

Study of Relationship between vitamin D and glucose homeostasis in patients on chronic hemodialysis

Thesis

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INTRODUCTION

Cardiovascular complications are the major cause of mortality & morbidity in patients with CKD. Vitamin D receptors have a broad tissue distribution that includes vascular smooth muscle, endothelium, and cardiomyocytes. A growing body of evidence suggests that vitamin D deficiency may adversely affect the cardiovascular system. Vitamin D deficiency is associated with increased cardiovascular risk, above and beyond established cardiovascular risk factors, (heart failure, fatal and nonfatal coronary heart disease, fatigue in the legs during activity, fatal or nonfatal cerebro vascular events). The higher risk associated with vitamin D deficiency was particularly evident among individuals with high blood pressure (*Wang et al., 2008*).

Vitamin D has also been recognized to have numerous non-calcemic functions. Specifically, there is increasing evidence that vitamin D metabolism affects the insulin resistance (IR), diabetes and the metabolic syndrome, although the underlying molecular mechanism of this association remains to be elucidated (*Chonchol & Scragg 2007*).

Human studies have shown diminished insulin-stimulated glucose uptake in uremic patients. Insulin resistance in patients with kidney disease is accompanied by hyperinsulinemia and glucose intolerance (*Sarafidis 2008*).

Chen et al., identified a significant relationship between IR, insulin levels, and risk of CKD defined as an estimated glomerular filtration rate (eGFR) <60 ml/min/1.73 m² among 6453 persons without diabetes (*Chen et al., 2003*).

Thus, kidney dysfunction appears to be associated with a syndrome of IR and several explanations have been proposed, including, vitamin D deficiency, anemia or uremic toxins (*Becker et al., 2005*).

Low vitamin D levels are associated with various degrees of impaired glucose metabolism and diabetes risk in people with normal kidney function, it seems reasonable to propose that IR in patients with CKD may also be associated with reduced levels of 25-hydroxyvitamin D (**Chonchol & Scragg 2007**).

Insulin resistance is characterized by the systemic impairment of insulin action and is usually the result of aging, obesity, chronic inflammation or another factor that may contribute to the inhibition of the insulin signaling pathway. Insulin resistance is accompanied by defects in lipid metabolism and blood coagulation, hypertension, obesity and vascular inflammation in a syndrome called syndrome X or metabolic syndrome. Metabolic syndrome is risk factor in the development of atherosclerosis with consequent cardiovascular complications including acute myocardial infarction, stroke and vascular disease, vitamin D acts as a negative regulator of the renin gene and that vitamin D deficiency is followed by increased renin-angiotensin II expression (**Rammos et al., 2008**).

Stage of kidney dysfunction and quartile of 25-hydroxyvitamin D were each inversely associated with levels of homeostasis model assessment of insulin resistance (HOMA-IR) and fasting insulin, adjusting for demographic variables and BMI, indicating that eGFR function and 25-hydroxyvitamin D have independent associations with insulin resistance (**Chonchol & Scragg 2007**).

Vitamin D [25(OH)D] deficiency appears to be widely prevalent in stage 5 CKD. Repletion with ergocalciferol may assist in improving glycemic control in the management of diabetes. Additional research is needed to confirm these results and determine the optimal levels of serum 25(OH)D (**Blair et al., 2008**).

The risk of post-transplant diabetes mellitus increases continuously with time post-transplant. There has been an increase in the incidence of post-transplant diabetes mellitus in patients transplanted recently and that increase can be explained partially by changes in the recipients' characteristics. Cosio et al postulate that this increase may be due to the introduction of better absorbed cyclosporine formulations that result in higher blood levels and higher cumulative exposure to this diabetogenic drug (*Cosio et al., 2001*).

An inverse association between vitamin D status and diabetes, possibly involving insulin resistance, in non-Hispanic whites and Mexican Americans. The lack of an inverse association in non-Hispanic blacks may reflect decreased sensitivity to vitamin D and/or related hormones such as the parathyroid hormone (*Scragg et al., 2004*).

Hepatic synthesis of 25 (OH)D3 is only loosely regulated, and blood levels of this molecule largely reflect the amount of vitamin D produced in the skin or ingested. In contrast, the activity of 1-alpha- hydroxylase in the kidney is tightly regulated and serves as the major control point in production of the active hormone. Parathyroid hormone is the major inducer of 1-alpha-hydroxylase. Low blood phosphate levels induce it as well. This means that 25 (OH)D3 is the best marker for vitamin D status because it best reflects vitamin D stores and is telling us how much the skin is making and how much we are consuming (*Chonchol & Scragg 2007*).

So measurements of vitamin D, blood glucose, insulin, and kidney function provides an ideal opportunity to determine whether vitamin D status is related to IR and/or insulin secretion in participants with CKD.

Aim of the study:

To evaluate the possible effect of vitamin D administration on glucose homeostasis and insulin resistance in patients with ESRD under hemodialysis.

Effect of vitamin D on glucose homeostasis

Vitamin D is a fat-soluble vitamin that is naturally present in few foods, added to others and available as a dietary supplement. It is also produced endogenously when ultraviolet rays from sunlight strike the skin and trigger vitamin D synthesis. Vitamin D obtained from sun exposure, food and supplements is biologically inert and must undergo two hydroxylations in the body for activation. The first occurs in the liver and converts vitamin D to 25hydroxyvitamin D, also known as calcidiol. The second occurs primarily in the kidney and forms the physiologically active 1,25dihydroxyvitamin D, also known as calcitriol (*Fineberg et al., 2010*).

Vitamin D promotes calcium absorption in the gut and maintains adequate serum calcium and phosphate concentrations to enable normal mineralization of bone and to prevent hypocalcemic tetany. It is also needed for bone growth and bone remodeling by osteoblasts and osteoclasts (*Cranney et al., 2007*). Without sufficient vitamin D, bones can become thin or brittle. Vitamin D sufficiency prevents rickets in children and osteomalacia in adults. Together with calcium, vitamin D also helps to protect older adults from osteoporosis (*Fineberg et al., 2010*).

Vitamin D has other roles in the body, including modulation of cell growth, neuromuscular and immune function and reduction of inflammation (*Holick et al., 2006 and Norman & Henry 2006*). Many genes encoding proteins that regulate cell proliferation, differentiation, and apoptosis are modulated in part by vitamin D. Many cells have vitamin D receptors and some convert 25hydroxyvitamin D to 1,25dihydroxyvitamin D (*Fineberg et al., 2010*).

Low vitamin D status can be caused by a number of factors, including insufficient cutaneous synthesis (due to limited sunlight exposure or aging), inadequate intake and absorption of vitamin D, obesity or darker skin (*Mosekilde 2005& Holick 2007*).

In Europe, there is a significant positive correlation between serum 25hydroxyvitamin D concentration and latitude (**Lips et al., 2001**). Latitude determines the available sunlight exposure, which affects 25hydroxyvitamin D concentration. Therefore, regional differences in 25hydroxyvitamin D concentration are a well-recognized phenomenon (**McKenna 1992**). People living at higher latitudes are especially at increased risk for vitamin D deficiency, because from November through February most of the ultra violet β radiation from sunlight, which is required for cutaneous vitamin D synthesis, is absorbed by the atmosphere and does not reach the earth's surface (**Johnson and Kimlin 2006**).

Serum concentration of 25hydroxyvitamin D is the best indicator of vitamin D status. It reflects vitamin D produced cutaneously and that obtained from food and supplements and has a fairly long circulating half-life of 15 days (**chiu et al., 2004 & Jones 2008**). 25hydroxyvitamin D functions as a biomarker of exposure, but it is not clear to what extent 25hydroxyvitamin D levels also serve as a biomarker of effect (i.e., relating to health status or outcomes) (**Fineberg et al., 2010**).

In contrast to 25hydroxyvitamin D, circulating 1,25dihydroxyvitamin D is generally not a good indicator of vitamin D status because it has a short half-life of 15 hours and serum concentrations are closely regulated by parathyroid hormone, calcium, and phosphate (**Jones 2008**). The concentration of 25hydroxyvitamin D, but not that of 1,25dihydroxyvitamin D, defines nutritional vitamin D status (**Gloth et al., 1995** and **Hollis 1996**). Levels of 1,25dihydroxyvitamin D do not typically decrease until vitamin D deficiency is severe (**Holick 2007&Cranney et al., 2007**).

As a result, the reference ranges defined with the use of the regional population samples lead to different range of lower limits

among various regions (*O'Shea and Carter 1992*). The definition using the regional population samples did not reflect the true body need because hypovitaminosis D causes secondary hyperparathyroidism (*Chiu et al., 2004*).

Another approach to defining hypovitaminosis D is based on the relation of 25hydroxyvitamin D and parathyroid hormone concentration (*Malabanan et al., 1998& Chapuy et al., 1996*).

Table 1: Serum 25-Hydroxyvitamin D Concentrations and Health*		
nmol/l**	ng/ml*	Health status
<30	<12	Associated with vitamin D deficiency, leading to rickets in infants and children and osteomalacia in adults
30–50	12–20	Generally considered inadequate for bone and overall health in healthy individuals
≥50	≥20	Generally considered adequate for bone and overall health in healthy individuals
>125	>50	Emerging evidence links potential adverse effects to such high levels, particularly >150 nmol/L (>60 ng/mL)

* Serum concentrations of 25hydroxyvitamin D are reported in both nanomoles per liter (nmol/l) and nanograms per milliliter (ng/ml).

** 1 nmol/l = 0.4 ng/ml

Based on its review of data of vitamin D needs, a committee of the Institute of Medicine concluded that persons are at risk of vitamin D deficiency at serum 25hydroxyvitamin D concentrations <30 nmol/L (<12 ng/mL). Some are potentially at risk for inadequacy at levels ranging from 30–50 nmol/L (12–20 ng/mL). Practically all people are sufficient at levels ≥50 nmol/L (≥20 ng/mL); the committee stated that 50 nmol/L is the serum

25hydroxyvitamin D level that covers the needs of 97.5% of the population. Serum concentrations >125 nmol/L (>50 ng/mL) are associated with potential adverse effects (*Fineberg et al., 2010*) (**Table 1**).

Most tissues have not only vitamin D receptors, but also the hydroxylase enzyme that is required to convert 25hydroxyvitamin D to the active form 1,25dihydroxyvitamin D. Therefore, vitamin D can affect tissues that are not involved in calcium homeostasis and bone metabolism. Vitamin D deficiency is a risk factor for hypertension, type 1diabetes and various cancers (*Holick 2002*).

Hypovitaminosis D has long been suspected as a risk factor for glucose intolerance. The 25hydroxyvitamin D concentration was lower in patients with type 2 diabetes than in the nondiabetic control subjects (*Scragg et al., 1995*). The 25hydroxyvitamin D concentrations were lower and associated with impaired insulin secretion in patients at risk for diabetes than in those who were not at risk for diabetes (*Boucher et al., 1995*).

There is accumulating evidence to suggest that poor vitamin D status may play a role in the development of type 2 diabetes mellitus (DM) (*Pittas et al., 2007*). Cross-sectional studies have consistently demonstrated that blood 25hydroxyvitamin D concentrations are lower in patients with DM (*Isaia et al., 2001, Scragg et al., 2004 and Targher et al., 2006*). Prospective studies have showed that lower vitamin D intakes or lower 25hydroxyvitamin D concentrations might increase the risk of type 2 DM (*Pittas et al., 2006 and Mattila et al., 2007*).

Furthermore, Insulin resistance is a recognized precursor in the development of type 2 DM. A few observational studies have reported the relationship between 25hydroxyvitamin D and insulin resistance represented by the homeostatic model assessment of insulin resistance (HOMA-IR) or insulin sensitivity derived from

hyperglycemic clamp (*Chiu et al., 2004* , *Pittas et al., 2006* and *Mattila et al., 2007*).

Therefore, vitamin D could play a role in the pathogenesis of type 2 diabetes, by affecting either insulin sensitivity or β cell function or both (*Baynes et al., 1997*).

Ethnic variation in vitamin D effect

The inverse association between vitamin D and diabetes risk in non-Hispanic whites and Mexican Americans is consistent with previous epidemiological studies in other ethnic groups (*Scragg et al., 1995 and Baynes et al., 1997*). The lack of confounding between vitamin D and leisure physical activity and also between vitamin D and BMI suggests that vitamin D affects diabetes risk by a mechanism separate from those of the other two risk factors (*Scragg et al., 2004*).

The contrasting lack of any inverse association of vitamin D with diabetes risk and insulin resistance in non-Hispanic blacks was unexpected, particularly given their low vitamin D levels, which has been reported by (*Looker et al., 2002*). The reasons for the lack of an inverse association with vitamin D in non-Hispanic blacks are unclear, but an explanation of this ethnic variation in vitamin D effect may provide new insights into any possible protective mechanisms related to vitamin D. One possibility is that there is a threshold effect that varies with ethnicity (*Scragg et al., 2004*).

Non-Hispanic blacks may have a decreased sensitivity to the effects of vitamin D and/or related hormones. Bone density is increased in blacks compared with whites (*Kleerekoper et al., 1994*), despite the former having elevated parathyroid hormone blood levels (*Bell et al., 1985 and Kleerekoper et al., 1994*), which should result in increased bone resorption and decreased bone mineral density. This suggests a decreased sensitivity among non-

Hispanic blacks to the effects of parathyroid hormone (**Cosman et al., 1997**).

Cosman et al. have proposed that whites may have developed an increased skeletal sensitivity to the effects of the parathyroid hormone and vitamin D in order to maintain calcium homeostasis in general body tissues by increasing calcium supply from the skeleton to compensate for increased urinary calcium excretion (**Cosman et al., 1997**).

Perhaps some other tissues, such as skeletal muscle, also show increased sensitivity to parathyroid hormone in non-Hispanic whites and in Mexican Americans who have similar bone mineral density to whites (**Morton et al., 2003**).

Vitamin D deficiency results in hyperparathyroidism (**Vieth 1999** and **Zitterman 2003**), through which it may influence glucose metabolism. Patients with hyperparathyroidism have an increased prevalence of diabetes and insulin resistance and parathyroidectomy improves their glucose intolerance (**Taylor and Khaleeli 2001**). Skeletal muscle, a key component of the insulin resistance syndrome (**DeFronzo 1988** and **Kashyap & DeFronzo 2001**), may also be involved since vitamin D receptors have been identified in that tissue (**Simpson et al., 1985**).

The role of serum 25hydroxyvitamin D in predicting the risk for type 2 DM and insulin resistance in non-Caucasian ethnic populations (African, Asian, and Indian-descent), is worth investigating, as these populations are at high risk for type 2 DM and low 25hydroxyvitamin D concentrations (**Alvarez & Ashraf 2010**).

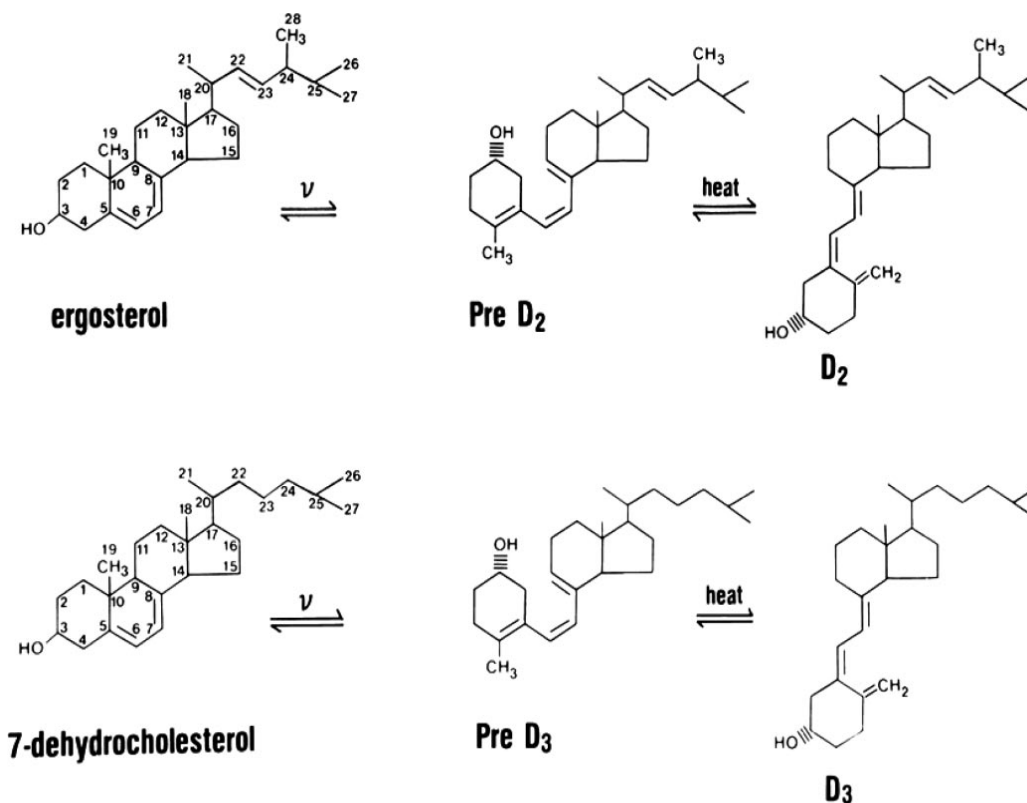


Figure 1: Production of vitamin D₂ and vitamin D₃. Ergosterol in plants and 7-dehydrocholesterol in skin are the precursors for vitamin D₂ and vitamin D₃, respectively. UV light B breaks the B chain of each molecule to form the pre-D isomer, which then undergoes isomerization to D. D₂ and D₃ differ only in the side chain in which D₂ has a double bond between C22–C23 and a methyl group at C24. These differences alter somewhat its binding to DBP and metabolism.

Sources of Vitamin D

• Food

Few foods in nature contain vitamin D. The flesh of fatty fish (such as salmon, tuna, and mackerel) and fish liver oils are among the best sources (**Vilsack 2011**). Small amounts of vitamin D are found in beef liver, cheese and egg yolks. Vitamin D in these foods is primarily in the form of vitamin D₃ and its metabolite 25hydroxyvitamin D₃ (**Ovesen et al., 2003**). Some mushrooms provide vitamin D₂ in variable amounts. Mushrooms with enhanced levels of vitamin D₂ from being exposed to ultraviolet

light under controlled conditions are also available (*Calvo et al., 2004*).

Fortified foods provide most of the vitamin D in the American diet (*Calvo et al., 2004*). For example, almost all of the U.S. milk supply is voluntarily fortified with 100 IU/cup while in Canada; milk is fortified by law with 35–40 IU/100 ml, as is margarine at ≥ 530 IU/100 g.) (*Fineberg et al., 2010*).

- **Sun exposure**

Most people meet at least some of their vitamin D needs through exposure to sunlight. Ultraviolet B radiation with a wave length of 290–320 nanometers penetrates uncovered skin and converts cutaneous 7-dehydrocholesterol to previtamin D₃, which in turn becomes vitamin D₃. Season, time of day, length of day, cloud cover, smog, skin melanin content, and sunscreen are among the factors that affect Ultraviolet radiation exposure and vitamin D synthesis (*Cranney et al., 2007*). Perhaps surprisingly, geographic latitude does not consistently predict average serum 25hydroxyvitamin D levels in a population. Ample opportunities exist to form vitamin D (and store it in the liver and fat) from exposure to sunlight during the spring, summer and fall months even in the far north latitudes (*Fineberg et al., 2010*).

Groups at Risk of Vitamin D Inadequacy

Patients with renal failure, breastfed infants, older adults, people with limited sun exposure, people with dark skin, people with fat malabsorption, people who are obese or who have undergone gastric bypass surgery.

Structure and Synthesis

Two forms of vitamin D exist: vitamin D₃ or cholecalciferol and vitamin D₂ or ergocalciferol. The former is produced in the skin under the influence of UVB radiation (UVR); the latter is produced by UVR in a variety of plant materials and yeast (**Fig.1**). Differences exist in their binding to the major transport protein in blood, vitamin D binding protein and in their metabolism because of the differences in the chemistry of their side chains, with the