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Potential Impact of the Steroid Hormone, Vitamin D, on the Vasculature:

Vitamin D-hormones and cardiovascular disease

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Abstract

The role of vitamin D in the cardiovascular system is complex because it regulates expression of genes involved in diverse metabolic processes. Although referred to as a vitamin, it is more accurately considered a steroid hormone, because it is produced endogenously in the presence of ultraviolet light. It occurs as a series of sequentially activated forms, here referred to as vitamin D-hormones. A little-known phenomenon, based on pre-clinical data, is that its biodistribution and potential effects on vascular disease likely depend on whether it is derived from diet or sunlight. Diet-derived vitamin D-hormones are carried in the blood, at least in part, in chylomicrons and lipoprotein particles, including LDL. Since LDL is known to accumulate in the artery wall and atherosclerotic plaque, diet-derived vitamin D-hormones may also collect there, and possibly promote the osteochondrogenic mineralization associated with plaque. Also, little known is the fact that the body stores vitamin D-hormones in adipose tissue with a half-life on the order of months, raising doubts about whether the use of the term “daily requirement” is appropriate. Cardiovascular effects of vitamin D-hormones are controversial, and risk appears to increase with both low and high blood levels. Since low serum vitamin D-hormone concentration is reportedly associated with increased cardiovascular and orthopedic risk, oral supplementation is widely used, often together with calcium supplements. However, meta-analyses show that oral vitamin D-hormone supplementation does not protect against cardiovascular events, findings that are also supported by a randomized controlled trial. These considerations suggest that prevalent

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recommendations for vitamin D-hormone supplementation for the purpose of cardiovascular protection should be carefully reconsidered.

Keywords

vitamin D; calcification; vascular; atherosclerosis

Types of vitamin D-hormones

Vitamin D is not a true vitamin[1] in most people because it can be synthesized in the presence of sunlight. It qualifies as a true vitamin only for those who receive no ultraviolet light. It is also not a single chemical but a group of lipid-soluble secosteroids that, once activated, work as steroid hormones.[2] Vitamin D_3 (D_3) is cholecalciferol (9,10-secocholesta-5,7,10(19)-trien-3beta-ol), a form that is synthesized in the skin of humans as well as herbivores and other omnivores.[2] Vitamin D_2 (D_2) is ergocalciferol (3β,5Z,7E,22E)-9,10-secoergosta-5,7,10(19),22-tetraen-3-ol), a form synthesized in plants, such as mushrooms,[3] and often used as a dietary supplement. Thus, humans may derive D_3 from either sun exposure or dietary intake of animal fats, oils, or liver (such as oily fish, cod liver oil, or blubber), and humans may obtain D_2 from plant-based food. Both of these forms are biologically inert,[2] absorbed with similar efficiency,[4] and are activated by sequential hydroxylation reactions in specific locations in the body.

Activation and storage of D vitamins

The initial step in the synthesis of vitamin D-hormones is in the skin, where ultraviolet light produces D_3 (cholecalciferol) from a non-enzymatic reaction with a blood-borne cholesterol derivative, 7-dehydrocholesterol (provitamin D3) as it passes through the microvessels of the skin.[2] Ultraviolet light also produces plant based D_2 from its reaction with ergosterol, the plant analog of cholesterol.[2] D_2 is also active in humans and is used in some supplements. The subsequent processing of D_2 and D_3 to active hormones requires stepwise hydroxylation that occurs in certain tissues, including liver, kidney, and the vasculature by the enzymatic activity of 1-alpha hydroxylase.[5]

For simplicity of terminology, we will refer to these as “D vitamins,” and we will use calcidiol to refer to the monohydroxylated forms of both D_2 and D_3 and calcitriol to refer to the dihydroxylated forms of both D_2 and D_3.

Due to the lipophilic nature of vitamin D-hormones, adipose tissue is a major site for their storage,[6–8] and some is also stored in skeletal muscle.[9] Studies tracking radiolabeled D_3 in rats showed that within 24 hours of administration, it appears in adipose tissue, and 80% of the radioactivity remains there after 6 weeks; about half remains there in pro-hormone form.[10] The mechanisms regulating deposition and release of vitamin D-hormones from adipose tissue are not well established. Release of vitamin D-hormones from adipose tissue is gradual with a reported half-life of about 2 months[11] and seems to occur in proportion to its concentration.[10]

Half-lives and levels of inactive and activated D vitamins

The monohydroxylated and partially active form, calcidiol, and the dihydroxylated active form, calcitriol, both have shorter half-lives than the unmodified form. Calcidiol has a higher affinity for D-binding protein (DBP) than the active hormone, and it is also better absorbed in the upper gastrointestinal tract than the non-hydroxylated vitamin D-hormones.[4] Thus, the former has a half-life in the circulation of 10–20 days and the latter, with its lower affinity for the carrier protein, a much shorter half-life of 10–20 hours.[12] Calcidiol is a partially active, intermediate pro-hormone, but because it has a longer half-life, its serum level is used as an indicator of vitamin D-hormone sufficiency.[5] The “normal” serum level is controversial, but 20–40 ng/ml is a commonly used range.[13] Calcitriol is the biologically active hormone, but its levels are low and tightly controlled through the actions of parathyroid hormone[14] and fibroblast growth factor-23,[15] making it unsuitable as a marker. Interestingly, the plasma level of calcitriol remains on the order of picomoles even under conditions of D toxicity in animals. A recent review summarizes the vitamin D-hormone levels from the meta-analyses of randomized controlled trials and observational studies.[16]

Dietary requirements: daily or seasonal?

An important implication of the long duration of vitamin D-hormone storage in adipose and other depots is that, contrary to the statements throughout public health recommendations, daily doses are not required. With a half-life on the order of months[17, 18] for both D_2 and D_3, summer sun exposure may provide enough supply to last through darker winter months, except in those living in places where sunlight is severely limited such as nursing homes or the arctic. For this reason, the use of the term “daily,” in the context of required or recommended allowances, warrants reconsideration. Otherwise, one is left with the impression that every cloudy day requires dietary supplements.

Dependence of carrier and bio-distribution on source

It is little known that the metabolism, biodistribution, and effects of vitamin D-hormones likely depend on their source – from sunlight or diet. Since vitamin D-hormones as well as other fat-soluble vitamins A, E, and K, are lipophilic, they are not soluble in the aqueous environment of blood. They must be carried in a protected manner in the blood stream. This carrier may be different for sunlight-derived vs. diet-derived vitamin D-hormones. When produced in the skin via sunlight, vitamin D-hormones are carried in the bloodstream by DBP.[19] When derived from the diet, 90% of the dose is absorbed from the intestine within chylomicrons along with other fat and fat-soluble nutrients.[5, 20] Rather than traveling directly into the bloodstream, chylomicrons travel as chyle through the mesenteric and central lymphatic systems to the thoracic duct where it is passed into the central venous circulation.[21] Thus, chylomicrons, the fats and dietary vitamin D-hormones that they carry, avoid the portal circulation and first-pass metabolism in the liver.[21] Instead, they are delivered to the peripheral circulation where endothelial lipoprotein lipase breaks down the triglycerides, delivering fats to the cells in adipose tissue and muscle.[21]

Dependence of hydroxylation rate on source

Although both dietary and endogenous vitamin D-hormones are eventually hydroxylated in the liver to form calcidiol, diet-derived vitamin D-hormones may undergo more rapid hydrolytic activation to calcidiol. This is because they are carried by chylomicrons and their derivative low-density lipoprotein (LDL) particles, which have specific uptake, through apoprotein receptors, whereas DBP carrying endogenous sunlight derived D3 has only nonspecific uptake in the liver. Whether some of the dietary vitamin D-hormones associated with the triglyceride core of the chylomicron is also transferred to cells at this stage is not clear. Once the triglycerides are largely depleted, the chylomicron remnants, as a result of their apolipoproteins, are taken up by the liver where they are converted to very low, and low-density lipoproteins (VLDL and LDL).[21] Vitamin D-hormones remaining in the chylomicron remnants is converted by liver cells to 25(OH)D and returned to the circulation as part of LDL particles[20] or associated with DBP.

Pleiotropic effects of vitamin D-hormones

Vitamin D-hormones have diverse genomic and non-genomic targets, affecting a vast array of physiological functions. A limited search of literature reveals that cellular and molecular targets of vitamin D-hormones are extensive due to VDR dimerizes with receptors (e.g. retinoid acid receptors[22] and retinoid X receptors[23]), interacts with factors (e.g. insulin-like growth factor binding protein-5,[24] ikappab kinase beta protein[25]), activates signaling pathways (protein kinase C-alpha,[26] cAMP,[27] p38 MAPK[28]) and enhances actions of glucocorticoid[29] and vitamin K metabolism.[30] Notably, estrogen pathway has been shown to regulate levels of vitamin D-hormones.[31–33] A more extensive targets of vitamin D-hormones are described elsewhere.[34]

Vitamin D-hormones supplementation and cardiovascular outcomes

Excess amounts of vitamin D-hormone pose significant health risks, such as hypercalcemia, hypercalciuria, and calcification of soft tissues (such as the vasculature and kidney), cardiac arrhythmias, and even death.[35–37,38] Symptoms of vitamin D-hormone toxicity include nausea, vomiting, dehydration, pain, constipation, pancreatitis, and loss of appetite.[39, 40] Here, we will focus on its effects on the cardiovascular system, where the question is whether gradual calcification may result from chronic over-use, even if below levels considered toxic.

Since low serum levels of calcidiol have been reported to associate with increased cardiovascular risk, oral vitamin D-hormone supplementation has been widely used, often together with calcium.[41–45] However, as noted by Michos et al.,[44] recent clinical studies raise doubts about any benefit to be gained from supplementation, given the failure to reduce mortality or cardiovascular events.[46–50] Meta-analyses also fail to show conclusive benefits of vitamin D-hormone supplementation on cardiovascular and non-cardiovascular outcomes.[51–54] One report, often cited as showing increased cardiovascular risk with low levels of calcidiol, actually showed increased cardiovascular risk for both high and low levels and an optimum level close to what has been considered deficient.[55] Preclinical

studies also support this biphasic relationship for both deficiency and excess of vitamin D-hormones.[56] Moreover, a nationwide, randomized controlled trial (VITAL) showed no reduction in cardiovascular events with supplementation in over 25,000 diverse patients over about 5 years.[41] As with other dietary nutrients, the risk has a J-shaped or U-shaped relationship with levels.[55, 57–59] In a randomized trial, Gallagher and colleagues found that the common dose of 1600–2400 IU/d of calcidiol in postmenopausal women raises the serum 25(OH)D levels to greater than 36 ng/mL (75 nmol/L), a range considered unsafe.[35] While the doses required to achieve appropriate levels depend on individual exposure to ultraviolet light, skin color, other dietary intake, and metabolic characteristics, the Institute of Medicine[60] recommended only 600 IU/d for adults under 70 years of age and 800 IU/d for those over 70. Determining what level is safe is not straightforward. Given this inter-individual variability and given the numerous targets of vitamin D-hormones in a variety of tissues,[34] adverse effects of excess intake are likely to be pleiotropic and, as with vascular calcification, may be invisible and, hence, unreported.

Effect of D vitamins on vascular cells and atherosclerotic calcification

A high impact question is whether vitamin D-hormones affect atherosclerosis and, in particular, given their role in biomineralization, atherosclerotic calcification. Based on findings from the preclinical and cell culture models, the three key issues are whether vitamin D-hormones are present in the normal or diseased artery wall, whether they are activated there, and whether they have biological effects on the cells in vascular calcification.

The first key issue, whether vitamin D-hormones access the artery wall, is evidenced by the possibility that they are carried into the wall in LDL particles. The chylomicrons that carry diet-derived vitamin D-hormones are eventually taken up in the liver, where they are converted into very low density (VLDL) and low-density lipoprotein (LDL) particles while the vitamin D-hormones they carry are hydroxylated to calcidiol.[20] It is well established that in atherosclerotic disease, these LDL particles pass through the endothelial layer and accumulate in the subendothelial space of the artery wall. Over time, the phospholipids and apoproteins undergo nonenzymatic oxidation into products that trigger inflammation, cytokine release, influx of monocyte-macrophages, and formation of foam cells all of which together lead to development of atherosclerotic plaque.[61] To the extent that vitamin D-hormones or their metabolites remain in the cholesterol ester core of the LDL particle, 25(OH)D would accumulate in the artery wall along with the LDL. The second key issue is whether vitamin D-hormones undergo activation in the artery wall. In vitro evidence suggests that they are activated by a variety of vascular cells. Alpha-hydroxylase, which converts 25(OH)D_3 to the active form, is present not only in the kidney, but also in vascular endothelial, smooth muscle, and resident immune cells.[34, 62–64] Thus, any calcidiol carried into the artery wall by lipoproteins may undergo activation.

The third key issue in determining the role of vitamin D-hormones in atherosclerotic calcification is whether they have biological activity in artery wall cells. The activated hormone is expected to have biological effects because, as with adipocytes, vascular cells (smooth muscle, endothelial, and resident immune) express vitamin D receptor (VDR),

which is expected to affect their growth, migration, differentiation, and cytokine expression. Aortic endothelial cells produce and respond to the active hormone in an autocrine manner, including inhibition of growth[65] and of angiogenic activities such as sprouting and formation of networks.[66] It has been proposed that vitamin D-hormones may suppress oxidative stress in endothelial cells.[62] In retinal pericytes, vitamin D-hormones regulate proliferation directly and migration and adhesion via upregulating the expression of vascular endothelial growth factor.[67] Vascular smooth muscle cells also express VDR[68] as well as the hydroxylase enzymes that activate vitamin D-hormones.[63] Effects are variable. In some VSMC culture systems, the active hormone $1,25(OH)_2D_3$ has proliferative effects,[69, 70] but in others, it is anti-proliferative.[70] Similarly, its effects on VSMC are pro- or anti-migratory depending on dosage.[71–73] Based on studies silencing VDR in VSMC, it also promotes expression of VDR, Runx2, and osteoblastic genes as well as mineralization.[74, 75] In monocytes and macrophages in vitro, $1,25(OH)_2D_3$ inhibits macrophage adhesion and migration,[76] suppresses LDL uptake,[77] and inhibits the production of inflammatory cytokines IL-6 and TNF-alpha.[78, 79]Altogether, this in vitro evidence supports the concept that vitamin D-hormones affect the biology of vascular cells. However, the nature of the effects is difficult to predict.

In vivo evidence also supports a role for vitamin D-hormones in atherosclerotic calcification. High-dose dietary D_3 has been used for decades to generate experimental models of vascular calcification in rats and rabbits.[80–82] It is not yet clear whether the effect is local or systemic. As evidence for a local effect, VDR deficiency significantly reduces vascular calcification in hyperlipidemic mice,[83] even with elevated serum calcium or alkaline phosphatase.[74] However, as evidence for a systemic effect, when VDR-deficient and control aortae were transplanted into wild-type mice, uremia caused the same degree of calcification.[84]

Vitamin D-hormone activity in adipose tissue

Since adipocytes express both VDR[85] and the activating hydroxylases,[86] vitamin D-hormones are likely to be biologically active in fat tissue as well. They are known to regulate gene expression and several cell processes including stimulation of lipogenesis and expression of adipokines, such as leptin and adiponectin, as well as inhibition of lipolysis.[87] In perivascular adipose tissue, vitamin D-hormones regulate, in part, the inflammatory and hypoxia signaling pathways.[88]

Influence of obesity on vitamin D-induced vascular calcification

In genetically obese (*ob/ob*) mice, sensitivity to vitamin D-hormone toxicity is increased, potentially because they have impaired downregulation of VDR by high doses of vitamin D-hormones.[89] These mice also show greater calcification, in the form of osteochondrogenesis in response to vitamin D-hormones.[89] The mice also develop thinning and expansive remodeling of the wall, presumably to compensate for the vascular lesions. These findings may warrant consideration in the use of vitamin D-hormones in obese and insulin-resistant patients.

In human obesity, possibly due to the capacitance of the larger storage capacity, vitamin D-hormone levels are often reduced,[87] especially in young white individuals.[90] Various hypotheses have been proposed, but evidence suggests that low vitamin D-hormone levels do not cause obesity.[91–93] One possibility is that greater adipose mass may bind the same amount of vitamin D-hormones in a lower concentration,[94] thus reducing the gradient driving its re-entry into the circulation.

Inhibition of osteoclastic resorption

Biomineralization involves a balance and coupling between mineral formation by osteoblasts and mineral resorption by osteoclasts. This relationship appears to apply as much to vascular calcification as to skeletal bone mineral. Osteoclast-like cells have been described in association with calcium deposits in human atherosclerosis.[95] In skeletal bone, the effects of vitamin D-hormones on bone mass appear to be through suppression of bone resorptive osteoclasts.[96–98] When the VDR is selectively eliminated from osteoblasts, mice still develop increased bone mass in response to the vitamin D-hormone analog eldecalcitol, indicating that the effect on bone is attributable to suppression of bone resorption.[99] If vitamin D-hormone inhibition of osteoclasts also occurs in the artery wall, it may prevent regression of calcified atherosclerotic plaque in humans.

Summary

In summary, vitamin D-hormones are actually steroid hormones that are produced endogenously in the skin by sunlight and that are also available from food. Since the former are carried in the blood on a binding protein and the latter in a lipoprotein, the source may determine the biological effects especially with respect to vascular calcification. Since lipoproteins accumulate in the artery wall to form plaque, they may bring vitamin D-hormones into a position to induce or promote vascular calcification. Clinically, effects of vitamin D-hormones are controversial. Cardiovascular risk appears to increase with both low and high levels; and a large randomized controlled trial found that supplementation provided no reduction in cardiovascular events. Thus, supplementation with vitamin D-hormones should not be with the intention to interfere with disease, as it has potential harmful effects on the cardiovascular system. Even in chronic kidney patients, who have reduced calcitriol levels, vitamin D-hormone supplementation needs to be carefully weighed to avoid overuse while maintaining the dictum “primum non nocere.”

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