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Vitamin D deficiency, change in kidney function, and genetic effect modifications in population-based studies

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Vitamin D deficiency, change in kidney function, and genetic effect modifications in population-based studies

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Abstract

Vitamin D deficiency, change in kidney function, and genetic effect modifications in population-based studies

By Idris Guessous

Molecular evidence suggest that sufficient 25[OH]D levels could protect against renal function loss, but population-based studies on the association of 25[OH]D with change in estimated glomerular filtration rate (eGFR) and incident chronic kidney disease (CKD) are limited and results discordant. No study explored the potential effect modification of *VDR* genetic variants on the relationship between 25[OH]D and change in kidney function.

We used cross-sectional data from the 2010-2011 Swiss Study on Salt intake to compare the 25[OH]D levels, deficiency status and 25[OH]D level determinants in populations without CKD and with CKD. We tested the interaction of CKD status with six a priori defined attributes on vitamin D. We used baseline (2003-2006) and 5-year follow-up data (CoLaus study) of adults from the general population to evaluate the association of serum 25[OH]D with change in kidney function, rapid decline in kidney function, and incidence of CKD. Ten genetic polymorphisms, of which five were within the *VDR* gene, were considered *a priori*.

We found that vitamin D insufficiency or deficiency (25(OH)D: <30 ng/ml) was frequent among participants with CKD (74.8%, 95%CI 58.4-92.8) but neither the prevalence of vitamin D insufficiency/deficiency nor the mean 25(OH)D levels were different in patients with and without CKD. CKD status did not interact with major determinants of vitamin D for its effect on vitamin D status or levels.

We found that annual eGFR change was associated with baseline vitamin D levels and that higher baseline vitamin D level was associated with decreased risk of rapid decline in eGFR. We found a significant *Cdx2 VDR* genotype – 25(OH)D interaction on the risk of rapid eGFR decline or incident CKD (P-value for interaction=0.022). Among participants with the *Cdx2 VDR* risk allele (CT/TT genotype), the adjusted ROR was 1.17 (95%CI: 1.03-1.34), no association was found among participants without the *Cdx 2 VDR* risk allele genotype (CC genotype).

Our results suggest that serum vitamin D may play an important role in the early stages of eGFR decline in adults from the general population. This association may vary with common genetic differences in the *VDR* gene.

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CHAPTER 1

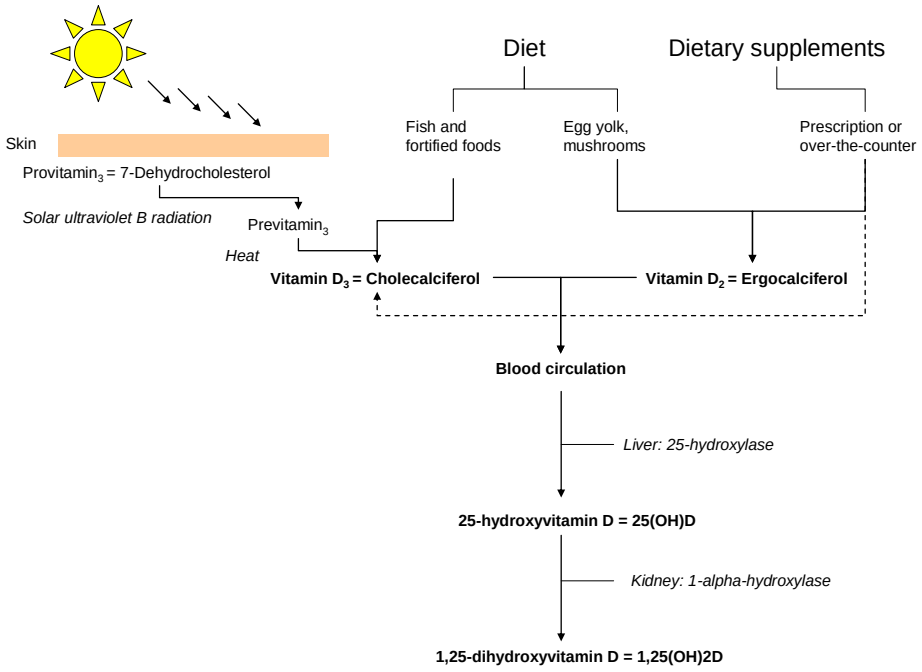
GENERAL INTRODUCTION

1.A VITAMIN D

1.A.1 SOURCES OF VITAMIN D AND FIRST STEPS IN VITAMIN D SYNTHESIS

Vitamin D is a steroid hormone. 1,25-dihydroxyvitamin D [1,25(OH)2D], calcitriol, is the hormonally active form of what is generally called "vitamin D". In humans, 1,25(OH)2D is derived from three sources: 1) sunlight, 2) diet, and 3) dietary supplements (**Figure 1**).[1, 2]

FIGURE 1 SOURCES OF VITAMIN D AND FIRST STEPS IN VITAMIN D SYNTHESIS



There are two precursors to active vitamin D hormones; vitamin D3 (cholecalciferol) and D2 (ergocalciferol). Vitamin D3 is synthesized in the skin after exposure to ultraviolet light. Solar ultraviolet B (UVB) radiation (wavelength, 290 to 315 nm) penetrates the skin and converts by photolysis 7-dehydrocholesterol to previtamin D3, which is rapidly converted to vitamin D3. Vitamin D3 may also be obtained from

some dietary sources and dietary supplements. Vitamin D2 (ergocalciferol) is derived solely from the diet (not from UVB). Both vitamin D3 and D2 enter the blood circulation and are attracted to the vitamin D binding protein (VDBP). In the literature as well as in the following chapters, D represents D2 or D3, unless otherwise specified.

Vitamin D in the circulation is transported to the liver, where vitamin D is converted by the vitamin D-25-hydroxylase to 25-hydroxyvitamin D [25(OH)D]. This first hydroxylation is made by the CYP27A1 enzyme.[3] This form of vitamin D is thought to be biologically "inactive" and must be converted in the kidneys by 25-hydroxyvitamin D-1α-hydroxylase to the biologically active form 1,25-dihydroxyvitamin D [1,25(OH)2D].[4] This second hydroxylation is made by CYP27B1. The CYP27B1 is located in the inner mitochondrial membrane of the proximal tubule cells of the kidney. As we will discuss later, the 25(OH)D is less active than 1,25(OH)D2 because of its lower affinity for the vitamin D receptor (VDR).

Extrarenal 1,25(OH)2D can also be produced and 1,25(OH)2D can act locally in the tissues where it is produced.[5] The 24-hydroxylation of both 25(OH)D and 1,25(OH)2D to form 24,25(OH)D and 1,24,25(OH)D is the primary mechanism and the first step to inactivate vitamin D metabolites.

1.A.2 Genomic And Non Genomic Vitamin D Functions

The actions of vitamin D are largely mediated by genomic functions. Vitamin D interacts with nuclear VDR. VDR is a ligand-induced nuclear receptor that regulates the expression of over 900 genes throughout the genome,[6, 7] among which *ABCB1,*[8-10] *CYP24A1,*[6] *CYP3A4,*[11] *CYP3A7,*[6] *FGF23,*[12] *SLC34A3,*[12] and *TRPV6.*[6, 13] VDR influences

the transcription of genes that are responsive to the complex VDR-vitamin D. 1,25(OH)2D dissociates from serum VDBP and enters the cell. Inside the cell, 1,25(OH)2D binds to the VDR, activates it and the VDR-vitamin D complex translocates from the cytosol to the nucleus where it is joined by the retinoid X receptor (RXR) partner. The 1,25(OH)2D-VDR-RXR complex binds to specific sequence in the promoter region of target genes, called the vitamin D response elements (VDRE). These lead to promotion and modulation of the expression of the targeted genes.

Several biological systems have VDRs and are responsive to vitamin D. Typical responses are in the intestine and in the kidney, where 1,25(OH)2D-VDR regulates genes to increase calcium and phosphate absorption. Another typical action is the suppression of parathyroid hormones (PTH) synthesis by 1,25(OH)2D-VDR in the parathyroid glands.

Recent research reported that the 1,25(OH)2D-VDR complex controls the expression of genes and mRNA synthesis unrelated to calcium homeostasis. For example, the complex control the expression of gene involved in the inhibition of the renin-angiotensin system (RAS) and thus genes that influence blood pressure (BP), as well as genes that promote the secretion of insulin, cell proliferation and differentiation.[14-17] The independent effect of vitamin D-VDR complex on other function such as kidney function is still unclear (discussed in Chapter 2).

Vitamin D has also some nongenomic “rapid response” functions. Rapid response means biologic responses that occur too rapidly to be explained by the interaction with the nucleus. In the nongenomic functions, vitamin D acts like a steroid hormone,

working through activation of signal transduction pathways linked to vitamin D receptors on cell membranes.

1.A.3 VITAMIN D RECEPTOR (VDR)

One of the reasons for the recent growing interests for vitamin D in health outcomes other than bone disease is the evidence that VDR is largely distributed in the human tissues. The list of tissues where VDR is distributed includes the hepato-gastrointestinal system (e.g. colon), the respiratory system (e.g. lung), the central nervous system (e.g. neurons), the cardiovascular system (e.g. cardiomyocyte), and the kidney.[18] Thus, tissue and cellular VDR distribution is wide. VDR abundance and activity also seem to play an important role in the individual responsiveness to 1,25(OH)2D; some of the VDR abundance and activity is determined by *VDR* polymorphisms.[19]

Polymorphisms are genetic variations which occur at a frequency of >1%.[20] The frequency of the rarer allele defines the minor allele frequency (MAF). Polymorphisms occur at 1 out of 300 DNA bases. Exons are DNA regions that create mRNA from DNA. Polymorphisms in exons can or cannot change the amino acid of the protein. Polymorphisms in introns, regions not involved in the synthesis of mRNA, can modify the stability of proteins.[20]

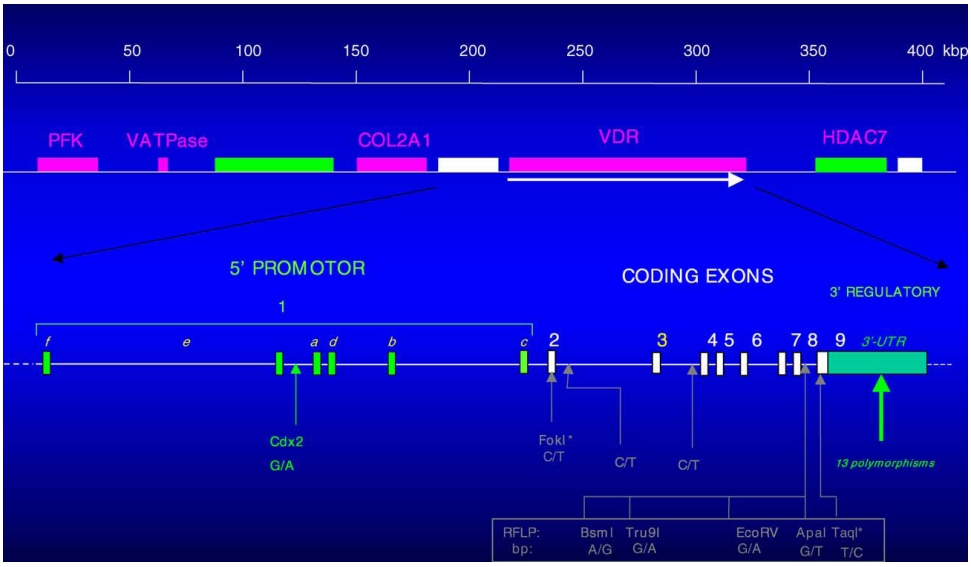
The *VDR* gene lies on chromosome 12 (**Figure 2**).[21] Eight exons comprise the coding sequence of the VDR protein. Several (>60) genetic polymorphisms have been identified (http://www.ncbi.nlm.nih.gov/sites/entrez),[19, 22] but six main genetic polymorphisms occur frequently in the population. The most frequently studied

polymorphisms are located at the 3' untranslated region in the intro: BsmI A/G, ApaI G/T, and TaqI T/C. These anonymous polymorphisms are in strong linkage disequilibrium (LD) with each other. LD measures describe the association of alleles of adjacent polymorphisms with each other. Thus, one single nuclear polymorphism (SNP) can predict the other adjacent linked one because very little recombination has occurred between them over the time of evolution. BsmI A/G, ApaI G/T, and TaqI T/C polymorphisms are unlikely to change the VDR structure. However, these polymorphisms might alter transcriptional activity and thus VDR abundance, which is an important mechanism for the modulation of cellular responsiveness to 1,25(OH)2D. There is also a polymorphic site at the 5' end of the VDR gene; FokI C/T. This polymorphism, in contrast to the other VDR variants, results in an altered amino acid sequence.[19, 22] The FokI C allele generates a shorter VDR protein than the T allele, and the shorter VDR protein is thought to be more active than the longer.[19]

The FokI polymorphism is also somewhat unique in the *VDR* gene since it is the only polymorphism that is not in LD with any other *VDR* polymorphisms and thus can be considered as an independent marker in the *VDR* gene. Because strong LD has been observed for the BsmI-ApaI- and TaqI, these latter polymorphisms are often studied as haplotypes. Haplotypes are blocks of linked alleles of adjacent SNPs.[20] Substantial differences in haplotypes and polymorphisms prevalence have been reported between ethnic groups: FokI MAF 34%, 51%, 24%; BsmI MAF 42%, 7%, 36%, ApaI MAF 44%, 74%, 31%, and TaqI MAF 43%, 8%, 31% in Caucasian, Asian, and African, respectively.[22] Africans seems to have a low prevalence of FokI C allele and therefore a higher prevalence of more active VDR protein form.

Yet another important polymorphism is the *Cdx2 VDR* polymorphism located in the promoter region of the *VDR* gene in exon 1. The *Cdx2 VDR* polymorphism has been associated with VDR transcriptional activity in the intestinal tract; the T allele showed up to 70% greater transcriptional activity than the C allele.[23]

FIGURE 2 GENOMIC STRUCTURE OF THE *VDR* LOCUS ON CHROMOSOME 12Q13 AND POSITION OF SOME POLYMORPHISMS IN THE VITAMIN D RECEPTOR GENE, from reference[22]



1.A.4 CALCIUM-RELATED ACTIONS OF VITAMIN D

One of the major biological functions of vitamin D is to maintain calcium homeostasis. Below, we briefly discuss the main calcium-related actions of vitamin D.

Calcium circulating in the blood represents less than 1 percent of total body calcium (about 1 Kg or 27.5 mol). Half of the circulating calcium is bound to proteins, mostly albumin, or complexed to phosphate and citrate. The other half is free ionized calcium,[24] which can cross plasma cell membranes and is therefore the metabolically active form. Extracellular and intracellular Ca^{2+} are involved in multiple key physiologic functions including the excitation-contraction in the neuromuscular system (e.g., heart), the synaptic transmission in the nervous system, the platelet aggregation and coagulation in the blood, the secretion of hormones, the maintenance of skeletal integrity, the Na^{+} channel voltage-gating, the regulation of cell division, the cell motility and the cell membrane trafficking.[24, 25] The metabolism of calcium is regulated by three hormones: PTH (produced by the parathyroid glands), 1,25(OH)2D and calcitonin (produced by the thyroid gland). These hormones target mainly three tissues: the bone, the kidney, and the intestine.

Dietary intake of calcium averages 1000 mg/day (25 mmol), of which only a third is absorbed in the intestinal tract.[25] Intestinal absorption of calcium occurs by transcellular and paracellular processes. Transcellular process is driven by vitamin D, which induced the synthesis of the intracellular calcium-binding protein (calbindin).

In the bone, PTH and vitamin D bind to osteoblasts leading to their maturation to osteoclasts, which increase bone resorption (i.e., process by which osteoclasts break down bone and release the minerals, resulting in a transfer of calcium from bone fluid to the blood) and release calcium into the circulation. Inversely, calcitonin inhibits osteoclasts activity and decrease bone resorption. The daily exchange of calcium between the bone and extracellular fluid is around 550 mg.[24, 25]

In the kidney, almost all (98–99%) the filtered calcium is reabsorbed in the proximal tubules (60%) and in the distal ascending limb of the loop of Henle and tubules. The proximal reabsorption of calcium is independent of hormonal regulation. PTH stimulates the distal reabsorption of calcium and stimulates the hydroxylation of 25(OH)D leading to the formation of 1,25(OH)2D. This is done by a stimulation of the 1-alpha hydroxylase by PTH. Calcitriol facilitates the reabsorption of calcium in the kidney. Calcitonin increases the renal excretion of calcium.

An increase in free ionized calcium stimulates negative feedbacks which lead to decreased release of PTH, decreased activation of vitamin D in the kidney, and increased release of calcitonin. 1,25(OH)2D functions as a regulator of calcium and phosphorus homeostasis, through its direct actions on gene expression in intestine, kidney, and bone.[26]

1.A.5 Non Calcium-Related Actions Of Vitamin D

Vitamin D deficiency is known to be associated with rickets in children, osteomalacia in adults, osteoporosis, and, although less consistently, with bone fracture. But recently, a growing body of evidence supports the role of vitamin D in the risk of many other chronic illnesses, including common cancers, autoimmune and infectious diseases.[17, 27] For example, vitamin D deficiency (25(OH)D < 20 ng per mililiter, ng/ml, conversion factor for 25(OH)D: 1 ng/ml = 2.496 nmol/l) seems to increase the risk (30 to 50%) of incident colon, prostate, and breast cancer, as well as mortality from these cancers.[28]

Vitamin D deficiency has also been associated with cardiovascular disease (CVD). The epidemiological evidence of a relationship between vitamin D and CVD has been discussed by Guessous et al.[3] For example, epidemiological studies suggest that vitamin D deficiency is associated with myocardial infarction, stroke, congestive heart failures, and hypertension.[1, 17]

Molecular evidence revealed actions of 1,25(OH)2D on mechanism related kidney function. These mechanisms include a direct inhibition of 1,25(OH)2D on the RAS and NF-kappaB (NF-kB) pathways.

NF-kB is a family of transcription factors that functions as a master regulator of immune response.[29] It regulates a wide range of genes involved in inflammation, proliferation and fibrogenesis and is known to have a key role in kidney disease.[30]

Mechanisms by which vitamin D might be associated with chronic kidney disease (CKD) have been summarized in the **figure 3** adapted from a recent review by Guessous et al.[17]

FIGURE 3. POSSIBLE MECHANISMS OF ASSOCIATIONS BETWEEN VITAMIN D AND CHRONIC KIDNEY DISEASE

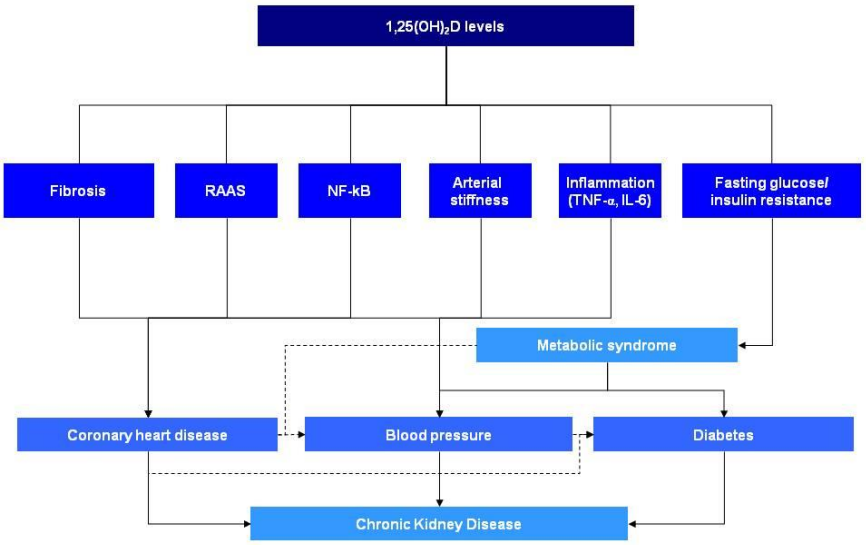


Figure adapted from reference[17]

Other vitamin D proprieties can influence the risk of CKD. Vitamin D seems to favor less inflammation by decreasing IL-6, IL-12, IFN-c, and TNF-alpha production and increasing IL-10.[31] Vitamin D modulates the expression of tissue matrix metalloproteinases (MMPs).[32] MMPs are connective tissue enzymes secreted by macrophages during inflammatory responses. MMPs break down collagen within the atherosclerotic lesion and cause lesion rupture, leading to thrombosis. We reported that plasma MMP-9 levels are inversely related to vitamin D status.[32]

Watson et al have reported an inverse correlation between serum vitamin D levels and coronary calcification,[33] which also suggests a protective role of vitamin D in atherogenesis.

The quasi-ubiquitous distribution of VDR in the human tissues and also the high prevalence of vitamin D deficiency worldwide have generated much enthusiasm about the opportunity of CVD prevention through vitamin D supplementation. However, as discussed by Guessous & Bochud,[3] reported associations between vitamin D and CVD present important limitations and are prone to confounding and reverse causation. In addition, the very large distribution of VDR has been recently challenged.[34]

1.A.6 Vitamin D Status

25(OH)D is the major circulating form of vitamin D. 25(OH)D levels reflect the overall vitamin D status from combined sunlight, diet and dietary supplement.[1] Sunlight exposure is the major (80%-90%) source of 25(OH)D. Diet contributes only between 10% and 20% to 25(OH)D levels, but becomes more important when sunshine exposure is low.[35] 1,25(OH)2D is the active form of vitamin D, but because 25(OH)D has a much longer circulating half life, circulates at higher concentrations, and is less under the influence of other hormones such as the PTH, 25(OH)D is used to determine vitamin D status. Because of its long half life in the circulation, 25(OH)D reflects vitamin D supply and usage over a period of time. Circulating 25(OH)D is also a better marker of vitamin D exposure than indirect estimates of vitamin D exposure based solely on diet, which does not take into consideration sunlight sources.[36]

1.A.7 Vitamin D Deficiency

Whenever there is little exposure to sun sufficient vitamin D levels cannot be reached without vitamin D fortification or supplements (e.g. 400 IU vitamin D supplement).[37, 38]

The definition of vitamin D deficiency is currently subject to debate.[34] There is a lack of consensus as to optimal vitamin D status as determined by 25(OH)D concentration. Because it seems that the sequelae of vitamin D deficiency occurs at a higher level than previously thought, many experts have proposed that minimum acceptable serum levels be increased to at least 20 ng/ml.[39, 40] For example, during the 2009 14th Workshop on Vitamin D the position was that an absolute minimum 25(OH)D level of 20 ng/ml (50 nmol/l) is necessary in all individuals in order to support and maintain all the classic actions of vitamin D on bone and mineral health and that, according to this criterion, a large proportion of the world's population is vitamin D deficient. In an editorial, Vieth et al. noted a frustrating and regrettable situation for nutrition researchers. In the early 1970s, a given serum 25(OH)D concentrations were thought to be indicative of "healthy" white adults in the United Kingdom. During those early years, the adequacy of 25(OH)D serum concentration was based simply on whether the concentration was enough to prevent osteomalacia or rickets. The authors of the editorial stressed that three decades later, several scientists think that 25(OH)D concentrations relate to many other aspects of health and that much higher concentrations of 25(OH)D are needed to prevent adverse outcomes.

Several definitions of vitamin D deficiency exist; some of them are presented in the **table 1**. The reasons for the differences in definition are, in part, due to the fact that the outcomes for which the cutoff levels were defined were different (e.g. bone fracture, PTH level, CV events, cancers). It is very unlikely that the risk of these outcomes increases at the same 25(OH)D level. Given the wide variation in the vitamin D deficiency definition, Pitz et al. suggested that the ideal 25(OH)D concentration for

overall health-related outcomes ranges between 40 nmol/l and 120 nmol/l. In fact, the range seems to be more between 40 and 80 nmol/l according to a recent work that suggests a J curve between vitamin D level and overall mortality.[41] The debate on the ideal vitamin D concentration has also increased after the 2011 Institute of Medicine (IOM) report. The IOM suggested that a lower level of 25(OH)D level should be used to define vitamin D deficiency.

TABLE 1. EXAMPLES OF VITAMIN D STATUS DEFINITIONS
Expressed in nmol/L (conversion factor for 25(OH)D: 1 ng/ml = 2.496 nmol/l)

Mayo clinic[42]		***Institute of Medicine (IOM)***[43]		***Pilz et al.***[44]		***Swiss Federal Office of Public Health***[45]	
Severe deficiency	<25	**At risk of deficiency**	<30	**Deficiency**	<50	**Acute deficiency**	<25
Moderate deficiency	25-59.9	**At risk of inadequate level**	30-49	**Insufficiency**	50-74.9	**Deficiency**	25-49
Optimal	60-200	**Sufficient**	50-125	**Optimal**	75-100	**Deficiency**	<50
Possible toxicity	>200	**Possible toxicity**	>125	**Sufficiency**	75-250	**Sufficiency**	50
				Intoxication	>375-500	**Target level to reduce fracture risk**	75

1.A.8 MAJOR FACTORS INFLUENCING VITAMIN D LEVELS

Below, major factors influencing vitamin D levels are discussed.

1) Age: skin production of vitamin D decreases with age[4]; 2) Body mass index: the reasons for the inverse association between body mass index (BMI) and 25(OH)D level are not completely understood. But fat in the skin seems to decrease the efficacy of vitamin D synthesis potentially because of a 7-dehydrocholesterol sequestration. Of note, the inverse association could also be confounded by a decrease in sun exposition (e.g. decrease in outdoor physical activity, comorbidities); 3) Latitude and seasons: UVB exposure decreases from equator towards the polar regions.[46] At around 0^{o} latitude (i.e., equator, e.g. The Republic of the Seychelles), a high level of vitamin D effective UV radiation is found and varies only slightly during the year. On the other hand, at around 40^{o} latitude (e.g. Switzerland has a latitude of 47^{o}) the level of vitamin D effective UV radiation varies greatly during the year and decrease substantially during the winter.[47] Theoretically, persons living in regions closer to the equator should present higher levels of vitamin D photosynthesis than persons in regions remote from the equator. In practice however, since more than 90% of vitamin D arises from sunlight (in absence of supplementation), vitamin D levels also depend on cultural behaviors (clothing, time spent outdoor, sunbathing habits). Overall, the effect of latitude on serum 25(OH)D is inconsistent.[48-50] A positive correlation between 25(OH)D and latitude was found (r^2=0.42) in a (25 European countries) pooled analysis, whereas a highest rate of 25(OH)D deficiency was observed in Scottish participants (highest latitude) in a British cross-sectional study.[49] A recent meta-regression did not find an influence of latitude on 25(OH)D levels;[50] 4) Skin Pigmentation: the packaging and size of melanosomes in the

keratocytes influence darkness of the skin. Dark pigment in the skin reduces the skin's ability to synthesize vitamin D from sunlight by up to 95%.[46] There is even a theory on the vitamin D synthesis capacity and human pigmentation variation and adaptation during human migration from sun-rich regions (Africa) to the less sun-intensive regions. Dark-skinned people need 5-10 times as much exposure to synthesize the same amount of 25(OH)D as light-skinned people. This likely explains why African-Americans have lower 25(OH)D than Non-Hispanic whites in the US.[46] The darker skin pigmentation in southern as compared to northern European countries may underlies the higher prevalence of 25(OH)D deficiency in southern Europe;[51] 5) Diet: diet contains only small amounts of vitamin D (i.e., vitamin D_3 or vitamin D_2). Fish is the major dietary source of vitamin D in humans. Three ounces of cooked salmon and 3.5 ounces of cooked mackerel provide respectively 90% and 86% of the recommended daily vitamin D intake (400-600 International Units /day), whereas 3.5 ounces of cooked beef only provide 4% of the recommended intake; 6) Occasional sunscreen: sunscreen use by children and young adults is unlikely to cause vitamin D deficiency, but chronic use of sunscreen by elderly has been shown to decrease 25(OH)D and to cause vitamin D deficiency. At higher altitudes, UVB radiations are stronger because the concentration of aerosols and particles are lower. Air pollution decreases vitamin D effective radiation. Ozone, time of the day, and cloud cover also influence vitamin D effective radiation and thus vitamin D photosynthesis.

1.B Chronic Kidney Disease

1.B.1 Definition of Chronic Kidney Disease

Chronic kidney disease (CKD) is defined as the persistence for 3 or more months of structural and/or functional abnormalities of the kidney (**Table 2**).[52] CKD is a

nonspecific term that does not include the cause for the injury or impaired kidney function. Structural abnormalities include i) overt proteinuria or microalbuminuria; ii) abnormal urinary sediment; or iii) abnormal findings on imaging tests. Proteinuria refers to increased concentration of albumin and other proteins. Microalbuminuria is a subclass of proteinuria due to increased albumin excretion that can be detected by more sensitive laboratory methods than urine dipstick. Functional abnormality relies on creatinine clearance estimates derived from the Modification of Diet in Renal Disease (MDRD) or the CKD-Epidemiology (CKD-EPI) glomerular filtration rate (GFR) estimation equations.[53, 54] GFR measured the volume of plasma filtered at the glomerulus per unit of time. GFR cannot be measured directly but is estimated by the clearance of nonmetabolized markers.

TABLE 2 CHRONIC KIDNEY DISEASE, DEFINITION

CKD is defined by the persistent presence of either
1) Kidney damage or structural abnormalities*
Or
2) An estimated GFR ≤60 ml/min/1.73m^2, even in the absence of evidence of kidney damage or structural abnormalities

*Kidney structural abnormalities are defined as either microalbuminuria or overt proteinuria, abnormal urinary sediment, or abnormal imaging.

Stages and progression of CKD are generally based on the estimated GFR (eGFR). Although other more accurate measurements (including inulin clearance, iothalamate clearance, cystatin C) exist, they are more expensive for use in clinical

routine or epidemiological studies. The MDRD and CKD-EPI equations are widely accepted as a measure of kidney function,[53, 54] and widely used in clinical practice. Interpretations of results should however always take into consideration the possible imprecision of creatinine-based GFR in the near-normal range and the dependence on laboratory performance while measuring serum creatinine.

Kidney functions should also be based on proteinuira when available. Proteinuria and albuminuria are powerful predictors of renal and cardiovascular outcomes, including cardiovascular deaths,[55] cardiac ischemic events, coronary artery disease,[56] survival after myocardial infarction, stroke and onset of type 2 diabetes.[57]

Albuminuria can be estimated by 24-hour urinary collection or by urinary spot. Microalbuminuria and macroalbuminuria are defined as a urine protein concentration between 30 mg/24h and 300 mg/24h and greater than 300 mg/24h, respectively. Using urinary spot, overt proteinuria is defined as a spot urine albumin-to-creatinine ratio (ACR) > 250 mg/g in men and >355 mg/g in women, and microalbumiuria is defined as an ACR of 17-250 mg/g in men and 25-355 mg/g in women. The American Diabetes Association proposed definitions that are not gender specific; spot urine ACR >30 and 300 mg/g for microalbuminuria and proteinuria, respectively.

MDRD and CKD-EPI do not provide precise estimates of GFR and thus CKD is best classified by stage rather than by derived value of GFR. The degree of impaired kidney function is generally ranked into stages 1 to 5 as described in **table 3** (K/DOQI guidelines).

TABLE 3. CHRONIC KIDNEY DISEASE, STAGES

Stage	eGFR (ml/min/1.73 m^2)	
1	>90	+ structural abnormalities for at least 3 months
2	60-90	+ structural abnormalities for at least 3 months
3	30-59 for at least 3 months	
4	15-29 for at least 3 months	
5	<15 for at least 3 months	

1.B.2 ETIOLOGIES OF CHRONIC KIDNEY DISEASE

Major risk factors of CKD are presented in the **table 4**. Risk factors for CKD include modifiable and non-modifiable factors. Below, major CKD risk factors are discussed and a more extensive review is presented elsewhere.[58]

TABLE 4. CHRONIC KIDNEY DISEASE, RISK FACTORS

Modifiable risk factors	**Non modifiable risk factors**
Hypertension	Age
Diabetes	Gender
NSAIDs use	Family history of kidney failure
Obesity	Ethnicity
Metabolic syndrome	
Smoking	
Nephrotoxoc agents (e.g., radiocontrast, aminoglycosides)	

1) Age: the risk of CKD increases with age. The mortality rate among older patients with CKD is about seven times the rate of older patients without CKD.[59, 60] Rate of decline in GFR is about 3-4 mL/min/year and this rate is quite similar across different baseline GFR. It is worth noting that at all levels of baseline GFR, some people have a stable or positive GFR slope; 2) Hypertension, including essential hypertension, is a major risk factor of CKD. The relative risk of CKD among patients with high BP compared to normal pressure is about 2.5;[61] 3) Diabetes increases the risk of CKD and the recent diabetes epidemic is assumed to be responsible for an important part of the increase in end-stage renal disease (ESRD) incidence.[62] The prevalence of diabetes is between 3% and 6.5% for CKD stage 1 and increases to around 20% in CKD stage 4.[62]

In a community-based prospective observational study of 20-year duration and involving 23,534 men and women in the US, the adjusted hazard ratio (95% confidence interval, 95%CI) of developing CKD among women was 2.5 (0.05 to 12.0) for normal BP, 3.0 (0.6 to 14.4) for high-normal BP, 3.8 (0.8 to 17.2) for stage 1 hypertension, 6.3 (1.3 to 29.0) for stage 2 hypertension, and 8.8 (1.8 to 43.0) for stages 3 or 4 hypertension compared with individuals with optimal BP. In men, the relationship was similar but somewhat weaker than in women, with corresponding hazard ratios of 1.4 (0.2 to 12.1), 3.3 (0.4 to 25.6), 3.0 (0.4 to 22.2), 5.7 (0.8 to 43.0), and 9.7 (1.2 to 75.6), respectively. The adjusted hazard ratio of developing CKD among women with diabetes was 10.7 (6.0 to 19.0) compared with women without diabetes. In men, the adjusted hazard ratio among men with diabetes was 5.0 (3.0 to 10.0) compared with men without diabetes;[63] 4) A family history of CKD increases the risk of both CKD and ESRD. The risk of ESRD increases by about 30% if a single first-degree relative with CKD is reported to a ten

time-fold if two or more first-degree relatives are reported; 5) In the US, African-American race and Hispanic ethnicity have been associated with an increased risk of CKD and ESRD when compared to non-Hispanic whites. Ethnicity-related increased risks are in part explained by higher prevalence of diabetes and hypertension among African-americans and/or Hispanics, but given that differences seemed to persist after controlling for hypertension and diabetes, other reasons including true biologic difference, socio-economic status and effect of low birth weight may contribute to this disparity;[62] 6) Increased BMI is associated with an increased risk of CKD and ESRD. An European study reported a threefold-increased risk of elevated serum creatinine in patients with BMI equal of greater than 25 kg/m^2.[64] More generally, each unit increase in BMI seems to be associated with a 5% increase in CKD risk; 7) Smoking is associated with albuminuria and abnormal renal function in both non-diabetic and diabetic persons;[63, 65] 8) Agents, such as antibiotics (e.g. aminoglycosides), NSAIDs, and contrast used in imaging, increase the risk of both acute and CKD; 9) Other factors like proteinuria can promote CKD and should ideally be considered when assessing kidney function. Of note, in industrialized countries, hypertension and diabetes account for 75% and 25% of the CKD etiologic fraction, respectively. The identification of modifiable risk factors suggests that progressive CKD is not inevitable and that mediating factors could modify the rate of CKD progression.

1.B.3 Health Consequences of Chronic Kidney Disease

Kidney disease is the 12th leading cause of death worldwide and the 9th leading cause of death in the USA.[66] CKD is associated with an increased risk of ischemic heart disease, stroke, peripheral vascular disease, anemia, bone disease, ESRD, and mortality.

The prevalence of these factors increased as eGFR declines. For example, the 3-year CVD risk increased from 2.1% to 14.1% for CKD stage 1 to 5. While the highest prevalence of CV risk factors (e.g. hypertension, diabetes), nutritional risk factors (e.g., hypoalbuminemia), and bone disease risk factors (e.g., hypocalcemia, hyperphosphatemia) are more frequent in advanced CKD, the prevalence of these risk factors among stage 3 –which includes 85% of CKD stage 3 to 5 patients are– is not negligible. Among patients with CKD stage 3, half have hypertension, 17% have diabetes, and 6% have hypoalbuminuria. In addition, the 5-year mortality rate among CKD stage 3 patients is as high as 25%. Moreover, clinical trials have also shown that even incipient renal failure is an important independent cardiovascular risk factor.[67-70]

1.B.4 Prevalence, Time Trends, And Risk Of Chronic Kidney Disease

Throughout the world, an epidemic of ESRD (defined as the cessation of effective kidney functions and the substitution of renal replacement therapy for native kidney functions to sustain life) has occurred.[62] This epidemic is associated with substantial decrease in life expectancy and quality of life and increase in cost impacts. In addition to an increase in renal replacement therapy and a decrease in competing risks, the exponential growing rate of ESRD incidence is probably due to an increase in CKD incidence.

Data on CKD trends are limited. Iseki et al. compared the prevalence of CKD from the 1993 (N=143,948) and 2003 (N=154,019) mass screenings in Okinawa, Japan. CKD prevalence was compared using eGFR calculated by the MDRD equation. The prevalence of CKD (eGFR<60 ml/min/1.73 m^2) was similar between the two periods,

being 15.7% in 1993 and 15.1% in 2003.[71] In the US, Coresh et al. compared prevalence of CKD from two surveys of NHANES III conducted from 1988 to 1994 and 1999 to 2000, separately.[72] They reported that the overall prevalence of CKD (GFR 15 to 59 ml/min per 1.73 m^2, corresponding to CKD stages 3 and 4 was high but similar in both surveys (4.5% from 1998 to 1994, 3.8% from 1999 to 2000) and the proportions of CKD were also comparable with respect to gender and ethnicity. However, in a more recent analysis, the same investigators reported that the prevalence of CKD in the US in 1999-2004 was actually meaningfully higher than it was in 1988-1994.[73] This increase was partly explained by the increasing prevalence of diabetes and hypertension. The prevalence of CKD stages 1 to 4 increased from 10.0% (95% confidence interval [CI], 9.2%-10.9%) in 1988-1994 to 13.1% (95% CI, 12.0%- 14.1%) in 1999-2004 with a prevalence ratio of 1.3 (95% CI, 1.2-1.4). The prevalence estimates of CKD stages in 1988-1994 and 1999-2004, respectively, were 1.7% (95% CI, 1.3%-2.2%) and 1.8% (95% CI, 1.4%-2.3%) for stage 1; 2.7% (95% CI, 2.2%- 3.2%) and 3.2% (95% CI, 2.6%-3.9%) for stage 2; 5.4% (95% CI, 4.9%-6.0%) and 7.7% (95% CI, 7.0%-8.4%) for stage 3; and 0.21% (95% CI, 0.15%-0.27%) and 0.35% (0.25%-0.45%) for stage 4. A higher prevalence of diagnosed diabetes and hypertension and higher BMI explained the entire increase in prevalence of albuminuria but only part of the increase in the prevalence of decreased eGFR. Experts have raised concerns about future increased incidence of kidney failure and other complications of CKD.

Although large ESRD surveillance system exists in the US (e.g., USRDS) and Europe (e.g., ERA-EDTA), there are few surveillance systems for stage 1-4 CKD. The largest CKD database is derived from the Kidney Early Evaluation Program (KEEP)

data, a National Kidney Foundation sponsored ongoing study started nine years ago. The study population of KEEP is, however, not the general population but a target population of adults with a history of diabetes or hypertension or a first-order relative with diabetes, hypertension, or kidney disease.

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CHAPTER 2

PROJECT-SPECIFIC STUDY AIMS AND LITERATURE REVIEW

PROJECT 1

1) TO DETERMINE THE CKD SPECIFIC-PREVALENCE AND DETERMINANTS OF VITAMIN D DEFICIENCY IN SWITZERLAND

- HYPOTHESIS #1A : THE PREVALENCE OF VITAMIN D DEFICIENCY IS HIGHER AMONG SUBJECTS WITH CKD THAN SUBJECTS WITHOUT CKD. HYPOTHESIS #1B : THE DETERMINANTS OF VITAMIN D DEFICIENCY IN SUBJECTS WITH AND WITHOUT CKD ARE DIFFERENT

PROJECT 2

1) TO DETERMINE THE CHANGE IN KIDNEY FUNCTION ASSOCIATED WITH BASELINE SERUM VITAMIN D

- HYPOTHESIS #1 : LOW BASELINE VITAMIN D LEVELS ARE ASSOCIATED WITH GREATER DECLINES IN EGFR THAN HIGH BASELINE VITAMIN D LEVELS

IN SECONDARY ANALYSES:

2) TO DETERMINE THE ASSOCIATION OF BASELINE VITAMIN D LEVELS WITH RISK OF RAPID EGFR DECLINE AND CKD, 3) TO DETERMINE THE ASSOCIATION OF BASELINE VITAMIN D LEVELS WITH CHANGE IN CKD STATUS

PROJECT 3

1) TO EXAMINE THE *VDR* GENE- VITAMIN D LEVEL INTERACTION ON THE CHANGE OF KIDNEY FUNCTION ASSOCIATED WITH BASELINE SERUM VITAMIN D

- HYPOTHESIS #1 *VDR* GENETIC VARIANTS MODIFY THE EFFECT OF BASELINE VITAMIN D LEVEL ON THE CHANGES IN KIDNEY FUNCTION

Current knowledge and evidence are specifically presented in the introduction and discussion sections of chapter 3 (project 1), 4 (project 2) and 5 (project 3). Below, general reviews related to the three projects are presented.

2.A Literature Review Related to Project 1

2.A.1 Prevalence of Vitamin D deficiency

25(OH)D deficiency is prevalent in different regions of the world,[1] but major differences in the prevalence of vitamin D deficiency has been reported across regions and populations; from 15% to 80%.[1-4]

The prevalence is also function of how deficiency is defined.[4] A definition frequently used in epidemiological studies is vitamin D deficiency: 25(OH)D level < 20 ng/ml (50 nmol/l)), and vitamin D relative insufficiency: 25(OH)D level between 20 and 30 ng/ml (50 to 75 nmol/l). Using these definitions, it is estimated that 1 billion people worldwide, 40 to 100% of U.S. and European elderly men and women, and more than 50% of postmenopausal women have vitamin D deficiency.[5] Children, and particularly obese children, are also at risk of vitamin deficiency. Vitamin D deficiency was identified in 74% of 127 obese children and adolescents (age, 6.0-17.9 years).[6] Bodnar et al. reported vitamin D deficiency and insufficiency occurred in 29.2% and 54.1% of black women and 45.6% and 46.8% black neonates, respectively.[7] Five percent and 42.1% of white women and 9.7% and 56.4% of white neonates are vitamin D deficient and insufficient, respectively.[7] Thus, black and white pregnant women and neonates residing in the northern US are clearly at high risk of vitamin D insufficiency. In Europe, the mean level of 25(OH)D varied by countries; 20 ng/ml in France, 18 ng/ml in Italy, 30

ng/ml in Norway, and 27 ng/ml in Sweden.[8] In addition to latitude and BMI, the variations are probably due to differences in diet and behaviour to sun exposition.

Although limited, additional data suggest that the prevalence of vitamin D deficiency and relative insufficiency have increased this last decade. Vitamin D status of the US population was assessed more than a decade ago and again in 2008. Looker et al. used data NHANES to compare serum 25(OH)D concentrations in the US population in 2000–2004 with those in 1988–1994 and to identify contributing factors.[9] Age-adjusted mean serum 25(OH)D concentrations were 5–20 nmol/L lower in NHANES 2000–2004 than in NHANES III (1988-1994). Adjustment for changes in the factors likely related to real changes in vitamin D status (i.e., BMI, milk intake, and sun protection) changed mean serum 25(OH)D concentrations by 1–1.6 nmol/L. Therefore, the authors concluded that in adults, combined changes in BMI, milk intake, and sun protection appeared to have contributed to a real decline in vitamin D status.[9]

2.A.2 Prevalence of Vitamin D deficiency in Switzerland

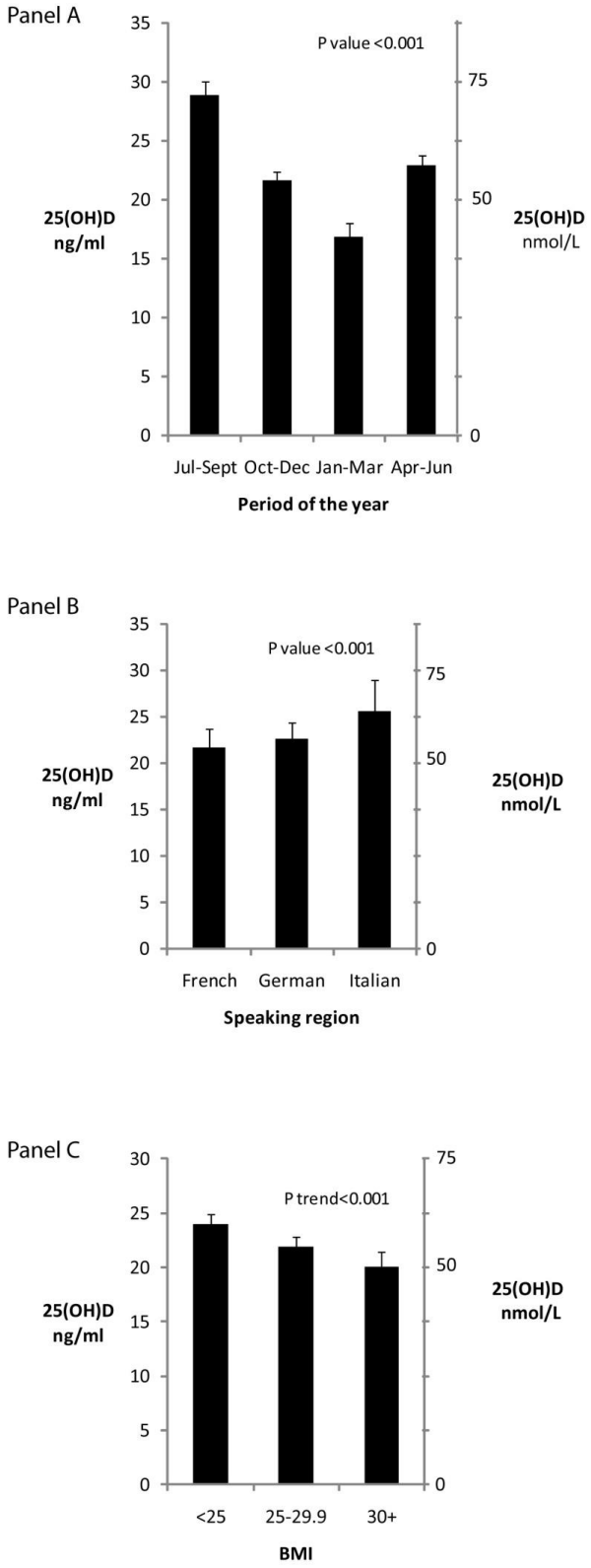
In Switzerland, a population-based estimation of vitamin D deficiency, based on 25(OH)D serum levels, was made more than 20-year ago (Swiss MONICA project, N=3276)).[10] At this time, 6% of the population were clearly deficient (ie, 25(OH)D ≤ 20 nmol/L), and a further 34% had a low concentration of vitamin D (ie, < 38 nmol/L).

Guessous et al. estimated the 25(OH)D distribution and updated the prevalence of vitamin D insufficiency (defined as 25(OH)D between 20 ng/ml and 29.9 ng/ml) and deficiency (defined as 25(OH)D <20 ng/ml) in the Swiss adult population. Factors associated with vitamin D status in the Swiss adult population were also identified.[11] Data

from the 2010-2011 Swiss Study on Salt intake, a population-based study in the Swiss population, were used. Vitamin D concentration in serum was measured by liquid chromatography- tandem mass spectrometry. A total of 1,309 subjects were included in the analysis. Major known factors that influence 25(OH)D levels, such as sunshine hours, altitude, latitude, ethnicity, physical activity, diet and supplements were taken into account. The adjusted 25(OH)D mean was associated with the period of the year and speaking regions (**Figure 1**). Overall, the adjusted 25(OH)D mean was 29.0 ng/ml (28.0-30.1), 22.1 (21.0-23.2), 17.2 (16.1-18.3), and 23.2 (22.3-24.1), respectively, in the July-September, October-December, January-March, and April-June periods (P-value<0.001)(**Figure 1**). Differences across periods of the year remained significant when restricted to participants without vitamin D supplements or treatments (28.9 ng/ml [27.8-30.0], 21.6 [20.5-22.8], 16.9 [15.8-18.0] and 22.9 [22.0-23.8], P value<0.001).

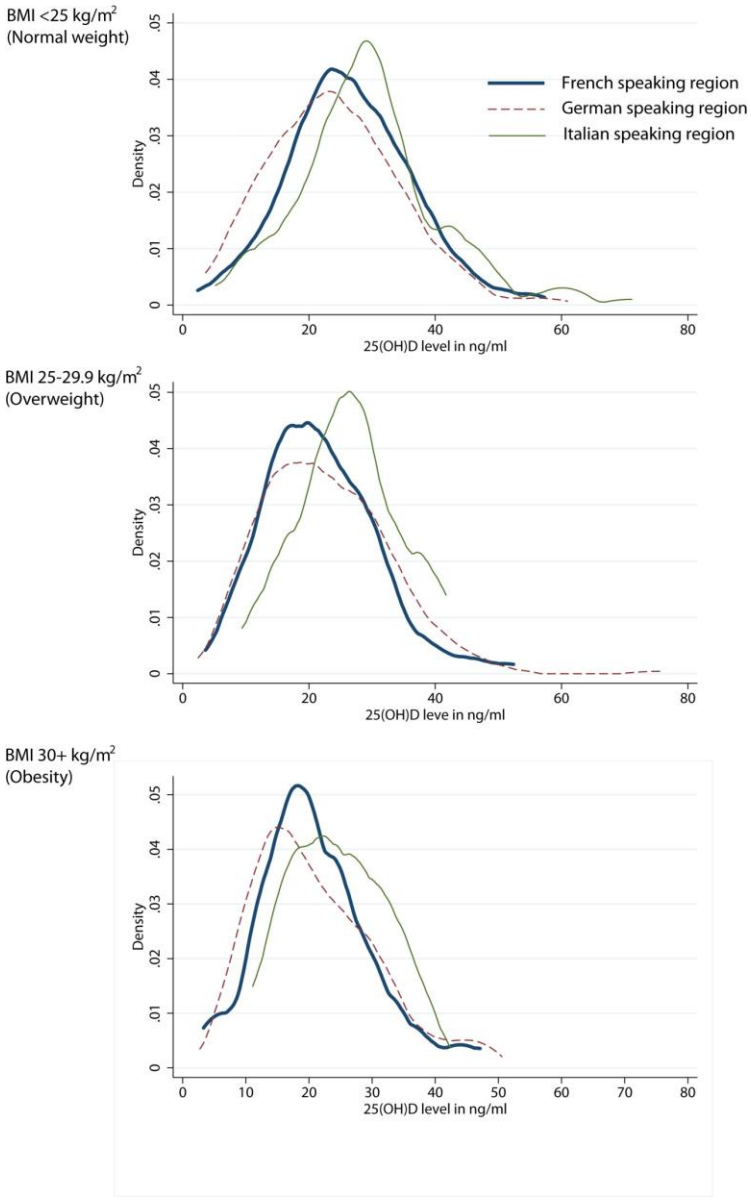
Adjusted 25(OH)D means also differed by BMI categories (**Figure 1**). Among all subjects, the adjusted mean of 25(OH)D was 24.3 ng/ml (23.6-25.1), 22.3 (21.3-23.2), and 20.3 (19.0-21.7), respectively in the normal, overweight, and obese categories (P-value<0.001).

FIGURE 1 ADJUSTED MEANS OF 25(OH)D BY PERIOD OF THE YEAR (PANEL A), SPEAKING REGION (PANEL B), AND BODY MASS INDEX CATEGORY (PANEL C) from reference [11]



For each BMI categories, the distribution of 25(OH)D levels in the Italian-speaking region was shifted to higher values when compared with the French and German-speaking regions' distributions (**Figure 2**).

FIGURE 2 DISTRIBUTIONS OF 25(OH)D BY SPEAKING REGION AND BODY MASS INDEX CATEGORY, from reference [11]



The prevalences of vitamin D insufficiency and deficiency were the highest in the January-March period; 26.4% (21.6-31.7) and 61.6% (56.0-67.0). The prevalence of vitamin D insufficiency or deficiency was highest among men throughout all period of the year; in the January-March period, more than 9 of ten men were vitamin D insufficient or deficient. Among participants with obesity (i.e. BMI $\geq$ 30 kg/m^2), the prevalence of vitamin D insufficiency and deficiency in the January-March period were 21.3% (11.8-35.2) and 68.1% (53.6-79.8), respectively. The Italian-speaking region stood up with the lowest prevalence of vitamin D insufficiency or deficiency (74.2%, 76.4%, and 59.8% in the French-, German-, and Italian-speaking regions, respectively, p value <0.001).

Determinants of vitamin D in the Swiss general population

Multivariate associations of participants' characteristics with vitamin D month-specific tertiles were conducted. The 25(OH)D concentrations fluctuation due to seasonal variation in sun exposure and the use of a single blood sample to estimate long term 25(OH)D average may lead to misclassification bias. To analyze the associations of covariates of interest with vitamin D status, month-specific percentiles (here tertiles) of 25(OH)D were used as recommended.[12] BMI was negatively associated with vitamin D month-specific tertiles; each unit increase of BMI was associated with an 8% decreased likelihood of being in a higher vitamin D tertile. Oral contraceptive, altitude, urinary excretion of calcium, use of vitamin D supplement or treatment, high wine consumption, physical activity were positively associated with vitamin D month-specific tertiles. Compared to the French-speaking region and after adjustment, the Italian-speaking

region was associated with a higher likelihood of being in a higher vitamin D tertile (OR: 1.66, 95%CI 1.14-2.43).

2.A.3 Prevalence and determinants of vitamin D deficiency in the Chronic Kidney Disease population

In summary, vitamin D deficiency is frequent in the general population and determinants of vitamin D deficiency have been identified. Some studies have reported that vitamin D deficiency is even more prevalent among populations with kidney failure than in the general population.[13, 14] Compared to the population without CKD, patients with CKD might also have decreased sunlight exposure and sub-optimal vitamin D intake from the diet.[15]

While prevalence and the determinants of vitamin D deficiency in the Swiss general population were determined with the previous Swiss study, it remains unknown whether the prevalence of vitamin D deficiency and / or the determinants of vitamin D deficiency are similar among people with CKD.

Thus, we aimed in the Project 1 to determine the CKD specific-prevalence and determinants of vitamin D deficiency in Switzerland.

2.B Literature Review Related to Project 2

2.B.1 Vitamin D And Kidney Function

Association between vitamin D and kidney function first came from therapeutic studies in patients with vitamin D deficiency secondary to kidney failure such as CKD. The kidney 1 alpha hydroxylation is decreased in kidney failure, and patients with ESRD typically suffered from 1,25(OH)2D vitamin D deficiency, while usually keeping a normal 25(OH)D level.[16] Vitamin D therapy is often prescribed to ESRD patients with secondary 1,25(OH)2D deficiency. Retrospective studies suggest that vitamin D therapy (such as alfacalcidol, doxercalciferol, paricalcitol, and calcitriol) improves outcomes in adults with CKD.[17-21]

An antiproteinuric effect of oral vitamin D therapy in CKD have been reported in RCTs,[22-24] but only one RCT reported a favorable effect of vitamin D therapy on GFR (The VITAL study).

In addition to the antiproteinuric effect (through preservation of glomerular podocyte structure),[25] a possible mechanism of the clinical renoprotective effect of vitamin D therapy is related to the renin-angiotensin system (RAS).[26, 27] More recently, low 25(OH)D levels have been associated with the development of CKD, [28] even after adjustment for CKD risk factors.

The potential causal role of vitamin D deficiency on kidney failure has only been explored these last years. In 2009, Ravani et al. showed that baseline low 25(OH)D levels were associated with an increase risk of ESRD[29] compared to patients with normal 25(OH)D levels**.** In a prospective cohort study of 9,000 hemodialysis patients, vitamin D therapy appeared to be associated with survival.[30]

Given the major relationship between BP and kidney function, the molecular evidences of vitamin D actions with both blood pressure (BP) and kidney function is discussed below:

1) Vitamin D and blood pressure (molecular evidences):

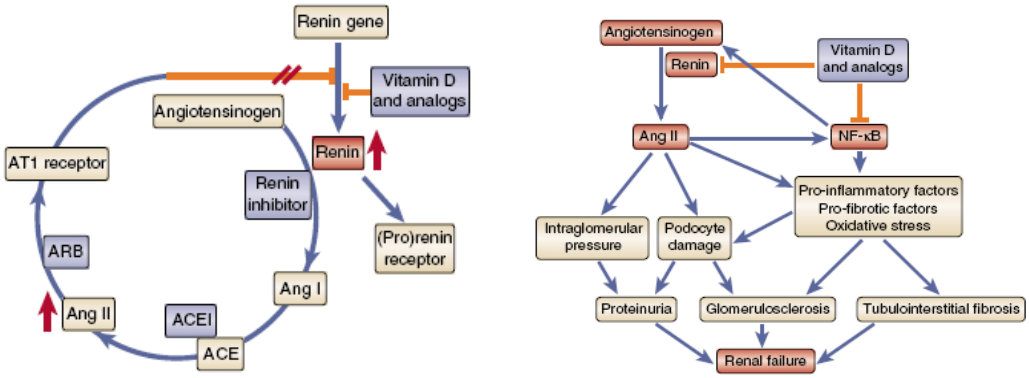
Molecular evidence revealed actions of 1,25(OH)2D on mechanism related to kidney function and BP (**Figure 3**). These mechanisms include a direct inhibition of 1,25(OH)2D on RAS and nuclear factor-kappaB (NF-kB) pathways. The RAS plays an important role in BP regulation.[25] The renin (synthetized in the juxtaglomerular cells of the kidney) cleaves angiotensinogen into angiotensin I, which is then converted into angiotensin II by the angiotensin-converting enzyme. Angiotensin II increases BP through multiple mechanisms including vasoconstriction, aldosterone and antidiuretic hormone synthesis.

The VDR is expressed in the juxtaglomerular apparatus and modulates renin synthesis. Mice in which VDR was abolished are hyperreninemic and present high BP and cardiac hypertrophy.[31] By contrast, when VDR was overexpressed in the mouse juxtaglomerular apparatus, hyporeninemia was noted.[32] Vitamin D could also potentially contribute to arterial stiffening and hypertension.[33]

NF-kB is a family of transcription factors that functions as a master regulator of immune response.[34] It regulates a wide range of genes involved in inflammation, proliferation and fibrogenesis and is known to have a key role in kidney disease.[35] Both RAS and the NF-kB promote the production of pro-fibrotic and pro-inflammatory factors, increase oxidative stress, and damage podocytes. In addition to the suppression of the

RAS, Vitamin D can regulate BP through the prevention of secondary hyperparathyroidism and effect on calcium metabolism.[36] Vitamin D seems to have a direct effect on vascular cells and endothelial function as well.[37, 38]

FIGURE 3 OUTLINE OF RENOPROTECTIVE AND BLOOD PRESSURE REGULATION MECHANISMS OF VITAMIN D
(Adapted from [39]). Note on the figure on the left: bold arrows highlight factors increasing BP.



2) Vitamin D and kidney function (molecular evidences):

Experimental studies suggest that vitamin D levels can directly or indirectly prevent kidney failure. Kidney failure typically results from three kidney lesions: 1) tubulointerstitial fibrosis, 2) glomerulosclerosis, and 3) proteinuria.[40]

The effects of paricalcitol (19-nor-1,25-hydoxy-vitamin D2), a synthetic vitamin D analogue, on tubulointerstitial lesions have been investigated in animal models. Compared with vehicle controls, paricalcitol significantly attenuated renal interstitial

fibrosis[41] and both 1,25(OH)2D and oxacalcitriol (another vitamin D analogue) ameliorates glomerulosclerosis with reduction of type I and IV collagenes in antibody-induced glomerulonephritis.[42] The reduction of albuminuria[22, 23, 43] or proteinuria[44] in CKD patients have been reported in three RCTs comparing placebo to vitamin D analogues. Oral calcitriol treatment also reduced proteinuria in patients with IgA nephropathy.[45] Yet, another mechanism by which vitamin D can presumably modify kidney function is through the suppression of the RAS. The RAS plays a major role in determining the rate of chronic renal progression.[46] In 1986, Resnick et al. already reported that serum level of 1,25(OH)2D was inversely associated with the plasma renin activity in normotensive and hypertensive subjects.[47] 1,25(OH)2D and paricalcitol were found to decrease angiotensinogen, renin, and renin receptor in animal models.[48, 49] Overall, this suggests that the beneficial effects of vitamin D analogues in chronic renal failure are due, in part, to down regulation of the RAS.

2.B.2 VITAMIN D AND BLOOD PRESSURE (EPIDEMIOLOGICAL STUDIES)

Given the close relationship between kidney function and BP, we reviewed the epidemiological studies on vitamin D, and BP. Molecular, animal and human studies have established that vitamin D is associated with CVD, including BP. This topic has been reviewed elsewhere.[50] Three RCTs assessed the efficacy of vitamin D supplementation on BP. Only one found a significant effect.[51] Compared with calcium alone (1,200 mg/day), vitamin D (800 IU/day) and calcium (1,200 mg/day) supplements resulted in 9.3% decreased systolic BP ($p = 0.02$) in a 8-week trial including 148 women (mean age 74 years) with a 25(OH)D level < 50 nmol/l.[51] In 2008, Wang et al.[52] investigated the associations of vitamin D intake with the incidence of hypertension in a

10-year prospective cohort of 28,886 US women aged 45 or more years. Vitamin D intake was assessed from food frequency questionnaire. The risk of hypertension decreased in the higher quintiles of dietary vitamin D, even after adjustment for dietary calcium intake. This observation was reported for vitamin D intake from diet, not from supplements. Most of the large cross-sectional studies show a significant inverse association between 25(OH)D levels and BP.[50] The number of prospective studies examining 25(OH)D levels and the incidence of hypertension or change in BP are limited and results are inconsistent. Most studies were small, had suboptimal BP measurement (e.g., single measure of BP), or did not control for potential confounders such as PTH.

In summary, the decline in renal function, such as observed in CKD, is associated with a progressive decrease in the ability of the kidney to produce 1,25-dihydroxyvitamin D. Vitamin D deficiency is therefore common in patients with kidney diseases, even in the early stages.[16] Growing evidence however suggest that the relation of vitamin D with renal function could also exist in the opposite direction, i.e. sufficient vitamin D levels could protect against renal function loss.[53]

So far, longitudinal studies that assessed the association of baseline circulating 25(OH)D with change in eGFR and incident CKD with conflicting results.[54, 55] Given the conflicting nature of results on the association of circulating vitamin D with change in eGFR and incident CKD, we aimed in the Project 2, to determine the change in kidney function associated with baseline serum vitamin D.

2.C Literature Review Related to Project 3

2.C.1 *VDR* Variants And End-Stage Renal Disease or Chronic Kidney Disease

The role of *VDR* genetic variants on kidney function has been explored only recently and current data are mostly limited to ESRD. The influence of *VDR* polymorphism on ESRD was notably investigated in 258 ESRD patients and 569 healthy controls (**Table 1**).[56] A significant difference in the frequencies of the ApaI, FokI and BsmI were found. In addition, the ApaI/TaqI/FokI/BsmI haplotype analysis revealed that subjects with a/t/F/b haplotype were at greater risk of ESRD (OR =11.0, 95%CI 1.38-87.7).[56] This study confirmed the B allele to be the risk allele as being previously reported in a case-control study including 222 subjects with and without ESRD.[57]

These results contrast with a previous report analyzing the influence of BsmI variants on PTH and 1,25(OH)2D in patient with different degrees of CKD before dialysis.[58] Most of the 248 patients included had moderate kidney failure (i.e., creatinine clearance 35-60 mL/min). In multivariate analyses, calcitriol levels were less reduced in the BB genotype and the progression of hyperparathyroidism was slower in patients with BsmI BB genotypes than in the other types.[58]

The large majority of *VDR* polymorphisms studies in CKD included hemodialysed patients. For example, De Souza et al. investigated the association between polymorphisms in the *VDR* gene and ESRD. Polymorphisms TaqI and BsmI in the *VDR* gene were analyzed. Allele G was associated with protection against ESRD. [Odds Ratio ESRD: (GA+AA) vs (GG) = 2.5, 95% CI = 1.4-4.6)].[57]

TaqI SNPs have been associated with iron status and hyperparathyroidism in ESRD patients.[59,60]

According to a search (March, 2013) on HuGE Navigator (http://www.hugenavigator.net), an online, curated and searchable knowledge base in human genome epidemiology, on the 14 eligible studies, only two assessed the association between *VDR* polymorphisms and non-ESRD CKD patients. The other 12 studies were limited to ESRD patients. In one of the two studies, the authors investigated the relationship between *VDR* FokI polymorphism and serum levels of PTH, 1,25(OH)2D, and calcium in 64 Spanish patients with CKD. The mean serum PTH level in the FF group was significantly higher (159.77+/-25.69 pg/mL) than in both the Ff and ff groups (106.67+/-19.07 and 77.55+/-15.85 pg/ml, respectively; $p<0.05$). However there were no significant differences in serum levels of 1,25(OH)2D or calcium among genotypes.[61] De Souza included patients with and without ESRD; they reported an association of BsmI with ESRD.[57]

2.C.2 *VDR* AND ALBUMINURIA

Albuminuria serves as an important predictive factor for the progression of kidney disease and for the development of CVD. The contribution of genetic variants, including *VDR*, to the development of albuminuria has been evaluated in 5321 participants from the second phase (1991-1994) of the Third National Health and Nutrition Examination Survey (NHANES III), a population-based and nationally representative survey of the United States.[62] Associations with albuminuria were reported for some genetic variants (*IL1B*, *CRP*, *NOS3*) but not for *VDR* variants.

2.C.3 *VDR* VARIANTS, VITAMIN SUPPLEMENTATION, AND KIDNEY FUNCTION

The pharmacogenomics of vitamin D and kidney function have been largely unexplored so far. To the best of our knowledge, there is no study that explored the *VDR* genetic effect modification of the association between 1,25(OH)2D and kidney function. Nor could we find study on the pharmacogenomics of vitamin D analogues with respect to kidney function. The lack of pharmacogenomics information on renal-related *VDR* pharmacogenomics is surprising given the association between vitamin D deficiency and CKD, and given the high prevalence of vitamin D supplementation / food fortification in North America.[63] There are inter-individual differences in vitamin D optimization and recent evidence of lack of response to oral vitamin D in subjects who harbor specific *VDR* variants. In postmenopausal women, *VDR* BmsI and TaqI polymorphisms were association with non response to vitamin D oral supplementation.[64] Given the ongoing debate on recommendations of daily allowance of vitamin D, notably for cardiovascular disease prevention, further pharmacogenomics and nutrigenomics data should be gathered including in patient with non ESRD CKD who might benefit from vitamin D.

2.C.4 VDR AND CKD MAJOR RISK FACTORS

Further information can be derived from the influence of *VDR* polymorphisms on CKD major risk factors; diabetes and hypertension. *VDR* polymorphisms have been associated with diabetes, fasting glucose, and blood pressure (**Table 1**).[65-70,71-75]

TABLE 1 SOME ASSOCIATIONS BETWEEN VDR POLYMORPHISMS AND DISEASE OR TRAITS RELATED TO KIDNEY FUNCTIONS

	Hypertension	**Diabetes**	**ESRD**
*Bsm*I			
	Muray et al.; 2003 (ref 74); N=590; bb genotype → ↑SBP in men, positive correlation between 25(OH)D and BP only in men with BB genotype	**Ortlepp et al.; 2003 (ref 65);** N=752; BB genotype → ↑ fasting glucose than Bb/bb	**De Souza et al.; 2007 (ref 57);** N=222; B allele → ↑ ESRD
	Lee et al.; 2001 (ref 75); N=933; BB/Bb genotype → ↑SBP and HTN in men	**Oh et al.; 2002 (ref 68);** N=1545; bb genotype → insuline resistance	**Tripathi et al.; 2010 (ref 56);** N=750; BB→ ↑ ESRD
*Apa*I			
		Hitman et al.; 1998 (ref 69); N=171, aa genotype → ↓ insulin secretion	
		Oh et al.; 2002 (ref 68); N=1545; aa genotype → ↑ diabetes	
*Fok*I			
		Ongunkolage et al.; 2002 (ref 70); N=143; FokI ff→ ↓ insulin secretion in subjects with 25(OH)D insufficiency; ff genotype → ↑ diabetes	**Tripathi et al.; 2010 (ref 56);** N=750; ff→ ↑ ESRD

2.C.5 *VDR*-VITAMIN D INTERACTION

Association studies performed so far have limitations, including the lack of statistical power to detect small effect of *VDR* polymorphism and the lack of gene-environment analyses. Environment factors like vitamin D exposure may meaningfully interact with *VDR* polymorphism and change the overall effect of *VDR* polymorphism on disease.

Large interindividual differences in vitamin D system exist. The vitamin D endocrine system could be the same across ethnic groups, but the variation could come from polymorphisms. Thus, one approach to understand interindividual differences in the vitamin D endocrine system is to study the influence of variation on the DNA sequence of important proteins of this system.

VDR BsmI, FokI and ApaI minor allele frequencies in Caucasian are approximately 42%, 34%, and 44%, respectively. These SNPs are therefore frequent in the Caucasian population. Although there is evidence of allelic heterogeneity, polymorphisms in the gene that encodes VDR have been associated with hypertension, insulin secretion and more recently with the risk of ESRD. While the exact mechanism is unknown, possible mechanisms include the synthesis of longer and less active VDR protein (FokI polymorphism), modification of the gene expression or VDR stability, and linkage disequilibrium with other causal polymorphism. Indeed, when a given allele of polymorphism is found to be associated with a trait or disease, it can be i) the effect of this allele, or ii) the effect of other alleles that happen to be linked to this allele for example with an haplotype. Thus, even though *VDR* polymorphisms are not causally associated with the trait, one expects them to be linked to truly functional polymorphisms

elsewhere in the *VDR* gene or nearby genes which can then explain the associations observed.

So far, no study explored the potential effect modification of *VDR* genetic variants on the relationship between vitamin D and change in kidney function. Thus, in the project 3, we aimed to examine the *VDR* gene- vitamin D level interaction on the change of kidney function associated with baseline serum vitamin D.

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CHAPTER 3

PROJECT 1

COMPARISONS OF SERUM VITAMIN D LEVELS, STATUS AND DETERMINANTS IN POPULATION WITH AND WITHOUT CHRONIC KIDNEY DISEASE NOT REQUIRING RENAL DIALYSIS: A 24-HOUR URINE COLLECTION POPULATION-BASED STUDY

SPECIFIC AIMS OF THE PROJECT 1

1) TO DETERMINE THE CKD SPECIFIC-PREVALENCE AND DETERMINANTS OF VITAMIN D DEFICIENCY IN SWITZERLAND

- HYPOTHESIS #1A : THE PREVALENCE OF VITAMIN D DEFICIENCY IS HIGHER AMONG SUBJECTS WITH CKD THAN SUBJECTS WITHOUT CKD

- HYPOTHESIS #1B : THE DETERMINANTS OF VITAMIN D DEFICIENCY IN SUBJECTS WITH AND WITHOUT CKD ARE DIFFERENT

Abstract

Background: Vitamin D deficiency is frequent in the general population and might be even more prevalent among populations with kidney failure. We compared the vitamin D levels, deficiency status and vitamin D level determinants in populations without chronic kidney disease (CKD) and with CKD not requiring renal dialysis.

Methods: Cross-sectional data from the 2010-2011 Swiss Study on Salt intake, a multicenter population-based study with 24-hour urine collection, was used. CKD was defined using estimated glomerular filtration rate and albuminuria. Serum vitamin D was measured by liquid chromatography- tandem mass spectrometry. We tested the interaction of CKD status with six *a priori* defined attributes (age, sex, BMI, walking activity, serum albumin-corrected calcium, and altitude) on vitamin D taking into account potential confounders. Individual interaction of kidney function (CKD status, CKD stages) with different anthropometric, socio-economic, behavioral, biological, and environmental attributes on vitamin D were also tested in an independent exploratory analysis. Survey statistical procedures were used.

Results: Overall, 11.8% (135/1145) participants had CKD. Vitamin D insufficiency or deficiency (serum 25(OH)D: <30 ng/ml) was frequent among participants with CKD (74.8%, 95%CI 58.4-92.8) but neither the prevalence of vitamin D insufficiency/deficiency nor the mean 25(OH)D levels were different in patients with and without CKD. CKD status did not interact with major determinants of vitamin D including age, sex, BMI, walking minutes, serum albumin-corrected calcium, or altitude for its effect on vitamin D status or levels. Exploratory analysis suggested that kidney function modifies the relationship between urinary excretion of Na and vitamin D.

Conclusions: People with CKD not requiring renal dialysis had similar prevalence of vitamin D deficiency as those without CKD.

INTRODUCTION

Advanced kidney failure is associated with a decline in the 1-alpha hydroxylation of 25(OH)D and with a reduction in the metabolically active form 1,25(OH)2D3.[1] Kidney failure is also associated with an increase urinary loss of vitamin D and vitamin D binding protein.[2] Renal retention of phosphorus and increased fibroblast growth factor-23 in kidney failure may also contribute to vitamin D deficiency in kidney failure.[3] The 2003 K/DOQI and 2009 KDIGO Clinical Practice Guidelines focus on the need to evaluate vitamin D status in patients with chronic kidney disease (CKD) stages 3 and 4 (stages 3 to 5 in the KDIGO) by measuring 25(OH)D levels.[4, 5] Supplementation is then recommended if 25(OH)D is < 30 ng/mL, which is also a definition of vitamin D insufficiency for the general population.[6, 7]

While the inverse associations of vitamin D levels with proteinuria have been observed with early stage of CKD,[8-10] the associations of vitamin D levels with less severe reductions of glomerular filtration rate (GFR) and earlier stages of CKD stages have been inconsistent.[11-15] Some studies have reported that vitamin D deficiency is even more prevalent among populations with kidney failure than in the general population.[16, 17] In contrast, other studies suggest that 25(OH)D levels are relatively stable in the earlier stages of CKD and only decrease with later stages of CKD (estimated GFR < 30 mL/min/1.73m^2).[15] Others have reported no correlation or an inverse correlation between 25(OH)D levels and GFR even among individuals with decreased levels of 1,25(OH)2D3.[11-15]

A previous analysis of the Swiss Study on Salt Intake showed a high prevalence of vitamin D insufficiency and deficiency in the Swiss general adult population.[18] This study extends these observations to assess the association between early stages of CKD and the prevalence of vitamin deficiency. Further, we determined the degree to which risk factors associated with vitamin D levels differed by CKD status.

METHODS

Swiss Study on Salt Intake (SSS)

We used the data from the 2010-2011 Swiss Study on Salt intake (SSS).[19] The SSS study is a population-based study including data in ten centers reflecting the geographical and cultural diversity of the Swiss adult population (≥15 years old). Its main objective was to estimate dietary salt/sodium intake using 24-hour urine collection in the Swiss population. The SSS complied with the Declaration of Helsinki and was approved by the local Institutional Ethics Committees. All participants gave written informed consent. For participants below age 18 years, written consent from one parent or a legal representative was obtained.

Sampling strategy

Sampling was stratified using eight age- (15-29 years, 30-44y, 45-59y, 60+y) and -sex strata. The Italian-speaking region was oversampled to allow a meaningful comparison with the two other major linguistic regions (i.e. French- and German-speaking regions). Recruitment began in January 2010 and ended in August 2011. Information letters were followed by phone calls with up to 3 attempts on different days, including evening hours, during which people are more likely to be at home. Participation

rate was low (10%). Because of important difficulties in recruiting young participants, we had to complete the study sample (aged 15-20 years old) by recruiting volunteers from schools and universities. Blood collection was not mandatory to participate in the study.

Subjects were identified by means of the Swiss Federal Office Statistics phone directory which is regularly updated by the major Swiss phone provider and cover 95% of non-institutionalized inhabitants in Switzerland. We performed a two-stage sampling strategy. Stage 1 identified households (primary unit) and stage 2 identified a single individual per household (secondary unit). We determined household characteristics by phone (household size, age, sex and nationality of its members) to identify the target population, from which a random sample was then drawn.

In the first stage, we contacted households by phone after having sent a letter of invitation to participate to the survey. A single person per household was randomly selected to respond to a first questionnaire by phone. In the second stage, the selected person came to the study center on two consecutive mornings for measurement by a trained health professional from the survey team and for urine collection. The following efforts were made to minimize the non-response rate: (1) multiple attempts to contact participants including outside of regular working hours, such as during evenings and week-ends; (2) home visits to provide and collect urine bottles; (3) small reward to participants at the end of the study (e.g. 30 Swiss franc CHF gift card, 1CHF≈1$ in July 2013). Invitation letters were sent to a random sample of 7500 households.

Assessment process and clinical data

Kidney function was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.[20] The CKD-EPI equation includes age, sex, and race as surrogates for non GFR determinants of serum creatinine. CKD-EPI (functional marker of the kidney lesion) and albuminuria (structural marker of the kidney lesion) estimated by 24-hour urine collection were used to stage CKD. Microalbuminuria and macroalbuminuria were defined as a urine albumin concentration between 30 mg/24h and 300 mg/24h and greater than 300 mg/24h, respectively. CKD stages 1 to 5 were defined as recommended in the K/DOQI guidelines.[17] Given the small number of participants with CKD stages 4 and 5, CKD stages 3 or greater were combined. The albumin-to-creatinine ratio (ACR) was also used to estimate albuminuria. ACR was log transformed in the statistical analysis to improve linearity. All urine samples were analyzed centrally in the laboratory of Lausanne University Hospital.

Blood pressure (BP) was measured five times at each visit on the left arm after at least 5 minutes rest in the seated position using a clinically validated automated oscillometric device (Omron® HEM-907, Matsusaka, Japan) with a standard cuff, or a large cuff if arm circumference was ≥33 cm.[21] The average of ten BP readings was used for analyses. Hypertension was defined as mean systolic BP (SBP) ≥140 mmHg or mean diastolic BP (DBP) ≥90 mmHg or presence of anti-hypertensive medication.

Weight and height were measured and body mass index (BMI) calculated as weight/height in m^2. Waist circumference was measured using standard procedures. Diabetes was considered as present whenever the use of antidiabetic drug treatment was

reported. Post-menopausal status, use of oral contraceptive or hormone replacement therapy, ethnicity, and smoking status were self-reported.

Information on diet (e.g. fish, wine, and beer consumptions) and daily exercise (minutes of walking) were collected using questionnaires. Participants were asked to report their medications and supplements. This list of reported medications/supplements was compared to the original medicines Compendium® of Switzerland database (http://www.compendium.ch/) to identify vitamin D supplements and treatments.

Biologic data

Total serum calcium was measured by O-cresolphtalein and albumin by bromocresol green; albumin-corrected calcium was then calculated. Creatinine was measured using Jaffe kinetic compensated method (Roche Diagnostics, Switzerland). 24-hour urinary sodium, urea, and potassium excretions were used to estimate their respective consumptions. We lack information on parathyroid hormone (PTH). We used 24-hour urinary calcium excretion as a proxy of PTH activity.

Altitude and sunshine hours data

Participants were geocoded by merging information on the participant's private address with latitude, longitude and altitude information using Python programming and Google Maps Find Altitude software https://developers.google.com/maps/documentation/elevation/). Data on sunshine hours were obtained from Meteoswiss which collects sunshine hours using meteorological stations distributed throughout Switzerland (http://www.meteoswiss.admin.ch/web/en.html). For each participant, data on sunshine

hours collected in the station nearest to the participant's address was used. The exposure period considered in this study was starting from the month before the participant's day of blood collection and was used to estimate the monthly mean sunshine hours.

Vitamin D

25(OH)D = 25(OH)D_3 + 25(OH)D_2 concentrations in serum were measured by liquid chromatography- tandem mass spectrometry (LC-MS/MS) in the laboratories of the Swiss Federal Office of Public Health. An in-house validated method with hexadeuterated 25(OH)D_3 as internal standard (IS) was developed. This takes into account the difficulty of measuring the serum 25(OH)D level over a long period of time ensuring the comparability of results.[22,23] NIST SRM 972 reference material[24] was used during validation of the method and again every 6th month during routine detection. Five different reference sera were used in each assay. During the whole measurement period the laboratory participated successfully in a quality control program (DEQAS).[25] The limits of quantification of the method were 1.5 ng/ml (conversion factor: 1 ng/ml=2.496 nmol/l) for 25(OH)D_3 and 1.0 ng/ml for 25(OH)D_2. Intra- and inter-assay CV for 25(OH)D_3 were 4% and 8%, respectively. If one of the reference sera showed more than 15% deviation from the target value the results were discarded and the assay repeated.

Vitamin D status

To describe the cohort, we used vitamin D status usually defined as sufficient, insufficient, and deficient respectively for 25(OH)D ≥30 ng/ml, 20-29.9 ng/ml, and <20 ng/ml.[6, 7]

Vitamin D month-specific tertiles

The 25(OH)D concentrations fluctuate due to seasonal variation in sun exposure and the use of a single blood sample to estimate long term 25(OH)D average may lead to misclassification bias. When considering 25(OH)D as a categorical variable, it has been shown that month-specific tertiles of 25(OH)D is preferable to adjusting for seasonal variation.[26] We therefore used month-specific tertiles of 25(OH)D as the dependent variable. For each month of the study period, the distribution of 25(OH)D is used to define the specific tertiles of 25(OH)D. This approach is considered as the most valid measure of associations when vitamin D is used as categorical variable.[26]

Vitamin D tertiles were considered as inherently ordered categories and cumulative logit models were used in our analyses. In this approach, the odds of vitamin D upper tertile is equal to the probability of vitamin D upper tertile divided by the probability of lower or middle vitamin D tertiles. In the cumulative logit models, we assume that the odds ratio is invariant to where the outcome categories are dichotomized (e.g., between lower and middle/upper or between lower/middle and upper tertiles). We tested the Proportional Odds Assumption using the Score test. For survey data we used a design-based Score test implemented in Stata in a user-written command *gologit2*.[27] The null hypothesis of proportional odds fails to be rejected whenever this Score test is not significant. No matter how many variables were considered in a given proportional odds model, the score test was not significant, which suggests that the use of a cumulative logit model is appropriate.

When considering 25(OH)D as continuous outcome, the failure to adjust for date of sample collection has been shown to create a bias toward the null. We therefore included the month of sample collection (11 dummy variables) as a covariate in linear model when vitamin D was entered as a continuous variable. Serum 25(OH)D levels were transformed using the square-root transformation.

STATISTICAL ANALYSES

Statistical analyses were performed using Stata 12.0 (Stata Corp, College Station, USA) and SAS 9.3 (SAS Institute, Cary NC). To account for the complex sampling design (i.e. stratification, clustering and unequal weighting), estimates (e.g. weighted mean, weighted frequencies, weighted parameters) and variances were calculated using design-based and Taylor expansion method procedures instead of using standard procedures.[27] Procedures based on Taylor expansion method take into account the sample design (e.g weight related to oversampling, covariance related to clustering and stratification) to estimate sampling errors of estimators.[27] Design-based test statistics were used for statistical inference. To test mean and proportion differences, we used the design-based Student t-test and the Rao-Scott test, respectively. The Rao-Scott likelihood ratio X^2 test is a design-adjusted version of the likelihood ratio test, which involves ratios between observed and expected frequencies. The Rao-Scott X^2 statistic is recommended for complex survey data.[28]

To test single and combination of regression parameters, we used design-based Student t-test and F tests (means, linear regression) or design-based Wald X^2 (logistic regressions) and generalized design-based Wald X^2 tests, respectively. These tests are design-based as they use the variance (usually increased in complex sampling compares

to simple sampling) and the degrees of freedom (decreased in complex sampling compared to simple sampling) that are specific to the complex design.[27] The Stata svy and SAS survey statistical software procedures were used.

Models and Modeling strategy

For our first hypothesis (the prevalence of vitamin D sufficiency, insufficiency, and deficiency are different in participants with and without CKD), we used a survey-adjusted cumulative logit model to compare weighted prevalences of sufficient, insufficient, and deficient vitamin D status, by CKD status. Rao-Scott X^2 tests were used to test this hypothesis.

Our second hypothesis was that the associations of determinants of vitamin D levels and status were different in patients with and without CKD as well as across CKD stages. In these analyses we hypothesized that CKD modified the effect of attributes on vitamin D. We considered six specific attributes of vitamin D: age, sex, BMI, walking activity, serum albumin-corrected calcium, and altitude. These attributes were *a priori* selected based on biological and epidemiological evidence.[29-32] Product terms of CKD status (no CKD *vs.* CKD stage 1 or greater) and the six attributes were integrated in models adjusting for potential confounders and ensuring that the models were hierarchically well-formulated (models 1 and 2)[1]. We considered only first degree interaction terms (e.g., a two factor product such as attribute x CKD) in our models.

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[1] **Model 1 Linear regression model: $E(Y) = \alpha + \beta_1(E_1) + \sum\gamma_i(MO_i) + \sum\gamma_j(V_j) + \sum\delta_j(E_1{*}V_j)$** where Y = 25[OH]D in ng/mL **(continuous, square-root transformed);** E_1=CKD status (0,1); MO=month of sampling with k=12 and k-1 dummy variables: January (category 1), …, December (category 12) where MO={1 if category i, 0 if otherwise for i=2,3,4,5,6,7,8,9,10,11,12 (referent : 1) V_j= potential confounders considered at the same time, the following Vs were considered as first degree interaction terms with CKD: age, gender, BMI, physical activity, calcium level, and altitude

As outcome variables, we used both the square-root transformed 25(OH)D) level (model 1) and the month-specific tertiles 25(OH)D levels (model 2) and used linear and ordinal logistic regressions accordingly.

VARIABLE SPECIFICATION

To identify factors included in the *initial* multivariate models 1 and 2, bivariate associations between vitamin D levels (continuous variable) / vitamin D status and attributes of interest were tested and compared between subjects with and without CKD as well as across CKD stages. Significances of P values for including the attribute in the *initial* model were set at <0.10. For the purpose of this analysis, BMI was categorized into <25.0 kg/m^2, 25.0-29.9 kg/m^2, ≥30 kg/m^2; self-described ethnicity was defined as Caucasian versus non-Caucasian; smoking status as never, current, and ex-smokers; high wine and fish consumptions were both defined as consumption of these items 3 or more days per week; tertiles of the reported daily average minutes of walk were used.

COLLINEARITY, INTERACTION, CONFOUNDING[2]

We used the SAS Collin macro (Emory University, Kleinbaum et al.) for assessing collinerarity. Conditions indexes (CIs), and variance decomposition proportions (VDP's) using the SAS Collin Survey macro were scrutinized and situations were CIs >30 and at least two VDPs ≥0.50 were not observed.

Model 2 Ordinal logistic regression model: logit $P(D \geq g) = \alpha_g + \beta_1(E_1) + \sum\gamma_i(V_i) + \sum\delta_i(E_1 * V_i)$, where D= month-specific tertiles 25[OH]D level **(ordinal outcome with g=1,2),** for each month of the study period, the distribution of 25(OH)D is used to define the specific tertiles of 25(OH)D; E_1=CKD status (0,1), V_i= potential confounders considered at the same time, the following Vs were also considered as first degree interaction terms with CKD: age, gender, BMI, physical activity, calcium level, and altitude

[2] Examples of collinearity and backward change in estimate elimination methods are presented in the Appendix

We considered only first degree interaction terms. We used both chunk tests and backward stepwise elimination procedures to test statistical interactions. Significance for interaction is often set at P value <0.10. Given the number of interaction tested, we used a more conservative threshold of P value <0.05. To reduce models 1 and 2, we used the backwards change in estimate elimination and precision approaches.[33] Full (gold standard) and reduced models yield similar results. To limit the number of models, only full (gold standard) models are presented in the Results section.

Independently of the approach described above, we conducted a more exploratory analysis by considering the effect modification of kidney function on several individual attributes (anthropometric, socio-economic, behavioral, biological, and environmental factors) considered separately and using CKD status as a binary variable (no CKD *vs.* CKD stage 1 or greater) (models 3 and 4)[3]. We repeated these analyses using CKD stages (no CKD, CKD stages 1 or 2, CKD stages 3 or more) as dummy variables (models 5 and 6)[4].

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[3] **Model 3 Linear regression model: $E(Y) = \alpha + \beta_1(E_1) + \gamma_1(V_1) + \sum\gamma_i(MO_i) + \delta_1(E_1*V_1)$**, where Y = 25[OH]D in ng/mL **(continuous, square-root transformed);** E_1= Each participants-level attributes of interest considered separately; V_1= CKD status (0,1); MO=month of sampling with k=12 and k-1 dummy variables: January (category 1), …, December (category 12) where MO={1 if category i, 0 if otherwise for i=2,3,4,5,6,7,8,9,10,11,12 (referent : 1)

Model 4 Ordinal logistic regression model: logit $P(D \geq g) = \alpha_g + \beta_1(E_1) + \gamma_1(V_1) + \delta_1(E_1*V_1)$, where D= month-specific tertiles 25[OH]D level **(ordinal outcome with g=1,2),** for each month of the study period, the distribution of 25(OH)D is used to define the specific tertiles of 25(OH)D; E_1= Each participants-level attributes of interest considered separately; V_1= CKD status (0,1)

[4] **Model 5 Linear regression model: $E(Y) = \alpha + \beta_1(E_1) + \sum\gamma_i(MO_i) + \sum\gamma_jV_j + E_1\sum\delta_{1j}V_j$** where Y = 25[OH]D in ng/mL **(continuous, square-root transformed);** E_1= Each participants-level attributes of interest considered separately; MO=month of sampling with k=12 and k-1 dummy variables: January (category 1), …, December (category 12) where MO={1 if category i, 0 if otherwise for i=2,3,4,5,6,7,8,9,10,11,12 (referent : 1); V=CKD stage with k=3 and k-1 dummy variables : CKD stage 0 (category 1), CKD stage 1 or 2 (category 2), and CKD stage 3 or more (category 3) where V={ 1 if category j, 0 if otherwise for j=2,3 (referent: category 1)

Model 6 Ordinal logistic regression model: logit $P(D \geq g) = \alpha_g + \beta_1(E_1) + \sum\gamma_iV_i + E_1\sum\delta_{1i}V_i$ where D= month-specific tertiles 25[OH]D level **(ordinal outcome with g=1,2),** for each month of the study period, the distribution of 25(OH)D is used to define the specific tertiles of 25(OH)D; E_1= Each participants-level attributes of interest considered separately; V=CKD stage with k=3 and k-1 dummy variable : CKD stage 0 (catgory 1), CKD stage 1 or 2 (category 2), and CKD stage 3 or more (category 3) where V={ 1 if category i, 0 if otherwise for i=2,3 (referent:category 1)

RESULTS

PARTICIPANTS' CHARACTERISTICS

A total of 1,145 subjects were included in the multivariate analysis, 11.8% (135/1145) of whom had CKD with CKD stages as follows: 48/1145 (4.2%) stage 1 or 2, 87/1145 (7.6%) stage 3 or 4. No participant had CKD stage 5. Characteristics for all participants and by CKD status are detailed in **Table 1**. In addition to the eGFR, participants with and without CKD statistically differed in the following characteristics: age, education, hypertension, diabetes, menopause, use of oral contraceptive or hormonal replacement therapy, BMI, waist circumference, calcium and urea urinary excretions, beer consumption and vitamin D supplementation. Systolic but not diastolic BP differed between participants with and without CKD (systolic BP: 124.8 vs. 133.0, p value<0.001; diastolic BP: 75.5 vs. 74.9, p value=0.554, respectively).

The mean 25(OH)D levels by CKD status and CKD stages are presented in the **Figure 1**. These means are derived from linear (full) models adjusted for sex, age, albumin-corrected calcium, BMI, waist circumference, daily minutes of walk, altitude, education, monthly mean hours of sunshine, latitude, smoking status, vitamin D supplement or treatment (when appropriate), linguistic-region, wine and beer consumption, oral contraceptive or hormonal replacement therapy, menopause, hypertension, diabetes, nationality, ethnicity, Na-, K-, urea-, and calcium urinary excretion, without interaction. Similar comparisons stratifying by vitamin D supplement and by period of the year (April-September vs. October-March) are also presented. The 25(OH)D adjusted means were 23.1 (22.6-23.7) and 23.5 (21.7-25.3) ng/mL in participants without and with CKD, respectively. In participants without CKD, CKD

stages 1 or 2, and CKD stages 3 or greater, the 25(OH)D adjusted means were 23.1 (22.5-23.7), 23.4 (22.3-24.5) and 23.7 (21.6-25.9) ng/mL, respectively. The unadjusted and adjusted 25(OH)D means were not associated with either CKD status or CKD stages (**Figure 1**). We found no association between 25(OH)D and CKD status or stages in analyses restricted to participants without vitamin D supplement or treatment (N=1100) (**Figure 1**). The prevalence of vitamin D insufficiency (20-29.9 ng/mL) or deficiency (<20 ng/mL) were high but similar in participants with and without CKD (75.3% [95%CI 69.3-81.5] and 69.1 [95%CI 53.9-86.1], p value=0.054) (**Table 2**).

In multivariate analyses, none of the six *a priori* interaction terms used in models 1 and 2 were statistically significant (**Table 3**).

Table 4 reports the p value for interaction for models 3 to 6 used to test whether determinants of vitamin D status differ according to CKD status. Using model 3, we found significant interactions (defined as a p value < 0.05) of CKD status (presence or absence) with education, tertiles of daily average minutes of walk, oral contraceptive, and urinary excretion of Na. Of these, only urinary excretion of Na and middle tertiles of daily average minutes of walk interacted significantly with CKD status using model 4. Using model 5, we found significant overall interactions of CKD stages (no CKD, CKD stage 1 or 2, CKD stage 3 or greater) with diabetes, urinary excretion of Na, and urinary excretion of Ca; significant interactions of CKD stages 1 or 2 with daily average minutes of walk, diabetes, and urinary excretion of Na; and significant interactions of CKD stages 3 or more with education. Finally, using model 6, we found significant overall interactions of CKD stages with urinary excretion of Na; significant interactions of CKD stages 1 or 2 with diabetes, urinary excretion of Na, and urinary

excretion of K; and significant interactions of CKD stage 3 or greater with education, middle tertile of daily average minutes of walk, and beer consumption.

DISCUSSION

In this population-based study, we found that the prevalence of vitamin D deficiency was high in people with CKD but not higher than in people without CKD. We found no evidence that vitamin D major determinants are different in subject with and without CKD (i.e. CKD does not modify the effect of major attributes on vitamin D). Vitamin D status and vitamin D levels (25(OH)D) did not differ by CKD status or stages.

Using 25(OH)D levels, we found a high prevalence of vitamin D insufficiency or deficiency in participants with CKD not requiring dialysis. While a previous report concluded that CKD had a higher risk of 25(OH)D deficiency,[16] we did not found that people with CKD had a higher risk of deficiency compared to people without CKD. Vitamin D insufficiency or deficiency is very high in the general population. In a recent study using the same source population, we showed that vitamin D insufficiency or deficiency were as high as 75% in the general population. [18] While the high prevalence of sub-optimal vitamin D is of concerns in general, it remained to be determined whether it is of even more concerns among the CKD population. In a recent meta-analysis of prospective studies (10 studies, N=6,853), higher 25(OH)D levels were associated with significantly improved survival in patients with CKD.[34] Of note, several meta-analyses have shown that higher 25(OH)D levels were also associated with significantly improved survival in the general population as well.[35, 36] Whether treatment of low 25(OH)D level

using vitamin D supplementation improves survival in patients with or without CKD (not requiring dialysis) remains to be shown in randomized controlled trials.

If vitamin D supplementation is proved to reduce the mortality among people with vitamin D deficiency in randomized controlled trials, population at risk of vitamin D deficiency should be well characterized. Few studies explored whether the determinants of vitamin D deficiency in population with and without CKD are the same.

In multivariate analyses, we assessed this by testing six *a priori* formulated interactions. CKD status did not interact with age, sex, BMI, walking minutes, serum albumin-corrected calcium, or altitude for its effect on vitamin D status or levels. This is consistent with a previous study that assessed these interactions.[9] Thus, major determinants of vitamin D seemed to be similar in populations with and without CKD not requiring dialysis.

When attributes such as education, daily walking minutes, oral contraceptive, and urinary excretion of Na were considered separately, we found some evidence that CKD modifies the association between these attributes and vitamin D. The modification by CKD of the relationship between urinary excretion of Na and vitamin D was the most consistent. Urinary excretion of Na was negatively associated with 25(OH)D levels and month-specific 25(OH)D tertiles among participants with CKD but not among participants without CKD. Because urinary excretion of Na estimates the Na intake, this could suggest that compared to participants with lower Na intake, participants with higher Na intake might also have lower vitamin D intake. We only observed this in

participants with CKD but the association was not statistically significant after adjustment for diet related factors such as BMI and fish consumption.

Strengths and limitations

With respect to exploring the association between 25(OH)D levels and kidney function, this is a large population-based study using 24-hour urine collection, measuring 25(OH)D by the gold standard technique (LC/MSMS), and taking into account geographical, meteorological, and nutritional information. Another strength is the use of a single laboratory for centralized urine and blood analyses. Information on major known potential confounders was available but despite these efforts, information is still incomplete. For example, we lack information on PTH and on the darkness of the skin.

We did not use a comprehensive food frequency questionnaire. Yet diet contains only small amounts of vitamin D (i.e., vitamin D_3 or vitamin D_2), and information on fish consumption was collected, which is the major dietary source of vitamin D in humans. We did not take air pollution, ozone, time of the day, and cloud cover into account. These weather-related factors do influence vitamin D effective radiation and thus vitamin D photosynthesis,[32] although the impact is likely to be minor. We did not use para-aminobenzoic acid (PABA) to check the completeness of urinary collection. However, the ratio urinary creatinine excretion/ kg body weight over 24-hour and urine volumes suggested a good quality of most urine collections.

We used definition of vitamin D deficiency and insufficiency that have been proposed by experts. This definition has been used in most epidemiological studies, but different definitions exist.[37] While the definition of vitamin D deficiency and

insufficiency is debated, this would not bias our results since we used the same definition when comparing people with and without CKD. GFR and CKD stages were based on the CKD-Epi equation and albuminuria. More accurate measurements (including inulin clearance, iothalamate clearance, cystatin C) exist.

The low participation rate limits the external validity of our findings. We explain this low participation rate by the unattractiveness of 24-hour urine collection, together with the two-stage sampling strategy, which implies that the person we contacted by phone was not automatically the one selected to enter the study. Blood collection was not mandatory to participate in the original study and we cannot exclude that this could have potentially introduced a bias. Blood was not collected in 55.4% of the participants excluded from the analyses (N=370). Among participants excluded from the analyses for other missing data than blood values, age, sex, the levels of 25(OH)D, eGFR, and albuminuria did not differ. Finally, some households were dropped from being included in the target population if certain household characteristics did not meet specific critieria.

CONCLUSIONS

People with CKD had similar prevalence of vitamin D deficiency and levels of 25(OH)D than people without CKD. CKD status did not modify the effect of major vitamin D determinants on vitamin D levels or status. Our results suggest that the deficiency in active vitamin D (1,25(OH)D) observed in people with CKD is not mainly due to a deficiency in 25(OH)D substrate.

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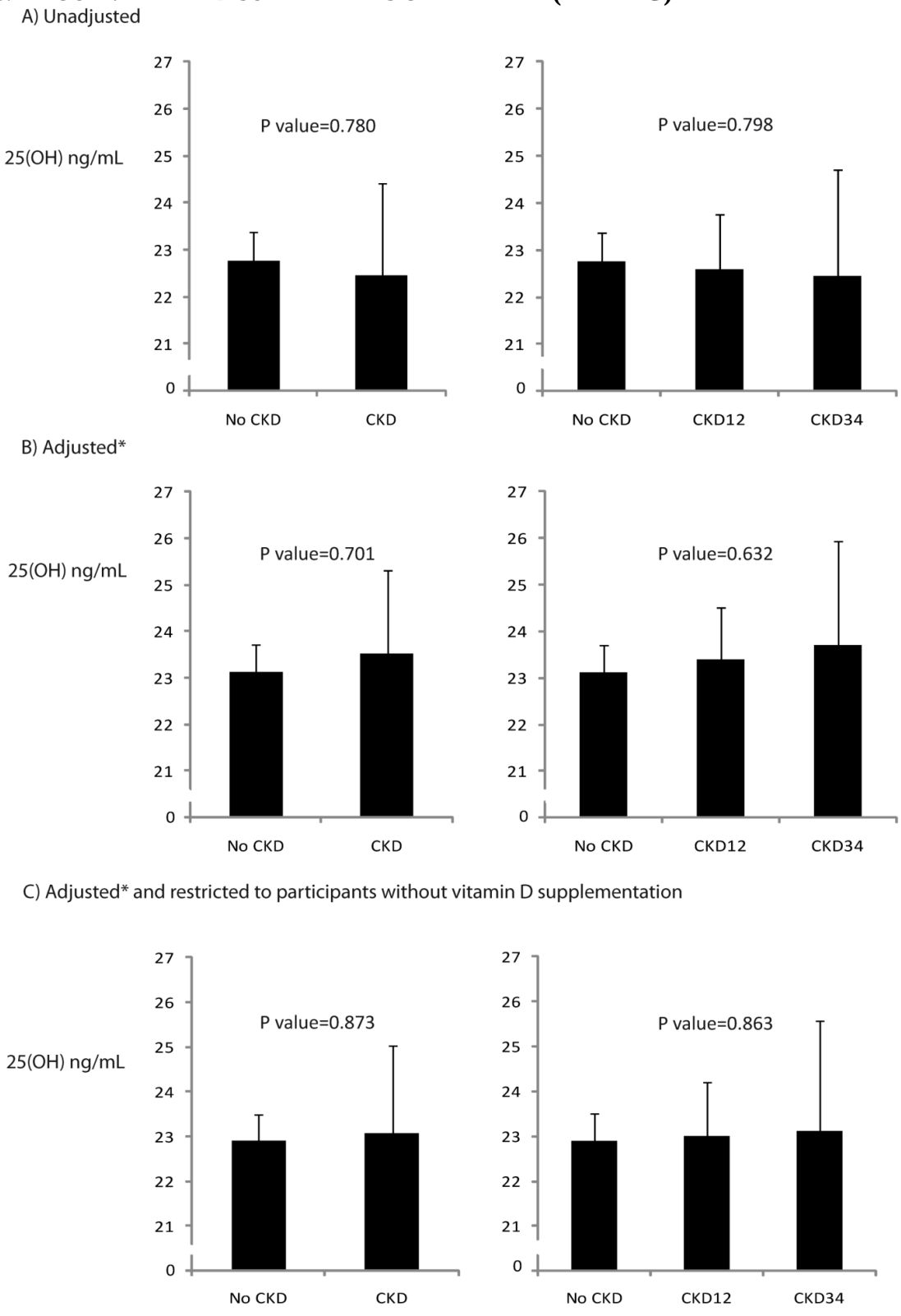
TABLE 1. CHARACTERISTICS OF THE SSS PARTICIPANTS, BY CHRONIC KIDNEY DISEASE (CKD) STATUS (N=1145)

	ALL (N=1145)		No CKD (N=1010)		CKD (N=135)		P value
Attributes	**Mean or %**	**95%CI**	**Mean or %**	**95%CI**	**Mean or %**	**95%CI**	
Male gender	48.9	47.3-50.5	49.0	47.0-50.9	48.5	40.7-56.4	0.710
Age (years)	48.5	47.9-49.2	46.4	45.6-47.1	64.7	61.7-67.8	<0.001
Education superior (university, diploma superior)	42.4	39.5-45.3	43.5	40.4-46.6	34.1	26.5-42.7	0.043
Linguistic regions							0.791
French speaking	26.8	24.2-29.6	27.0	24.3-30.0	25.1	18.3-33.5	
German speaking	66.8	64.0-69.5	66.5	63.5-69.4	68.9	60.5-76.1	
Italian speaking	64.1	55.0-74.6	6.5	5.5-7.6	6.0	3.8-9.4	
Swiss citizenship	85.4	83.2-87.3	85.1	82.8-87.2	87.2	80.2-92.0	0.536
Caucasian ethnicity	98.5	97.5-99.1	98.5	97.4-99.1	98.4	97.4-99.1	0.261
Smoking status							0.081
Never smokers	53.6	50.6-56.5	53.6	50.5-56.7	53.2	44.6-61.7	
Smokers	17.1	15.0-19.5	18.0	15.6-20.5	11.1	6.8-17.9	
Former smokers	29.3	26.7-32.0	28.4	25.7-31.3	35.6	27.8-44.2	
Tertiles of daily average minutes of walk, minutes							0.312
Lower tertile	54.8	51.8-57.7	55.6	52.4-58.7	48.5	40.0-57.2	
Middle tertile	24.6	22.1-27.2	24.2	21.6-27.0	27.3	20.3-35.7	
Upper tertile	20.6	18.3-23.1	20.2	17.7-22.8	24.1	17.4-32.4	
Hypertension	31.1	28.6-33.6	27.2	24.7-29.9	59.9	51.2-67.9	<0.001
Diabetes	2.8	2.0-3.9	2.6	1.0-2.7	11.2	6.8-17.9	<0.001

Menopause(among women)	44.6	41.8-47.5	41.4	38.2-44.7	68.9	57.1-78.6	<0.001
Oral contraceptive(among women)	35.0	31.2-39.0	33.3	29.4-37.6	47.2	35.4-59.2	0.003
eGFR*	89.8	88.8-90.7	92.8	91.9-93.7	67.4	63.1-71.7	<0.001
BMI, kg/m^2	25.2	25.0-25.5	25.1	24.9-25.4	26.2	25.3-27.1	0.032
Waist circumference, cm	90.2	89.5-90.9	89.7	88.9-90.4	94.0	91.3-96.8	0.003
Systolic BP, mmHg	125.8	124.9-126.6	124.8	124.0-125.7	133.0	129.7-135.7	<0.001
Diastolic BP, mmHg	75.4	74.8-76.0	75.5	74.9-76.1	74.9	73.1-76.7	0.554
Albumin-corrected calcium	2.31	2.30-2.31	2.31	2.30-2.31	2.32	2.31-2.34	0.100
Urinary excretion, (mmol/24h)							
Na	158.4	154.5-162.3	159.3	155.3-163.4	151.3	137.9-164.8	0.269
K	67.4	66.0-68.8	67.8	66.3-69.0	64.5	59.7-69.2	0.195
Ca	4.05	3.91-4.18	4.18	4.04-4.33	3.02	2.60-3.45	<0.001
Urea	365.9	358.6-373.3	370.5	362.7-378.4	331.8	307.2-356.4	0.004
High Fish**	5.8	4.6-7.4	5.9	4.5-7.6	5.7	2.7-11.4	0.929
High Wine**	35.4	32.7-38.2	35.8	32.9-38.8	32.4	24.8-40.9	0.447
High Beer**	16.1	14.1-18.3	16.9	14.7-19.3	10.0	5.9-16.6	0.049
VitD supplement or Rx	4.1	3.1-5.4	3.2	2.2-4.5	10.8	6.5-17.5	<0.001
Altitude, meters	468.6	460.1-477.0	470.0	460.5-478.7	460.8	438.5-483.2	0.476
Latitude, degrees	47.0	47.0-47.1	47.0	47.0-47.1	47.1	47.0-47.1	0.423
Mean monthly sunshine hours	5.41	5.28-5.55	5.45	5.31-5.59	5.16	4.79-5.52	0.136

* estimated using the CKD-epi equation; ** defined as consumption of these items 3 or more days per week; BMI: body mass index; BP: blood pressure; Na: sodium; K: potassium; Ca: calcium; Rx: treatment

FIGURE 1. VITAMIN D LEVELS AND CHRONIC KIDNEY DISEASE (CKD), UNADJUSTED (PANEL A), FULLY ADJUSTED* (PANEL B), AND RESTRICTED TO PARTICIPANTS WITHOUT VITAMIN D SUPPLEMENTS OR THERAPY (PANEL C)



*Adjusted for sex, age, albumin-corrected calcium, BMI, waist circumference, daily minutes of walk, altitude, education, monthly mean hours of sunshine, latitude, smoking status, vitamin D supplement or treatment (when appropriate), linguistic-region, wine and beer consumption, oral contraceptive or hormonal replacement therapy, menopause, hypertension, diabetes, nationality, ethnicity, Na-, K-, urea-, calcium urinary excretion, and month of sampling. Interaction term not considered.

TABLE 2. RAW NUMBERS AND WEIGHTED PREVALENCES OF SUFFICIENT (≥30 NG/ML), INSUFFICIENT (20-29.9 NG/ML), AND DEFICIENT (<20 NG/ML) VITAMIN D STATUS, BY CHRONIC KIDNEY DISEASE (CKD) STATUS (N=1145)

CKD status	Vitamin D status	N	% (95%CI)
No CKD	**Sufficient**	263/1010	24.7 (22.1-27.5)
	Insufficient	387/1010	38.1 (35.1-41.2)
	Deficient	360/1010	37.2 (34.2-40.3)
CKD	**Sufficient**	43/135	30.9 (23.6-39.3)
	Insufficient	40/135	27.5 (20.5-35.7)
	Deficient	52/135	41.6 (33.4-50.4)
Rao-Scott Chi-square test comparing participants with and without CKD: p value			0.0537

TABLE 3. ADJUSTED* A PRIORI INTERACTION TESTS OF CHARACTERISTICS WITH TRANSFORMED 25(OH)D LEVELS USING LINEAR REGRESSION, CHUNCK TEST, AND BACKWARD ELIMINATION PROCEDURES (STEP 1 TO 6), N=1145.

		P value step 1	P value step 2	P value step 3	P value step 4	P value step 5	P value step 6
Backward elimination elimination	**Linear regression, transformed 25(OH)D (model 1)**						
	CKD x gender	0.435	0.457	0.442	0.398	dropped	dropped
	CKD x age	0.617	0.626	dropped	dropped	dropped	dropped
	CKD x BMI	0.927	dropped	dropped	dropped	dropped	dropped
	CKD x tertiles of daily average minutes of walk	0.199	0.198	0.218	0.172	0.195	0.151
	CKD x corrected calcium	0.497	0.499	0.454	dropped	dropped	dropped
	CKD x altitude	0.110	0.115	0.119	0.111	0.128	dropped
	Chunck test	0.436	0.366	0.275	0.235	0.152	0.151
Backward elimination	**Ordinal logistic regression, month-specific tertiles of 25(OH)D (model 2)**						
	CKD x gender	0.809	0.777	dropped	dropped	dropped	dropped
	CKD x age	0.846	dropped	dropped	dropped	dropped	dropped
	CKD x BMI	0.686	0.671	0.714	dropped	dropped	dropped
	CKD x tertiles of daily average minutes of walk	0.382	0.361	0.347	0.347	0.289	0.316
	CKD x corrected calcium	0.363	0.399	0.413	0.432	dropped	dropped
	CKD x altitude	0.384	0.365	0.343	0.360	0.341	dropped
	Chunck test	0.766	0.674	0.554	0.396	0.360	0.316

Footnote: Both chunck tests and backward stepwise elimination procedures were used to test statistical interactions. Significance for interaction was set at P value <0.05.

Model 1 Linear regression model: $E(Y) = \alpha + \beta_1(E_1) + \sum\gamma_i(MO_i) + \sum\gamma_j(V_j) + \sum\delta_j(E_1*V_j)$ where Y = 25[OH]D in ng/mL **(continuous, square-root transformed);** E_1=CKD status (0,1); MO=month of sampling with k=12 and k-1 dummy variables: January (category 1), …, December (category 12) where MO={1 if category i, 0 if otherwise for i=2,3,4,5,6,7,8,9,10,11,12 (referent : 1) V_j= potential confounders considered at the same time, the following Vs were considered as first degree interaction terms with CKD: age, gender, BMI, physical activity, calcium level, and altitude

Model 2 Ordinal logistic regression model: logit $P(D \geq g) = \alpha_g + \beta_1(E_1) + \sum\gamma_i(V_i) + \sum \delta_i(E_1*V_i)$, where D= month-specific tertiles 25[OH]D level **(ordinal outcome with g=1,2),** for each month of the study period, the distribution of 25(OH)D is used to define the specific tertiles of 25(OH)D; E_1=CKD status (0,1), V_i= potential confounders considered at the same time, the following Vs were also considered as first degree interaction terms with CKD: age, gender, BMI, physical activity, calcium level, and altitude

* Adjusted for gender, age, education, linguistic regions, Swiss citizenship, Caucasian ethnicity, smoking status, tertiles of daily average minutes of walk, hypertension, diabetes, menopause, oral contraceptive, BMI, waist circumference, corrected-calcium, VitD Rx or supll., urinary excretion of Na, urinary excretion of K, urinary excretion of Ca, urinary excretion of urea, wine consumption, beer consumption, altitude, latitude, month of sampling, and mean monthly sunshine hours.

TABLE 4. P VALUES FOR INTERACTION OF SELECTED ATTRIBUTES WITH CKD STATUS AND STAGES ON VITAMIN D LEVELS AND MONTH-SPECIFIC TERTILES (MODELS 3 TO 6)

	MODELS							
Model	**Model 3 (Linear regression, square-root 25OHD, CKD yes/no)**	**Model 4 (Ordinal logistic regression, month-specific 25(OH)D tertiles, CKD yes/no)**	**Model 5 (Linear regression, square-root 25OHD, No CKD, CKD stages 1-2, CKD stage 3 or greater)**			**Model 6 (Ordinal logistic regression, month-specific 25(OH)D tertiles, No CKD, CKD stages 1-2, CKD stage 3 or greater)**		
All CKD stages or CKD stage-specific interaction	-	-	**All CKD stages**	**CKD stages 1-2**	**CKD 3+ stages**	**All CKD stages**	**CKD stages 1-2**	**CKD 3+ stages**
Male gender	NS	NS	NS	NS	NS	NS	NS	NS
Age (years)	NS	NS	NS	NS	NS	NS	NS	NS
Education superior (university, diploma superior)	**0.033**	NS	NS	NS	**0.025**	NS	NS	**0.037**
Linguistic regions	NS	NS	NS	NS	NS	NS	NS	NS
French speaking	**Ref**	**Ref**	**Ref**	**Ref**	**Ref**	**Ref**	**Ref**	**Ref**
German speaking	NS	NS	NS	NS	NS	NS	NS	NS
Italian speaking	NS	NS	NS	NS	NS	NS	NS	NS
Swiss citizenship	NS	NS	NS	NS	NS	NS	NS	NS
Caucasian ethnicity	NS	--	NS	NS	--	--	--	--
Smoking status	NS	NS	NS	NS	NS	NS	NS	NS
Never smokers	**Ref**	**Ref**	**Ref**	**Ref**	**Ref**	Ref	**Ref**	**Ref**

Smokers	NS	NS	NS	NS	NS	NS	NS	NS
Former smokers	NS	NS	NS	NS	NS	NS	NS	NS
Tertiles of daily average minutes of walk, minutes	**0.018**	NS	NS	NS	NS	NS	NS	NS
Lower tertile	**Ref**	**Ref**	**Ref**	**Ref**	**Ref**	**Ref**	**Ref**	**Ref**
Middle tertile	**0.023**	**0.048**	NS	NS	NS	NS	NS	**0.035**
Upper tertile	NS	NS	NS	**0.046**	NS	NS	NS	NS
Hypertension	NS	NS	NS	0.319	NS	NS	NS	NS
Diabetes	NS	NS	**0.007**	**0.002**	NS	NS	0.021	NS
Menopause	NS	NS	NS	NS	NS	NS	NS	NS
Oral contraceptive	**<0.001**	NS	NS	NS	NS	NS	NS	NS
BMI, kg/m^2	NS	NS	NS	NS	NS	NS	NS	NS
Waist circumference, cm	NS	NS	NS	NS	NS	NS	NS	NS
Systolic blood pressure, mmHg	NS	NS	NS	NS	NS	NS	NS	NS
Diastolic blood pressure, mmHg	NS	NS	NS	NS	NS	NS	NS	NS
Corrected-calcium	NS	NS	NS	NS	NS	NS	NS	NS
VitD Rx or supll.	NS	NS	NS	NS	NS	NS	NS	NS
Urinary excretion of Na, (mmol/24h)	**0.033**	**0.012**	**0.046**	**0.017**	NS	**0.027**	**0.018**	NS
Urinary excretion of K, (mmol/24h)	NS	NS	NS	NS	NS	NS	**0.038**	NS
Urinary excretion of Ca, (mmol/24h)	NS	NS	**0.029**	NS	NS	NS	NS	NS
Urinary excretion of Urea, (mmol/24h)	NS	NS	NS	NS	NS	NS	NS	NS
High Fish	NS	NS	NS	NS	NS	NS	NS	NS
High Wine	NS	NS	NS	NS	NS	NS	NS	NS

High Beer	NS	NS	NS	NS	NS	NS	NS	**0.036**
Altitude, meters	NS	NS	NS	NS	NS	NS	NS	NS
Latitude, degrees	NS	NS	NS	NS	NS	NS	NS	NS
Mean monthly sunshine hours	NS	NS	NS	NS	NS	NS	NS	NS

[1]**Model 3 Linear regression model: $E(Y) = \alpha + \beta_1(E_1) + \gamma_1(V_1) + \sum\gamma_i(MO_i) + \delta_1(E_1*V_1)$**, where Y = 25[OH]D in ng/mL **(continuous, square-root transformed);** E_1= Each participants-level attributes of interest considered separately; V_1= CKD status (0,1); MO=month of sampling with k=12 and k-1 dummy variables: January (category 1), …, December (category 12) where MO={1 if category i, 0 if otherwise for i=2,3,4,5,6,7,8,9,10,11,12 (referent : 1)

Model 4 Ordinal logistic regression model: logit $P(D \geq g) = \alpha_g + \beta_1(E_1) + \gamma_1(V_1) + \delta_1(E_1*V_1)$, where D= month-specific tertiles 25[OH]D level **(ordinal outcome with g=1,2),** for each month of the study period, the distribution of 25(OH)D is used to define the specific tertiles of 25(OH)D; E_1= Each participants-level attributes of interest considered separately; V_1= CKD status (0,1)

[1]**Model 5 Linear regression model: $E(Y) = \alpha + \beta_1(E_1) + \sum\gamma_i(MO_i) + \sum\gamma_jV_j + E_1\sum\delta_{1j}V_j$** where Y = 25[OH]D in ng/mL **(continuous, square-root transformed);** E_1= Each participants-level attributes of interest considered separately; MO=month of sampling with k=12 and k-1 dummy variables: January (category 1), …, December (category 12) where MO={1 if category i, 0 if otherwise for i=2,3,4,5,6,7,8,9,10,11,12 (referent : 1); V=CKD stage with k=3 and k-1 dummy variables : CKD stage 0 (category 1), CKD stage 1 or 2 (category 2), and CKD stage 3 or more (category 3) where V={ 1 if category j, 0 if otherwise for j=2,3 (referent: category 1)

Model 6 Ordinal logistic regression model: logit $P(D \geq g) = \alpha_g + \beta_1(E_1) + \sum\gamma_iV_i + E_1\sum\delta_{1i}V_i$ where D= month-specific tertiles 25[OH]D level **(ordinal outcome with g=1,2),** for each month of the study period, the distribution of 25(OH)D is used to define the specific tertiles of 25(OH)D; E_1= Each participants-level attributes of interest considered separately; V=CKD stage with k=3 and k-1 dummy variable : CKD stage 0 (catgory 1), CKD stage 1 or 2 (category 2), and CKD stage 3 or more (category 3) where V={ 1 if category i, 0 if otherwise for i=2,3 (referent: category 1)
NS: not statistically significant (P value ≥0.05), Ref: reference, --: not enough data

CHAPTER 4

PROJECT 2

VITAMIN D SERUM LEVEL IS ASSOCIATED WITH CHANGE IN EGFR AND RAPID EGFR DECLINE IN THE GENERAL ADULT POPULATION

PROJECT 2 SPECIFIC AIMS

TO DETERMINE THE CHANGE IN KIDNEY FUNCTION ASSOCIATED WITH BASELINE SERUM VITAMIN D

HYPOTHESIS #1 : LOW BASELINE VITAMIN D LEVELS ARE ASSOCIATED WITH GREATER DECLINES IN EGFR THAN HIGH BASELINE VITAMIN D LEVELS

IN SECONDARY ANALYSES:

TO DETERMINE THE ASSOCIATION OF BASELINE VITAMIN D LEVELS WITH RISK OF RAPID EGFR DECLINE AND CKD

TO DETERMINE THE ASSOCIATION OF BASELINE VITAMIN D LEVELS WITH CHANGE IN CKD STATUS

ABSTRACT

Background: Molecular evidence suggests that sufficient vitamin D levels protect against renal function loss. Population-based studies on the association of circulating vitamin D with change in eGFR and incident CKD are limited and results discordant.

Methods: We used baseline (2003-2006) and 5-year follow-up data of adults from the general population to evaluate the association of serum vitamin D with change in kidney function, rapid decline in kidney function, and incidence of CKD. Serum vitamin D was measured at baseline using liquid chromatography-tandem mass spectrometry. Estimated glomerular filtration rate (eGFR) and albuminuria information were collected at baseline and follow-up. Rapid decline in eGFR was defined as an annual loss greater than 3 mL/min/1.73m^2. Multivariate linear and logistic regressions models were used considering major factors known to influence vitamin D levels.

Results: A total of 4,474 subjects were included in the analysis (mean follow-up 5.6 years). The mean (SD) annual eGFR change was -0.574 (1.77) mL/min/1.73m^2. Three hundred and three (6.8%) participants presented a rapid decline in eGFR. Of the participants without CKD at baseline, 282 (7.2%) presented a new CKD (defined as proteinuria or eGFR<60 mL/min/1.73m^2) at follow-up. Baseline vitamin D level was not associated with CKD incidence. The adjusted mean annual eGFR change was associated with baseline vitamin D levels. For each 10 ng/mL increase in baseline 25(OH)D, the adjusted mean annual eGFR change was higher by 0.094 mL/min/1.73m^2 (95%CI 0.026, 0.162, P value=0.006). Higher baseline vitamin D level was associated with decreased risk of rapid decline in eGFR, Odds Ratio=0.82, 95%CI 0.70, 0.97, p value=0.019 for each 10 units increase in baseline vitamin D.

Conclusions: Our study suggests that serum vitamin D may play an important role in the early stages of eGFR decline in adults from the general population.

INTRODUCTION

The kidney plays a major key role in the metabolism of vitamin D, in particular by converting 25-hydroxyvitamin D (25(OH)D) into the biologically active 1,25-dihydroxyvitamin D. The decline in renal function is associated with a progressive decrease in the ability of the kidney to produce 1,25-dihydroxyvitamin D.[1] Vitamin D deficiency is therefore common in patients with impaired kidney function, as measured by the glomerular filtration rate, even in the early stages of impairment.[2] In contrast, there is growing evidence that sufficient vitamin D levels could protect against renal function loss.[3]

An inverse association of vitamin D levels with proteinuria has also been reported.[4-6] but the associations of vitamin D levels with glomerular filtration rate (GFR) and CKD stages are less clear.[2, 7-10] Most of the studies conducted so far were cross-sectional and were subject to bias including temporality bias. Indeed, the product of 25(OH)D metabolism (24,25(OH)D) seems to be decreased in CKD leading to a state of stagnant vitamin D metabolism.[11] Thus, the level of 25(OH)D could paradoxically be higher among people with early stages of CKD than people with adequate kidney function.[11]

So far, three longitudinal studies assessed the association of baseline circulating 25(OH)D with change in estimated GFR (eGFR) and incident CKD.[9, 12, 13] In the Cardiovascular Health Study (United States) including 1705 people aged 65 years and more, serum 25(OH)D was associated with both increased change in eGFR and increased risk of rapid decline in eGFR.[9] In 1442 adults from the Framingham Heart Study (United States), plasma 25(OH)D was neither associated with incident CKD nor with incident

CKD defined as eGFR <60 mL/min and a 25% decline in eGFR.[12] Vitamin D deficiency was associated with a higher annual incidence of albuminuria but not with reduced eGFR in an Australian cohort of 6,180 adults.[13]

Given the high prevalence of vitamin D deficiency in the general adult population[14] as well as the paucity, and the conflicting nature, of results on the association of circulating vitamin D with change in eGFR and incident CKD, we used baseline (2003-2006) and 5-year follow-up data of 4'474 adults from the general population to evaluate the association of serum 25(OH)D with change in kidney function, rapid decline in kidney function, and incidence of CKD after controlling for major factors known to influence 25(OH)D levels, such as sunshine hours, altitude, physical activity, and supplements.

METHODS

COLAUS

We used the data from the CoLaus study. The primary aim of the CoLaus study was to assess the prevalence of cardiovascular risk factors in the Caucasian population of Lausanne, Switzerland.[15] The CoLaus study complied with the Declaration of Helsinki and was approved by the local Institutional Ethics Committees. All participants gave written informed consent. The sampling procedure of the CoLaus study has been described elsewhere.[15] Briefly, the CoLaus study was population-based and included participants aged 35 to 75 years. The recruitment took place in the city of Lausanne in Switzerland, a town of 117,161 inhabitants, of which 79,420 are of a Swiss nationality. The complete list of the Lausanne inhabitants aged 35–75 years (n = 56,694 in 2003) was

provided by the population register of the city and served to sample the participants to the study. A simple, non-stratified random sample of 35% of the overall population was drawn. The following inclusion criteria applied: a) written informed consent; b) aged 35-75 years; c) willingness to take part in the examination and donate blood. Recruitment began in June 2003 and ended in May 2006. The sample of 8,121 subjects who agreed to participate represented 41% of the initially sampled population. Participants were asked to attend the outpatient clinic at the Centre Hospitalier Universitaire Vaudois (CHUV) in the morning after an overnight fast. Between 2009 and 2012, all CoLaus participants have been invited for a follow-up (CoLaus 2). 4,679/6,188 (75.6%) subjects participated to the follow-up. The follow-up included standardized questionnaires, medication, physical examination, and blood exams.

Assessment process and clinical data

Data were collected by trained field interviewers using standardized questionnaires. Questionnaires recorded information on demographic data, socio-economic status, and several lifestyle factors such as tobacco and physical activity. A questionnaire, administered during a face-to-face meeting with the recruiter, focused on personal and family history of disease and cardiovascular risk factors. Self-reported prescription and self-prescribed drugs, vitamin and mineral supplements were recorded. Use of oral contraception and hormonal replacement therapy was self-reported.

Blood pressure (BP) was measured thrice on the left arm after at least 10 minutes rest in the seated position using a clinically validated oscillometric device (Omron® HEM-907, Matsusaka, Japan).[16] The average of the last two BP readings was used for

analyses. Hypertension was defined as mean systolic BP (SBP) ≥140 mmHg or mean diastolic BP (DBP) ≥90 mmHg or presence of anti-hypertensive medication. Diabetes was defined as a fasting glucose ≥7 mmol/L and/or presence of antidiabetic drug treatment (insulin or oral drugs). In addition, weight, height, and waist and hip circumferences were measured using standardized procedures. Body mass index (BMI) was defined as weight/height^2.[17]

Biologic data

Venous blood samples were drawn after an overnight fast. Glucose was measured by Glucose dehydrogenase (Roche Diagnostics, CH; CV 2.1%-1.0%), total serum cholesterol, HDL-cholesterol and serum triglycerides were measured by glycerol-3-phosphate oxidase – phenol aminophenazone (GPO-PAP) (Roche Diagnostics, CH; CV 3.6%-0.5%). Total serum calcium was measured by O-cresolphtalein (Roche Diagnostics, CH; CV 2.1%-1.5%) and albumin by bromocresol green (Roche Diagnostics, CH; CV 2.5%-0.4%). Albumin-corrected calcium was calculated using the following formula: Ca_c = Serum total calcium - 0.012 (serum albumin / 0.9677 - 39.55). This formula was derived by the central laboratory of the University Hospital of Lausanne based on data from 320 consecutive outpatients without disorders of phosphocalcic metabolism. In CoLaus, Ca_c calculated using this formula presented no residual correlation with serum albumin. Ultrasensitive protein C reactive (hsCRP) was measured by Immunoassay and latex HS (Agilent 1100 apparatus, CH; CV 4.6%-1.3%).

A urine sample was collected for the assessment of creatinine and albumin and the albumin-to-creatinine ratio (ACR) was calculated. To obtain a more linear relationship

with vitamin D levels, ACR was log-transformed. Kidney function was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation using both functional (GFR) and structural (urine albumin-to-creatinine ratio) information.[18] CKD stages were defined according to K/DOQI guidelines.[26, 27] Given the small number of participants with CKD stages 4 and 5, CKD stages 3 or greater were combined.

Altitude, sunshine hours

Participants were geocoded by merging information on the participant's private address with latitude, longitude and altitude information using Python programming and Google Maps Find Altitude software (https://developers.google.com/maps/documentation/elevation/).

Data on sunshine hours were obtained from MeteoSwiss which collects sunshine hours using meteorological stations distributed throughout Switzerland (http://www.meteoswiss.admin.ch/web/en.html). For each participant, data on sunshine hours collected in the station nearest to the participants's home address was used. The exposure period considered in this study was starting from the month before the participant's day of blood collection and was used to estimate the monthly mean sunshine hours.

25-hydroxyvitamin D

A fast, accurate and reliable method for the quantification of the vitamin D metabolites, 25-hydroxyvitamin D2 (25OH-D2) and 25-hydroxyvitamin D3 (25OH-D3) in human serum samples was developed and validated for this project. An ultra-high

pressure liquid chromatography-tandem mass spectrometry (LC-MS/MS) system was developed. The development and validation of this system is described elsewhere.[19]

Vitamin D level was expressed in ng/mL (Conversion factor for 25(OH)D: 1 ng/mL= 2.496 nmol/L). Vitamin D status was categorized into sufficiency, insufficiency, and deficiency defined respectively as 25(OH)D ≥30 ng/mL, 20–29.9 ng/mL, and <20 ng/mL according to experts recommendations.[20-22] The 25(OH)D concentrations fluctuation due to seasonal variation in sun exposure and the use of a single blood sample to estimate long term 25(OH)D average may lead to misclassification bias. With a continuous outcome, not adjusting for date of sample collection has been shown to create a bias toward the null. We therefore used the month of sample collection (11 dummy variables) in model when vitamin D was entered as a continuous variable.

STATISTICAL ANALYSES

Statistical analyses were performed using Stata 12.0 (Stata Corp, College Station, USA) and SAS 9.3 (SAS Institute, Cary NC). Annual change in eGFR was defined as the difference between eGFR (estimated using the CKD-EPI equation) at follow-up and eGFR at baseline, standardized to 1 year. In addition to annual change in eGFR, we also considered rapid decline in eGFR and CKD incidence as dependent outcomes. Rapid decline in eGFR was defined as an annual loss greater than 3 mL/min/1.73m^2.[9, 23] CKD incidence was defined as new CKD (CKD stage 1 or more) at follow-up among participants without CKD at baseline. To take into account possible regression to the mean, we ran additional models adjusting for both eGFR and ACR at baseline.

To explore the associations of kidney function with vitamin D, we alternatively used 1) vitamin D levels (continuous variable) and 2) vitamin D status (sufficient, insufficient, deficient), as the independent variable of interest.

Hypotheses and models

In this Project, kidney function –and specifically the yearly change in eGFR – was considered as the outcome and 25(OH) level (alternatively vitamin D status) the main exposure.

HYPOTHESIS #1 : LOW BASELINE VITAMIN D LEVELS ARE ASSOCIATED WITH GREATER DECLINES IN EGFR THAN HIGH BASELINE VITAMIN D LEVELS

To test this hypothesis, we conducted the following models:

We first considered kidney function as a continuous variable: the annual change in eGFR in mL/min/1.73 m^2 calculated as the difference between eGFR at baseline and at follow-up standardized to 1 year. The exposure variable was the baseline 25(OH)D level, considered as a continuous variable as well as an ordinal variable. Linear regression models were used (models 1 and 2)[5].

[5] **Model 1 Linear regression model: $E(Y) = \alpha + \beta_1(25[OH]D) + \sum \gamma_i (MO_i) + \sum \gamma_n(C_n)$,** where Y = change in eGFR= $eGFR_{baseline} - eGFR_{follow\text{-}up}$; 25[OH]D = baseline 25(OH)D in ng/mL; MO=month of sampling with k=12 and k-1 dummy variables: January (category 1), …, December (category 12) where MO={1 if category i, 0 if otherwise for i=2,3,4,5,6,7,8,9,10,11,12 (referent : 1); C=potential confounders

Model 2 Linear regression model: $E(Y) = \alpha + \sum \beta_i(vitD\ status) \sum \gamma_i (MO_i) + \sum \gamma_n(C_n)$, where Y = change in eGFR= $eGFR_{baseline} - eGFR_{follow\text{-}up}$; vitD status = baseline vitamin D status (2 dummy variables variable with sufficiency (=ref), insufficiency, and deficiency); MO=month of sampling with k=12 and k-1 dummy variables: January (category 1), …, December (category 12) where MO={1 if category i, 0 if otherwise for i=2,3,4,5,6,7,8,9,10,11,12 (referent : 1); C=potential confounders

Then, rapid decline in eGFR (binary variable) and incidence of CKD (binary variable) were modeled *separately* as a function of vitamin D at baseline (models 3 and 5)[6]. Additional models adjusting for CKD stages (defined using by both baseline eGFR and ACR) at baseline (in annual change in eGFR and rapid decline in eGFR models) or eGFR and ACR at baseline (in CKD incidence model) were run (model 4 and model 6)[7]. Unconditional logistic regression models were used for the rapid decline outcome as well as for the incidence of CKD outcome (Models 3-6).

We then considered the outcome as CKD status (presence vs. absence) at baseline and at follow-up and conducted a correlated binary logistic regression model. In the analysis of the association of vitamin D at baseline with CKD status at baseline and at follow-up, CKD status at baseline and at follow-up might be correlated. Repeated classification of CKD status in the same subject might be more correlated to each other than the observations on different subjects. The use of repeated observations in a longitudinal study needs to be considered as clustered data. Here the clusters=subjects

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[6] **Model 3 Unconditional logistic regression model: logit $P(D=1|X) = \alpha + \beta_1(25[OH]D) + \sum \gamma_i (MO_i) + \sum \gamma_n(C_n)$,** where D=1 if rapid loss in eGFR= annual eGFR loss >3mL/min/1.73 m^2, D= 0 otherwise; 25[OH]D = baseline 25(OH)D in ng/mL; MO=month of sampling with k=12 and k-1 dummy variables: January (category 1), …, December (category 12) where MO={1 if category i, 0 if otherwise for i=2,3,4,5,6,7,8,9,10,11,12 (referent : 1); C=potential confounders

Model 5 Unconditional logistic regression model: logit $P(D=1|X) = \alpha + \beta_1(25[OH]D) + \sum \gamma_i (MO_i) + \sum \gamma_n(C_n)$, where D=1 if new CKD=CKD at follow-up among participants without CKD at baseline, D=0 otherwise; 25[OH]D = baseline 25(OH)D in ng/mL; MO=month of sampling with k=12 and k-1 dummy variables: January (category 1), …, December (category 12) where MO={1 if category i, 0 if otherwise for i=2,3,4,5,6,7,8,9,10,11,12 (referent : 1); C=potential confounders

[7] **Model 4 Unconditional logistic regression model: logit $P(D=1|X) = \alpha + \beta_1(25[OH]D) + \sum \gamma_i (MO_i) + \sum \gamma_n(C_n) + \gamma_{n+1}$(CKD stage at baseline),** where D =1 if rapid loss in eGFR= annual eGFR loss >3mL/min/1.73 m^2, D=0 otherwise; 25[OH]D = baseline 25(OH)D in ng/mL; MO=month of sampling with k=12 and k-1 dummy variables: January (category 1), …, December (category 12) where MO={1 if category i, 0 if otherwise for i=2,3,4,5,6,7,8,9,10,11,12 (referent : 1); C=potential confounders; CKD stage at baseline is defined using by both eGFR and ACR at baseline.

Model 6 Unconditional logistic regression model: logit $P(D=1|X) = \alpha + \beta_1(25[OH]D) + \sum \gamma_i (MO_i) + \sum \gamma_n(C_n) + \gamma_{n+1}$(eGFR baseline) γ_{n+2}(logACR baseline), where D = 1 if new CKD=CKD at follow-up among participants without CKD at baseline, D=0 otherwise; 25[OH]D = baseline 25(OH)D in ng/mL; MO=month of sampling with k=12 and k-1 dummy variables: January (category 1), …, December (category 12) where MO={1 if category i, 0 if otherwise for i=2,3,4,5,6,7,8,9,10,11,12 (referent : 1); C=potential confounders

(number of clusters = number of subjects) with 2 observations within each cluster, and the dataset was balanced. The covariance between the classification on the two occasions (baseline and follow-up) for the same subject was considered as a marginal covariance. We considered the exchangeable covariance structure. Of note, with exactly 2 observations per subject (at baseline study and at follow-up study), the only correlation to consider is the correlation between the two responses for the same subject. As a result, using an autoregressive 1, exchangeable, or unstructured correlation yields the same 2x2 working correlation matrix.[24] Covariates were considered as time-dependent (e.g., age, hypertension, etc) or time-independent (e.g., sex, education). We used model without (model 7) and with (model 8)[8] adjustment for kidney function at baseline.

VARIABLES SPECIFICATION

Covariates were considered given their reported or potential influence on kidney function and/or vitamin D level. To identify factors to be included in the *initial* multivariate models, bivariate associations between kidney function, respectively vitamin D, and attributes of interest were tested. Significances of *p*-values for including the attribute in the *initial* full model were set at <0.10.

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[8] **Model 7 Correlated binary logistic regression: logit $P(D=1|X) = \alpha + \beta_{11}(25[OH]D) + \sum \gamma_{11i}(MO_i) + \sum \gamma_{nj}(C_{nj})$,** where D= CKD binary outcome considered at baseline and at follow-up; CKD baseline is correlated with CKD at follow-up; Correlation structure= exchangeable; dataset=balanced; 25(OH)D=vitamin D in ng/mL; MO=month of sampling with k=12 and k-1 dummy variables: January (category 1), …, December (category 12) where MO={1 if category i, 0 if otherwise for i=2,3,4,5,6,7,8,9,10,11,12 (referent : 1); C_{nj}= N potential confounders considered at baseline and at follow-up (j=1,2). Some covariates were considered as time-dependent (e.g., age, hypertension, etc) and others as time-independent (e.g., sex, education)

Model 8 Correlated binary logistic regression: logit $P(D=1|X) = \alpha + \beta_{11}(25[OH]D) + \sum \gamma_{11i}(MO_i) + \gamma_{n+1}$(eGFR baseline) + γ_{n+2}(logACR baseline) + $\sum \gamma_{nj}(C_{nj})$, where D= CKD binary outcome considered at baseline and at follow-up; CKD baseline is correlated with CKD at follow-up; Correlation structure= exchangeable; dataset=balanced; 25(OH)D=vitamin D in ng/mL; MO=month of sampling with k=12 and k-1 dummy variables: January (category 1), …, December (category 12) where MO={1 if category i, 0 if otherwise for i=2,3,4,5,6,7,8,9,10,11,12 (referent : 1); C_{nj}= N potential confounders considered at baseline and at follow-up (j=1,2). Some covariates were considered as time-dependent (e.g., age, hypertension, etc) and others as time-independent (e.g., sex, education)

COLLINEARTIY, CONFOUNDING

In the full model, collinearity problem between latitude and altitude was diagnosed (CI >30 and VDPs >0.50 for both latitude and altitude). Because latitude did not vary across subjects in the city of Lausanne (46.5^{o}), latitude was dropped. A second collinearity problem between BMI and waist circumference was diagnosed (CI >30 and VDPs >0.50 for both BMI and waist circumference). Based on biological criteria (e.g, waist circumference is known to be a better determinant of obesity-related disease - including kidney failure - than BMI), we decided to drop BMI. Collinearity problem was resolved.

To build the reduced models, we used the backwards change in estimate elimination and precision approaches.[25] Both full (gold standard) and reduced models are presented.

Supplementary analyses

The 25(OH)D concentrations fluctuation due to seasonal variation in sun exposure and the use of a single blood sample to estimate long term 25(OH)D average may lead to misclassification bias. To explore the robustness of our findings, we also used month-specific quintiles of 25(OH)D as the dependent variable. Monthly-specific quintiles of 25(OH)D were computed as follows: For each month of the study period, the distribution of 25(OH)D is used to define the specific quintiles of 25(OH)D. The quintiles of the 25(OH)D distribution restricted to blood samples conducted during the month of August

were computed. Similarly, the quintiles of the 25(OH)D distribution were computed for each month of the year so that each month has specific quintiles of 25(OH)D.

RESULTS

PARTICIPANTS' CHARACTERISTICS

A total of 4,474 subjects were included in the analysis (95.6% of the cohort). The mean and median follow-up were 5.6 years and 5.5 years (SD 0.46 and IQR 5.4-5.7). Characteristics by vitamin D status are detailed in **Table 1**. The prevalence of vitamin D sufficiency, insufficiency, and deficiency were 13.0%, 32.7%, and 54.3%, respectively, with mean 25(OH)D levels of 35.6 (5.0), 24.4 (2.8), and 13.0 (4.2) ng/mL, respectively. At baseline, 10.9% presented CKD (2.9% CKD stage 1, 3.7% CKD stage 2, and 4.3% CKD stage 3+). The groups defined by vitamin D status statistically differed by all characteristics apart from gender, education, altitude, and triglycerides.

The mean annual eGFR change was -0.574 (1.77) mL/min/1.73m^2. Three hundred and three (6.8%) participants presented a rapid decline in eGFR. Out of the participants without CKD at baseline, 282 (7.2%) presented a new CKD (defined as proteinuria or eGFR<60 mL/min/1.73m^2) at follow-up (1.1%, 2.1%, 4.0% stage 1, 2, and 3 or greater, respectively).

Table 2 reports the mean change in eGFR and proportions of rapid decline and new onset of CKD, by vitamin D status.

The adjusted mean annual eGFR change was associated with baseline 25(OH)D. For each 10 ng/mL increase baseline 25(OH)D, the adjusted mean annual eGFR change

increased by 0.084 mL/min/1.73m^2 (95%CI 0.014, 0.154, P value=0.018) in the full model, and 0.094 mL/min/1.73m^2 (95%CI 0.026, 0.162, P value=0.006) in the reduced model (**Table 3**). Baseline 25(OH)D level was negatively associated with the risk of rapid decline in eGFR. The risk decreased by 16% (OR=0.84, 95%CI 0.71, 0.99, p value=0.041) and 28% (OR=0.82, 95%CI 0.70, 0.97, p value=0.019) for each 10 units increase in baseline 25(OH)D in full and reduced models, respectively (**Table 3**). These negative associations persisted after adjustment for CKD stages at baseline (**Table 3**). Baseline vitamin D level was not associated with CKD incidence (**Table 3**). Further adjustment for kidney function at baseline did not change meaningfully the magnitude of the associations or the statistical significance (**Table 3**).

Vitamin D status (sufficiency, insufficiency, deficiency) was independently associated with mean annual eGFR change. A dose-response effect was observed between vitamin D status and mean annual eGFR change. Compared to subjects with vitamin D sufficiency at baseline, the fully adjusted difference in annual eGFR change was -0.118 (95%CI -0.219, 0.0555, P value 0.183) and -0.254 (95%CI -0.433, -0.074, P value =0.006) among subjects with vitamin D insufficiency and deficiency, respectively (**Table S1**). Similar results were observed using the reduced model.

We found no association between baseline 25(OH)D level and the presence or absence of CKD using CKD information at baseline and at follow-up (**Table S2**).

DISCUSSION

In this prospective population-based study, we show that 25(OH)D in the adult population with predominantly normal baseline kidney function is associated with change

and risk of rapid decline in eGFR. These inverse associations are independent of major potential confounders including CKD stages defined by eGFR and proteinuria at baseline and cover the entire range of vitamin D levels.

Circulating vitamin D has been associated with kidney function.[1] This association is mediated by different mechanisms including a decline in the 1-alpha hydroxylation of 25(OH)D to the metabolically active form of vitamin D (1,25(OH)2D3),[26] an increase in urinary loss of vitamin D and vitamin D binding protein,[27] and an increase in renin secretion and renin-angiotensin system activity.[28] Renal retention of phosphorus and increased fibroblast growth factor-23 in kidney failure may also contribute to vitamin D deficiency in kidney failure.[29] Compared to people without CKD, patients with CKD might also have decreased sunlight exposure and sub-optimal vitamin D intake from the diet.[30]

These associations are found with 25(OH) vitamin D considered as concentration levels (ng/mL), status (sufficiency, insufficiency, deficiency), and month-specific quintiles (**Supplementary material**). The significant increased risk in rapid decline in eGFR in people with low vitamin D levels suggests that the effect is large enough to have clinical significance. Lower GFR and rapid decline in eGFR are associated with increased risks of cardiovascular disease and death.[22, 23, 31]

Depending on the definition of low vitamin D used (using either vitamin D status or month-specific quintiles), the risk of rapid decline in eGFR increases up to about 60%. The increased risk was statistically significant only in subjects with vitamin deficiency (<20ng/mL) or subjects in the lowest month-specific quintile, although the risk is

intermediate in the three middle quintiles. The upper limit of the lowest quintile (fifth quintile range: 1.7-20.3 ng/mL) was 20ng/mL. Twenty ng/mL was recently found to be the "optimal" concentrations of 25(OH)D because the risk for relevant clinical disease events increased below this level.[22] This level was also previously recommended by the Institute of Medicine for bone health.[21] Our results suggest that, similarly to other clinical disease events, the risk in rapid eGFR decline significantly increase when the serum 25(OH)D concentration is falling below this level.

Our results are in line with, and expand further, the recent findings reported by de Boer et al. in older adults.[9] Here show that low vitamin D is also associated with kidney function in adult younger than 65 years population. As expected, the prevalence of rapid decline in eGFR was lower in our younger study population than the prevalence reported by de Boer et al. using data on older adults (6% vs. 12%). Thus, vitamin D seems to increase the risk of rapid decline in eGFR even in populations at lower risk of rapid decline in eGFR. The consequences of low vitamin D on health outcomes related to kidney failure could therefore be greater than previously reported by de Boer et al. De Boer et al. lacked information on proteinuria and acknowledged this limitation and the associated risk of confounding. We measured and calculated the urinary ACR to classify all subjects into CKD stages. We then ran additional analyses and adjusted our estimates for CKD stages. We show that low vitamin D is also associated with kidney function independently of baseline kidney function. Our findings also add to the current evidence as we collected information on a large sample size for a long period (5-year) and on known, yet rarely considered as potential confounders, factors of both 25(OH)D and kidney function such as altitude.[32]

We found no association between vitamin D and CKD incidence. This is in line with findings reported by O'Seaghdha et al. [12] We suggest that the lack of association with incident CKD in the presence of a clear association with the continuous eGFR and rapid decline in eGFR can be explained by the fact that vitamin D deficiency seems to be mostly a risk factor of eGFR decline in pre-CKD stage. In our study, 86.1% of subjects with rapid decline in eGFR had no CKD, while only 4.9% and 6.6% had CKD stage 1 and 2, respectively.

Strengths and limitations

These results should be interpreted in the light of the study's strengths and limitations. This is the largest prospective study assessing the effect of circulating vitamin D on kidney function in a general adult population. We measured 25(OH)D by the gold standard technique (LC-MS/MS). Information on major known potential confounders was available. However, despite these efforts, information is still incomplete. We lack information on potential confounders such as parathyroid hormone and fibroblast growth factor-23. GFR was estimated using the CKD-Epi equation. More accurate measurements (including inulin clearance, iothalamate clearance, cystatin C) exist. Participants were all Caucasians and our findings may not be generalizable to non-Caucasians populations. The prevalence of vitamin D deficiency and insufficiency depends on the definition used. We have based our analyses on the most recent definition of vitamin D deficiency by the 2011 US Report on Dietary Reference Intakes for Calcium and Vitamin D from the IOM.[21] The IOM committee concluded that a serum 25-hydroxyvitamin D level of 20 ng/mL is desirable for bone and overall health. We

acknowledge that the definition of vitamin D insufficiency is more controversial. Fasting serum was collected from CoLaus participants at the 2003-2006 study visit and stored at -80^{o}C before 25(OH)D was measured. Yet, 25(OH)D is stable for long periods at this temperature.[33] To represent the participant's typical level of vitamin D, we relied on a single 25(OH)D measurement. It has been shown that a single baseline 25(OH)D serum measurement provide a reasonably representative measure of vitamin D in adults, confirming its utility as epidemiologic biomarkers in prospective studies.

CONCLUSIONS

Our study further suggests that 25(OH)D may play an important role in the early stages of eGFR decline in adults. A concentration below 20 ng/mL, which has been reported as a critical concentration for clinical disease events and bone health, seems to be associated with the risk of rapid decline in eGFR. To determine if this relationship is causal, randomized controlled trials exploring whether correcting vitamin D deficiency slows down the age-related decline in renal function. This could represent a major opportunity to mitigate the growing burden of chronic kidney disease and end-stage renal disease.

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TABLE 1. BASELINE CHARACTERISTICS OF THE COLAUS PARTICIPANTS, BY VITAMIN D STATUS (N=4474)

	ALL	Sufficiency	Insufficiency	Deficiency	**P value**
Number (%)	4474 (100.0)	584 (13.0)	1464 (32.7)	2426 (54.3)	
Age, years (SD)	52.6 (10.6)	54.2 (10.7)	52.7 (10.6)	52.2 (10.5)	0.001
Female gender (%)	2403 (53.7)	331 (56.7)	808 (55.2)	1264 (52.1)	0.053
Education					0.123
Low (%)	2437 (54.5)	306 (52.4)	768 (52.5)	1363 (56.2)	
Middle (%)	1136 (25.4)	161 (27.6)	392 (26.8)	583 (24.0)	
High (%)	901 (20.1)	117 (20.0)	304 (20.8)	480 (19.8)	
Smoking status					0.014
Never smokers (%)	1809 (40.4)	239 (40.9)	610 (41.7)	950 (39.6)	
Former smokers (%)	1517 (33.9)	216 (37.0)	506 (34.6)	795 (32.8)	
Smokers (%)	1148 (25.7)	129 (22.1)	348 (23.8)	671 (27.7)	
Physical activity					<0.001
Never or don't know (%)	1466 (32.8)	141 (24.1)	384 (26.2)	941 (38.8)	
1x/week (%)	430 (9.6)	37 (6.3)	124 (8.5)	269 (11.1)	
2x/week (%)	2578 (57.6)	941 (69.5)	269 (65.3)	1216 (50.1)	
Hypertension (%)	1493 (33.4)	182 (31.2)	433 (29.6)	878 (36.2)	<0.001
Diabetes (%)	243 (5.4)	16 (2.7)	63 (4.30)	164 (6.8)	<0.001

Oral contraceptive (among women N=2403) (%)	200 (8.3)	43 (13.0)	59 (7.3)	98 (7.8)	0.004
Waist circumference, cm (SD)	88.6 (13.2)	85.3 (12.0)	87.6 (12.7)	90.0 (13.5)	<0.001
Albumin-corrected calcium, mmol/L (SD)	2.21 (0.08)	2.23 (0.09)	2.21 (0.08)	2.20 (0.08)	<0.001
VitD supllements or Rx (%)	190 (4.2)	74 (13.0)	75 (5.1)	41 (1.7)	<0.001
Altitude, meters (SD)	534.7 (91.2)	527.7 (85.3)	535.9 (91.4)	535.6 (92.4)	0.143
Mean monthly sunshine hours (SD)	5.1 (2.2)	5.9 (2.1)	5.5 (2.3)	4.6 (2.1)	<0.001
usCRP, mg/L (SD)	2.36 (3.3)	2.24 (3.3)	2.19 (3.20)	2.50 (3.42)	0.0122
Triglycerides, mmol/L (SD)	1.36 (1.04)	1.34 (1.12)	1.33 (1.14)	1.37 (0.95)	0.405
HDL-cholesterol, mmol/L (SD)	1.64 (0.43)	1.74 (0.45)	1.67 (0.44)	1.61 (0.42)	<0.001

TABLE 2. KIDNEY PHENOTYPES AND OUTCOMES, BY VITAMIN D STATUS (N=4474)

	ALL	Sufficiency	Insufficiency	Deficiency	**P value**
eGFR, mL/min/1.73m^2 (SD)	85.5 (14.9)	80.1 (14.2)	84.0 (14.6)	87.7 (14.8)	<0.001
Albumin-to-creatinine ratio, mg/g	4.9 (2.4)	4.6 (2.4)	4.8 (2.3)	5.0 (2.5)	<0.001
Annual change in eGFR, mL/min/1.73m^2 (SD)	-0.575 (1.77)	-0.265 (1.80)	-0.456 (1.68)	-0.721 (1.80)	<0.001
CKD stages					<0.001
NO CKD, %	89.1	87.2	89.8	89.1	
CKD stage 1, %	2.9	2.4	2.0	3.6	
CKD stage 2, %	3.7	3.2	3.5	4.0	
CKD stage 3 or greater, %	4.3	7.2	4.6	3.3	
Rapid decline in eGFR, %	6.8	4.6	5.3	8.2	<0.001
Incident CKD, %	7.2	7.4	7.4	7.1	0.923

Table 3. Associations of baseline 25(OH)D level with annual change in eGFR, rapid loss in eGFR, and incident CKD

Baseline 25(OH)D (per 10 ng/mL unit)	Annual change in eGFR			Rapid loss in eGFR			Incident CKD		
	Beta coefficients	95%CI	P value	OR	95%CI	P value	OR	95%CI	P value
Full model*	0.084[a]	0.014, 0.154	0.018	0.84[b]	0.71, 0.99	0.041	1.02[c]	0.86, 1.22	0.780
Reduced model**	0.094[a]	0.026, 0.162	0.006	0.82[b]	0.70, 0.97	0.019	0.95[c]	0.81, 1.11	0.27
Full model* + adjusted for CKD stage at baseline***	0.080[d]	0.001, 0.148	0.026	0.84[e]	0.71, 0.99	0.042	-	-	-
Reduced model** + adjusted for CKD stage at baseline***	0.090[d]	0.022, 0.158	0.009	0.82[e]	0.70, 0.97	0.019	-	-	-
Full model* + adjusted for eGFR and albuminuria at baseline	-	-	-	-	-	-	0.98	0.82, 1.17	0.856
Reduced model** + adjusted for eGFR and albuminuria at baseline	-	-	-	-	-	-	0.91[f]	0.77, 1.08	0.308

* Adjusted for age, gender, education, smoking status, physical activity), hypertension, diabetes, oral contraceptive, waist circumference, albumin-corrected calcium, VitD supplements or Rx, altitude, mean monthly sunshine hours, usCRP, triglycerides, HDL-cholesterol, and month of sampling.

**Adjusted for age, albumin-corrected calcium, mean monthly sunshine hours, diabetes, physical activity, smoking status, oral contraceptive, and month of blood sampling

*** CKD stage at baseline is defined using by both eGFR and ACR at baseline.

[a]**Model 1 Linear regression model: $E(Y) = \alpha + \beta_1(25[OH]D) + \sum\gamma_i (MO_i) + \sum\gamma_n(C_n)$,** where Y = change in eGFR= $eGFR_{baseline} - eGFR_{follow\text{-}up}$; 25[OH]D = baseline 25(OH)D in ng/mL; MO=month of sampling with k=12 and k-1 dummy variables: January (category 1), …, December (category 12) where MO={1 if category i, 0 if otherwise for i=2,3,4,5,6,7,8,9,10,11,12 (referent : 1); C=potential confounders

[b]**Model 3 Unconditional logistic regression model: logit $P(D=1|X) = \alpha + \beta_1(25[OH]D) + \sum\gamma_i (MO_i) + \sum\gamma_n(C_n)$,** where D=1 if rapid loss in eGFR= annual eGFR loss >3mL/min/1.73 m^2, D= 0 otherwise; 25[OH]D = baseline 25(OH)D in ng/mL; MO=month of sampling with k=12 and k-1 dummy variables: January (category 1), …, December (category 12) where MO={1 if category i, 0 if otherwise for i=2,3,4,5,6,7,8,9,10,11,12 (referent : 1); C=potential confounders

[c] **Model 5 Unconditional logistic regression model: logit $P(D=1|X) = \alpha + \beta_1(25[OH]D) + \sum\gamma_i (MO_i) + \sum\gamma_n(C_n)$,** where D=1 if new CKD=CKD at follow-up among participants without CKD at baseline, D=0 otherwise; 25[OH]D = baseline 25(OH)D in ng/mL; MO=month of sampling with k=12 and k-1 dummy variables: January (category 1), …, December (category 12) where MO={1 if category i, 0 if otherwise for i=2,3,4,5,6,7,8,9,10,11,12 (referent : 1); C=potential confounders

[d]**Model 1b Linear regression model: $E(Y) = \alpha + \beta_1(25[OH]D) + \gamma_1$(month of sampling) $+ \sum\gamma_n(C_n) + \gamma_{n+1}$(CKD stage at baseline),** where Y = change in eGFR= $eGFR_{baseline} - eGFR_{follow\text{-}up}$; 25[OH]D = baseline 25(OH)D in ng/mL; C=potential confounders; CKD stage at baseline is defined using by both eGFR and ACR at baseline.

$E(Y) = \alpha + \beta_1(25[OH]D) + \sum\gamma_i (MO_i) + \sum\gamma_n(C_n) + \gamma_{n+1}$(CKD stage at baseline), where Y = change in eGFR= $eGFR_{baseline} - eGFR_{follow\text{-}up}$; 25[OH]D = baseline 25(OH)D in ng/mL; MO=month of sampling with k=12 and k-1 dummy variables: January (category 1), …, December (category 12) where MO={1 if category i, 0 if otherwise for i=2,3,4,5,6,7,8,9,10,11,12 (referent : 1); C=potential confounders; CKD stage at baseline is defined using by both eGFR and ACR at baseline.

[e] **Model 4 Unconditional logistic regression model: logit $P(D=1|X) = \alpha + \beta_1(25[OH]D) + \sum\gamma_i (MO_i) + \sum\gamma_n(C_n) + \gamma_{n+1}$(CKD stage at baseline),** where D =1 if rapid loss in eGFR= annual eGFR loss >3mL/min/1.73 m^2, D=0 otherwise; 25[OH]D = baseline 25(OH)D in ng/mL; MO=month of sampling with k=12 and k-1 dummy variables: January (category 1), …, December (category 12) where MO={1 if category i, 0 if otherwise for i=2,3,4,5,6,7,8,9,10,11,12 (referent : 1); C=potential confounders; CKD stage at baseline is defined using by both eGFR and ACR at baseline.

[f] **Model 6 Unconditional logistic regression model: logit $P(D=1|X) = \alpha + \beta_1(25[OH]D) + \sum\gamma_i (MO_i) + \sum\gamma_n(C_n) + \gamma_{n+1}$(eGFR baseline) γ_{n+2}(logACR baseline),** where D = 1 if new CKD=CKD at follow-up among participants without CKD at baseline, D=0 otherwise; 25[OH]D = baseline 25(OH)D in ng/mL; MO=month of sampling with k=12 and k-1 dummy variables: January (category 1), …, December (category 12) where MO={1 if category i, 0 if otherwise for i=2,3,4,5,6,7,8,9,10,11,12 (referent : 1); C=potential confounders

TABLE S1. MULTIVARIATE ASSOCIATION OF VITAMINE D STATUS AT BASELINE WITH ANNUAL CHANGE IN ESTIMATED GFR (N=4474), FULL AND REDUCED MODELS

	Beta coefficients	**95%CI**	**P value**
Full* model[a]			
Vitamin D sufficiency (30+)	Ref	--	--
Vitamin D insufficiency (20-30)	-0.118	-0.291, 0.055	0.183
Vitamin D deficiency (<20)	-0.254	-0.433, -0.074	0.006
Reduced model[a]**			
Vitamin D sufficiency (30+)	Ref	--	--
Vitamin D insufficiency (20-30)	-0.121	-0.293, 0.0502	0.166
Vitamin D deficiency (<20)	-0.267	-0.442, -0.092	0.003
Full* model + adjusted for CKD stage at baseline*[b]**			
Vitamin D sufficiency (30+)	Ref	--	--
Vitamin D insufficiency (20-30)	-0.106	-0.278, 0.066	0.227
Vitamin D deficiency (<20)	-0.241	-0.419, -0.062	0.008
Reduced model + adjusted for CKD stage at baseline***[b]**			
Vitamin D sufficiency (30+)	Ref	--	--
Vitamin D insufficiency (20-30)	-0.109	-0.280, 0.061	0.208
Vitamin D deficiency (<20)	-0.255	-0.429, -0.081	0.004

* Adjusted for age, gender, education, smoking status, physical activity, hypertension, diabetes, oral contraceptive, waist circumference, albumin-corrected calcium, VitD supplements or Rx, altitude, mean monthly sunshine hours, usCRP, triglycerides, HDL-cholesterol, and month of blood sampling

**Adjusted for age, albumin-corrected calcium, mean monthly sunshine hours, diabetes, physical activity, smoking status, oral contraceptive, and month of blood sampling

*** CKD stage at baseline is defined using by both eGFR and ACR at baseline.

[a]**Model 2 Linear regression model: $E(Y) = \alpha + \sum\beta_i(\text{vitD status}) + \sum\gamma_i (MO_i) + \sum\gamma_n(C_n)$,** where Y = change in eGFR= $eGFR_{baseline} - eGFR_{follow\text{-}up}$; vitD status = baseline vitamin D status (2 dummy variables variable with sufficiency (=ref), insufficiency, and deficiency); MO=month of sampling with k=12 and k-1 dummy variables: January (category 1), …, December (category 12) where MO={1 if category i, 0 if otherwise for i=2,3,4,5,6,7,8,9,10,11,12 (referent : 1); C=potential confounders

[b]**Model 2 further adjusted for kidney function at baseline: Linear regression model: $E(Y) = \alpha + \sum\beta_i(\text{vitD status}) + \sum\gamma_i (MO_i) + \sum\gamma_n(C_n) + \sum\gamma_{n+1}(\text{CKD stage at baseline})$,** where Y = change in eGFR= $eGFR_{baseline} - eGFR_{follow\text{-}up}$; vitD status = baseline vitamin D status (2 dummy variables variable with sufficiency (=ref), insufficiency, and deficiency); MO=month of sampling with k=12 and k-1 dummy variables: January (category 1), …, December (category 12) where MO={1 if category i, 0 if otherwise for i=2,3,4,5,6,7,8,9,10,11,12 (referent : 1); C=potential confounders; CKD stage at baseline is defined using both eGFR and ACR at baseline

TABLE S2. MULTIVARIATE ASSOCIATION (CORRELATED BINARY LOGISTIC REGRESSION) OF VITAMINE D LEVEL AT BASELINE WITH CKD STATUS AT BASELINE AND AT FOLLOW-UP (N=4474), FULL AND REDUCED MODELS

Baseline 25(OH)D (per 10 ng/mL unit)	**OR**	**95%CI**	**P value**
Full* model[a]	0.97	0.86, 1.09	0.584
Full* model + adjusted for baseline eGFR (time-independent) and baseline albuminuria (time-independent)[b]	0.94	0.79, 1.11	0.498
Reduced model[a]**	0.93	0.83, 1.04	0.210
Reduced model + adjusted for baseline eGFR (time-independent) and baseline albuminuria (time-independent)[b]**	0.89	0.76, 1.05	0.169

* Adjusted for age (time-dependent), gender (time-independent), education (time-independent), smoking status (time-dependent), physical activity (time-dependent), hypertension (time-dependent), diabetes (time-dependent), oral contraceptive (time-dependent), waist circumference (time-dependent), albumin-corrected calcium (time-dependent), VitD supplements or Rx (time-independent), altitude (time-independent), mean monthly sunshine hours (time-independent), usCRP (time-dependent), triglycerides (time-dependent), HDL-cholesterol (time-dependent), and month of blood sampling (time-independent).

**Adjusted for age (time-dependent), smoking status (time-dependent), physical activity (time-dependent), diabetes (time-dependent), oral contraceptive (time-dependent), albumin-corrected calcium (time-dependent), and month of blood sampling (time-independent).

[a]**Model 7 Correlated binary logistic regression: logit** $\mathbf{P(D=1IX) = \alpha + \beta_{11}(25[OH]D) + \sum\gamma_{11i}(MO_i) + \sum\gamma_{nj}(C_{nj})}$, where D= CKD binary outcome considered at baseline and at follow-up; CKD baseline is correlated with CKD at follow-up; Correlation structure= exchangeable; dataset=balanced; 25(OH)D=vitamin D in ng/mL; MO=month of sampling with k=12 and k-1 dummy variables:

January (category 1), …, December (category 12) where MO={1 if category i, 0 if otherwise for i=2,3,4,5,6,7,8,9,10,11,12 (referent : 1); ; C_{nj}= N potential confounders considered at baseline and at follow-up (j=1,2). Some covariates were considered as time-dependent (e.g., age, hypertension, etc) and others as time-independent (e.g., sex, education)

[b]**Model 8 Correlated binary logistic regression: logit P(D=1IX) = $\alpha + \beta_{11}$(25[OH]D) + $\sum\gamma_{11i}$ (MO_i) + γ_{n+1}(eGFR baseline) + γ_{n+2}(logACR baseline) + $\sum\gamma_{ni}(C_{ni})$,** where D= CKD binary outcome considered at baseline and at follow-up; CKD baseline is correlated with CKD at follow-up; Correlation structure= exchangeable; dataset=balanced; 25(OH)D=vitamin D in ng/mL; MO=month of sampling with k=12 and k-1 dummy variables: January (category 1), …, December (category 12) where MO={1 if category i, 0 if otherwise for i=2,3,4,5,6,7,8,9,10,11,12 (referent : 1); C_{nj}= N potential confounders considered at baseline and at follow-up (j=1,2). Some covariates were considered as time-dependent (e.g., age, hypertension, etc) and others as time-independent (e.g., sex, education)

Table S3. Difference in Annual Change in estimated GFR (ml/min/1.73m^2) (95%CI), by vitamin D month-specific quintiles

	Difference in Annual Change in estimated GFR (ml/min/1.73m^2) (95%CI)				
	N (%)	**Full* model[a]**	**Reduced** model[a]**	**Full* model + adjusted for CKD stage at baseline***[b]**	**Reduced** model + adjusted for CKD stage at baseline***[b]**
Vitamin D month-specific					
quintile 1 (highest)	892 (19.9)	0 (ref)	0 (ref)	0 (ref)	0 (ref)
quintile 2	894 (20.0)	-0.182 (-0.347, -0.0172)†	-0.182 (-0.347, -0.0172)†	-0.184 (-0.348, -0.021)†	-0.187 (-0.349, -0.024)†
quintile 3	894 (20.0)	-0.206 (-0.72, -0.0396)‡	-0.205 (-0.371, -0.0396)†	-0.191 (-0.356, -0.025)†	-0.205 (-0.368, -0.042)†
quintile 4	896 (20.0)	-0.273 (-0.442, -0.105)‡	-0.273 (-0.441, -0.104)‡	-0.263 (-0.430, -0.095)‡	-0.281 (-0.445, -0.117)‡
quintile 5 (lowest)	898 (20.1)	-0.214 (-0.386, -0.043)‡	-0.214 (-0.386, -0.0429)†	-0.209 (-0.380, -0.039)†	-0.229 (-0.396, -0.062)‡

*Adjusted for age, gender, education, smoking status, physical activity, hypertension, diabetes, Oral contraceptive, waist circumference, albumin-corrected calcium, VitD supplements or Rx, altitude, mean monthly sunshine hours, usCRP, triglycerides, and HDL-cholesterol

**Adjusted for age, smoking status, physical activity, diabetes, oral contraceptive, albumin-corrected calcium

*** CKD stage at baseline is defined using by both eGFR and ACR at baseline.

† P value< 0.05; ‡ P values<0.01

[a]**Model S1 Results of the following Linear regression model: $E(Y) = \alpha + \sum\beta_i$(vitD month-specific quintiles) + $\sum\gamma_n(C_n)$,** where Y = change in eGFR= $eGFR_{baseline} - eGFR_{follow\text{-}up}$; **vitD month-specific quintiles** = month-specific quintiles 25[OH]D level **(4 dummy variables with highest quintiles=ref),** for each month of the study period, the distribution of 25(OH)D is used to define the specific quintiles of 25(OH)D; C=potential confounders;

[b]**Model S2 Results of the following Linear regression model: $E(Y) = \alpha + \sum\beta_i$(vitD month-specific quintiles) + γ_n(CKD stage at baseline) + $\sum\gamma_{n+1}(C_{n+1})$,** where Y = change in eGFR= $eGFR_{baseline} - eGFR_{follow\text{-}up}$; **vitD month-specific quintiles** = month-specific quintiles 25[OH]D level **(4 dummy variables with highest quintiles=ref),** for each month of the study period, the distribution of 25(OH)D is used to define the specific quintiles of 25(OH)D; C=potential confounders; CKD stage at baseline is defined using by both eGFR and ACR at baseline.

Table S4. Risk of rapid decline in eGFR, Risk Odds Ratio (95%CI), by vitamin D month-specific quintiles

		Rapid decline in eGFR			
	N (%)	**Full* model[a]**	**Reduced** model[a]**	**Full* model + adjusted for CKD stage at baseline[b]**	**Reduced** model + adjusted for CKD stage at baseline[b]**
Vitamin D month-specific					
quintile 1 (highest)	892 (19.9)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
quintile 2	894 (20.0)	1.23 (0.81-1.86)	1.21 (0.80-1.82)	1.23 (0.81-1.86)	1.21 (0.80-1.82)
quintile 3	894 (20.0)	1.20 (0.90-2.03)	1.21 (0.80-1.82)	1.19 (0.79-1.80)	1.21 (0.80-1.82)
quintile 4	896 (20.0)	1.35 (0.90-2.03)	1.37 (0.92-2.04)	1.35 (0.90-2.02)	1.37 (0.92-2.05)
quintile 5 (lowest)	898 (20.1)	1.60 (1.07-2.38)†	1.67 (1.13-2.46)†	1.60 (1.07-2.38)†	1.67 (1.13-2.46)‡

*Adjusted for age, gender, education, smoking status, physical activity, hypertension, diabetes, oral contraceptive, waist circumference, albumin-corrected calcium, VitD supplements or Rx, altitude, mean monthly sunshine hours, usCRP, triglycerides, and HDL-cholesterol

**Adjusted for age, smoking status, physical activity, diabetes, oral contraceptive, albumin-corrected calcium

† P value< 0.05; ‡ P value<0.01

[a]**Model S3 Unconditional logistic regression model: logit P(D=1 I X) = $\alpha + \sum\beta_i$(vitD month-specific quintiles)+ $\sum\gamma_n(C_n)$,** where D=1 if rapid loss in eGFR= annual eGFR loss >3mL/min/1.73 m^2, D=0 otherwise; **vitD month-specific quintiles** = month-specific quintiles 25[OH]D level **(4 dummy variables with highest quintiles=ref),** for each month of the study period, the distribution of 25(OH)D is used to define the specific quintiles of 25(OH)D; C=potential confounders

[b]**Model S4 Unconditional logistic regression model: logit P(D=1 I X) = $\alpha + \sum\beta_i$(vitD month-specific quintiles)+ $\sum\gamma_n(C_n) + \gamma_{n+1}$(CKD stage at baseline) ,** where D=1 if rapid loss in eGFR= annual eGFR loss >3mL/min/1.73 m^2, D=0 otherwise; **vitD month-specific quintiles** = month-specific quintiles 25[OH]D level **(4 dummy variables with highest quintiles=ref),** for each month of the study period, the distribution of 25(OH)D is used to define the specific quintiles of 25(OH)D; C=potential confounders; CKD stage at baseline is defined using by both eGFR and ACR at baseline.

Table S5. Risk of CKD, Risk Odds Ratio (95%CI), by vitamin D month-specific quintiles

		Risk Odds Ratio of CKD			
	N (%)	**Full* model[a]**	**Reduced** model[a]**	**Full* model + adjusted for eGFR and ACR at baseline[b]**	**Reduced** model + adjusted for eGFR and ACR at baseline[b]**
Vitamin D month-specific					
quintile 1 (highest)	778 (20.0)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
quintile 2	779 (20.0)	1.05 (0.84-1.31)	1.05 (0.84-1.31)	1.19 (0.79-1.80)	1.29 (0.86-1.93)
quintile 3	803 (20.6)	1.03 (0.82-1.29)	1.02 (0.82-1.28)	0.94 (0.61-1.45)	1.04 (0.68-1.59)
quintile 4	785 (20.2)	0.91 (0.72-1.14)	0.88 (0.70-1.11)	1.03 (0.67-1.59)	1.20 (0.79-1.82)
quintile 5 (lowest)	749 (19.2)	0.90 (0.71-1.14)	0.90 (0.72-1.14)	1.30 (0.84-1.99)	1.52 (1.01-2.30)†

*Adjusted for age, gender, education, smoking status, physical activity, hypertension, diabetes, oral contraceptive, waist circumference, albumin-corrected calcium, VitD supplements or Rx, altitude, mean monthly sunshine hours, usCRP, triglycerides, and HDL-cholesterol

**Adjusted for age, smoking status, physical activity, diabetes, oral contraceptive, albumin-corrected calcium

†P value< 0.05

[a]**Model S5 Unconditional logistic regression model: logit P(D=1 I X) = α + $\sum\beta_i$(vitD month-specific quintiles)+ $\sum\gamma_n(C_n)$,** where D=1 if new CKD= CKD at follow-up among participants without CKD at baseline, D=0 otherwise; **vitD month-specific quintiles** = month-specific quintiles 25[OH]D level **(4 dummy variables with highest quintiles=ref),** for each month of the study period, the distribution of 25(OH)D is used to define the specific quintiles of 25(OH)D; C=potential confounders

[b]**Model S6 Unconditional logistic regression model: logit P(D=1 I X) = α + $\sum\beta_i$(vitD month-specific quintiles)+ γ_n(eGFR baseline) γ_{n+1}(logACR baseline) $\sum\gamma_{n+2}(C_{n+2})$ +,** where D= 1 if new CKD= CKD at follow-up among participants without CKD at baseline, D=0 otherwise; **vitD month-specific quintiles** = month-specific quintiles 25[OH]D level **(4 dummy variables with highest quintiles=ref),** for each month of the study period, the distribution of 25(OH)D is used to define the specific quintiles of 25(OH)D; C=potential confounders

CHAPTER 5

PROJECT 3

VDR VARIANTS MODIFY THE EFFECT OF VITAMIN D SERUM LEVEL ON THE RISK OF eGFR OUTCOME IN THE GENERAL ADULT POPULATION

SPECIFIC AIMS PROJECT 3

1) TO EXAMINE THE *VDR* GENE-VITAMIN D LEVEL INTERACTION ON THE CHANGE OF KIDNEY FUNCTION ASSOCIATED WITH BASELINE SERUM VITAMIN D

- HYPOTHESIS #1 *VDR* GENETIC VARIANTS MODIFY THE EFFECT OF BASELINE VITAMIN D LEVEL ON THE CHANGES IN KIDNEY FUNCTION

Abstract

Background: No study explored the potential effect modification of *VDR* genetic variants on the relationship between vitamin D and change in kidney function. We evaluated whether *VDR* variants modify the association of serum vitamin D with annual change in kidney function, rapid decline in kidney function, and incidence of chronic kidney disease.

Methods: We used baseline (2003-2006) and 5-year follow-up data from the Colaus population-based cohort. Serum vitamin D was measured at baseline using liquid chromatography-tandem mass spectrometry. Estimated glomerular filtration rate (eGFR) and albuminuria information were collected at baseline and follow-up. Rapid decline in eGFR was defined as an annual loss greater than 3 mL/min/1.73m^2. Ten single-nucleotide polymorphisms, of which five were within the *VDR* gene, were considered *a priori*. Multivariate linear and logistic regressions models were used controlling for major factors known to influence 25(OH)D levels.

Results: Among the ten candidate polymorphisms, the *Cdx2 VDR* rs11568820 was selected for further analyses and categorized as CC vs CT/TT genotype. A total of 3,954 subjects were included in the analysis. The mean follow-up was 5.6 years (SD 0.46). Serum vitamin D was negatively associated with annual change in eGFR and rapid decline in participants with the *Cdx2 VDR* CT/TT genotype (N=1355, 39.5%), whereas no such association was found among CC participants (p value for interaction >0.05). We found a significant *Cdx2 VDR* genotype - vitamin D interaction on the risk of rapid eGFR decline or incident CKD (adjusted P-value for interaction=0.022). Among participants with the *Cdx2 VDR* risk allele, the adjusted ROR was 1.17 (95%CI: 1.03-1.34), no association was found among participants without the *Cdx 2 VDR* risk allele genotype (CC genotype).

Conclusions: 25(OH) D is associatied with adverse kidney function and this association varies with common genetic differences in the *VDR* gene.

INTRODUCTION

25-hydroxyvitamin D (25[OH]D) is the major circulating form of vitamin D and reflects the overall vitamin D status from combined sunlight, diet and dietary supplement.[1] For full biological activity, 25(OH)D must be converted to 1,25-dihydroxyvitamin D (1,25[OH]2D) whose actions are largely mediated by genomic functions through an initial action on the nuclear vitamin D receptor (VDR).[2, 3] VDR is a ligand-induced nuclear receptor that regulates the expression of over 900 genes throughout the genome.[4, 5] VDR abundance and activity seems to play an important role in the individual responsiveness to 1,25(OH)2D;[3] some of the VDR abundance and activity is determined by *VDR* genetic polymorphisms.[6]

Studies have suggested that 1,25(OH)2D presents some renoprotective effects[7] and observational studies have reported associations of circulating 25(OH)D with glomerular filtration rate (GFR)[8-14] and proteinuria.[15-17] However, results have been inconsistent when associations of baseline circulating 25(OH)D with change in eGFR and incident CKD have been assessed prospectively.[12, 18] Some of this inconsistency might be explained by *VDR* genetic heterogeneity. The role of *VDR* genetic variants on kidney function has been explored only recently and current data are limited to end-stage renal disease (ESRD).[19, 20] While known associations of low 25(OH)D with major health outcomes have recently been shown to vary according to common genetic differences in the *VDR*,[21] so far no study explored the potential effect modification of *VDR* genetic variants on the relationship between vitamin D and change in kidney function.

We used baseline (2003-2006) and 5-year follow-up data of 3,954 adults from the general population to evaluate prospectively whether *VDR* variants modify the association of serum 25(OH)D with annual change in kidney function, rapid decline in kidney function, and incidence of CKD. We took major factors known to influence 25(OH)D levels into account, such as sunshine hours, altitude, physical activity, and supplements.

METHODS

CoLaus

We used the data from the CoLaus study. The primary aim of the CoLaus study was to assess the prevalence of cardiovascular risk factors in the Caucasian population of Lausanne, Switzerland.[22] The CoLaus study complied with the Declaration of Helsinki and was approved by the local Institutional Ethics Committees. All participants gave written informed consent. The sampling procedure of the CoLaus study has been described elsewhere.[22] Briefly, the CoLaus study was population-based and included participants aged 35 to 75 years. The recruitment took place in the city of Lausanne in Switzerland, a town of 117,161 inhabitants, of which 79,420 are of a Swiss nationality. The complete list of the Lausanne inhabitants aged 35–75 years (n = 56,694 in 2003) was provided by the population register of the city and served to sample the participants to the study. A simple, non-stratified random sample of 35% of the overall population was drawn. The following inclusion criteria applied: a) written informed consent; b) aged 35-75 years; c) willingness to take part in the examination and donate blood. Recruitment began in June 2003 and ended in May 2006. The sample of 8,121 subjects who agreed to participate represented 41% of the initially sampled population. Participants were asked to attend the outpatient clinic at the Centre Hospitalier Universitaire Vaudois (CHUV) in

the morning after an overnight fast. Between 2009 and 2012, all CoLaus participants have been invited for a follow-up (CoLaus 2). 4,679 out of 6,188 (75.6%) subjects participated to the follow-up. The follow-up included standardized questionnaires, medication, physical examination, and blood exams.

Assessment process and clinical data

Data were collected by trained field interviewers using standardized questionnaires. Questionnaires recorded information on demographic data, socio-economic status, and several lifestyle factors such as tobacco and physical activity. A questionnaire, administered during a face-to-face meeting with the recruiter, focused on personal and family history of disease and cardiovascular risk factors. Medicine use, including prescription and self-prescribed drugs, vitamin and mineral supplements were collected. Use of oral contraception and hormonal replacement therapy was self-reported.

Blood pressure (BP) was measured thrice on the left arm after at least 10 minutes rest in the seated position using a clinically validated oscillometric device (Omron® HEM-907, Matsusaka, Japan).[23] The average of the last two BP readings was used for analyses. Hypertension was defined as mean systolic BP (SBP) ≥140 mmHg or mean diastolic BP (DBP) ≥90 mmHg or presence of anti-hypertensive medication. Diabetes was defined as a fasting glucose ≥7 mmol/L and/or presence of antidiabetic drug treatment (insulin or oral drugs). In addition, weight, height, and waist and hip circumferences were measured using standardized procedures. Body mass index (BMI) was defined as weight/height^2.[24]

Biologic data

Venous blood samples were drawn after an overnight fast. Glucose was measured by Glucose dehydrogenase (Roche Diagnostics, CH; CV 2.1%-1.0%), total serum cholesterol, HDL-cholesterol and serum triglycerides were measured by glycerol-3-phosphate oxidase – phenol aminophenazone (GPO-PAP) (Roche Diagnostics, CH; CV 3.6%-0.5%). Total serum calcium was measured by O-cresolphtalein (Roche Diagnostics, CH; CV 2.1%-1.5%) and albumin by bromocresol green (Roche Diagnostics, CH; CV 2.5%-0.4%). Albumin-corrected calcium was calculated using the following formula: Ca_c = Serum total calcium - 0.012 (serum albumin / 0.9677 - 39.55). This formula was derived by the central laboratory of the University Hospital of Lausanne based on data from 320 consecutive outpatients without disorders of phosphocalcic metabolism. In CoLaus, Ca_c calculated using this formula presented no residual correlation with serum albumin. Ultrasensitive protein C reactive (hsCRP) was measured by Immunoassay and latex HS (Agilent 1100 apparatus, CH; CV 4.6%-1.3%).

A urine sample was collected for the assessment of creatinine and albumin and the albumin-to-creatinine ratio (ACR) was calculated. Kidney function was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation using both functional (GFR) and structural (urine albumin-to-creatinine ratio) information.[25] CKD stages were defined according to K/DOQI guidelines.[26, 27]

Altitude, sunshine hours

Participants were geocoded by merging information on the participant's private address with latitude, longitude and altitude information using Python programming and Google Maps Find Altitude software

(https://developers.google.com/maps/documentation/elevation/). Data on sunshine hours were obtained from MeteoSwiss which collects sunshine hours using meteorological stations distributed throughout Switzerland (http://www.meteoswiss.admin.ch/web/en.html). For each participant, data on sunshine hours collected in the station nearest to the participants' home address was used. The exposure period considered in this study was starting from the month before the participant's day of blood collection and was used to estimate the monthly mean sunshine hours.

25-hydroxyvitamin D

A fast, accurate and reliable method for the quantification of the vitamin D metabolites, 25-hydroxyvitamin D2 (25OH-D2) and 25-hydroxyvitamin D3 (25OH-D3) in human serum samples was developed and validated for this project. An ultra-high pressure liquid chromatography-tandem mass spectrometry (LC-MS/MS) system was developed. The development and validation of this system is described elsewhere.[28]

Vitamin D level was expressed in ng/mL (conversion factor for 25(OH)D: 1 ng/mL = 2.496 nmol/L). To describe the cohort, vitamin D status was categorized into sufficiency, insufficiency, and deficiency defined respectively as 25(OH)D ≥30 ng/mL, 20–29.9 ng/mL, and <20 ng/mL according to experts' recommendations.[1, 29, 30] To explore the associations of vitamin D with kidney function, we used month-specific quintiles of 25(OH)D (independent variable) as previously published.[31] For each month of the study period, the distribution of 25(OH)D is used to define the specific quintiles of 25(OH)D. This approach is considered as the most valid measure of associations when vitamin D is used as categorical variable.[32] 25(OH) month-specific quintiles were treated

as a single ordinal variable coded from the highest (reference group) to the lowest quintile.

Genotyping and *VDR* variants

Nuclear DNA was extracted from whole blood for whole genome scan analysis, and genotyping was performed using the Affimetrix 500 K SNP chip, as recommended by the manufacturer. Five single-nucleotide polymorphisms (SNPs) genotyped or imputed within the *VDR* gene were considered *a priori*: Cdx2 (rs11568820), FokI (rs2228570), BsmI (rs1544410), ApaI (rs7975232), and TaqI (rs7975128). Cdx2 rs11568820 was genotyped while the other four were imputed with different quality levels; r^2 values were 87.6%, 98.3%, and 87.8% for BsmI (rs1544410), ApaI (rs7975232), and TaqI (rs7975128), respectively. R^2 value for Fok I (rs2228570) was only 25.2%. These *VDR* variants were chosen *a priori* because they are the most frequent *VDR* variants reported in Caucasian populations and are the VDR variant most commonly reported in epidemiological studies.[6, 33, 34] In order to determine a specific VDR variant to include in our primary analysis, we first performed a preliminary analysis that identified several candidate VDR variants and then assessed the extent to which each variant was associated with both annual change in eGFR and vitamin D level.

We also considered five additional variants that have been reported in a recent meta-analysis[21]: rs7968585 and rs2239179 in *VDR*, rs1801222 and rs12766939 in *CUBN* (cubilin gene on chromosome 10), and rs703842 in *CYP27B1* (chromosome 12). R^2 for rs7968585 and rs2239179 (*VDR*) were 99.8% and 89.2%; r^2 for the rs1801222 and rs12766939 in *CUBN* were 72.5% and 47.9%. Rs703842 in *CYP27B1* was genotyped.

Minor allele frequencies in the CoLaus study were as follows: Cdx2 (rs11568820, minor/major alleles: T/C) 21.9%, FokI (rs2228570, minor/major alleles: A/G) 49.4%, BsmI (rs1544410, minor/major alleles: T/C) 40.5%, ApaI (rs7975232, minor/major alleles: C/A) 45.9%, TaqI (rs7975128, minor/major alleles: A/G) 40.3%, rs7968585, minor/major alleles: C/T 47.7%, rs2239179, minor/major alleles: C/T 40.2%, rs1801222, minor/major alleles: A/G 27.8%, rs12766939, minor/major alleles: G/A 29.4%, and rs703842, minor/major alleles: G/A 29.3%. Among these ten candidate polymorphisms, we selected the polymorphism that was the most strongly (in terms of magnitude of association and statistical significance) associated with i) change in annual eGFR and ii) 25(OH)D in crude and month-, age-, and sex- adjusted linear regression models. The ten candidate polymorphisms were tested separately.

The pair-wise LD (r^2) structure across these SNPs in the CoLaus study is presented in the Supplementary material (**Table S1**). The highest LD was between rs11804171 and rs1544410 (r^2=0.99). Allele frequencies were estimated by the gene counting method, and departures from Hardy–Weinberg equilibrium (HWE) were tested using a χ^2 goodness-of-fit test.

Statistical methods

Statistical analyses were performed using Stata 12.0 (Stata Corp, College Station, TX, USA) and SAS 9.3 (SAS Institute, Cary NC). Regression diagnotics were performed including check for linearity. Annual change in eGFR was defined as the difference between eGFR (estimated using the CKD-EPI equation) at follow-up and eGFR at

baseline, standardized to 1 year. In addition to annual change in eGFR, we also considered rapid decline in eGFR and CKD incidence as dependent outcomes. Rapid decline in eGFR was defined as an annual loss greater than 3 mL/min/1.73m^2.[12, 35] CKD incidence was defined as new CKD (CKD stage 1 or more) at follow-up among participants without CKD at baseline. In addition, we used a combined kidney function outcome defined as rapid loss in eGFR or CKD incidence. To take into account possible regression to the mean, we ran additional models adjusting for both eGFR and ACR at baseline.

Per-allele risk analyses (additive coding using 0, 1 and 2) were performed using linear regression to select the best variant among the ten candidate polymorphisms. Among the ten candidate polymorphisms tested, the *Cdx2 VDR* rs11568820 presented the greatest association and the lowest P value for both annual change in eGFR and vitamin D (whether considered as month-specific quintiles or as continuous level) (**Tables S2 and S3**). The Cdx2 rs11568820 was therefore considered for further analyses. Upon selection of the best variant (i.e., Cdx2 rs11568820) among the ten candidate polymorphisms, we then carried out mathematical modeling using linear and logistic models to predict CKD outcome as a function of vitamin D level, the selected VDR variant, and interaction of vitamin D level with the VDR variant, controlling for known determinants of vitamin D level, and allowing for possible interaction between vitamin D level and the determinants selected for control.

Genetic dominant models approach was then conducted. Dominant models assume that carriers of one or two copies of a specific risk allele have the same genetic

susceptibility. Carrier status is dichotomized participants with (coded as 1) or without (coded as 0) the high-risk allele. Accordingly, participants were categorized as either *Cdx2 VDR* CC (60.4%, 2389/3954) or CT/TT genotypes (39.6%, 1565/3954). The rs11568820 genotype frequencies did not significantly deviate from HW proportions (χ^2 test P value for HWE =0.218).

Hypotheses and models

In this report, kidney function –and specifically the yearly change in eGFR – was considered as the outcome and month-specific quintiles of 25(OH)D level and *Cdx2 VDR* genotype as two main exposures.

HYPOTHESIS #1 : VDR GENETIC VARIANTS MODIFY THE EFFECT OF BASELINE VITAMIN D LEVEL ON THE CHANGES IN KIDNEY FUNCTION

To test this hypothesis, we conducted the following models:

First, annual change in eGFR was modeled using linear regression with two exposures (model 1)[9]; *Cdx2 VDR* variants and 25(OH)D; *Cdx2 VDR* genotypes and month-specific quintiles of 25(OH). The initial model included exposure-exposure as well as two-way exposure-covariates interaction terms. In addition to *Cdx2 VDR* genotype-vitamin D interaction term, we included the following interaction terms: diabetes x vitamin D, hypertension x vitamin D; BMI x vitamin D; diabetes x *Cdx2 VDR*

[9] **Model 1 Linear regression with 2 exposures and interaction terms: $E(Y) = \alpha + \beta_1(25[OH]D) + \beta_i\,(Cdx2) + \gamma_1(HTN) + \gamma_2(Diabetes) + \gamma_3(BMI) + \Sigma\gamma_n(C_n) + \delta_1(25[OH]D \times HTN) + \delta_2(25[OH]D \times Diabetes) + \delta_3(25[OH]D \times BMI) + \delta_4(Cdx2 \times HTN) + \delta_5(Cdx2 \times Diabetes) + \delta_6(Cdx2 \times BMI) + \Sigma\delta_i(25[OH]D \times Cdx2)$,** where $Y = eGFR_{baseline} - eGFR_{follow\text{-}up}$; 25[OH]D = month-specific quintiles 25[OH]D level **(a single ordinal variable with highest quintile=0, lowest quintile=4),** for each month of the study period, the distribution of 25(OH)D is used to define the specific quintiles of 25(OH)D; C=other potential confounders

Results from model 1 further adjusted for baseline CKD stage (defined using both baseline eGFR and baselin ACR) are presented in the Table 2

genotype, hypertension x *Cdx2 VDR* genotype; and BMI x *Cdx2 VDR* genotype. These interaction terms were determined *a priori* based on the biology and on knowledge from the scientific literature. We used a modified selection algorithm proposed by Kleinbaum & Klein [36] to build the final model; diabetes x vitamin D, hypertension x vitamin D; BMI x vitamin D; diabetes x *Cdx2 VDR* genotype, hypertension x *Cdx2 VDR* genotype; and BMI x *Cdx2 VDR* genotype (i.e., EWs) were assessed first, then the potential confounders, then the *Cdx2 VDR* genotype x 25(OH)D product term (i.e., EEs) and finally *Cdx2 VDR* genotype and 25(OH)D (i.e, the Es).[36]

Then, rapid decline in eGFR (model 2)[10], incident CKD (model 3)[11], and the combined kidney outcome (rapid decline in eGFR or incident CKD[12], model 4) were modelled using unconditional logistic regression with again two exposures; *Cdx2 VDR* genotype and month-specific 25(OH)D quintiles. Initial models also included exposure--exposure as well as two-way exposure-covariates interaction terms.

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[10] **Model 2 Unconditional binary logistic binary regresion with 2 exposures: logit $P(D=1|X) = \alpha + \beta_1(25[OH]D) + \beta_2(Cdx2) + \gamma_1(HTN) + \gamma_2(Diabetes) + \gamma_3(BMI) + \Sigma\gamma_n(C_n) + \delta_1(25[OH]D \times HTN) + \delta_2(25[OH]D \times Diabetes) + \delta_3(25[OH]D \times BMI) + \delta_4(Cdx2 \times HTN) + \delta_5(Cdx2 \times Diabetes) + \delta_6(Cdx2 \times BMI) + \delta_7(25[OH]D \times Cdx2)$,** where D=1 if rapid eGFR decline, D=0 otherwise; 25[OH]D = 25[OH]D = month-specific quintiles 25[OH]D level **(a single ordinal variable with highest quintile=0, lowest quintile=4),** for each month of the study period, the distribution of 25(OH)D is used to define the specific quintiles of 25(OH)D; C=other potential confounders

Results from model 2 further adjusted for baseline CKD stage (defined using both baseline eGFR and ACR) are presented in the Table 3

[11] **Model 3 Unconditional binary logistic binary regresion with 2 exposures: logit $P(D=1|X) = \alpha + \beta_1(25[OH]D) + \beta_2(Cdx2) + \gamma_1(HTN) + \gamma_2(Diabetes) + \gamma_3(BMI) + \Sigma\gamma_n(C_n) + \delta_1(25[OH]D \times HTN) + \delta_2(25[OH]D \times Diabetes) + \delta_3(25[OH]D \times BMI) + \delta_4(Cdx2 \times HTN) + \delta_5(Cdx2 \times Diabetes) + \delta_6(Cdx2 \times BMI) + \delta_7(25[OH]D \times Cdx2)$,** where D=1 if incident case of CKD, D=0 otherwise; 25[OH]D = 25[OH]D = month-specific quintiles 25[OH]D level **(a single ordinal variable with highest quintile=0, lowest quintile=4),** for each month of the study period, the distribution of 25(OH)D is used to define the specific quintiles of 25(OH)D; C=other potential confounders

Results from model 3 further adjusted for baseline eGFR and baseline log(ACR) are presented in the Table 3

[12] **Model 4 Unconditional binary logistic binary regresion with 2 exposures: logit $P(D=1|X) = \alpha + \beta_1(25[OH]D) + \beta_2(Cdx2) + \gamma_1(HTN) + \gamma_2(Diabetes) + \gamma_3(BMI) + \Sigma\gamma_n(C_n) + \delta_1(25[OH]D \times HTN) + \delta_2(25[OH]D \times Diabetes) + \delta_3(25[OH]D \times BMI) + \delta_4(Cdx2x HTN) + \delta_5(Cdx2 \times Diabetes) + \delta_6(Cdx2 \times BMI) + \delta_7(25[OH]D \times Cdx2)$,,** where D=1 if rapid eGFR decline or incidental case of CKD, D=0 otherwise; 25[OH]D = 25[OH]D = month-specific quintiles 25[OH]D level **(a single ordinal variable with highest quintile=0, lowest quintile=4),** for each month of the study period, the distribution of 25(OH)D is used to define the specific quintiles of 25(OH)D; C=other potential confounders

Results from model 4 further adjusted for baseline eGFR and baseline log(ACR) are presented in the Table 3

Additional models adjusting for CKD stages (defined using by both baseline eGFR and ACR) at baseline (in annual change in eGFR and rapid decline in eGFR models) or eGFR and ACR at baseline (in CKD incidence model) were run.

VARIABLES SPECIFICATION

Covariates were considered given their reported or potential influence on kidney function and/or vitamin D level. To identify factors to be introduced in the *initial* multivariate models, bivariate associations between kidney function, respectively vitamin D, and attributes of interest were tested. The *P*-value cut-off for introducing the attribute in the *initial* full model was set at <0.10.

COLLINEARITY, INTERACTION AND CONFOUNDING[13]

We used the SAS Collin macro (Emory University, Kleinbaum et al.) to assess collinearity.

A collinearity problem involving the two main effects BMI and waist circumference was diagnosed (CI >30 and VDPs >0.50 for both BMI and waist circumference.). The collinearity problem was resolved by dropping the BMI term.

Statistical interactions were tested using Wald test or likelihood ratio test with 1 degree of freedom in the genotype-dominant approach (2 genotypes). Significances of *P*-values for interaction test are often set at <0.10. Given the number of interaction terms tested, we used more conservative cutoff for significances of P values (P value<0.05). Using both backward elimination and chunck test approaches, we found none of the

[13] Examples of collinearity and backward change in estimate elimination methods are presented in the appendix

exposure-covariate interaction terms to be statistically significant, thus these terms were dropped from the models.

To build the reduced models, we used backwards change in estimate elimination and precision approaches.[37] Full (gold standard) models and reduced models yield similar results. To limit the number of models, only full (gold standard) models are presented in the Results section.

RESULTS

Participants' characteristics

A total of 3,954 subjects were included in the analysis (84.5% of the cohort). The mean and median follow-up were 5.6 and 5.5 years (SD 0.46 and IQR 5.4-5.7), respectively. Participants' characteristics by vitamin D status are detailed in **Table 1**. The prevalence of vitamin D sufficiency, insufficiency, and deficiency were 13.2%, 33.0%, and 53.8%, respectively, with mean 25(OH)D levels of 35.5 (5.0), 24.5 (2.9), and 13.0 (4.2) ng/mL, respectively. At baseline, the overall mean eGFR was 85.0 (14.9) ml/min /1.73m^2, and ranges from 79.7 (14.1), 83.6 (14.7), 87.2 (14.8) in participants with vitamin D sufficiency, insufficiency, and deficiency, respectively. The groups defined by vitamin D status statistically differed by all characteristics apart from gender, education, altitude, and serum triglyceride levels. The mean 25(OH)D (SD) levels by month-specific vitamin D quintiles were: 31.6 (6.5), 23.2 (4.9), 18.9 (4.9), 15.0 (4.6), and 10.0 (4.0) ng/mL. The mean annual change in eGFR was -0.566 (1.77) mL/min/1.73m^2.

Compared to participants with sufficient vitamin D at baseline, participants with vitamin D insufficiency and deficiency presented greater eGFR declines (-0.260 [1.77], -

0,453 [1.67], and -0.711 [1.81] mL/min/1.73m^2, respectively, P value <0.001). Overall, 6.6% (260/3954) of the participants presented a rapid decline in eGFR. Out of the participants without CKD at baseline (3429/3954), 250 (7.3%) presented a new CKD at follow-up.

We found no significant interaction between 25(OH)D month-specific quintiles and *Cdx2 VDR* genotype. In contrast, 25(OH)D month-specific quintiles were negatively associated with annual change in eGFR in participants with the *Cdx2 VDR* CT/TT genotype (N=1355, 39.5%) (fully adjusted beta coefficient= -0.0923 (SE=0.033), P value <0.01), whereas no such association was found among CC participants (**Table 2**). The association between vitamin D month-specific quintiles and the annual change in eGFR among participants with the CT/ TT genotype persisted after further adjustment for CKD stages at baseline.

25(OH)D month-specific quintiles were associated with the risk of rapid decline in eGFR (adjusted risk odds ratio (ROR): 1.11; 95%CI, 1.02-1.21) (**Table 3**). We did not find a significant Cdx 2 genotype - 25(OH)D month-specific quintiles interaction on the risk of rapid decline in eGFR (adjusted P-value for interaction= 0.090). Decreasing 25(OH)D month-specific quintiles were associated with an increased risk of rapid decline in eGFR among participants with the Cdx 2 risk allele (CT or TT genotype); the adjusted ROR was 1.28 (95%CI: 1.09-1.50). No association was found between 25(OH)D month-specific quintiles and the risk of rapid decline in eGFR among participants without the risk allele (CC genotype) (**Table 3**). The association between 25(OH)D month-specific quintiles and the risk of rapid decline persisted among participants with the Cdx 2 risk allele (CT or TT genotype) after further adjustment for CKD stages at baseline.

We found a significant *Cdx2 VDR* genotype - 25(OH)D month-specific quintiles interaction on the risk of incident CKD (adjusted P-value for interaction=0.049). After adjustment for baseline eGFR and baseline ACR, the association between 25(OH)D month-specific quintiles and the risk of CKD approached significance among participants with the Cdx 2 risk allele genotype (CT or TT genotype); the adjusted ROR was 1.18 (95%CI: 0.99-1.40), whereas the adjusted ROR remained centered around the nulle value (ROR: 0.96; 95%CI: 0.84-1.10) among participants without the Cdx 2 risk allele genotype (CC genotype) (**Table 4**).

When rapid decline of eGFR and CKD were considered as combined kidney outcome among adults without CKD at baseline, the *Cdx2 VDR* genotype - 25(OH)D month-specific quintiles interaction was stronger (adjusted P-value for interaction<0.04) (**Table 4**). The adjusted ROR varied between 1.08 and 1.36 among participants with the Cdx 2 risk allele genotype (CT or CT genotype) and were significant. No association was found among participants without the *Cdx2 VDR* risk allele genotype (CC genotype). Adjustment for baseline kidney function did not change meaningfully these associations (**Tables 3 and 4**).

DISCUSSION

In this prospective population-based study including adults with predominantly normal baseline kidney function, we show that the Cdx2 *VDR* polymorphism modifies the association of circulating vitamin D with the risk of rapid eGFR decline or the 5-year risk of incident CKD. Compared to adults carrying the *Cdx2 VDR* CC genotype, those

carrying the *Cdx2 VDR* T allele are at greater risk of rapid eGFR decline or incident CKD associated with low level of 25(OH)D. These associations and effect modifications are independent of major potential confounders including kidney function at baseline.

In previous studies, *VDR* polymorphisms have been associated with clinical outcomes related to kidney function such as ESRD,[19, 20, 38, 39] diabetes or insulin secretion,[40-43] hypertension or blood pressure,[44, 45] and BMI.[46] Studies that assessed the role of *VDR* polymorphisms directly on kidney function have previously been limited to CKD patients.[20, 47] Consistent with our findings in the general population, they have found associations of *VDR* polymorphisms (*BsmI* and *FokI VDR*) and risk of ESRD.[19, 20]

This is the first study that explored the *VDR* genetic effect modification on the association between circulating vitamin D level and change in kidney function. Vitamin D month-specific quintiles are associated with the risk of rapid decline in eGFR among subjects with the *Cdx2 VDR* T risk allele. Each quintile is associated with an increased risk of 28% of rapid decline in eGFR, independently of major potential confounders. The direction of the associations between vitamin D month-specific quintiles and the risk of CKD differ statistically between subjects with and without the *Cdx2 VDR* T risk allele. Among subjects with the *Cdx2 VDR* T risk allele, the association of vitamin D month-specific quintile with the risk of CKD approaches significance.

The mechanisms by which *Cdx2 VDR* polymorphism may interact with vitamin D circulating level to influence the change in kidney function are not clear. *The VDR* is located on chromosome 12 at q12-14 and includes five main exons. The domains of the *VDR* are involved in different functions including DNA binding, receptor dimerization,

gene transactivation, and cofactor activation.[48, 49] The *Cdx2 VDR* polymorphism (rs11568820) is located in the promoter region of the *VDR* gene in exon 1. The *Cdx2 VDR* polymorphism has been associated with *VDR* transcriptional activity in the intestinal tract; the T allele showed up to 70% greater transcriptional activity than the C allele.[50] The intestinal VDR content of the *Cdx2 VDR* -CC genotype is lower than those of the *Cdx2 VDR* -TT genotype.[50] This could increase the transcription of calcium transport proteins (such as calbindin 9K and 28K,TRPV5, TRPV6) and enhance the intestinal absorption of calcium.[6] In line with this, the *Cdx2 VDR* polymorphism has been associated with both bone mineral density and osteoporotic fractures.[51, 52]

Because the *Cdx2 VDR* polymorphism is in the DNA binding portion of the gene, it is possible that it influences other transcriptional processes. In addition, our findings might reflect a true effect of the *Cdx2 VDR* allele or an effect mediated by other alleles linked to the *Cdx2 VDR* allele. The *Cdx2 VDR* rs11568820 was not highly correlated with the other nine candidate *VDR* polymorphisms (highest r^2=0.65). A non *a priori* selected VDR variant, GATA rs4516035, resides with *Cdx2 VDR* rs11568820 in the VDR promoter region and a LD block including *Cdx2 VDR* rs11568820 and GATA rs4516035 has been described. Although *Cdx2 VDR* rs11568820 and GATA rs4516035 were not highly correlated in the CoLaus study (r^2=0.20), we found that the associations between 25(OH)D month-specific quintiles and the different phenotypes of kidney function were also modified by GATA rs4516035; the GATA rs4516035 T allele being the risk allele. The C allele of this variant has been shown to eliminate the GATA binding site and to confer a lower VDR promoter activity.[53-56] Subjects with allele T of GATA have a 1.9 fold higher VDR promoter activity compared to subjects with allele C.[53, 54]

In a recent population-based prospective cohort of older adults (Longitudinal Aging Study Amsterdam), the Cdx2-GATA haplotype was associated with increased mortality risk. The increased risk was not affected by adjustment for cardiovascular risk factors or 25(OH)D levels but was partly explained by osteoporotic fractures.[57] In a study that examined the effect of *VDR* polymorphisms on growth and treatment response of 1-alpha-hydroxyvitamin D3 derivatives, children who were carriers of the low transcription activity Cdx2/GATA haplotype, had a lower urinary calcium/creatinine level compared to non-carriers.[58] This study suggests that the Cdx2/GATA haplotype associated with low transcription activity may protect from developing hypercalciuria during vitamin D supplementation.[53] Overall, it seems that an increased *VDR* transcription activity could mediate, in part, the increased risk of rapid eGFR decline associated with low level of vitamin D.

We found no synergistic effect when combining Cdx2 and GATA variants thought to be associated with increased *VDR* transcription activity. Haplotype analysis might further our understanding of the modification effect of Cdx2 and GATA *VDR* polymorphisms on the association between vitamin D and kidney function.

We have previously shown that vitamin D is associated with change in kidney function in adults with predominantly normal baseline kidney function (Guessous et al. *Project 2*). Here, we show that the association is even larger among carriers of the *Cdx2 VDR* T allele. We previously found no association between vitamin D and CKD incidence, which was in line with previous findings. [18] Here, we show that this association might actually be blunted by VDR genetic polymorphisms; while our current study was not optimally powered to detect an effect on CKD incidence, the association

between vitamin D level and CKD incidence approaches significance among adults carrying the *Cdx2 VDR* T allele.

Strengths and limitations

These results should be interpreted in the light of the study's strengths and limitations. This is the first prospective study assessing the *VDR*-circulating vitamin D interaction on kidney function in a general adult population. We measured 25(OH)D by the gold standard technique (LC-MS/MS). Information on major known potential confounders was available. However, despite these efforts, information is still incomplete. We lack information on potential confounders such as parathyroid hormone and fibroblast growth factor-23. GFR was estimated using the CKD-Epi equation and CKD was defined using both eGFR and urinary spot-based ACR. Yet, more accurate measurements of renal function (including inulin clearance, iothalamate clearance, cystatin C, 24-hour urinary protein collection) exist. Participants were all Caucasians and our findings cannot be generalized to non-Caucasians. Fasting serum was collected form CoLaus participants at the 2003-2006 study visit and stored at -80^{o}C before 25(OH)D was measured. Yet, 25(OH)D is stable for long periods at this temperature.[59] To represent the participant's typical level of vitamin D, we relied on a single 25(OH)D measure. It has been shown that a single baseline 25(OH)D serum measurement provides a reasonably representative measure of vitamin D in adults, confirming its utility as epidemiologic biomarkers in prospective studies.[60]

CONCLUSIONS

These findings add further evidence that in addition to major health outcomes such as hip fracture, myocardial infarction, cancer and mortality, associations of low 25(OH) D with kidney function may vary according to common genetic differences in the VDR.

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Table 1. Baseline Characteristics of the CoLaus participants, by vitamin D status (N=3954)

	ALL	Sufficiency (25OHD≥30mg/mL)	Insufficiency (20≤25OHD<30mg/mL)	Deficiency (25OHD<20mg/mL)	**P value**
Number (%)	3954 (100.0)	524 (13.2)	1303 (33.0)	2127 (53.8)	
Age, years (SD)	53.0 (10.5)	54.7 (10.5)	53.3 (10.6)	52.5 (10.5)	<0.001
Female gender (%)	2131 (53.9)	299 (57.1)	719 (55.2)	1113 (52.3)	0.079
Education					0.366
Low (%)	2148 (54.3)	278 (53.)	687 (52.7)	1183 (55.6)	
Middle (%)	1011 (25.6)	141 (26.9)	353 (27.1)	517 (24.3)	
High (%)	795 (20.1)	105 (20.0)	263 (20.2)	427 (20.1)	
Smoking status					0.043
Never smokers (%)	1613 (40.8)	212 (40.5)	546 (41.9)	855 (40.2)	
Former smokers (%)	1345 (34.0)	199 (38.0)	446 (34.2)	700 (32.9)	
Smokers (%)	996 (25.2)	113 (21.6)	311 (23.9)	572 (26.9)	
Physical activity					<0.001
Never or don't know (%)	1293 (32.7)	123 (23.5)	345 (26.5)	825 (38.8)	
1x/week (%)	378 (9.6)	34 (6.5)	112 (8.6)	232 (10.9)	
2x/week (%)	2283 (57.7)	367 (70.0)	846 (64.9)	1070 (50.3)	
Hypertension (%)	1337 (33.8)	168 (32.1)	387 (29.7)	782 (36.8)	<0.001
Diabetes (%)	222 (5.6)	16 (3.0)	61 (4.7)	145 (6.8)	0.001

Oral contraceptive (among women N=2131) (%)	175 (8.2)	36 (12.0)	51 (7.1)	88 (7.9)	0.028
BMi, kg/m^2 (SD)	25.6 (4.3)	24.3 (3.7)	25.2 (4.0)	26.2 (4.5)	<0.001
Albumin-corrected calcium, mmol/L (SD)	2.21 (0.08)	2.23 (0.09)	2.21 (0.08)	2.20 (0.08)	<0.001
VitD supplements or Rx (%)	177 (4.5)	67 (12.8)	72 (5.5)	38 (1.8)	<0.001
Altitude, meters (SD)	534.4 (90.9)	527.0 (83.2)	535.8 (91.7)	535.3 (92.3)	0.137
Mean monthly sunshine hours (SD)	5.1 (2.3)	5.9 (2.1)	5.5 (2.3)	4.7 (2.1)	<0.001
usCRP, mg/L (SD)	2.37 (3.3)	2.18 (3.2)	2.20 (3.22)	2.52 (3.40)	0.008
Triglycerides, mmol/L (SD)	1.35 (1.01)	1.29 (0.81)	1.34 (1.17)	1.38 (0.95)	0.160
HDL-cholesterol, mmol/L (SD)	1.65 (0.44)	1.77 (0.45)	1.67 (0.44)	1.62 (0.43)	<0.001
25(OH)D, ng/mL (SD)	19.8 (9.0)	35.5 (5.0)	24.5 (2.9)	13.0 (4.2)	<0.001

TABLE 2 ADJUSTED ASSOCIATION OF 25(OH)D MONTH-SPECIFIC QUINTILES WITH ANNUAL CHANGE IN ESTIMATED GFR, BY *CDX2 VDR* GENOTYPE

	change in eGFR, beta coefficient (95%CI)		
***Cdx2 VDR* genotype**	**CC**	**CT or TT**	**Interaction p value**
N (%)	2074 (60.5)	1355 (39.5)	
Model			
Unadjusted	-0.0586 (0.025)**	-0.1209 (0.031)***	0.124
Full model[#]	-0.0325 (0.0267)	-0.0923 (0.033)**	0.180
Full model[#] + CKD stage at baseline	-0.0275 (0.0264)	-0.0927 (0.0331)**	0.164

[#]adjusted for age, sex, waist circumference, albumin-corrected calcium, sunshine mean hours, hsCRP, triglycerides, HDL-cholesterol, hypertension, diabetes, physical activity, smoking status, altitude, education, vitamin D supplementation or therapy, and oral contraceptive

* P value< 0.05; ** P values<0.01; *** P values<0.001

Table 3. Adjusted association of 25(OH)D month-specific quintiles with the risk of rapid decline in estimated GFR, the risk of chronic kidney disease (CKD), and the risk of rapid decline in estimated GFR or CKD, by *Cdx2 VDR* genotypes (rs11568820)

	Risk of rapid eGFR decline, Risk Odds Ratio (95%CI)		
***Cdx2 VDR* genotype**	**CC**	**CT or TT**	**Interaction P value**
N (%)	2389 (60.4)	1566 (39.6)	
Model			
Unadjusted	1.11 (0.99-1.24)	1.31 (1.13-1.52)**	0.083
Full model[#]	1.04 (0.92-1.18)	1.28 (1.09-1.50)**	0.092
Full model[#] +CKD stages at baseline	1.04 (0.92-1.17)	1.27 (1.09-1.50)**	0.090

[#] adjusted for age, sex, waist circumference, albumin-corrected calcium, sunshine mean hours, hsCRP, triglycerides, HDL-cholesterol, hypertension, diabetes, physical activity, smoking status, altitude, education, vitamin D supplementation or therapy, and oral contraceptive.

* P value< 0.05; ** P values<0.01; *** P values<0.001

Table 4. Adjusted association of 25(OH)D month-specific quintiles with the risk of chronic kidney disease (CKD), and the risk of rapid decline in estimated GFR or CKD, by *Cdx2 VDR* genotypes (rs11568820)

	Risk of incident CKD, Risk Odds Ratio (95%CI)			**Risk of rapid eGFR decline or incident CKD, Risk Odds Ratio (95%CI)**		
***Cdx2 VDR* genotype**	**CC**	**CT or TT**	**P value†**	**CC**	**CT or TT**	**P value†**
N (%)	2074 (60.5)	1355 (39.5)		2074 (60.5)	1355 (39.5)	
Model						
Unadjusted	0.96 (0.86-1.08)	1.16 (1.00-1.34)	0.057	1.02 (0.93-1.13)	1.21 (1.08-1.36)**	0.031
Full model#	0.92 (0.81-1.04)	1.14 (0.96-1.33)	0.050	0.99 (0.90-1.10)	1.20 (1.06-1.36)**	0.027
Full model#+ eGFR and log ACR	0.96 (0.84-1.10)	1.18 (0.99-1.40)	0.049	0.97 (0.88-1.08)	1.17 (1.03-1.34)*	0.022

adjusted for age, sex, waist circumference, albumin-corrected calcium, sunshine mean hours, hsCRP, triglycerides, HDL-cholesterol, hypertension, diabetes, physical activity, smoking status, altitude, education, vitamin D supplementation or therapy, and oral contraceptive.

†P value for 25(OH)D month-specific quintiles x *Cdx2 VDR* genotypes interaction

* P value< 0.05; ** P values<0.01; *** P values<0.001

Table S1. Candidate polymorphisms pair-wise correlations, R^2

	rs11568820	**rs2228570**	**rs1544410**	**rs7975128**	**rs7975232**	**rs7968585**	**rs2239179**	**rs1801222**	**rs12766939**	**rs703842**
rs11568820	1	0.0002	0.005	0.0054	0.0065	0.0056	0.0017	<0.0001	0.0001	<0.0001
rs2228570		1	0.011	0.0114	0.009	0.009	0.0341	<0.0001	<0.0001	0.0002
rs1544410			1	0.9951	0.6561	0.6809	0.7364	<0.0001	<0.0001	<0.0001
rs7975128				1	0.6489	0.6627	0.7517	<0.0001	<0.0001	<0.0001
rs7975232					1	0.8142	0.4678	<0.0001	0.0001	0.0001
rs7968585						1	0.4438	0.0001	0.0001	0.0002
rs2239179							1	<0.0001	<0.0001	<0.0001
rs1801222								1	0.1649	<0.0001
rs12766939									1	0.0004
rs703842										1

Table S2. **Association between candidate variants considered separately and mean annual change in eGFR**

	Difference in Annual Change in estimated GFR (ml/min/1.73m^2) (P value)	
Polymorphism (minor allele)	**Unadjusted***	**Age and sex adjusted***
Rs11568820 (T allele)	-0.0843 (0.082)	-0.0916 (0.058)
Rs2228570 (A allele)	-0.0742 (0.350)	-0.0777 (0.326)
Rs1544410 (T allele)	0.0354 (0.412)	0.0306 (0.477)
Rs7975232 (C allele)	-0.0217 (0.585)	-0.0171 (0.666)
Rs7975128 (A allele)	0.0379 (0.381)	0.0333 (0.444)
Rs7968585 (C allele)	-0.0307 (0.438)	-0.0265 (0.504)
Rs2239179 (C allele)	0.0236 (0.582)	0.0194 (0.650)
Rs1801222 (A allele)	0.0264 (0.612)	0.0206 (0.692)
Rs12766939 (G allele)	0.0298 (0.639)	0.0335 (0.596)
Rs703842 (G allele)	-0.0362 (0.397)	-0.0321 (0.451)

***Models used : $\mathbf{E(Y) = \alpha + \beta_n(VDR\ allele_n)}$** where $Y = eGFR_{baseline} - eGFR_{follow\text{-}up,}$ and $\mathbf{E(Y) = \alpha + \beta_n(VDR\ allele_n) + \Sigma\ \gamma_n\ (C_n),}$ where $Y = eGFR_{baseline} - eGFR_{follow\text{-}up,}$ C= age, sex

Table S3. Association between candidate variants and vitamin D phenotypes

	Vitamin D level (P value)		Month-specific vitamin D quintiles, highest to lowest (P value)	
SNP	**Ajusted for month of sampling***	**Month-, age- and sex-adjusted***	**Unadjusted****	**Age- and sex-adjusted****
Rs11568820 (T allele)	-0.4488 (0.539)	-0.4076 (0.448)	0.0481 (0.215)	0.0458 (0.237)
Rs2228570 (A allele)	-0.2001 (0.821)	-0.1951 (0.825)	0.0117 (0.854)	0.0116 (0.855)
Rs1544410 (T allele)	0.1540 (0.747)	0.1459 (0.760)	-0.0072 (0.835)	-0.0064 (0.853)
Rs7975232 (C allele)	-0.1457 (0.742)	-0.1083 (0.806)	0.0085 (0.789)	0.0059 (0.851)
Rs7975128 (A allele)	0.1281 (0.790)	0.1175 (0.806)	-0.0060 (0.861)	-0.0051 (0.882
Rs7968585 (C allele)	-0.0241 (0.956)	-0.0110 (0.980)	-0.0104 (0.742)	-0.0128 (0.686)
Rs2239179 (C allele)	0.2999 (0.530)	0.3026 (0.525)	-0.0230 (0.505)	-0.0229 (0.505)
Rs1801222 (A allele)	0.4700 (0.417)	0.4233 (0.464)	-0.06144 (0.142)	-0.0584 (0.162)
Rs12766939 (G allele)	-0.8422 (0.232)	-0.7516 (0.285)	0.0609 (0.231)	0.0549 (0.279)
Rs703842 (G allele)	-0.1532 (0.747)	0.1444 (0.760)	-0.0220 (0.520)	-0.0215 (0.530)

***Models used: $E(Y) = \alpha + \beta_n(\text{VDR allele}_n) + \sum\gamma_i\ (MO_i)$** where Y = baseline 25(OH)D level in ng/mL; MO=month of sampling with k=12 and k-1 dummy variables: January (category 1), …, December (category 12) where MO={1 if category i, 0 if otherwise for i=2,3,4,5,6,7,8,9,10,11,12 (referent : 1); **and $E(Y) = \alpha + \beta_n(\text{VDR allele}_n) + \sum\gamma_i\ (MO_i) + \Sigma\ \gamma_{n+1}(C_{n+1})$,** where Y = baseline 25(OH)D level in ng/mL; MO=month of sampling with k=12 and k-1 dummy variables: January (category 1), …, December (category 12) where MO={1 if category i, 0 if otherwise for i=2,3,4,5,6,7,8,9,10,11,12 (referent : 1); C= age, sex

**** $E(Y) = \alpha + \beta_n(\text{VDR allele}_n)$,** where Y = month-specific 25(OH)D quintiles and **$E(Y) = \alpha + \beta_n(\text{VDR allele}_n) + \Sigma\ \gamma_i\ (C_i)$,** where Y = month-specific 25(OH)D quintiles; C= age, sex

CHAPTER 6

GENERAL DISCUSSION

Using population-based data, we explored the CKD-specific prevalence and determinants of vitamin D deficiency (project 1), the changes in kidney function associated with baseline serum vitamin D (project 2), and the VDR gene-vitamin D baseline level interaction on the change of kidney function (project 3).

Each project has been specifically discussed in the corresponding discussion sections. Below, we present a general discussion and highlight the public health importance of our findings.

In project 1, we found that the prevalence of vitamin D deficiency was high in people with CKD but not higher than in people without CKD. We found no evidence that vitamin D major determinants such as age, BMI, and altitude are different in subject with and without CKD (i.e. CKD does not modify the effect of attributes on vitamin D). Vitamin D status and vitamin D levels (serum 25(OH)D) did not differ by CKD status or stages. We did not find that people with lower kidney function had lower 25(OH)D levels than people with higher kidney function.

More generally, we found that the level of vitamin D was low and the prevalence of vitamin D deficiency was high in Swiss adults with or without CKD. This is of concerns given the impact of vitamin D deficiency on bone- and muscle-related diseases. The high prevalence of vitamin D deficiency and the potential increase in vitamin D deficiency have conducted, in some regions (e.g North America) to public health measures such as the vitamin D food fortification.

Our results suggested that this should be considered in Switzerland as well. The Swiss Office of Public Health recently published recommendation on vitamin D food intake.[1] Beside food fortification, one might recommend to increase sun exposition to correct vitamin D deficiency. It is worth stressing that the high prevalence of vitamin D deficiency should be interpreted in the light of the indisputable increased risk of squamous and basal cell skin cancers caused by excessive unprotected sun exposure (the major source of vitamin D).[2] More specifically, individuals who photosynthesize vitamin D3 most effectively (e.g., fair-skinned persons) have the highest risk of skin cancer; and darker skinned persons, who have lower cutaneous vitamin D3 synthesis have lower skin cancer risk. Skin cancers are the most frequent cancer in the world and account for about one-half of the human cancers in the U.S. Although no clear cutoff of sun exposure have been reported yet, there might be behaviors that may balance the risk benefit of sun exposure.

While considering the potential public health burden of vitamin D deficiency, it should be stressed that between vitamin D status and health outcomes, including cardiovascular health outcomes, in observational studies could merely indicate that vitamin D is a «simple» indicator of health status; compared to healthier subjects, sicker subjects would present lower vitamin D levels or status.[3] The diversity of biological systems with which vitamin D deficiency has been associated (cardiovascular, diabetes, depression, neurodegenerative diseases, cancers, etc) could further suggest that vitamin D is a marker of health status rather than a predictor of health outcomes. Yet, both the large distribution of vitamin D receptor in the human organism[4] and the influence of vitamin D on more than 3% of the human genome[5] could explain the broad influence of vitamin D

influence on health. The recent report from the Institute of Medicine highlights the lack of robust evidence on the association between vitamin D deficiency and diseases other than bone- and muscle-related diseases.[6]

Randomized clinical trials testing the efficacy of vitamin D supplementation to reduce cardiovascular events are ongoing. The Vitamin D and Omega 3 Trial (VITAL, US clinical trial registry number : NCT01169259) is investigating in 20,000 U.S adults whether taking daily dietary supplements of vitamin D3 (2000 IU) or omega-3 fatty acids for 5 years reduces the risk of developing cancer and cardiovascular. The Vitamin D Assessment Study (ViDa, ANZCTR clinical trial registry number : ANZCTR12611000402943) is investigating in 5100 adults in New Zealand whether taking vitamin D3 200,000 IU at baseline and 100,000 IU monthly for 4 years reduces the risk of cardiovascular disease. Both trials are still recruiting participants and first results will not be available before year 2016. Meanwhile, the previously reported associations of vitamin D with cardiovascular disease should not be interpreted as causal.

In project 2, we found that 25(OH)D in the adult population with predominantly normal baseline kidney function was associated with change and odds of rapid decline in eGFR. One way to underscore the public health impact of our findings, is to estimate the population attributable fraction (PAF) for rapid decline in eGFR associated with vitamin D deficiency. PAF indicates the proportion of cases that would not occur if the factor were eliminated. Here, we computed the PAF of rapid decline in eGFR burden due to vitamin D insufficiency and deficiency and compared it with PAFs due to hypertension and diabetes.

According to our resutls, 19% of the rapid decline in eGFR burden could be avoided if 25(OH)D level was corrected to ≥20 ng/mL in this population. In comparison, the PAF of rapid decline in eGFR due to hypertension and diabetes are 7% and 5%. Thus, the PAF of rapid decline in eGFR due to vitamin D deficiency is greater than the PAF of rapid decline in eGFR due to hypertension and diabetes combined.

Hypertension and diabetes are both major risk factors for kidney failure, but the magnitude of the PAF depends both on the strength of the relative association between the risk factor and the disease under investigation and on the prevalence of the risk factor in the population.[7] In the project 2, we found that vitamin D deficiency is both meaningfully associated with the odds of rapid decline in eGFR and highly prevalent in the population. This translates into a high PAF. In a population-based context, vitamin D deficiency can be corrected by sun exposure and/or diet (e.g. supplementation, food fortification). This highlights the potential for modifying, at both the individual and population levels, a potentially major risk factor of kidney failure. Of note, PAF assumes that the relationship between vitamin D and rapid decline in eGFR is causal. Causality cannot be inferred from our observational project. Therefore, evidence of a causal association between vitamin D deficiency and renal function decline should first be available from RCTs before translating this opportunity into clinical and public health recommendations.

In project 3, we found that the *Cdx2 VDR* polymorphism modifies the association of circulating vitamin D with the odds of rapid eGFR decline or the 5-year odds of incident CKD. Compared to adults carrying the *Cdx2 CC* genotype, those carrying the

Cdx2 T allele are at greater odds of rapid eGFR decline or incident CKD associated with low level of 25(OH)D.

Lower eGFR, rapid decline in eGFR, and CKD are associated with increased risks of cardiovascular disease and death.[8-10] Our study suggests that 25(OH)D and *VDR* genetic variants interact on the early stages of eGFR decline in adults. These *VDR* genetic variants are common in European population.[11] This suggests that a large population with specific 25(OH)D metabolism genotypes may be particularly susceptible to the potential adverse renal effects of low vitamin D. This is in line with the recent finding showing that known associations of low 25(OH)D with major health outcomes (hip fracture, myocardial infarction, cancer, death) have vary according to common genetic differences in the VDR.[12]

It has been suggested that the use of genetic information in research studies might lead to individualized preventive advice and personalized treatment.[13] Our results also suggest that randomized controlled trials exploring whether correcting vitamin D deficiency slows down the age-related decline in renal function should take into consideration genetic information. Conversely, our results suggest that failing to control for vitamin D level can mask important associations between genetic variants and kidney function or misestimate the magnitude of their effects. Considering both vitamin D level and genetic variants seem to be necessary when exploring new opportunities to mitigate the growing burden of chronic kidney disease and end-stage renal disease.

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APPENDIX

Collinearity

To diagnose the presence of collinearity we considered both condition indices (CIs) and variance decompostion proportions (VDP's). Two or more VDP's (other than on the intercept) greater than 0.50 and a condition index of 30 or more are considered as indication of collinearity problem. We resolved any collinearity problems by sequentially eliminating predictors involved in the collinearity problems and the choice of the predictor eliminated was based on scientific point of view (rather than on p values). An example is provided in the collinearity diagnostic table (with CIs and VDPs) below. We illustrated collinearity diagnostic using model without interactions. A similar approach was used when considering models with interaction terms.

Linear regression model: $E(Y) = \alpha + \beta_1(25[OH]D) + \sum\gamma_i (MO_i) + + \sum\gamma_n(C_n)$, where Y = change in eGFR= $eGFR_{baseline} - eGFR_{follow\text{-}up}$; 25[OH]D = baseline 25(OH)D in ng/mL; MO=month of sampling with k=12 and k-1 dummy variables: January (category 1), …, December (category 12) where MO={1 if category i, 0 if otherwise for i=2,3,4,5,6,7,8,9,10,11,12 (referent : 1); C=potential confounders

Cs= age, BMI, waist circumference, albumin-corrected calcium, mean monthly sunshine hours, usCRP, triglycerides, HDL-cholesterol, hypertension, diabetes, gender, physical activity, smoking status, latitude, altitude, education, VitD supplements or Rx, and oral contraceptive.

	Condition Indexes CIs)							
	43839.6	**126.37**	**74.96**	**37.61**	26.96	24.68	22.41	18.10
intercept	1.00	0.00	0.00	0.00	0. 00	0.00	0.00	0.00
25[OH]D	0.00	0.02	0.00	0.01	0. 00	0.01	0.00	0.05
age	0.00	0.00	0.03	0.05	0.00	**0.65**	0.23	0.00
BMI	0.00	0.01	**0.81**	0.11	0.01	0.04	0.01	0.02
waist circumference	0.00	**0.96**	**0.95**	0.01	0.00	0.01	0.00	0.01
albumin-corrected	0.00	0.00	0.01	0.03	0.00	0.00	0.00	0.00
mean monthly sunshine hours	0.00	0.00	0.01	0.04	0.89	0.02	0.00	0.00
usCRP	0.00	0.00	0.00	0.01	0.00	0.01	0.01	0.05
triglycerides	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.13
HDL-cholesterol	0.00	0.00	0.00	0.19	0.03	0.12	0.00	0.58
hypertension	0.00	0.01	0.00	0.04	0.00	0.04	0.07	0.01
diabetes	0.00	0.00	0.00	0.01	0.00	0.00	0.02	0.01
gender	0.00	0.00	0.29	0.00	0.00	0.00	0.00	0.12
physical activity	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00
smoking status	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00
latitude	**1.00**	0.00	0.00	0.00	0.00	0.00	0.00	0.00
altitude	**0.70**	0.00	0.00	0.06	0.00	0.04	0.19	0.00
education	0.02	0.00	0.01	0.00	0.00	0.00	0.00	0.03
VitD supplements or Rx	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
oral contraceptive	0.00	0.00	0.00	0.01	0.00	0.02	0.02	0.00
MO2	0.00	0.00	0.00	0.01	0.10	0.00	0.00	0.01
MO3	0.00	0.00	0.00	0.00	0.32	0.00	0.00	0.01
MO4	0.00	0.00	0.00	0.00	0.37	0.00	0.00	0.01
MO5	0.00	0.01	0.01	0.00	**0.55**	0.00	0.00	0.00
MO6	0.00	0.00	0.01	0.00	**0.70**	0.02	0.00	0.00
MO7	0.00	0.00	0.00	0.01	**0.65**	0.02	0.00	0.01
MO8	0.00	0.00	0.00	0.00	**0.57**	0.01	0.00	0.01
MO9	0.00	0.01	0.00	0.00	0.53	0.02	0.00	0.01
MO10	0.00	0.00	0.00	0.00	0.21	0.01	0.00	0.02
MO11	0.00	0.00	0.00	0.01	0.09	0.01	0.00	0.02
MO12	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.01

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Decision #1 : Because latitude did not vary across subjects in the city of Lausanne (latitude 46.5^{o} ; altitude range: 374m-873m), latitude was dropped.

	Condition Indexes (CIs)							
	142.11	**72.55**	**41.34**	26.04	23.87	21.65	17.51	13.40
intercept	**0.95**	0.00	0.05	0.00	0.00	0.00	0.00	0.00
25[OH]D	0.00	0.00	0.02	0.00	0.01	0.00	0.05	0.01
age	0.01	0.03	0.07	0.01	**0.64**	0.23	0.00	0.01
BMI	0.00	**0.79**	0.12	0.01	0.04	0.03	0.03	0.00
waist circumference	0.02	**0.95**	0.00	0.00	0.01	0.01	0.01	0.00
albumin-corrected calcium	**0.92**	0.01	0.07	0.00	0.00	0.00	0.00	0.00
mean monthly sunshine hours	0.01	0.01	0.04	0.89	0.02	0.04	0.05	0.01
usCRP	0.00	0.00	0.01	0.00	0.00	0.01	0.00	0.00
triglycerides	0.00	0.00	0.01	0.00	0.00	0.00	0.13	0.00
HDL-cholesterol	0.01	0.00	0.18	0.03	0.13	0.07	0.56	0.07
hypertension	0.01	0.00	0.04	0.03	0.03	0.01	0.01	0.00
diabetes	0.00	0.00	0.01	0.00	0.00	0.0	0.01	0.00
gender	0.00	0.29	0.00	0.00	0.01	0.00	0.12	0.00
physical activity	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.05
smoking status	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00
altitude	0.02	0.00	0.18	0.00	0.12	**0.65**	0.01	0.02
education	0.00	0.01	0.00	0.00	0.00	0.00	0.03	0.53
VitD supplements or Rx	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00
oral contraceptive	0.00	0.00	0.01	0.00	0.04	0.02	0.00	0.16
MO2	0.00	0.00	0.01	0.10	0.01	0.00	0.01	0.12
MO3	0.00	0.00	0.00	0.32	0.01	0.00	0.01	0.10
MO4	0.00	0.00	0.00	0.37	0.02	0.00	0.01	0.05
MO5	0.01	0.01	0.01	**0.55**	0.02	0.00	0.00	0.03
MO6	0.00	0.01	0.01	**0.70**	0.02	0.00	0.00	0.00
MO7	0.00	0.00	0.00	**0.65**	0.02	0.00	0.01	0.03
MO8	0.00	0.00	0.00	**0.57**	0.01	0.00	0.01	0.04
MO9	0.00	0.00	0.00	**0.53**	0.02	0.00	0.01	0.05
MO10	0.00	0.00	0.00	0.21	0.01	0.00	0.02	0.10
MO11	0.00	0.00	0.01	0.09	0.01	0.00	0.02	0.17
MO12	0.00	0.00	0.03	0.00	0.00	0.00	0.01	0.21

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Decision #2: Because waist circumference is known to be a better determinant of obesity-related disease (including kidney failure) than BMI, BMI was dropped from the model.

	Condition Indexes (CIs)						
	136.80	**45.89**	27.39	24.51	21.15	18.22	13.03
intercept	**0.95**	0.05	0.00	0.00	0.00	0.00	0.00
25[OH]D	0.00	0.02	0.01	0.00	0.00	0.02	**0.66**
age	0.01	0.00	0.32	0.17	0.48	0.01	0.00
waist circumference	0.02	**0.73**	0.19	0.02	0.01	0.02	0.00
albumin-corrected calcium	**0.92**	0.07	0.01	0.00	0.00	0.00	0.00
mean monthly sunshine hours	0.01	0.01	0.32	**0.63**	0.00	0.00	0.45
usCRP	0.00	0.02	0.01	0.00	0.02	0.03	0.00
triglycerides	0.00	0.00	0.02	0.00	0.01	0.11	0.00
HDL-cholesterol	0.01	0.14	0.00	0.00	0.03	0.69	0.01
hypertension	0.01	0.03	0.01	0.01	0.09	0.01	0.00
diabetes	0.00	0.01	0.00	0.00	0.02	0.00	0.00
gender	0.00	0.09	0.04	0.01	0.00	0.12	0.00
physical activity	0.00	0.01	0.00	0.00	0.00	0.00	0.04
smoking status	0.00	0.02	0.00	0.00	0.00	0.00	0.00
altitude	0.02	0.09	0.22	0.11	0.42	0.12	0.00
education	0.00	0.00	0.00	0.00	0.00	0.04	0.01
VitD supplements or Rx	0.00	0.00	0.00	0.00	0.01	0.00	0.03
oral contraceptive	0.00	0.00	0.01	0.01	0.04	0.00	0.00
MO2	0.00	0.01	0.01	0.10	0.00	0.00	0.01
MO3	0.00	0.01	0.05	0.28	0.00	0.02	0.01
MO4	0.00	0.00	0.07	0.31	0.00	0.00	0.01
MO5	0.01	0.00	0.14	**0.43**	0.00	0.02	0.00
MO6	0.00	0.00	0.18	**0.54**	0.00	0.02	0.00
MO7	0.00	0.00	0.15	**0.52**	0.00	0.01	0.01
MO8	0.00	0.00	0.14	**0.44**	0.00	0.01	0.01
MO9	0.00	0.00	0.12	**0.43**	0.00	0.02	0.01
MO10	0.00	0.00	0.02	0.19	0.00	0.02	0.02
MO11	0.00	0.01	0.01	0.10	0.00	0.02	0.02
MO12	0.00	0.02	0.02	0.00	0.00	0.03	0.01

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Although the highest CI was 134.94, only two VDPs were considered as high, one of which was on intercept. Therefore, collinearity problems resolved

Backwards change in estimate elimination approach

Given the large number of potential confounders (e.g., variables associated with exposure and/or outcomes in our bivariate analyses), the number of possible combinations to compare to the full (gold standard) model was very large. Instead, we used backwards change in estimate elimination approach. Least confounders, defined by the change in the magnitude of the association between kidney function and vitamin D, were sequentially removed from the full model until the magnitude of the association between kidney function and vitamin D (levels and month-specific tertiles) differed by 10% or more from the gold standard model (i.e., initial model prior to backward elimination). Precision was assessed by adding back variables that were dropped. An example is provided below:

Example:

Linear regression model: $E(Y) = \alpha + \beta_1(25[OH]D) + \sum\gamma_i (MO_i) + \sum\gamma_n(C_n)$, where Y = change in eGFR= $eGFR_{baseline} - eGFR_{follow\text{-}up}$; 25[OH]D = baseline 25(OH)D in ng/mL; MO=month of sampling with k=12 and k-1 dummy variables: January (category 1), …, December (category 12) where MO={1 if category i, 0 if otherwise for i=2,3,4,5,6,7,8,9,10,11,12 (referent : 1) ; C=potential confounders

Models and steps	**Variables dropped**	**Beta (95%CI)**	**Comments**
Gold standard initial full model*	None	0.0836 (0.0136-0.1534)	beta range using the 10% rule: 0.0752-0.0919
	age	0.0754	
	gender	0.0767	
	education	0.0829	
	Smoking status	0.0894	
	physical activity	0.0942	
	hypertension	0.0883	
	diabetes	0.0888	
	oral contraceptive	0.0956	
	waist circumference	0.0762	
	albumin-corrected calcium	0.0847	
	VitD supplements or Rx	0.0845	
	altitude	0.0840	*Least confounder*
	mean monthly sunshine hours	0.0875	
	usCRP	0.0847	
	triglycerides	0.0826	
	HDL-cholesterol	0.0896	
Model A (Full model without altitude)			
	age	0.0772	
	gender	0.0785	
	education	0.0845	*Least confounder*
	Smoking status	0.0908	
	physical activity	0.0939	
	hypertension	0.0854	
	diabetes	0.0902	
	oral contraceptive	0.0972	
	waist circumference	0.0777	

	albumin-corrected calcium	0.0977	
	VitD supplements or Rx	0.0862	
	mean monthly sunshine hours	0.0893	
	usCRP	0.0862	
	triglycerides	0.0814	
	HDL-cholesterol	0.0919	
Model B (Model A without education)			
k similar steps (in this example 7 more variables were dropped (gender, hypertension, waist circumference, VitD supplements or Rx, usCRP, triglycerides, HDL-cholesterol, thus k=7)	…	…	…
Model** after the k =7 steps			
	oral contraceptive	0.1075	
	smoking status	0.1010	
	physical activity	0.1093	
	diabetes	0.1048	
	mean monthly sunshine hours	0.0982	
	albumin-corrected calcium	0.1052	
	age	0.0977	
			No more variable eligible for being dropped

*adjusted for age, gender, education, smoking status, physical activity, hypertension, diabetes, oral contraceptive, waist circumference, albumin-corrected calcium, VitD

supplements or Rx, altitude, mean monthly sunshine hours, usCRP, triglycerides, HDL-cholesterol, and month of blood sampling.

** adjusted for age, albumin-corrected calcium, mean monthly sunshine hours, diabetes, physical activity, smoking status, oral contraceptive, and month of blood sampling

After all eligible variables were dropped the estimate was beta=0.094 (95%CI 0.027-0.162).

In this example, the gold standard estimate was slightly less precise than the estimate resulting from backward changes in estimate steps (CI ranges 0.135 versus 0.140). This difference was however not meaningful.