃ and D₂ was presented [43].

Importantly, subclinical (30–50 nmol/L) and clinical vitamin D deficiency (<30 nmol/L) in the general population has become a global problem worldwide [44–48]. Several physiological factors may influence vitamin D status, such as age, body mass index (BMI), skin type, pregnancy, and exclusive breastfeeding [49–53]. The genetic polymorphisms of some genes involved in skin pigmentation (*TYR*, *TYRP1*, *EXOC2*, and *DCT*) are also associated with 25(OH)D₃ serum concentration [54]. Many environmental factors contribute to vitamin D deficiency, such as the winter season, inadequate sun exposure, and high latitude location [55,56]. Sun avoidance and air pollution are the main factors leading to insufficient UVB exposure. Moreover, ozone and particulate matter (PM) can directly affect the cutaneous production of vitamin D [57]. Additionally, air pollutants, persistent organic pollutants, and heavy metals can behave like endocrine-disrupting chemicals (EDCs), which may cause vitamin D deficiency directly or indirectly. The latter would be secondary to a weight gain, the dysregulation of parathyroid hormone and calcium homeostasis, and a thyroid dysfunction [57,58]. Increasing evidence suggests that smoking can also decrease the serum levels of 25(OH)D₃ [57].

With advancing age, the capacity of the skin to produce vitamin D₃ decreases (irrespective of the season), and the degradation of its active forms increases [59,60]. It was found that the concentration of the precursor of vitamin D₃ in the skin, 7-dehydrocholesterol (7-DHC), declines approximately by 50% from age 20 to age 80 years [59]. Several other factors contribute to the vitamin D deficiency state in accelerated age, including limited sun exposure, insufficient dietary intake of vitamin D, or diseases causing malabsorption. The vitamin D deficiency, which is common in advanced age, can decrease the important physiological functions of the skin such as protection from the environment and prevention of cancer development [25,39,61–66].

In this review, we aimed to discuss the significance of vitamin D in the skin aging process.

2. Skin Aging—Your Skin Can Reveal Stories

Skin aging is a complex process that is influenced by the total exposure of both intrinsic and extrinsic factors over the human lifespan (skin exposome), which is responsible for the progressive morphological and functional alterations of the aged skin [1,67–69]. The main internal factors affecting the chronological (physiological) skin aging include a hormonal decline and changes in gene expression with advancing age [1]. In addition, the cutaneous regenerative potential decreases with the age due to the excessive senescence of keratinocytes, fibroblasts, and melanocytes over time, which contributes to skin aging [70–72]. The aged skin is characterized by fine wrinkles and atrophy with reduced elasticity. This chronological aging affects all skin areas but shows phenotypic differences among different anatomical regions, and it varies significantly within different populations [67,73]. The single nucleotide polymorphisms (SNPs) of the *MC1R* gene (main regulator of melanin pigmentation [74]) are significantly linked to a perceived facial age, providing a new molecular base of youthful looks [75].

The most prominent external stressors affecting skin and causing its premature aging include ultraviolet (UV) radiation [76,77], ambient pollutants [78–82], and smoking [57,83]. The continuous exposure of the skin to these environmental insults stimulates the production of reactive oxygen species (ROS) and generate oxidative stress [84,85]. The environmental factors can also cause an impairment of the epidermal barrier function [69] and alterations in skin microflora [86,87], leading to significant morbidity [2,88].

Ultraviolet radiation (UVR) is the most harmful external factor contributing to the cutaneous photodamage on the sun-exposed areas. Photoaged skin is presented as a dry, deep-wrinkled skin with rough texture, dyschromia and senile lentigines, vascular complications, etc. [68,89]. UVR decreases the expression of filaggrin that contributes to epidermal hydration, so its downregulation from UVR could explain the skin xerosis in photoaging [90]. Both UVA (315–400 nm) and UVB (280–315 nm) have been shown

to contribute to photoaging, either by imbalanced ROS production or by direct DNA damage [83]. However, UVA is considered to play a major role in the aging process. UVA represents more than 80% of total daily UV irradiation and can penetrate 5–10 times deeper into the reticular dermis, where it can damage the extracellular matrix (ECM) more significantly than UVB [91]. Moreover, UVA exposure increases the expression of matrix metalloproteinases (MMPs), especially the expression of the collagenolytic enzyme MMP-1 in dermal fibroblasts, which acts as an important regulator in photoaging [92,93]. Additionally, the chronic UVA irradiation inhibits hyaluronan synthesis, thus altering the composition of proteoglycans in the dermis [94]. A long-term exposure of UVA is related to photoaging and photocancer due to an overproduction of ROS and reactive nitrogen species (RNS), which can disrupt both the nuclear and mitochondrial DNA [95,96]. UVB can penetrate only through the epidermis but is biologically more active. It also induces the transformation of 7-DHC to vitamin D₃ [97,98]. UVB absorbed by DNA and RNA induces a formation of cyclobutane pyrimidine dimers (CPDs) and other photoproducts [99], thus inducing various solar signature mutations in specific genes, including the tumor suppressor gene p53 [100,101]. UVR induces an accumulation of p53 protein in the nucleus that in turn activates the transcription of genes responsible for cell cycle arrest allowing DNA repair, as well as causing an induction of apoptosis of the cells with unrepaired DNA damage [102,103]. Specific p53 mutations can be found in high rates, not only in actinic keratosis (precancerous state) and squamous cell carcinomas (60–90%) with typical UV signature, but also in the normal appearance of UV-exposed skin (about 75%), compared with a much lower rate of such mutations in healthy sun-protected skin (5% of all cases) [104].

Chronic sunlight exposure, together with the persistence of cellular senescence, can drive an impaired regenerative capacity of the skin, chronic inflammation, and photoaging, which correlates with enhanced cancer risk [77,105–107]. Thereby, photoaging results in premature skin aging. Although some aging mechanisms share several similarities or overlapping, photoaged skin differs from physiologically aged skin in the ECM changes. Photoaged skin is characterized by degraded collagen and accumulated aberrant elastin fibers and glycosaminoglycans, whereas physiologically aged skin is presented by the atrophy of dermal structures [108].

The negative impact of ambient pollutants on human health and the human skin is of growing concern [109]. Ozone (O₃) from the smog and PM, primarily contacting with the skin, is capable to stimulate ROS production and generate oxidative stress, leading to phenotypic features of extrinsic aging [69]. It was found that chronic exposure to PM leads to pigment spots and deep nasolabial folds [110,111]. Moreover, ultrafine particles (<0.1 µm) can penetrate tissues and localize in the mitochondria, causing an aberrant mitochondrial function because of the oxidative processes [112]. Additionally, the photo-pollution exposure may aggravate UVR-mediated skin aging [113].

UVR, predominantly UVA, by the excessive amount of ROS activates the mitogen-activated protein kinases (MAPKs) and transcription factors such as nuclear factor erythroid 2-like (Nrf2), c-Jun-N-terminal kinase (JNK), and nuclear factor-κβ (NF-κB), and increases the transcription of MMPs [114]. Activated MMPs, together with the decreased expression of MMP inhibitors (TIMP), cause a dysregulation of the ECM homeostasis and a progressive damage of collagen and elastin [115]. Additionally, UVR impairs the endogenous antioxidant enzymes, leading to an increased oxidative damage of collagen. The destruction of ECM integrity is visualized as wrinkle appearance in photo-damaged skin [93]. The activation of redox-sensitive transcription factors, the activator protein-1 (AP-1) and NF-κβ, involved in wrinkle formation and inflammation, plays crucial roles in skin aging [88]. Both factors, NF-κβ and AP-1, are elevated within hours after cutaneous exposure to low-dose UVB. The upregulation of AP-1 suppresses the transforming growth factor β (TGF-β) receptors, which further blocks the synthesis of procollagen [116,117]. Additionally, activated AP-1 stimulates the degradation of collagen by MMPs and triggers the main activator of the inflammatory response NF-κβ [118]. NF-κβ signaling is a

well-known regulator of tissue homeostasis, and its central role in skin aging was recently underlined [119]. The ROS-induced activation of NF- κ B drives an increase of proinflammatory cytokines and MMPs and decreases TGF- β and type I collagen synthesis [119]. The proinflammatory cytokines (interleukin (IL)-1, IL-6, and tumor necrosis factor (TNF)- α) stimulate inflammatory responses and enhance the activation of NF- κ B [93]. It was found that NF- κ B expression could increase in mitochondrial DNA (mtDNA)-depleted mice and after restoring the mitochondrial function, the NF- κ B expression could be reduced. These data confirm that NF- κ B signaling is a key mechanism contributing to the skin and hair follicle pathologies [120]. Due to the longer wavelength, UVA reaches the dermal fibroblasts in vivo with the activation of the Nrf2-mediated expression of antioxidant genes. Unlike UVA, UVB does not activate Nrf2 in skin cells or even appears to have an inhibitory effect [121]. However, vitamin D₃ derivatives, which are products of UVB action, do activate Nrf2 signaling [122]. The endogenous Nrf2 is essential for the protection of skin cells against oxidative insults and for regulating the redox balance during skin aging [123,124]. Many in vitro and in vivo studies confirmed the importance of the transcription factor Nrf2 and its downstream signaling in UV protection [125,126].

Indeed, the human skin aging is mainly driven by oxidative events. An extensive ROS production and insufficient scavenging activity or a mitochondrial dysfunction are crucial events in oxidative stress-induced skin aging. The high levels of ROS lead to oxidative damage of lipids, proteins, genomic, and mtDNA, and also can deplete and damage the antioxidant defense systems of the skin (both non-enzymatic and enzymatic one) [85,127].

Accumulating evidence support a strong link between the mitochondrial dysfunction and the aging process [126]. Many studies demonstrate a decrease in mtDNA content and mitochondrial number during aging. It is thought that mitochondrial dysfunction plays a role in accelerated cellular senescence, seen in advancing age [128–130]. Furthermore, mitochondria are believed to contribute to 90% of generated ROS in the cells [95]. mtDNA, as an important target for ROS, is highly vulnerable to the oxidative damage and possesses inefficient DNA repair mechanisms [96,131]. The functional decline of the mitochondria leads to vicious cycle effect contributing to further enhancement of ROS production [127,132].

3. Effects of Vitamin D₃ on the Skin

3.1. Impact Paths on the Skin

Excessive exposure to solar UVR accelerates skin aging and could trigger cutaneous cancerogenesis [133]. However, UVR plays a beneficial role in the regulation of many skin functions [56,77,134]. The same UVB, responsible for the increase of non-melanoma skin cancer, is required for vitamin D₃ production in the skin that supplies more than 90% of the vitamin D₃ body's requirement [44,55,135]. In the skin, vitamin D₃ is essential for the formation of the epidermal barrier and hair follicles, and its deficiency has been linked to many proliferative and inflammatory cutaneous disorders [20,29,44,136].

Upon the absorption of UVB, 7-DHC is transformed to vitamin D₃ in the skin, a process accelerated by thermal energy. Prolonged UVB exposure can also generate tachysterol (T₃) and lumisterol (L₃) [24,97]. These reactions are non-enzymatic and dependent on the UVB dose and the temperature. Vitamin D₃ can be activated through canonical and non-canonical pathways with similar activation of L₃ to biologically active metabolites (Figure 1). In the classical pathway, vitamin D₃ is hydroxylated to 25-hydroxyvitamin D₃ (25(OH)D₃) by CYP2R1 and/or CYP27A1 in the liver with further hydroxylation by CYP27B1 in the kidney, skin, and other tissues to its biologically active metabolite 1,25(OH)₂D₃ [20,21,137].

In the alternative (non-canonical) pathway, vitamin D₃ can be activated by CYP11A1 with further modification by other cytochrome enzymes leading to production of large number of metabolites in humans [21,36,138–142] (Figure 1), some of which are non- or low-calcemic at high, therapeutic, doses [143–146]. The major CYP11A1-derived vitamin D₃ products are 20(OH)D₃ and 20,23(OH)₂D₃ [23,139,147,148]. In addition, 20(OH)D₃ can be defined also as a natural product because its presence in the honey [149]. The L₃ can

also be metabolized to biologically active derivatives [150–152], which are not recognized by the 7-DHC reductase [153].

The main genomic effects and biological responses of vitamin D metabolites in the skin are mediated through their binding to the nuclear VDR [32,61,154–156]. Notably, VDR has been reported to regulate about 3% of mammalian genome due to its broad expression in all tissues [4,34,157,158]. The skin also expresses the VDR and serves not only as a source but also as a site for the action of vitamin D₃ [28,39]. Additionally, the VDR activated by classical 1,25(OH)₂D₃ can induce rapid response signaling through a non-genomic, membrane-associated mechanism based on an alternative ligand-binding site [159] or through action on 1,25D₃-MARRS receptor [156,160,161]. Similar non-genomic activities for CYP11A1-derived hydroxyderivatives are still not established. SNPs can affect VDR activity favoring a development of melanoma and non-melanoma skin tumors [162,163]. VDR functions as a tumor suppressor [164] and a decrease in its expression is associated with progression of cutaneous melanoma [165,166]. On the opposite, the nuclear VDR expression has been found significantly elevated (moderate to strong) in squamous cell carcinomas (SCCs) and basal cell carcinomas (BCCs) compared to in normal skin [167,168]. Thus, targeting VDR with vitamin D secosteroids (especially low calcemic ones) would be rational in skin cancer prevention, attenuation, or therapy [62,64,169].

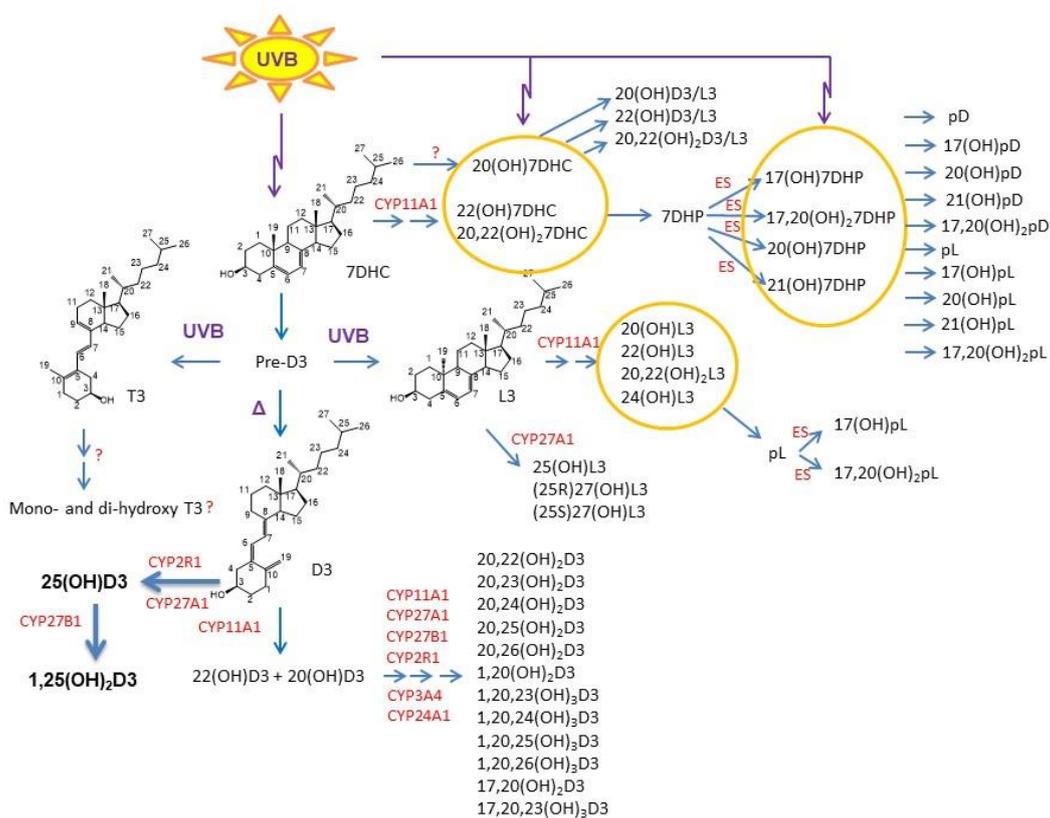


Figure 1. Noncanonical pathways of vitamin D₃ and lumisterol (L₃) activation (reprinted from [61] with the permission from Springer). D₃, L₃, and 7-DHC are substrates for CYP11A1 that produces the corresponding hydroxyderivatives. In the case of L₃ and 7-DHC, the side chain can be cleaved by CYP11A1 to produce 7DHP or pL that can be further metabolized by steroidogenic enzymes (ES). In the skin, UVB acting on 5,7-dienes can lead to the production of D₃, L₃, and T₃ derivatives with a full-length side chain and pD, pL, and pT derivatives with a shortened side chain. While the cut-off for UVC/UVB is 280 nm, we show the range of 290–315 nm because wavelengths below 290 nm are filtered by the ozone layer and no additional pre-D₃ is produced above 315 nm [170]. 7DHC, 7-dehydrocholesterol; 7DHP, 7-dehydropregnenolone; pD, pregnacalciferol; pL, preganalumisterol; D₃, vitamin D₃; L₃, lumisterol; T₃, tachysterol; OH, hydroxyl group; number before OH, carbon number with OH; number in subscripts after (OH), number of hydroxyl groups.

CYP11A1-derived hydroxyderivatives can regulate some skin functions through other nuclear receptor such as retinoic acid-related orphan receptors (ROR) α and γ , which expressed in the skin [171]. The endogenously produced nonclassical vitamin D₃ hydroxyderivatives, 20(OH)D₃ and 20,23(OH)₂D₃ can act as weak ROR α and ROR γ inverse agonists [155,171,172]. Moreover, these hydroxyderivatives could exert anti-inflammatory effect and could inhibit tumor progression in the skin via ROR γ -mediated mechanism [173].

Alternative, the classical 1,25(OH)₂D₃ and CYP11A1-initiated vitamin D₃ derivatives can act as agonists on aryl hydrocarbon receptor (AhR) [174] and liver X receptors (LXR) [175]. Moreover, the activation of AhR is the top canonical pathway for 20,23(OH)₂D₃ [174]. This receptor regulates cellular proliferation, inflammation, and melanogenesis in the skin [176]. Although many different ligands can target AhR, some functional studies and molecular modeling can predict that secosteroidal signal transduction further leads to the downregulation of proinflammatory responses [177], detoxification, and antioxidative action [61,174].

Summarizing, the biologically active classical and novel vitamin D₃ metabolites exert different affinities to multiple receptors in the skin and through their modulation they can influence different cutaneous pathologies. In addition to act on the VDR, the active forms of vitamin D can act on alternative nuclear receptors including RORs, AhR, LXR, and 1,25D₃-MARRS receptor. The active forms of vitamin D₃ have various functions, which partially overlap in their anti-inflammatory, antimicrobial, antiproliferative, prodifferentiation, antifibrotic, and antioxidative effects on the skin [20,38,63,141,145,178]. Along with the best characterized 1,25(OH)₂D₃, CYP11A1-derived products of vitamin D₃ and L₃ exhibit photoprotective properties against UVR-induced skin damage (Figure 2) [37,61,179–183].

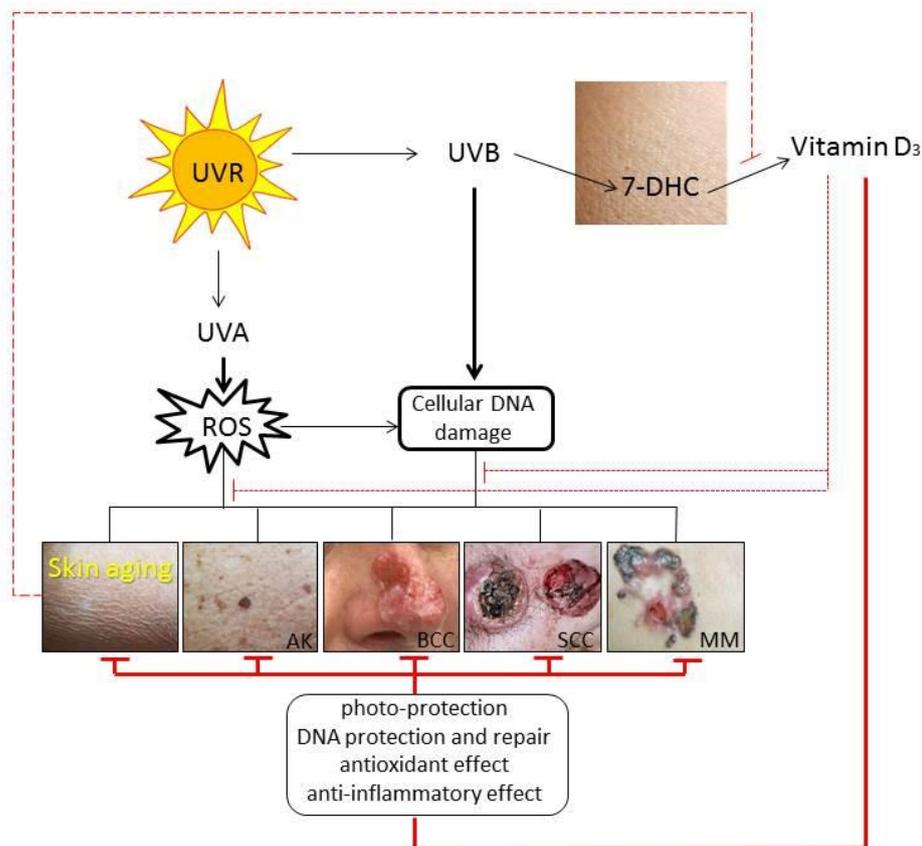


Figure 2. Photoprotective effects of vitamin D₃ in premature skin aging and cutaneous cancerogenesis. Abbreviations: 7-DHC, 7-dehydrocholesterol; AK, actinic keratosis; BCC, basal cell carcinoma; SCC, squamous cell carcinoma; MM, malignant melanoma.

3.2. Effects on the Cutaneous Immune Function

Vitamin D₃ and its analogues and precursors play an important complex role in the regulation of both the innate and adaptive immune systems, including in the skin [8,184,185]. There is a clear connection between vitamin D deficiency and incidences of infections and immune-mediated skin diseases [31,186–188]. The expression of the VDR has been found in almost all immune cells including T- and B-lymphocytes (Lym), macrophages, mast cells, natural killer (NK) cells, and regulatory T cells (Tregs), but it is differently regulated [189]. Monocytes, for example, lose VDR expression levels during differentiation towards macrophages and dendritic cells (DCs) [190,191]. Immunomodulatory actions of active vitamin D₃ metabolites include the induction of Tregs [192] and Thelper-2 (Th2)-Lym, together with the downregulation of proinflammatory Th1/Th17/Th9-Lym [193]. 1,25(OH)₂D₃ can have both direct and indirect effects on T-Lym [194]. The indirect effect is based on DC-derived cytokines, which modulate Th-Lym response [30]. Directly, 1,25(OH)₂D₃ can suppress the immune cell production of inflammatory cytokines such as interferon gamma (IFN-γ), TNF-α, and IL-2 (Th1 cytokines), IL-17/21 (Th17 cytokines), and Th9 cytokines [193,195,196], while it enhances either the levels of anti-inflammatory IL-10 from Tregs [197] or Th2-derived IL-4 [198]. As a result, vitamin D shifts Th1 inflammatory response towards more tolerogenic Th2 response with an increase of CD4 + CD25 + Tregs reflected to a change in cytokine profile in the skin [199]. Additionally, 1,25(OH)₂D₃ influences the activated B-Lym by inducing apoptosis [200], suppressing immunoglobulin E (IgE)-dependent mast cell activation [201,202], and upregulating IL-10 production [203]. Enhanced IL-10 synthesis contributes to a suppressed mast cell-mediated inflammation and IgE-related allergic reactions [201]. 1,25(OH)₂D₃ and its analogues directly regulate antimicrobial peptide (AMP) gene expression in innate immune cells [204,205]. Moreover, it has been found that vitamin D is able to induce essential for antimicrobial defense, production of cathelicidin (LL37) [204] and to modulate the phagocytic activity of macrophages and NK cells [193]. Additionally, vitamin D exerts immunosuppressive effects through the modulation of epidermal Langerhans cells [206] and the proliferation of the Tregs number [184,185,197].

CYP11A1 is expressed also in immune cells [207], where vitamin D can be further metabolized to biologically active hydroxyderivatives [31,39]. Via the activation of VDR or through the inhibition of RORγ-mediated activation, 20(OH)D₃ and 20,23(OH)₂D₃, like 1,25(OH)₂D₃, can attenuate Th17 differentiation, as well as the formation and activity of inflammatory cytokine IL-17 by immune cells [155,208,209]. Thus, Th17-related cutaneous inflammation could be successfully modulated via RORγ inverse agonists such as CYP11A1-derived D₃-hydroxyderivatives, causing the regulation of the immune system and a resistance against autoimmunity [210,211]. The most recently inhibition of collagen-induced autoimmune arthritis by CYP11A1-derived 20(OH)D₃ was reported [212].

3.3. Impact on Skin Aging

The normal vitamin D₃ status is important for a general prevention of premature aging maintaining a healthful skin aging [213,214]. Vitamin D₃ metabolites including its classical (1,25(OH)₂D₃) and novel (CYP11A1-intitiated) D₃ hydroxyderivatives exert many beneficial protective effects on the skin, which could influence the process of premature aging via many different mechanisms, leading to a delay or attenuation of both chronological skin aging and photoaging. Skin-resident cells (keratinocytes, fibroblasts, and sebocytes) are capable of locally activating vitamin D₃ [23,36,215] and exhibiting a diverse biological effect such as photoprotection and immunosuppression, similar to the UVR-induced one [179,216].

The process of chronological aging is associated with immunological alteration and the imbalance between inflammatory and anti-inflammatory mechanisms, leading to a chronic low-grade inflammation, known as “inflammaging” state [217,218]. The “inflammaging” phenotype of the skin and hair follicles is a result of both chronic antigen stimulation and continued exposure to oxidative stress caused by ROS and RNS [219,220]. With advancing

age, skin is affected by the profound remodeling of the immune system, leading to a decline in its adaptive capacity [221,222]. Th1- and Th17-related markers, together with the number of epidermal DCs are increased as a function of age [223–225]. DCs during aging appear to be functionally impaired, which contributes to an initiation of inflammatory and autoimmune skin disorders and a loss of their protective role against cutaneous infections. The active forms of vitamin D₃ are able to decrease the proliferation and cytotoxicity of T-Lym, as well as to suppress the differentiation of B-Lym and the maturation of DCs [193]. Therefore, vitamin D₃ hydroxyderivatives exert potent anti-inflammatory activities including the inhibition of TNF- α , INF- γ , and IL-1/6/9/17 production [4,38,185], suggesting their implication in the modulation of skin inflammation. Moreover, the noncalcemic and nontoxic 20(OH)D₃ has shown a similar anti-inflammatory property in vivo to 1,25(OH)₂D₃ (hypercalcemic in high doses) through the suppression of the immune responses by T- and B-lym [155,212].

Active vitamin D₃ metabolites can protect skin against the hazardous effects of skin aging-triggering agents, including UVR, pollution, and microbial infections [179,226–230]. It has been shown that the oral administration of high-dose vitamin D₃ shortly after UVB exposure could reverse rapidly the photo-induced cutaneous damage by decreasing the inflammation and induction of the repair mechanisms of the epidermal barrier [38]. There is strong experimental evidence that active vitamin D₃ and L₃ hydroxyderivatives can induce, in a dose-dependent manner, antioxidative responses and reverse the UVB-mediated ROS production in keratinocytes by the activation of Nrf2 that works for cytoprotection and detoxification, thus attenuating photoaging [122]. Therefore, they serve as protective agents against UVB-induced oxidative stress in cells, pre-treated with each of these active metabolites for 24 h prior to UVB irradiation (50 mJ/cm²) [122]. These hydroxyderivatives stimulate the expression of antioxidant-response genes downstream of Nrf2 (*GR*, *HO-1*, *CAT*, *SOD-1*, and *SOD-2*) as well as the expression of HO-1, CAT, and MnSOD at the protein level [122]. The transcription factor Nrf2 plays an important role in the detection of excessive ROS and RNS and in the induction of mechanisms counteracting the oxidative damage and skin pigmentations produced by UVA [121,125,231].

Chronic UVR irradiation, mainly UVB [232] and UVA [233], induces DNA damage and the formation of CPDs that potentially lead to premature skin aging and carcinogenesis. CYP11A1-derived D₃ and L₃ hydroxyderivatives, along with 1,25(OH)₂D₃, demonstrate photoprotective and reparative properties by increasing the expression and phosphorylation of p53 with its translocation to the nucleus [61,229,234,235]. The P53 gene family, in particular its isoform p63, might be an important molecular target for vitamin D action in premature aging and cancer [236], which are promoted by similar mechanisms [237].

Moreover, 1,25(OH)₂D₃ and 1,25(OH)₂L₃ inhibit DNA damage and facilitate DNA repair by the reduction of CPDs [182,235,238,239] and RNS [178,234]. The photoprotection by 20(OH)D₃ and 20,23(OH)₂D₃ is comparable to 1,25(OH)₂D₃ reduction of UVB-induced CPDs and DNA fragmentation in vivo [181,182] and in vitro [178]. In addition, both 20(OH)D₃ and 20,23(OH)₂D₃ stimulate differentiation, inhibit proliferation and downregulate proinflammatory responses in keratinocytes via the decrease of NF κ B activity [240,241]. It was shown recently that not only the pretreatment, but also the post-treatment of keratinocytes with CYP11A1-derived D₃ and L₃ derivatives can reverse their UVB-induced damage [37,230].

Additionally, 1,25(OH)₂D₃ can induce rapid and dose-dependent reduction in skin cell apoptosis, and it can increase CPDs repair and decrease the oxidative DNA damage through non-genomic energy-conserving autophagy and mitophagy [227], thus contributing to the intrinsic skin photoprotection mechanism [242].

4. Conclusions and Future Perspectives

Vitamin D₃ and its active metabolites exert a variety of antiaging and (photo) protective effects on the skin. These are achieved through immunomodulation that include anti-inflammatory actions and regulation of keratinocytes proliferation and differentiation

program to build the epidermal barrier necessary to maintain skin homeostasis. In addition, they induce antioxidative responses, inhibit DNA damage and induce DNA repair mechanisms to attenuate premature skin aging and cancerogenesis. Similar actions can be assigned to lumisterol metabolites. Therefore, active forms of vitamin D₃ including its canonical (1,25(OH)₂D₃) and noncanonical (CYP11A1-intitaded) D₃-hydroxyderivatives as well as L₃-derivatives are promising agents for the prevention, attenuation, or treatment of premature skin aging, when applied topically. It is expected that they will attenuate photoaging and perhaps repair the existing damage induced by external stressors. The mechanism of action would involve interaction with nuclear receptors including VDR, AhR, LXR, reverse agonism on ROR α and ROR γ , and nongenomic actions through 1,25D₃-MARRS receptor and interaction with the nongenomic binding site of the VDR. The regulatory mechanism affected by D₃ and L₃ derivatives would include the activation of Nrf2 and p53 and the downregulation of NF κ B signaling pathways or the regulation of mitochondrial functions. To prevent skin aging, vitamin D₃ and lumisterol or their derivatives could be administrated orally and/or topically. Other forms of parenteral application of the vitamin D₃ precursor should be considered to avoid channeling its metabolism to 25(OH)D₃, which is not recognized by CYP11A1 enzyme [243]. The efficacy of topically applied vitamin D₃ and L₃ derivatives needs further clinical evaluation in future trials.

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