ROLE OF VITAMIN D IN MITOCHONDRIAL HEALTH

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Abstract

Vitamin D, a secosteroid hormone, appears to have significant beneficial effects on various physiological systems, including the musculoskeletal system. Vitamin D assists in the regulation of numerous critical biological functions and physiological processes in humans, including inflammation, oxidative stress, and mitochondrial respiration, and is also linked to cardiac diseases. It is also reported that vitamin D plays a central role in molecular and cellular mechanisms, which reduce oxidative stress, and tissue damage and regulate cellular health. On the other side, hypovitaminosis D reduces mitochondrial activity and increases oxidative stress and inflammation in the body. Hypervitaminosis D increases the prevalence and severity of cellular damage. It has also been reported that vitamin D is involved in many functions of the reproductive system in human and critically play an important role in the reproductive tissues of women and men. Its role is very well defined, starting from female menarche to menopause, pregnancy, and lactation, and finally in male fertility. Hence, the appropriate amount of vitamin D is necessary to maintain the normal function of cell organelles. Based on recent studies, it is understood that vitamin D is involved in the biological activities of mitochondria in cells, especially in cardiomyocytes. In this review, we emphasized the role of vitamin D in mitochondrial respiration, which could significantly influence heart health and human reproduction.

Keywords: Vitamin D, mitochondrial dysfunction, oxidative stress, cell damage, inflammation, cardiac diseases

Introduction

Vitamin D is fat-soluble, linked to bone metabolism, and is well-known for its essential role in bones and skeletal muscle health (Halfon et al., 2015(1)). Vitamin D is present in bread, milk, fatty fish, mushrooms, and dietary supplements. As we know that there are two main forms of dietary or supplemental vitamin D. These are vitamin D2 and D3. Vitamin D2 is derived from plants, while vitamin D3 comes from animals (fatty fish or sheep's lanolin). Both the forms are significant for overall vitamin D levels. The biological roles of vitamin D are very well defined. Cholecalciferol (known as vitamin D3) is bound to serum vitamin D-binding protein (DBP). For the biological activation of vitamin D, two-step enzymatic pathways are necessary. These are involving 25-hydroxylase of the liver and 1α-hydroxylase (CYP27B1) of the kidney and extra-renal tissues. The majority of the functions are mediated by the VD receptor (VDR), which is a ligand-dependent transcription factor. This transcription factor is primarily localized in the nuclei of target cells. VDR works as a mediator for the genomic action of the biologically active hormone calcitriol ($1,25(OH)_2D3$). Calcitriol is an active form of vitamin D, which is produced by the hydroxylation of 25(OH)D in kidneys under the regulation of parathyroid hormone (PTH) and serum calcium. This form acts as an inducer for the transcription of more than 900 genes (Minghetti and Norman, 1988(2)). VDR receptor is widely distributed over various tissues and organs including skeletal muscles, parathyroid glands, and the reproductive tissues.

Vitamin D levels of 50 nmol/L were found to be insufficient in various clinical investigations and were linked to muscular atrophy and faintness (Tagliafico et al., 2010 (3); Van Langenberg et al., 2014(4)). When exposed to ultraviolet B rays, the human skin converts 7-dehydrocholesterol to vitamin D (Jäpelt and Jakobsen, 2013(5). The *CYP P450* gene family has been expanded to include numerous genes that play pivotal roles in vitamin D activation and degradation (*CYP2R1*, *CYP27B1*, and *CYP24A1*). Vitamin D initially hydroxylated in the liver predominantly by the CYP2R1 enzyme to produce 25-hydroxyvitamin D and then in the kidneys by another enzyme, CYP27B1, to produce 1α,25-dihydroxyvitamin D, which is the hormonally active form (Griffin et al., 2003; Bouillon et al., 2008; Saponaro et al., 2020).

Vitamin D is attached to its serum carrier, vitamin D binding protein (DBP) in the bloodstream. DBP is a highly polymorphic protein that has at least 120 different isoforms. Gc1f, Gc1s, and Gc2 are the three primary isoforms that have sparked the most interest. Their structural differences have an impact on DBP function, which is linked to a large number of clinical aspects. Studies confirm that polymorphism of specific DBP isoforms leads to a lower level of circulating DBP (San-

tos et al., 2019). Serum calcium levels have been reported to be normal in patients with low levels of circulating DBP. The primary role of DBP is to keep a stable reservoir of circulating extracellular vitamin D metabolites in place. DBP is more effective in this role than albumin due to its stronger affinity for vitamin D metabolites, even though both DBP and albumin are filtered into urine and retrieved by megalin.

Under normal physiological conditions, 1α,dihydroxyvitamin D binds to its nuclear receptor, the vitamin D receptor (VDR), and then to a retinoid X receptor (RXR), forming a VDR-RXR heterodimer that interacts with regulatory elements in the genome and regulates the transcription (Haussler et al., 1997). VDR is a transcription factor that has been shown to influence the expression patterns of many genes (Khammissa et al., 2018). The interaction of 1α,25-dihydroxyvitamin D with its intracellular receptors is also known to affect vitamin D-dependent gene transcription and activation of vitamin D-responsive elements and trigger various second messenger systems (Gil et al., 2018). Vitamin D deficiency raises the risk and severity of several diseases, including obesity, insulin resistance, type 2 diabetes, hypertension, pregnancy issues, memory difficulties, osteoporosis, autoimmune diseases, malignancies, and systemic inflammatory illnesses (Garcia-Bailo et al., 2011; Berridge, 2017; Szymczak-Pajor et al., 2020).

The Endocrine Society recommends a daily dose of 400 IU of vitamin D for children aged 0 to one year and 600 IU for children aged one to eighteen years. The Society advises 1500-2000 IU for men and women over 18 years old, including nursing and pregnant women whose newborns are not getting enough vitamin D (Endocrine Society, 2017). Vitamin D deficiency/insufficiency is a public health problem because it is independently connected with a greater risk of all-cause mortality. Vitamin D3 therapy with a daily dose of 500 U reduced the frequency of respiratory infections by two-thirds in patients with 25-hydroxyvitamin D levels less than 20 ng/ml, which typically leads to impaired absorption of vitamin D. Vitamin D sufficiency, a serum 25-hydroxyvitamin D level of at least 30 ng/mL reduced the risk for adverse clinical outcomes in patients with COVID-19 infection (Maghbooli et al., 2020). Hypovitaminosis D was associated with a decline in muscular function and performance and increased disability (Berridge, 2017; D'Amelio and Quacquarelli, 2020). Vitamin D supplementation has been shown to boost muscle strength and speed in aged people (Halfon et al., 2015; Berridge, 2017). Vitamin D supplementation has been linked to a lower risk of falls due to direct effects on muscle cells (Ramasamy, 2020; Wilson-Barnes et al., 2020). On the other hand, excessive vitamin D levels might have harmful effects, such as kidney stones, renal impairment, malignancy, and possibly some indications of cardiovascular disease (CVD), especially when combined with a high calcium intake (Brouwer-Brolsma et al., 2013).

The research focus on vitamin D has expanded beyond its recognized classic bone health benefits, including diabetes and cardiovascular, neurological, pulmonary, renal, and liver illnesses. Yet, several contradictory discoveries continue to emerge (Stokes and Lammert, 2016). However, some controversies and uncertainties still exist in certain aspects related to a daily dose of vitamin D required in the general population to maintain normal levels of 25-hydroxyvitamin D, supplementation for metabolic bone diseases, ultraviolet-B induced cutaneous production of vitamin D, regulation of 25-hydroxyvitamin D metabolites in the liver, the definition of hypervitaminosis of D, hypovitaminosis in acute illness, requirements of vitamin D during reproduction, cellular and organ activities under vitamin D receptor influence, and possible links between vitamin D and major diseases (Minisola et al., 2019; Giustina et al., 2020). Keeping vitamin D levels in an optimal range allows it to improve several processes while avoiding the complications associated with overdose. The vitamin D fluctuation is widely associated with several diseases, including cardiovascular diseases (Ohsawa et al., 2000; Brewer et al., 2011), cancer (Hammad et al., 2013; Weinstein et al., 2015), immune system disorders (Jeffery et al., 2015; Wang et al., 2017), diabetes (Al-Timimi and Ali, 2013), neuropsychiatric disorders (Kesby et al., 2011) and several other diseases.

Role of vitamin D in mitochondrial function and disfunction

Mitochondria are cell organelles with outer and inner membranes, though the inner membrane forms many folds known as cristae (Ashcroft et al., 2020). The intermembrane space refers to the space between the outer and inner membranes, while the matrix refers to the space within the inner membrane (Ashcroft et al., 2020). The mitochondria produce energy in the form of ATP. The reduction in ATP production is independent of changes in many parameters of mitochondrial machinery, including electron transport system (ETS) complexes I-V, citrate synthase, and cytochrome C oxidase (Ashcroft et al., 2020). After the step-by-step transmission of electrons, the mitochondrial matrix actively pumps hydrogen ions into the intermembrane space. ATP synthase returns protons from intermembrane space to the mitochondrial matrix by passing them across an electrochemical gradient process. ATP is synthesized by coupling proton translocation with phosphorylation of ADP.

In addition to impacting muscle mass and functionality, evidence suggests that vitamin D in skeletal muscles may influence mitochondrial activity. In the case of vitamin D deficiency, mitochondrial respiration diminishes with a reduction in nuclear mRNA and protein (Kim et al., 2014). When 1,25-dihydroxyvitamin D3 was administered to human primary myoblasts, mitochondrial activity improved, and the number of mRNAs encoding mitochondrial proteins increased by almost 80 percent (Ryan et al., 2016). Vitamin D is needed to sustain the functioning of the mitochondrial respiratory chain (Consiglio et al., 2015). The synthesis of the uncoupling protein (UCP), which regulates thermogenesis on the internal mitochondrial membrane, is similarly affected by vitamin D (Abbas, 2017). In particular, the synthesis of ATP is reduced because of the decrease in vitamin D-dependent development of the electron transport chain complex I. Ashcroft et al., (2020) found that VDR is required to maintain mitochondrial respiration at an optimal level in myoblasts

and myotubes (Ashcroft et al., 2020). The reduction in mitochondrial respiration in VDR-deleted myoblasts and myotubes as a result of reduced ATP. Further, vitamin D has no effect on mitochondrial ETC subunit I-V, citrate synthase, or cytochrome-c protein content in VDR-KD myoblasts and myotubes (Ashcroft et al., 2020). The decrease in maximum oxidant ability without any alterations in ETS I-V protein expression was reported in an *in vivo* study with mice deprived of vitamin D (Habib et al., 2020).

Most of the vitamin D research in humans focuses on protein synthesis and breakdown. There is growing evidence that vitamin D supplementation improves mitochondrial density and function (Sinha et al., 2013; Rana et al., 2014). Vitamin D supplementation enhances the balance between synthesis and breakdown of muscle protein, as well as mitochondrial density in an *in vivo* rat model study (Gogulothu et al., 2020). To accomplish the immediate and intensive energy demands during exercise, the muscle stores phospho-creatinine (P-creatinine) (Bouillon and Verstuyf, 2013). P-creatinine is the muscle's inorganic phosphate content. In vitamin D deficient patients, P-creatinine levels were decreased. However, it was restored after vitamin D treatment and exercise in randomized clinical trials (Wagner et al., 2013). The slower rate of energy generation in the mitochondria of skeletal muscle could lead to a loss of muscle strength and a fast feeling of exhaustion during moderate activity (Latham et al., 2021). P-creatinine is broken down during muscle contraction and generates creatine. The P-creatinine system generates a lot of ATPs, which is crucial when metabolic demand is high, such as during intense aerobic exercise, and other metabolic pathways cannot keep up with the need. In essence, P-creatinine fosters the short-term high level of energy needed during intense exercise. Ye et al. (2001) observed a decrease in the P-creatinine /ATP ratio in a pig model of congestive heart failure (Ye et al., 2001). Further, research shows that P-creatinine system damage occurs before contractile dysfunction, which reduces the energy reserve (Ingwall and Weiss, 2004). Vitamin D could reverse P-creatinine fluctuation in contractile function by restoring mitochondrial function, and this could be a potential research target for further investigation. Furthermore, the activation of the vitamin D receptor protein has been confirmed to increase serum creatinine. Concern regarding this fact is that while serum creatinine levels are increased, the glomerular filtration rate decreases. However, a study found results to the contrary. The short-term activation of vitamin D receptors increases serum creatinine levels along with overall creatinine production and generates no detrimental effects on the glomerular filtration rate (Agarwal et al., 2011).

The role of vitamin D receptors in mitochondrial function

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The role of vitamin D receptors in mitochondrial function

The ubiquitous nature of VDR suggests the possibility of extensive impacts, prompting further research into the effects of vitamin D in several tissues in the human body (Omdahl et al., 2002). In genomic results, VDR activation in the nucleus leads to cellular differentiation and proliferation (Sirajudeen et al., 2019). Nongenomic effects leading to fast calcium influx within muscle cells could be attributed to a potential transmembrane receptor (Rebas et al., 2017).

1,25-dihydroxyvitamin D controls the oxidative capacity by avoiding substantial changes in the density or amount of ETS protein. A recent study revealed that VDR knock-down (VDR-KD) in myotubes of C2C12 enhances the synthesis of mitochondrial fusion protein optic atrophy 1 (OPA1), which has been proposed as a compensatory strategy for restoring mitochondrial function (Ashcroft et al., 2020). In mitochondria, OPA1 causes internal membrane fusion, resulting in improved mitochondrial oxidative capacity (Kushnareva et al., 2013). The OPA1 protein expression was elevated with 1,25-dihydroxyvitamin D treatment in vitamin D deficient mice with statin-induced myopathy and human skeletal muscle cells (Ryan et al., 2016; Ren et al., 2020). Ricca et al. (2018) studied the *in vitro* function of mitochondria by silencing VDR and reported the enhanced respiratory activity in silenced cells that was associated with increased reactive oxygen generation (ROS). The absence of the receptor eventually led to mitochondrial malfunction and cell death and slowed down cellular proliferation.

Furthermore, a similar study by Ricciardi et al. (2015) found that vitamin D and VDR signaling decreases mitochondrial respiration, serving as an important regulator for many essential bioenergetic metabolic pathways. The ability of vitamin D/VDR to regulate mitochondrial respiration allows the mitochondria to adapt to various metabolic states that arise during times of cell growth, signaling, and proliferation (Silvagno and Pescarmona, 2017). To continue, the study conducted by Silvagno and Pescarmona (2017) found that KO VDR mice have shown deficient calcium absorption leading to hypocalcemia, hypophosphatemia, secondary hyperparathyroidism, osteomalacia, and rickets. The same study also identified VDR as a mitochondrial energy expenditure regulator due to the finding that VDR KO mice have shown higher basal energy expenditure rates and increased levels of energy upcoupling in the form of the electron transport chain in mitochondria. Additionally, VDR KO mice were found to have a debilitating effect on the skin in regards to decreasing the efficiency of the barrier to a host of pathogens via disruption in the processes of lipid composition and secretion that help make the barrier effect.

The VDR protein was discovered with two anti-VDR antibodies, and the mitochondrial VDR disappeared by the VDR gene silencing in immortalized human keratinocytes (Consiglio et al., 2014). VDR localization in mitochondria needs a specific mitochondrial import mechanism involving the import of cholesterol or the export of cytochrome C (Silvagno et al., 2013). It's unclear how mitochondrial VDR affects gene expression, cation control, oxidative function, and tissue-specific activity in platelets or muscles. It also regulates several other nuclear receptors in mitochondria including estrogens, glucocorticoids, and thyroid hormone receptors (Psarra et al., 2006). Mitochondria are generally acknowledged as having a function in forming reactive nitrogen, reactive oxygen species (ROS), and antioxidants. The antioxidants' defense mechanisms were shown to be enhanced in rickets (Doğan et al., 2012). Both metabolic and osteoporosis syndrome is associated with oxidative stress (Manolagas, 2010).

The lack of VDR leads to a decrease in cell proliferation, which is highly required during the cells that acquired higher VDR in the G0 and G1 phases of the cell cycle than in the M phase (Consiglio et al., 2014). Consiglio et al. (2014) found that cancer cells with silenced VDR have decreased the rates of proliferation, leading to the hypothesis that reduced VDR expression could be a possible cancer treatment. Silencing of VDR increased cytochrome C oxidase subunits II and IV transcript. VDR silencing inhibited the mevalonate pathway and histone acetylation levels, which are acetyl CoA-dependent biosynthetic pathways (Consiglio et al., 2014). Inhibiting histone acetylation decreases gene transcription levels, and since acetyl CoA is a key molecule in metabolism, various pathways have the potential to be affected by VDR. These findings suggest that VDR regulates the mitochondrial respiratory chain activity, the acetyl-CoA pathway, the TCA cycle, and biosynthetic pathways of cell development.

Researchers investigated the significance of vitamin D in maintaining the *in vivo* activity of mitochondria by utilizing a known diet-induced vitamin D deprivation model in mice C57BL/6J. The study with a diet-induced vitamin D deficiency model of mice(C57BL/6J) results in reduced mitochondrial respiration in skeletal muscle that could lead to muscle fatigue and performance deficits (Ashcroft et al., 2021). Vitamin D (calcitriol) increases intramyocellular lipid (IMCL) accumulation and oxygen consumption rate, which is driven by mitochondrial complex II in C2C12 myotubes, and this increase is at least partially mediated by a protein, Perilipin 2 (PLIN2) (Schnell et al.,

2019). Kolleritsch et al. (2020) reported that the low cardiac perilipin is linked to reduced mitochondrial fission and could be used to inhibit the emergence of lipotoxic cardiomyopathy (Kolleritsch et al., 2020). An increase in myocardial lipid storage and decreased cardiac performance were observed after myocardial infarction in people with PLIN2 deficiency(Mardani et al., 2019).

Conclusion

There is significant evidence that lack of vitamin D contributes to the development of heart failure (Zittermann et al., 2006). Vitamin D promotes mitochondrial homeostasis and prevents protein oxidation, lipid peroxidation, and DNA damage caused by oxidative stress. Autophagy, mitochondrial malfunction, inflammation, oxidative stress, epigenetic modifications, DNA abnormalities, and calcium and ROS signaling changes are all known to be regulated by vitamin D. The excess vitamin D may lead to calcification. Therefore, proper dosages of vitamin D are required to treat patients in clinics. In several patients, low blood levels of 25-hydroxyvitamin D lead to increased mortality, especially sudden cardiac death and coronary illness. In this review, we emphasized the potential role of vitamin D in critical biological processes and the functions of explorations of the biological components in mitochondrial-associated cardiac diseases.

In summary, vitamin D supplementation in humans plays a significant role in supporting mitochondrial health and regulating cardiac disease progressions. Detailed mechanical inquiries are necessary to light the manifestation of vitamin D in mitochondrial function and cardiac health.

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