



## Review

## Vitamin D metabolism and cardiovascular risk factors in postmenopausal women

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## ABSTRACT

**Objectives:** The purpose of this review is to focus on the association of vitamin D and cardiovascular risk factors in postmenopausal women.

**Methods:** Review of the relevant literature and results from recent clinical studies as well as critical analysis of published results concerning the association of vitamin D and cardiovascular risk factors in postmenopausal women obtained from both a PubMed and individual searches.

**Results and discussion:** Both basic science and clinical studies support the protective role of vitamin D on cardiovascular health, although there are controversial results in the literature. Hypovitaminosis D is associated with disturbed glucose metabolism and pancreatic  $\beta$ -cell dysfunction, lipoprotein alterations, hypertension, overweight and obesity. The evidence highlights the importance of improving vitamin D status in the general population for the prevention of adverse long-term health risks, including cardiovascular health. The optimal vitamin D dose remains to be determined. However, correction of low vitamin D itself does not guarantee the prevention of these conditions.

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**Abbreviations:** 1,25(OH)<sub>2</sub>D, 1,25-dihydroxyvitamin D; 25(OH)D, 25-hydroxyvitamin D; Apo, apolipoprotein; ATPIII, Third Adult Treatment Panel; BMD, bone mineral density; BMI, body mass index; BP, blood pressure; CI, confidence interval; CRP, C-reactive protein; CVD, cardiovascular disease; HbA1c, glycated hemoglobin; HPFS, Health Professionals' Follow-up Study; HR, hazard ratio; HDL, high density lipoprotein; IDF, International Diabetes Federation; IGF, insulin growth factor; IHD, ischemic heart disease; LDL, low density lipoprotein; NHANES, National Health and Nutrition Examination Survey; NHS, Nurses' Health Study; OR, odds ratio; PAD, peripheral arterial disease; PTH, parathyroid hormone; RAS, renin-angiotensin system; RR, relative risk; S.D., standard deviation; S.E.M., standard error of the mean; TNF, tumor necrosis factor; UV, ultraviolet; VEGF, vascular endothelial growth factor; VDR, vitamin D receptor; WHI, Women's Health Initiative.

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## 1. Introduction

Vitamin D is, in fact, a group of molecules that function as hormones. There are two different forms important in humans: ergocalciferol (vitamin D<sub>2</sub>) and cholecalciferol (vitamin D<sub>3</sub>). Vitamin D<sub>2</sub> is synthesized by plants. Vitamin D<sub>3</sub> is considered the sunshine vitamin because the major source of human vitamin D is endogenous synthesis under ultraviolet (UV) B exposure. In short, the skin transforms a derivative of cholesterol – dehydrocholesterol – normally found in the skin into vitamin D<sub>3</sub>. The liver, kidneys and other tissues further activate this molecule into 25-hydroxyvitamin D (25(OH)D)<sup>1</sup> or calcidiol and the biological main active hormonal form 1,25-dihydroxyvitamin D (1,25(OH)<sub>2</sub>D) or calcitriol [1]. Recent studies have shown that sunshine levels in far from equator countries are so weak during the winter months that the body makes little to no vitamin D at all, leading to widespread deficiencies of the vitamin. Increased skin pigmentation also reduces the effect of ultraviolet B radiation [2]. Changes in lifestyle, such as working indoors, wearing occlusive clothing, and increasing the use of sunscreen creams, have increased the number of individuals with low vitamin D levels. Vitamin D is also an essential micronutrient found in foods like eggs, liver, fortified milk and orange juice, cod liver oil, sardines, salmon, other oily fish, some cereals, mushrooms, egg yolk, beef liver, cheese and others.

Serum 25(OH)D levels are the best indicator of vitamin D status, although some controversy remains regarding “normal” and “abnormal” values. Optimal levels of 25(OH)D should be at least in the range of 30–50 ng/ml, and possibly higher to maintain general health [3,4]. It is well established that 25(OH)D serum levels below 5–7 ng/ml induce osteomalacia, serum levels below 10–12 ng/ml induce secondary hyperparathyroidism and osteoporosis, and serum levels above 18–20 ng/ml are usually considered normal or adequate. Low vitamin D levels are associated to a high prevalence of secondary hyperparathyroidism [5,6]. Aside of this endocrine feedback mechanism, mild degrees of vitamin D insufficiency seems to alter many cell processes since the adequate cell active vitamin D (1,25(OH)<sub>2</sub>D) level depends on the precursor concentration (25(OH)D). It seems quite plausible that vitamin D insufficiency should be placed at 25(OH)D serum levels below 30 ng/ml (or 75 nmol/l). A level less than 20 ng/dl indicates deficiency. However, definitions of vitamin D deficiency and insufficiency are hampered by the fact that large interlaboratory differences exist in assays for serum 25(OH)D [5]. In addition, there are differing recommendations on defining 25(OH)D deficit since there is not a consensus for its cut-off value for general health [3,4,7,8]. Neither is there any consensus on the serum concentration of 25(OH)D required to maintain the cardiovascular health.

Vitamin D is a regulator of calcium homeostasis, and helps maintain normal cell function in many organs and tissues. When vitamin D receptor (VDR) or 25(OH)D 1 $\alpha$ -hydroxylase – the rate-limiting enzyme responsible for the synthesis of 1,25(OH)<sub>2</sub>D – calcium homeostasis is impaired, leading to hypocalcemia and secondary hyperparathyroidism which induce bone alterations. However, the broad VDR distribution suggests that the vitamin D endocrine system might have additional functions on the immune system, cardiovascular system, and reproductive system, depending on the individual genetic background [3,7–10]. Cardiovascular disease (CVD) is the leading cause of death among postmenopausal women in Western countries. Although causes are unclear, there are several risk factors including increased age, gender, heredity and race, cigarette smoking, high blood cholesterol, high blood pressure (BP), physical inactivity, obesity and overweight, diabetes mellitus, and

individual response to stress. The influences of ovarian hormones as well as adipocyte factors, leptin, and ghrelin have been reviewed in relation to the menopause transition [11–14]. Low bone mineral density (BMD) has been reported associated to both a high risk of cardiovascular disease that is proportional to the severity of osteoporosis at the time of the diagnosis [15], and non-cause-specific and cardiovascular mortality [16]. Insufficiency of vitamin D is common among postmenopausal women and is associated with osteoporosis [4]. Low 25(OH)D levels have been also associated with cardiovascular disease events [17]. My objective is to review the evidence published during the recent years concerning vitamin D and cardiovascular risk factors in postmenopausal women.

## 2. The metabolic syndrome, other risk conditions and vitamin D

The metabolic syndrome or syndrome X is a clustering of metabolic abnormalities that increases the risk of developing atherosclerotic disease and type 2 diabetes with subsequent complications including acute ischemic heart disease (IHD) and stroke [18,19]. There are currently two major definitions for metabolic syndrome provided by the International Diabetes Federation (IDF) and the US National Institute of Health Third Adult Treatment Panel (ATPIII) [19,20]. The revised ATPIII and IDF definitions of metabolic syndrome are very similar and they will identify many of the same individuals as having metabolic syndrome. The ATPIII defined the metabolic syndrome as 3 or more of 5 risk determinants: abdominal obesity (waist circumference > 88 cm), increased serum triglycerides ( $\geq 150$  mg/dl), decreased high density lipoprotein cholesterol (HDL-C < 50 mg/dl), high fasting glucose ( $\geq 110$  mg/dl) and increased blood pressure ( $\geq 130/85$  mmHg) [20]. There are two differences between the ATPIII and the IDF classifications: the IDF excludes any subject without increased waist circumference, while in the ATPIII definition metabolic syndrome can be diagnosed based on other criteria and the IDF uses geography-specific cut points for waist circumference, while ATPIII uses only one set of cut points for waist circumference regardless of geography. The metabolic syndrome affects a great number of people, and prevalence increases with age. Some studies estimate the prevalence to be up to 25% of the population [21]. Diabetes risk increases in women with metabolic syndrome, and the development of IHD in women aged <65 years occurs primarily in those with metabolic syndrome or multiple IHD risk factors [22]. However, it is also asserted that diagnosis of the metabolic syndrome has a negligible association with the risk of heart disease [23].

The metabolic syndrome exact etiology remains unclear, but it is known to be a complex interaction between genetic, metabolic, and environmental factors. Insulin resistance is considered a likely mechanism underlying the metabolic syndrome [24]. There is debate concerning whether insulin resistance or obesity is the cause of the metabolic syndrome, or even these are consequences of a not yet discovered metabolic derangement. However, metabolic syndrome is not diagnosed in the absence of insulin resistance, while obesity is not constant in many subjects with metabolic syndrome [23,25]. In postmenopausal women, the metabolic syndrome rate increases with weight gain and obesity [12,14].

Among participants from the third US National Health and Nutrition Examination Survey (NHANES) cohort, the prevalence of metabolic syndrome was 21.9%, and the mean 25(OH)D concentration was significantly lower among those with the metabolic syndrome (67.1 nmol/l) as compared with those without the syndrome (75.9 nmol/l) (Table 1). Among non-diabetic individuals, the adjusted odds ratios (ORs) for having the metabolic syndrome relative to the 1st quintile for the 2nd through 5th quintiles of

<sup>1</sup> 25(OH)D equivalence: 1 ng/ml = 2.5 nmol/l.

**Table 1**  
Vitamin D levels or vitamin D intake and metabolic syndrome prevalence, glucose levels or insulin resistance.

Authors	Population	Outcome
Ford et al. [26]	Study of serum 25(OH)D in 8421 men and nonpregnant women aged $\geq 20$ years from the third NHANES cohort and the associations between the MS and its components and concentrations of 25(OH)D.	The mean level of 25(OH)D was 67.1 nmol/l (range 12.5–192.2) among those with the MS and 75.9 nmol/l (range 8.7–227.9) among those without the MS ( $P < 0.001$ ). The adjusted ORs for having the MS relative to the 1st quintile for the 2nd through 5th quintiles of level of 25(OH)D were 0.85 (95% CI 0.61–1.17), 0.75 (0.54–1.03), 0.62 (0.46–0.86), and 0.46 (0.32–0.66) ( $P < 0.001$ ). Significant inverse associations were present for quintiles of level of 25(OH)D and abdominal adiposity, hypertriglyceridemia, and hyperglycemia.
Reis et al. [27]	Cross-sectional study of 25(OH)D and PTH in 834 men and 820 women aged $\geq 20$ years from the third NHANES cohort with MS and without diagnosed diabetes.	The OR for MS in the highest quintile of 25(OH)D compared with the lowest quintile was 0.27 (0.15, 0.46; $P$ (trend) $< 0.001$ ). This relation was unchanged after adjustment for PTH level and did not differ by sex or age ( $< 50$ or $\geq 50$ years).
Hyppönen et al. [28]	Study of 25(OH)D and IGF-1 in 6810 British White subjects in the 1958 cohort, surveyed during 2002–2004 (age 45 years).	MS prevalence was lowest for subjects with the highest concentrations of both 25(OH)D and IGF-1 (OR for highest vs. lowest third of both 0.26 (95% CI 0.17–0.40).
Scragg et al. [29]	Measurements of 25(OH)D serum levels in 6228 participants from the third NHANES population with fasting and/or 2-h plasma glucose and serum insulin.	ORs for diabetes (fasting glucose $\geq 7.0$ mmol/l) varied inversely across quartiles of 25(OH)D in a dose-dependent pattern (OR 0.25; 95% CI 0.11–0.60) for non-Hispanic Whites and (0.17; 0.08–0.37) for Mexican Americans in the highest vitamin D quartile (25(OH)D $\geq 81.0$ nmol/l) compared with the lowest 25(OH)D ( $\leq 43.9$ nmol/l).
Need et al. [30]	Study of 753 postmenopausal women attending an outpatient clinic and not on any treatment known to affect glucose metabolism.	Fasting serum glucose had a positive correlation function of age ( $P < 0.05$ ), weight ( $P < 0.001$ ) and BMI ( $P < 0.001$ ) and a negative function of serum 25(OH)D ( $P < 0.001$ ). After adjustment, serum glucose increased as 25(OH)D levels fell throughout the range of serum 25(OH)D measured, being the greatest increase in women with 25(OH)D below 40 nmol/l.
Chiu et al. [31]	Study of 25(OH)D and ISI and $\beta$ -cell function in 126 healthy, glucose-tolerant subjects, assessed by a hyperglycemic clamp.	The 25(OH)D level was positively correlated with ISI ( $P < 0.0001$ ) and negatively correlated with first-phase ( $P = 0.0045$ ) and second-phase insulin responses ( $P < 0.0001$ ). Multiple regression analyses confirmed an independent correlation between 25(OH)D concentration and ISI ( $P = 0.0007$ ).
Targher et al. [32]	Comparison of 25(OH)D in 390 type 2 diabetic patients and 390 non-diabetic controls. Common carotid IMT was measured with ultrasonography in diabetic patients by a single trained operator blinded to subjects' details.	25(OH)D $\leq 37.5$ nmol/l is highly prevalent in type 2 diabetic adults and is strongly and independently associated with increased carotid IMT. Low 25(OH)D concentrations independently predicted carotid IMT ( $P < 0.001$ ) in people with type 2 diabetes after adjustment for risk factors.
Liu et al. [33]	Calcium and vitamin D intakes were related to the MS in 10,066 women aged $\geq 45$ years participating in the Women's Health Study who were free of cardiovascular disease, cancer, or diabetes and who never used postmenopausal hormones.	Dietary vitamin D was inversely associated with prevalence of MS but was not independent of total calcium intake. Similar strong relations between intakes of dairy products and MS were also observed. After adjustment, the multivariable ORs comparing highest with lowest intake categories were 0.66 (0.55–0.80) ( $P$ for trend $< 0.0001$ ) for total dairy products and 0.85 (0.71–1.02) ( $P$ for trend = 0.05) for total milk intake.
Pittas et al. [34]	Calcium and vitamin D supplement intake in 83,779 women from the prospective NHS who had no history of diabetes, cardiovascular disease, or cancer at baseline for the development of type 2 diabetes followed up during 20 years for the risk of type 2 diabetes.	The RR of type 2 diabetes was 0.87 (95% CI 0.75–1.00; $P$ for trend = 0.04) comparing the highest with the lowest category of vitamin D intake from supplements. The multivariate RRs of type 2 diabetes were 0.79 (0.70–0.90; $P$ for trend $< 0.001$ ) comparing the highest with the lowest category of calcium intake from all sources and 0.82 (0.72–0.92; $P$ for trend $< 0.001$ ) comparing the highest with the lowest category of calcium intake from supplements. A combined daily intake of $> 1200$ mg calcium and $> 800$ IU vitamin D was associated with a 33% lower risk of type 2 diabetes with RR of 0.67 (0.49–0.90) compared with an intake of $< 600$ mg and 400 IU calcium and vitamin D, respectively.
Pittas et al. [35]	Review and meta-analysis through January 2007 for observational studies and clinical trials in adults with outcomes related to glucose homeostasis.	Observational studies show a relatively consistent association between low vitamin D status, calcium or dairy intake, and prevalent type 2 DM or MS [OR (95% CI)]: type 2 DM prevalence, 0.36 (0.16–0.80) among non-Blacks for highest vs. lowest 25(OH)D; MS prevalence, 0.71 (0.57–0.89) for highest vs. lowest dairy intake]. There are also inverse associations with incident type 2 DM or MS: type 2 DM incidence, 0.82 (0.72–0.93) for highest vs. lowest combined vitamin D and calcium intake; 0.86 (0.79–0.93) for highest vs. lowest dairy intake.
De Boer et al. [36]	WHI cohort randomly assigned to receive 1000 mg elemental calcium plus 400 IU of vitamin D3 daily, or placebo. Among 33,951 participants without self-reported diabetes at baseline, new diagnoses of diabetes treated with oral hypoglycemic agents or insulin were ascertained.	WHI cohort suggest that calcium (1 g/day) and vitamin D (400 IU/day) supplementation did not reduce the risk of developing diabetes over 7 years of follow-up in postmenopausal women.

Table 1 (Continued).

Authors	Population	Outcome
Mattila et al. [37]	Longitudinal follow-up of a Finnish cohort measuring serum 25(OH)D at baseline and subsequent risk of type 2 diabetes identified from a nationwide registry of patients receiving diabetes medication reimbursement.	A high serum 25(OH)D concentration may reduce the risk of type 2 diabetes. The association was attenuated in the multivariate analysis, adjusting for potential risk factors of type 2 diabetes.
Tai et al. [38]	Thirty-three adults with serum 25(OH)D $\leq$ 50 nmol/l and without diabetes (12 with impaired glucose tolerance) were given two oral doses of 100,000 IU of cholecalciferol, 2 weeks apart. Before the first dose and 2 weeks after the second dose, a 75-g oral glucose tolerance test was performed.	Mean serum 25(OH)D increased from $39.9 \pm 1.5$ (S.E.M.) to $90.3 \pm 4.3$ nmol/l ( $P < 0.0001$ ) whereas PTH decreased from $6.7 \pm 1.2$ to $4.5 \pm 0.6$ pmol/l ( $P = 0.055$ ). There was no change in blood mean glucose or insulin levels, and no change in insulin sensitivity after vitamin D treatment. Results did not differ between subjects, with and without, impaired glucose tolerance.
Forouhi et al. [39]	Prospective study of 524 non-diabetic men and women, aged 40–69 years at baseline, with measurements for serum 25(OH)D and IGF-1, glycemic status, lipids, insulin, anthropometry, and blood pressure.	Baseline 25(OH)D was associated inversely with 10-year risk of hyperglycemia, insulin resistance, and metabolic syndrome after adjustment for age, sex, smoking, BMI, season, and baseline value of each metabolic outcome variable. Associations with 2-h glucose, insulin, and HOMA-IR remained significant after further adjustment for IGF-1, PTH, calcium, physical activity, and social class.

CI = confidence interval; HOMA-IR = homeostasis model assessment of insulin resistance; IMT = intimal medial thickening; ISI = insulin sensitivity index; MS = metabolic syndrome; NHANES = National Health and Nutrition Examination Survey; NHS = Nurses' Health Study; OR = odds ratio; RR = relative risk; S.E.M. = standard error of the mean.

concentration of 25(OH)D were 0.85 (95% confidence limits = CI 0.61–1.17), 0.75 (0.54–1.03), 0.62 (0.46–0.86), and 0.46 (0.32–0.66) ( $P < 0.001$ ). There were not significant differences between men and women or among the three major racial or ethnic groups for the association between 25(OH)D and the metabolic syndrome [26]. The combined effect of both endogenous 25(OH)D and parathyroid hormone (PTH) levels on metabolic syndrome have been determined in individuals, without diagnosed diabetes, as a representative sample of the third NHANES cohort. After adjustment for confounding factors, the OR for metabolic syndrome in the highest quintile of 25(OH)D (median 88.0 nmol/l) compared with the lowest quintile (median 26.8 nmol/l) was 0.27 (CI = 0.15–0.46;  $P(\text{trend}) < 0.001$ ). The OR was similar after additional adjustment for PTH, sex or age [27]. Therefore, it seems that there is an inverse association between serum 25(OH)D with metabolic syndrome.

In a British Caucasian cohort at age 45 years, 25(OH)D serum levels are also inversely associated with the metabolic syndrome, being the metabolic syndrome prevalence the lowest when the highest concentrations of both 25(OH)D and insulin growth factor (IGF) 1 adjusted for confounding factors. IGF-1 was not significantly associated with metabolic syndrome among those with the lowest levels of 25(OH)D, whereas higher 25(OH)D was significantly associated with the lowest metabolic syndrome prevalence at all IGF-1 concentrations. The association was attenuated after adjustments for body mass index (BMI), leisure-time exercise, smoking, and education [28]. Therefore, these results suggest some kind of protective effect of vitamin D against the metabolic syndrome in mid-aged women.

### 2.1. Insulin resistance and vitamin D

The vitamin D endocrine system is involved in glucose homeostasis and in insulin release mechanisms. Experimental and epidemiological studies suggest that calcium and vitamin D may reduce the risk of developing diabetes. Epidemiological evidence has indicated that vitamin D deficiency increased the risk of insulin resistance in metabolic syndrome. In humans, there are correlations between serum concentrations of vitamin D metabolites, and plasma glucose and insulin secretion (Table 1). Thus, plasma levels of 25(OH)D have been inversely related to the prevalence of type 2 diabetes, glucose concentration, and insulin resistance, although fasting glucose was not significantly related to either serum 1,25(OH)<sub>2</sub>D or PTH [29,30]. Low vitamin D has a small but significant impact on blood glucose metabolism and type 2 diabetes risk. Thus, subjects with vitamin D levels below 20 ng/ml

had a greater prevalence of components of the metabolic syndrome than did those with higher concentrations [31]. The prevalence of hypovitaminosis D, as measured by 25(OH)D  $\leq$  37.5 nmol/l, is significantly higher among type 2 diabetic adults than in non-diabetic controls (34.% vs. 16.4%) who are compared for age and sex. Among diabetic patients, those with hypovitaminosis D had a marked increase in common carotid intimal medial thickening – an indirect index of preclinical atherosclerosis – as compared to their vitamin D sufficient counterparts ( $1.10 \pm 0.15$  mm vs.  $0.87 \pm 0.14$  mm). In multivariate analysis, hypovitaminosis D predicted carotid intimal medial thickening in individuals with type 2 diabetes after adjustment for confounding factors such as diabetes duration, glycated hemoglobin (HbA1c), calcium, renal function tests, inflammatory markers, use of medications, and presence of the metabolic syndrome [32].

Intake of calcium and vitamin D are related to the metabolic syndrome in 10,066 middle-aged or older women from the Women's Health Study who are free of cardiovascular disease, cancer, or diabetes and who never used postmenopausal hormones [33]. Dietary vitamin D was inversely associated with the prevalence of metabolic syndrome when considering with calcium intake. Thus, women with lower amounts of calcium and vitamin D in their diets were more likely to have high glucose levels and diabetes, and lower amounts of vitamin D were shown to affect the body's ability to produce and secrete insulin. The results from the Nurses' Health Study (NHS) cohort including 83,779 women who had no history of diabetes, cardiovascular disease, or cancer at baseline for the development of type 2 diabetes reported an inverse association for the intake of vitamin D supplements and the risk of type 2 diabetes during a follow-up period of 20 years. Although there was no association between total vitamin D intake and type 2 diabetes, the relative risk (RR) of type 2 diabetes, was 0.87 (95% CI 0.75–1.00;  $P$  for trend = 0.04) comparing the highest with the lowest category of vitamin D intake from supplements [34].

A meta-analysis including the results from trials with vitamin D and/or calcium supplementation published through January 2007 suggests that combined vitamin D and calcium supplementation may have a role in the prevention of type 2 diabetes in populations at high risk [35]. However, the meta-analysis has limitations since some trials were observational or cross-sectional, the intervention studies were of short-term, including few individuals, using varied formulations of vitamin D and calcium, or were based on post hoc analyses.

The results from the Women's Health Initiative (WHI) cohort suggest that calcium (1 g/day) and vitamin D (400 IU/day) sup-

**Table 2**  
Plasma lipoproteins and triglyceride levels and vitamin D.

Authors	Population	Outcome
Auwerx et al. [40]	Measurement of 25(OH)D and Apo A-I in 185 men and 173 women.	Significant positive correlation between 25(OH)D and Apo A-I. 25(OH)D also had a positive correlation with high HDL-cholesterol levels ( $P < 0.05$ in men and $P < 0.005$ in women).
John et al. [41]	Fasting 25(OH)D and lipid concentrations in 170 British Bangladeshi adults, 69 men and 101 women, who were free of known diabetes or chronic disorders.	Total cholesterol, LDL cholesterol, and both Apo A-I and Apo B concentrations correlated directly with serum 25(OH)D levels. A multiple regression analysis, showed that the 25(OH)D was an independent predictor of increasing Apo A-I concentrations but not of fasting lipid concentrations.
Major et al. [42]	Overweight or obese women ( $n = 63$ ) with a daily calcium intake of $< 800$ mg/day were assigned to consume 1200 mg elemental calcium and 400 IU vitamin D or placebo; both groups observed a 700 kcal/day energy restriction.	After a 15-week intervention, greater decreases in total:LDL and LDL:HDL ( $P < 0.01$ for both) and of LDL cholesterol ( $P < 0.05$ ) were observed in the calcium + D group than in the placebo group. The differences in total:HDL and LDL:HDL were independent of changes in fat mass and in waist circumference. Mean 25(OH)D levels were lower in women, elderly persons ( $\geq 60$ years), ethnic minorities, and participants with obesity, hypertension, and diabetes mellitus. The adjusted prevalence of hypertension (OR, 1.30), diabetes mellitus (OR, 1.98), obesity (OR, 2.29), and high serum triglyceride levels (OR, 1.47) was significantly higher in the first than in the fourth quartile of serum 25(OH)D levels ( $P < 0.001$ for all).
Martins et al. [43]	The association between serum levels of 25(OH)D and select cardiovascular disease risk factors in US adults (7186 male and 7902 female).	There was a significant ( $P < 0.03$ ) positive association between 25(OH)D and Apo A-I and lipoprotein A-I. The ratio of LDL:HDL was significantly ( $P \leq 0.044$ ) negatively correlated with 25(OH)D levels. The levels of 25(OH)D increased significantly in the treated compared with control group ( $P < 0.05$ ). Overall, there were no significant differences between the treated and control groups in any lipoproteins or apolipoproteins after administration of UV irradiation.
Carbone et al. [44]	To determine the relationship of 25(OH)D to cholesterol and lipoprotein particles and to determine whether increasing 25(OH)D through UV irradiation (twice weekly for 12 weeks) impacted on these parameters in healthy young subjects as compared to a control group.	

Apo = apolipoprotein; OR = odds ratio; UV = ultraviolet.

plementation did not reduce the risk of developing diabetes over 7 years of follow-up in postmenopausal women [36]. Nevertheless, the WHI did not give information about leisure activities and sun exposure, had some limitations concerning the dose of vitamin D which is low to maintain adequate serum levels, and some women from the placebo group used supplements which might have attenuated the placebo effect as compared to the active group [4]. Therefore, although the WHI cohort includes a significant number of women and it is a randomized study, its value is limited due to those methodological bias. In addition, there were not vitamin D metabolite measurements, and the dose of vitamin D used was lower than the presently recommended [3,4,7]. Based on these limitations, the results from the WHI study does not discard the value optimal endogenous vitamin D levels. Furthermore, there is no data to determine if a higher dose of vitamin D supplementation might prevent or delay the apparition of diabetes.

The measurement of 25(OH)D reflects vitamin D acquired from the diet, cutaneous synthesis and additional supplements. Mattila et al. [37] reported the 25(OH)D serum levels in a Finish cohort followed during 17 years in relation with the subsequent risk of type 2 diabetes. After adjustment for confounding factors, there was a statistically significant inverse association between serum 25(OH)D concentration and diabetes incidence. The association was attenuated after further adjustments for body mass index, leisure-time exercise, smoking, and education.

In a small number of individuals with known vitamin D deficiency (serum 25(OH)D  $\leq 50$  nmol/l) without diabetes, were given two oral doses of 100,000 IU of cholecalciferol, 2 weeks apart, to study the effect of normalization of vitamin D on plasma glucose and serum insulin levels. Serum 25(OH)D significantly increased from  $39.9 \pm 1.5$  (S.E.M.) to  $90.3 \pm 4.3$  nmol/l whereas PTH levels decreased. However, there was no change in blood glucose or insulin mean concentrations, and no change in insulin sensitivity as measured by different indices. In addition, the results did not differ between individuals, with and without, impaired glucose tolerance [38]. It seems that short-term normalization of serum

vitamin D levels is not associated with changes of glucose–insulin homeostasis.

The predictive value of serum 25(OH)D for future glycemic status and insulin resistance was prospectively studied in a cohort of 524 randomly selected non-diabetic individuals, aged 40–69 years at baseline, with measurements for glycemic status (oral glucose tolerance), lipids, insulin, anthropometry, and blood pressure measured and metabolic syndrome risk (derived at baseline and at 10 years of follow-up). Baseline 25(OH)D was inversely associated with 10-year risk of hyperglycemia, insulin resistance, metabolic syndrome after adjustment for confounding factors [39].

## 2.2. Lipoproteins and vitamin D

Hypovitaminosis D has been reported to be associated not only with lowered insulin secretion and sensitivity but also with adverse effects on both total cholesterol and low density lipoprotein (LDL) cholesterol concentrations in studies of healthy men and women from several racial and ethnic groups (Table 2). A highly significant positive and independent correlation has been reported between serum 25(OH)D and apolipoprotein (Apo) A-I and with high density lipoprotein (HDL) cholesterol levels, being the relations independent of calcium [40]. In addition, there is a positive relation of Apo A-I concentrations to serum 25(OH)D concentrations, independent of other risk factors for type 2 diabetes and IHD after multiple regression analyses [41]. Therefore, it seems plausible that hypovitaminosis D may be associated with adverse effects on lipid profiles independent of any adverse effects that might result from the increased risk of type 2 diabetes. On the contrary, the calcium and vitamin D supplement treatment during a 15-week weight-loss intervention was associated with significantly greater decreases in total cholesterol:LDL, LDL:HDL and of LDL cholesterol as compared to the placebo group. The differences in total:HDL and LDL:HDL were independent of changes in fat mass and in waist circumference [42].

**Table 3**  
Vitamin D intake or 25(OH)D serum levels and incident hypertension.

Authors	Population	Outcome
Forman et al. [50]	Intake of vitamin D and the risk of incident hypertension among participants of three cohorts: NHS I ( $n = 77,436$ ), NHS II ( $n = 93,803$ ), and HPFS ( $n = 38,074$ ) that were followed for $\geq 8$ years.	Vitamin D intake was not associated with the risk of hypertension. The multivariable RR estimates for the highest compared with lowest quintile of intake were 0.98 (0.93–1.04) in NHS I, 1.13 (0.99–1.29) in NHS II, and 1.03 (0.93–1.15) in HPFS. No association was found among participants who consumed $\geq 1600$ to $<400$ IU/day and those who consumed $\geq 1000$ to $<200$ IU/day.
Forman et al. [51]	The association between plasma 25(OH)D levels and risk of incident hypertension was studied in 613 men from the HPFS and 1198 women from the NHS. Subjects were followed for 4–8 years. In addition, two prospective cohort studies including 38,388 men and 77,531 women with predicted 25(OH)D levels were followed for 16–18 years.	During 4 years of follow-up, the RR of incident hypertension among men whose plasma 25(OH)D levels were $<15$ ng/ml compared with those whose levels were $\geq 30$ ng/ml was 6.13 (95% CI: 1.00–37.8). Among women, the same comparison yielded a RR of 2.67 (95% CI: 1.05–6.79). Using predicted 25(OH)D levels in the larger cohorts, the RRs comparing the lowest to highest deciles were 2.31 (95% CI: 2.03–2.63) in men and 1.57 (95% CI: 1.44–1.72) in women.
Wang et al. [52]	In a prospective cohort of 28,886 US women aged $\geq 45$ years the associations of intake of dairy products, calcium, and vitamin D with the incidence of hypertension ( $n = 8710$ ) were identified during 10 years of follow-up.	After adjusting for major hypertension risk factors, the RRs of hypertension across increasing quintiles of low-fat dairy product intake were 1.00 (reference), 0.98, 0.97, 0.95, and 0.89 ( $P$ for trend: 0.001). The risk of hypertension decreased in the higher quintiles of dietary calcium (RR in the highest quintile: 0.87) and dietary vitamin D (RR in the highest quintile: 0.95), but did not change with calcium or vitamin D supplements.
Margolis et al. [53]	The effect of calcium plus vitamin D supplementation on BP and the incidence of hypertension in 36,282 postmenopausal women from the WHI cohort that received 1000 mg of elemental calcium plus 400 IU of vitamin D3 daily or placebo in a double-blind fashion.	Over a median follow-up time of 7 years, there was no significant difference in the mean change over time in systolic BP and diastolic BP between the active and placebo treatment groups. In 17,122 nonhypertensive participants at baseline, the hazard ratio for incident hypertension associated with calcium/vitamin D treatment was 1.01 (95% CI: 0.96–1.06).
Scragg et al. [55]	The association between serum 25(OH)D and BP in 12,644 Black people aged 20 years or more from the third NHANES, after excluding those on hypertensive medication.	Adjusted mean serum 25(OH)D was lowest in non-Hispanic Blacks (49 nmol/l), intermediate in Mexican Americans (68 nmol/l), and highest in non-Hispanic Whites (79 nmol/l). Systolic BP was 3.0 (0.7) mm Hg lower ( $P = 0.0004$ ) and diastolic BP was 1.6 (0.6) mmHg lower ( $P = 0.011$ ) for participants in the highest 25(OH)D quintile ( $\geq 85.7$ nmol/l) compared with the lowest ( $\leq 40.4$ nmol/l), after adjustment. The inverse association between 25(OH)D and systolic BP was stronger in participants aged $\geq 50$ years than younger ( $P = 0.021$ ).
Judd et al. [56]	The associations between vitamin D concentrations and systolic BP were determined in the third NHANES.	Lower 25(OH)D levels were associated with a higher BP category in Whites ( $P < 0.001$ ); however, when controlling for age, the association was no longer significant. Levels of 25(OH)D $> 80$ nmol/l decreased the age-related increase in systolic BP by 20% compared with subjects having 25(OH)D levels $< 50$ nmol/l ( $P < 0.001$ ).
Forman et al. [57]	Among 1484 women aged 32–52 years who did not have hypertension at baseline, the association between plasma levels of 25(OH)D and the odds of incident hypertension using a nested case-control study design was analyzed.	Median plasma 25(OH)D levels were lower in the cases (25.6 ng/ml) than in the controls (27.3 ng/ml; $P < 0.001$ ). Women in the lowest compared with highest quartile of plasma 25(OH)D had an adjusted OR for hypertension of 1.66 (95% CI: 1.11–2.48; $P$ for trend = 0.01). Compared with women with sufficient levels, those with vitamin D deficiency ( $< 30$ ng/ml) had a multivariable OR of 1.47 (95% CI: 1.10–1.97). Plasma 25(OH)D levels are inversely and independently associated with the risk of developing hypertension.

BP = blood pressure; HPFS = Health Professionals' Follow-up Study; NHANES = National Health and Nutrition Examination Survey; NHS = Nurses' Health Study; OR = odds ratio; RR = relative risk.

The analysis of the third NHANES showed that people with vitamin D deficiency were more likely to have high serum triglyceride levels. After adjustment for age, gender, and race, 32.9% of people in the first quartile of vitamin D levels ( $< 21$  ng/ml) had triglyceride levels of 150 mg/dl or above, compared with 23.8% of those in the fourth quartile ( $\geq 37$  ng/ml). The results give a significant OR of 1.47 for high triglyceride levels in people with low, compared to high, vitamin D levels [43].

Individuals exposed to suberythemal doses of whole-body irradiation using ultraviolet lamps ultraviolet irradiation, twice weekly for 12 weekly, to increase 25(OH)D synthesis showed a significant elevation of 25(OH)D as compared with a control group not irradiated. Positive associations between 25(OH)D and Apo A-I and lipoprotein A-I were found as compared to the control group. The ratio LDL lipoprotein:HDL lipoprotein was significantly negatively correlated with 25(OH)D levels. In individuals with 25(OH)D insufficiency (levels  $< 75$  nmol/l) had decreases in Apo A-II in the UV treated group and increases in the control group that were statistically significantly different between the groups [44]. The effects of different degrees of natural UV sunlight irradiation

should be further studied to determine the precise effect on lipid metabolism.

### 2.3. Vitamin D and hypertension

Hypertension increases fourfold the risk of cardiovascular disease in comparison with normotensive women and accounts for approximately 35% of all cardiovascular events [22,45]. Hypertension could be often associated with other risk factors, such as the metabolic syndrome, diabetes mellitus, obesity and dyslipidemia. The presence of hypertension had a significantly greater effect on coronary disease in women than men [46]. Sun exposure, as an indirect index of vitamin D skin synthesis, has been reported to be inversely associated to blood pressure and the prevalence of hypertension. In addition, ultraviolet light exposure lowers BP [47,48]. Many vitamin D actions are exerted by paracrine and autocrine regulatory mechanisms which are alternative to endocrine action changing calcium homeostasis. Thus, the  $1\alpha$ -hydroxylase enzyme that converts 25(OH)D to  $1,25(\text{OH})_2\text{D}$  is expressed in human endothelial and vascular smooth muscle cells

**Table 4**  
Vitamin D levels in overweight, obese and morbidly obese subjects and the influence of the skin colour.

Authors	Population	Outcome
Parikh et al. [63]	Serum PTH, 25(OH)D, and 1,25(OH) <sub>2</sub> D were measured in 302 healthy adults. Results from the 154 obese subjects (BMI 37.3 ± 5.8 kg/m <sup>2</sup> ) were compared with those from 148 nonobese (BMI 25.6 ± 2.9 kg/m <sup>2</sup> ) matched participants.	Serum 25(OH) D was negatively correlated with BMI ( $r = -0.4$ ; $P < 0.0001$ ) and body fat mass ( $r = -0.41$ ; $P < 0.0001$ ). Serum 1,25(OH) <sub>2</sub> D was also negatively correlated with BMI ( $r = -0.26$ ; $P < 0.0001$ ) and body fat mass ( $r = -0.25$ ; $P = 0.0001$ ). Serum 1,25(OH) <sub>2</sub> D was significantly lower in obese than nonobese subjects (105.7 ± 41.1 pmol/l vs. 124.8 ± 36.7 pmol/l; $P < 0.0001$ ).
Looker [64]	The relationship between serum 25(OH)D and %BF by race in 6042 women (3567 non-Hispanic Whites and 2475 non-Hispanic Blacks) from the third NHANES.	The negative relationship between serum 25(OH)D and %BF was noticeably stronger in Whites than in Blacks of the same age. Within race, the relationship was stronger in younger than older individuals.
Yanoff et al. [62]	To determine the effects of race and adiposity on 25(OH)D and PTH in a cross-sectional study of 379 Black and White adults which BMI ranged from 19.9 to 58.2 kg/m <sup>2</sup> .	The prevalence of hypovitaminosis D increased with increasing BMI, and was greater ( $P < 0.001$ ) in Blacks than Whites within all BMI categories examined. Among subjects with BMI ≥ 35 kg/m <sup>2</sup> , 59% of Blacks vs. 18% of Whites had hypovitaminosis D (OR 6.5, 95% CI 3.0–14.2). PTH was negatively correlated with 25(OH)D ( $r = -0.31$ , $P < 0.0001$ ).
McGill et al. [65]	Relationships of vitamin D3 with measures of fat mass, MS markers, HbA1c and MS in a cross-sectional sample of 250 overweight and obese adults of different ethnicities.	There were modest inverse associations of vitamin D3 with body weight ( $r = -0.21$ , $P = 0.0009$ ), BMI ( $r = -0.18$ , $P = 0.005$ ), waist ( $r = -0.14$ , $P = 0.03$ ), and HbA1c ( $r = -0.16$ , $P = 0.01$ ). Multivariable regression carried out separately for BMI and waist showed a decrease of 0.74 nmol/l ( $P = 0.002$ ) in vitamin D3 per 1 kg/m <sup>2</sup> increase in BMI and a decrease of 0.29 nmol/l ( $P = 0.01$ ) per 1 cm increase in waist.
Caan et al. [66]	Placebo-controlled trial in 36,282 women, aged 50–79 years, enrolled in the WHI trial that were randomized at their first or second annual visit to receive a dose of 1000 mg of elemental calcium plus 400 IU of cholecalciferol or placebo daily. Follow-up for an average of 7 years.	Women receiving calcium plus cholecalciferol supplements vs. women receiving placebo had a minimal but consistent favourable difference in weight change (mean difference, -0.13 kg; 95% CI, -0.21 to -0.05; $P = 0.001$ ). Calcium plus cholecalciferol supplementation has a small effect on the prevention of weight gain.
Harris and Dawson-Hughes [67]	A group of 381 healthy volunteers age 65 and older participated in the study to determine whether sun exposure habits differ according to %FAT and to what extent they explain the inverse association of adiposity with 25(OH)D.	25(OH)D decreased across %FAT quartiles ( $P < 0.05$ ) and was about 20% lower in the highest compared with the lowest quartile of %FAT after adjustments for age, sex, season, and vitamin D intake. In older adults, sun exposure habits do not vary according to adiposity and do not appear to explain lower 25(OH)D concentrations with increasing adiposity.
Hyppönen and Power [68]	Data are from the survey of the 45-year-old 1958 British birth cohort (2002–2004). Information on A1C, 25(OH)D, and BMI was collected from 7198 Caucasian subjects.	25(OH)D was <75 nmol/l in 80% of the obese subjects vs. 68% of the other subjects ( $P < 0.0001$ ). Serum 25(OH)D decreased and A1C increased by increasing BMI ( $P < 0.0001$ ). After adjustment, percent change in A1C by 10-nmol/l increase in 25(OH)D was -0.21 (95% CI -0.31 to -0.11) for BMI < 25 kg/m <sup>2</sup> , -0.25 (-0.37 to -0.13) for BMI 25–29.9 kg/m <sup>2</sup> , -0.65 (-0.95 to -0.34) for BMI 30–34.9 kg/m <sup>2</sup> , and -1.37 (-2.09 to -0.64) for BMI ≥ 35 kg/m <sup>2</sup> .
Aasheim et al. [69]	Prospective cross-sectional study of 110 patients (76 women) and 58 healthy controls (30 women). The mean (±S.D.) BMI (in kg/m <sup>2</sup> ) was 45 ± 7 in the patients and was 24 ± 3 in the controls. Patients with vitamin D levels lower than 2 S.D. below the sex-specific mean in controls were considered to have inadequate vitamin status.	The morbidly obese women and men had significantly lower concentrations of vitamin 25(OH)D than did the healthy controls ( $P < 0.01$ for each). Low concentrations of 25(OH)D adjusted for lipids are prevalent in morbidly obese Norwegian patients seeking weight-loss treatment.
Botella-Carretero et al. [70]	Observational study including 73 morbidly obese patients (BMI 40 kg/m <sup>2</sup> ), recording anthropometric variable, blood 25(OH)D levels, lipid profiles, glucose and insulin levels, and insulin resistance was estimated by homeostasis model assessment.	Vitamin D deficiency was more prevalent in morbidly obese patients presenting with the MS, compared with those who did not achieve the criteria for this syndrome (60.9% vs. 33.3% respectively, $P = 0.023$ ). When serum levels of 25(OH)D were categorized in tertiles, there was an association of the prevalence of the MS with the former ( $P = 0.038$ ). Serum HDL-cholesterol levels were lower (37.0 ± 7.8 mg/dl vs. 44.9 ± 8.7 mg/dl, $P = 0.003$ ), and triglycerides levels were higher (163.3 ± 81.5 mg/dl vs. 95.1 ± 24.2 mg/dl, $P = 0.001$ ) in the vitamin D-deficient group.

%BF = body fat; BMI = body mass index; HbA1c = glycated hemoglobin; MS = metabolic syndrome; WHI = Women's Health Initiative; NHANES = National Health and Nutrition Examination Survey; S.D. = standard deviation.

which have special relevance in the genesis of hypertension. This local conversion into the active hormone has been also reported in other processes like cancer and inflammation [3,10,49].

The relation between vitamin D intake and the risk of incident hypertension has been reported among the participants in three large prospective North American cohorts followed for at least 8 years: Nurses' Health Study I, NHS II, and Health Professionals' Follow-up Study (HPFS) [50]. The participants were classified by quintiles and there was not any significant association between the amount of vitamin D consumed and hypertension (Table 3). However, in participants from the NHS and HPFS followed for 4–8 years, plasma 25(OH)D levels were inversely associated with the risk of

incident hypertension, independent of age, BMI, physical activity, race, menopausal status, and other variables [51].

The intake of dairy products, calcium and vitamin D has been associated with the incidence of hypertension as prospectively determined in a cohort of mid-aged women followed during 10 years [52]. The risk of hypertension decreased in the higher quintiles of dietary calcium and dietary vitamin D intake. The more dairy products and food containing vitamin D the women ate, the lower their risk of developing high blood pressure. The women who drank two or more servings of fat-free milk or low-fat dairy products daily reduced their high blood pressure risk by 10% compared to women who consumed the low-fat dairy products less than once a month.

However, the study showed no benefit from taking supplements containing calcium or vitamin D.

In the randomized WHI cohort, daily dietary supplement of calcium (1000 mg) plus vitamin D (400 IU) taken for 7 years neither reduces BP nor alters the risk of developing hypertension, in older postmenopausal women (50–79 years) [53]. In the accompanying editorial, Geleijnse [54] points out that nearly half of the women already had high BP prior to the study and close to a third were using hypotensor treatments. In the WHI cohort there was a high number of older postmenopausal women (>65 years) in which the low dose of vitamin D would be ineffective to normalize endogenous vitamin D levels. It seems quite likely that the daily 400 IU of vitamin D is an insufficient dose to maintain adequate levels [3,4,7]. In addition, in the WHI study there were not vitamin D measurements.

BP and 25(OH)D were studied in people from the third NHANES after excluding those on hypertensive medication [55]. When participants were divided into serum 25(OH)D quintiles, both systolic and diastolic BP were significantly lower for participants in the highest quintile (25(OH)D  $\geq$  85.7 nmol/l) compared with the lowest (25(OH)D  $\leq$  40.4 nmol/l). The inverse relation between 25(OH)D and systolic BP was significantly stronger in participants aged  $\geq$  50 years as compared to younger individuals. In addition, it was found that optimal vitamin D status reduces the age-related increase in systolic BP in the White population. Lower 25(OH)D levels were associated with a significant higher BP category, although the association was not significant when controlling for age, and 25(OH)D levels higher than 80 nmol/l reduced the age-related increase in systolic BP [56].

A prospective nested case-control study in 1484 middle-aged women (median age 43 years) from the second NHS who did not have hypertension at baseline were matched to controls and 25(OH)D measured and the association with incident hypertension determined. Overall, 65.7% of the women had vitamin D deficiency (<30 ng/ml), whereas cases had a significant lower median plasma levels of 25(OH)D than controls (25.6 ng/ml vs. 27.3 ng/ml). In women who were vitamin D deficient the OR to develop hypertension was 1.47 (95% CI: 1.10–1.97) compared to those with adequate levels [57].

Although there are some doubts and contradictory results, it seems plausible that endogenous adequate vitamin D levels or related factors – presently unidentified – may regulate BP. However, randomized trials are needed to determine whether vitamin D supplementation could reduce blood pressure.

#### 2.4. Obesity and vitamin D

Overweight/obesity may be involved in the pathways to hypertension and lipid abnormalities, and worsens cardiovascular risk profiles independently of hyperinsulinemia [14,58]. Gender differences in cardiovascular disease have been explained by the different patterns of body fat distribution. Women increase total body fat content, predominantly central body fat, due to aging and menopausal effects [12,59]. In postmenopausal women, low vitamin D status is associated with greater bone turnover, bone loss, and obesity [60]. Hypovitaminosis D associated with obesity is likely to be functionally relevant since compensatory hyperparathyroidism has been reported in these patients [61,62]. Although there is an inverse association between BMI and the serum levels of 25(OH)D and 1,25(OH)<sub>2</sub>D (Table 4), it is unlikely that circulating 1,25(OH)<sub>2</sub>D contributes to the development of obesity since there is a negative association between 1,25(OH)<sub>2</sub>D concentrations and adiposity [63,64].

25(OH)D concentrations are associated with body composition variables, especially body fat, independently of seasonal variability [63]. Links between hypovitaminosis D and obesity have been reported when obesity is defined using both BMI and waist circum-

ference [20,29]. In a multivariate analysis it has been calculated a decrease of 0.74 nmol/l in vitamin D per 1 kg/m<sup>2</sup> increase in BMI, and a decrease of 0.29 nmol/l per 1 cm increase in waist [65]. In addition, vitamin D supplementation has a small effect on the prevention of weight gain [66]. The body fat excess makes vitamin D, which is stored in adipose tissue, less available for use by the body. It is sequestered in the large adipose mass from obese and overweight people and these individuals may feel ashamed about their body, minimising sunlight exposure [61]. However, in a cross-sectional study, sun exposure habits did not differ according to adiposity, and do not explain low 25(OH)D with increasing body fat mass [67]. The body fat composition and vitamin D relationship is influenced by the skin characteristics. Results from the third NHANES cohort suggest that the negative relationship between serum 25(OH)D and percentage of body fat seems stronger in Whites than in Blacks of the same age [64].

The inter-relationship between vitamin D status and glucose homeostasis, has been studied measuring HbA1c in a large British cohort aged 45 years. 25(OH)D was <75 nmol/l in 80% of the obese subjects (BMI  $\geq$  30 kg/m<sup>2</sup>) as compared to 68% of the other subjects, and vitamin D decreased and HbA1c increased as BMI increased [68].

Morbid obesity is associated with low circulating concentrations of 25(OH)D [69]. Vitamin D insufficiency is more prevalent in morbidly obese patients suffering the metabolic syndrome as compared to those that do not have the criteria of the syndrome. In addition, HDL-cholesterol levels were lower and triglycerides levels were higher in the vitamin D-deficient group [70]. In the mixed-ethnicity participants from the NHANES cohort, abdominal obesity as measured by waist alone was related to metabolic syndrome and to low vitamin D, notably affecting mixed-ethnicity participants equally [26].

Both weight loss and diet modifications are associated with an improved plasma lipid profile in obese postmenopausal women. In healthy overweight or obese women supplementation with calcium and vitamin D (400 IU/day) during a 15-week weight-loss programme enhanced the beneficial effect of weight loss on the lipid and lipoprotein levels. Thus, the intervention produced significantly greater decreases in total cholesterol:LDL cholesterol, LDL cholesterol:HDL cholesterol and LDL cholesterol were obtained as compared to the placebo group [42].

The metabolic and endocrine status in obese women is quite complex, and under the influence of different fat hormones [12,13] for which we do not yet know the biochemical links and feedback with vitamin D. Body size is a strong determinant for 25(OH)D, with concentrations being suboptimal in most obese individuals. Obese Blacks are at particularly high risk for vitamin D deficiency and secondary hyperparathyroidism. Physicians should consider routinely supplementing such patients with vitamin D, using dosages sufficient to improve 25(OH)D, or screening them for hypovitaminosis D. In addition, randomized controlled trials are required to determine whether clinically relevant improvements in glucose metabolism can be obtained by the serum level vitamin D normalization.

#### 2.5. Inflammation and vitamin D

There is evidence that low-grade systemic inflammation and insulin resistance are associated events that contribute to the development atherosclerosis and obesity. Vitamin D has been implicated in immune function and inflammation, and some evidence is accumulating that vitamin D deficiency could lead to immune malfunction. The relationship between low serum 25(OH)D concentrations and autoimmune disease (especially multiple sclerosis, type 1 diabetes and rheumatoid arthritis) has been appreciated for some time [3]. Polymorphisms of both vitamin D 25-hydroxylase (CYP2R1) gene – that catalyses the conversion of vitamin D<sub>3</sub> to

25(OH)D – and 25(OH)D 1 $\alpha$ -hydroxylase (CYP27B1) have been associated to the risk of type 1 diabetes [71,72]. These results fit well with epidemiological studies that link vitamin D deficiency with the susceptibility to type 1 diabetes, and with those suggesting that 1,25(OH)<sub>2</sub>D – the active hormone – could protect the  $\beta$ -pancreatic cells from immune destruction [73,74].

The VDR is found in T lymphocytes and macrophage cells [75], and the 1 $\alpha$ -hydroxylase is expressed by the activated macrophages, allowing these cells to synthesize and secrete 1,25(OH)<sub>2</sub>D [76]. In addition, the major degrading enzyme 24-hydroxylase is also expressed in monocytes/macrophages [77]. Vitamin D deficiency may lead to immune dysregulation, including defective macrophage function, such as impaired chemotaxis, phagocytosis, and increased cytokine levels [80]. A proinflammatory state probably contributes to the metabolic syndrome, since a number of markers of systemic inflammation, including C-reactive protein (CRP), are often increased, as are fibrinogen, interleukin 6, tumor necrosis factor (TNF)- $\alpha$  and others [78,79].

VDR polymorphisms identified by the restriction enzymes BsmI, ApaI, TaqI and FokI and CRP serum level were studied in hemodialysis patients routinely treated with active vitamin D. The results showed that the *b*, *a*, and *T* alleles were more frequent in patients with elevated serum level of CRP compared with patients with normal CRP level, suggesting an inflammation-related, atherosclerosis-dependent cardiovascular disease risk in uremic patients [80].

Serum vitamin D levels have been reported to preserve leukocyte telomere length in normal women aged 18–79 years, a predictor of aging-related disease. Thus, serum vitamin D levels were positively associated with leukocyte telomere length after adjustments for different cofactors. The difference between the highest and lowest tertiles of measured vitamin D was equivalent to 5 years of telomeric aging, being the difference greater by increased levels of CRP [81].

Healthy women with regular UV B exposure had serum 25(OH)D concentrations that were significantly higher and PTH concentrations that were significantly lower than women without regular UV B exposure whereas 25(OH)D status is inversely related to TNF- $\alpha$  concentrations [82]. However, participants in the Framingham Offspring Study did not show a consistent association between vitamin D and inflammation [83]. In addition, a randomized, controlled trial, in Caucasian adults without diabetes who were assigned to receive either 500 mg calcium citrate and 700 IU vitamin D or placebo daily for 3 years, showed no differences in CRP or interleukin-6 between the two arms of the study. However, supplementation with calcium and vitamin D attenuated increases in glycemia and insulin that occur over time [84]. On the other hand, in a double-blind, randomized, placebo-controlled trial vitamin D supplementation improved cytokine profiles in patients with congestive heart failure [85].

The mechanisms by which vitamin D may affect inflammation are not clear. Low-grade inflammation is an important risk factor for developing glucose intolerance, endothelial dysfunction and cardiovascular disease. However, circulatory inflammatory markers are difficult to be studied, and probably do not express the pathophysiology process to explain the putative association between vitamin D and inflammation.

### 3. Experimental evidence on vitamin D metabolism and the cardiovascular system

The action of the vitamin D endocrine system is mediated by both genomic and non-genomic pathways. The former is activated by the binding of 1, 25(OH)<sub>2</sub>D<sub>3</sub> to a specific cytosolic/nuclear VDR, whereas non-genomic pathways are activated via a putative mem-

brane vitamin D receptor and might be responsible for rapid effects of vitamin D [86].

#### 3.1. Glucose homeostasis and insulin function

The vitamin D endocrine system plays an important role in the glucose homeostasis and in insulin release mechanisms. The low serum 25(OH)D is known to alter cellular function in many tissues. In isolated perfused pancreas from vitamin D-deficient rats, glucose and arginine perfusion induced a 48% reduced response in insulin secretion as compared to that from rat pancreases that had been replenished with vitamin D [87]. Experimental studies have previously reported that vitamin D is essential for normal insulin in both perfused rat pancreas and in *in vivo* conditions. Thus, rat vitamin D deficiency impairs plasma glucose clearance which does not appear to be related to the increase caloric intake associated with vitamin D depletion. In addition normalization of plasma calcium and phosphorus levels fail to improve glucose clearance or insulin secretion [88].

Molecular pathways by which 1,25(OH)<sub>2</sub>D regulates insulin synthesis are not precisely defined. However, it is known that vitamin D promotes general activation of protein synthesis in pancreatic  $\beta$ -cells, enhances Ca<sup>2+</sup> influx into  $\beta$ -cells and stimulates the conversion of proinsulin to insulin [89–91]. It is likely a beneficial genomic influence of 1,25(OH)<sub>2</sub>D that occurs progressively within the islets of Langerhans and which may prepare the  $\beta$ -cells for an enhanced response to glucose stimulation.

In mice lacking a functional VDR which express a functionally inactive mutant VDR, baseline blood glucose levels were unchanged in fasting conditions. However, blood glucose was elevated after oral or subcutaneous glucose loading, and maximum serum insulin levels were reduced by approximately 60% in VDR mutants vs. normal mice. In addition, insulin mRNA levels were decreased in VDR mutant mice suggesting that vitamin D has a significant role in pancreatic insulin synthesis and secretion. In these VDR mutant mice,  $\beta$ -cell mass, islet architecture, and islet neogenesis were normal [92].

Adequate vitamin D levels also reduce insulin resistance at the peripheral target cell level. Thus, in muscle C2C12 cells treated with free fatty acids, was associated with 70% reduction in insulin-mediated uptake of glucose, a fivefold increase in serine phosphorylation of insulin substrate receptor 1. However, 1,25(OH)<sub>2</sub>D supplement significantly improved the free fatty acid-induced inhibition of glucose in both a dose-dependent and time-dependent manner [93].

In rats rendered diabetic, 1,25(OH)<sub>2</sub>D was unable to correct the hyperglycemia, hypoinsulinemia, glycosuria or ketonemia caused by the diabetes, although it partially reversed the over-expression of the insulin receptor gene in the liver and adipose tissue. At the same time, there was a normalization of the number of insulin receptors without altering receptor affinity but improving the insulin response to glucose transport in adipocytes from diabetic animals. These results suggest a genomic effect of 1,25(OH)<sub>2</sub>D by transcriptional activation of the rat insulin receptor gene [94].

All these results suggest that 1,25(OH)<sub>2</sub>D may act at different regulatory levels to protect the body and to preserve the normal insulin metabolic function.

#### 3.2. Cardiovascular effects

Vitamin D's antiproliferative, antiangiogenic and antioxidant properties have a significant protective effect on the cardiovascular system. It is very well known the cardiomyopathy secondary to nutritional rickets. Experimental vitamin D depletion is associated to cardiomegaly that is not accompanied by an increase in myocardial water content and was not caused by myocardial

cell hypertrophy. The histological examination shows a significant reduction in myofibrillar and a significant increment in extracellular space that alter the myocardial contractile function seen under vitamin D deficiency [95]. Vitamin D treatment normalizes the altered myocardial contractility observed in both experimental and clinical vitamin D deficiency [96–98].

VDR,  $1\alpha$ -hydroxylase and  $24$ -hydroxylase are present in both the myocytes and fibroblasts of the heart, as well as in the intact ventricular myocardium. Therefore, both the synthesis of vitamin D and the receptor are involved in the putatively antihypertrophic system through natriuretic peptide gene promoter which is a marker of the response to hypertrophy [99]. Rat heart cells are sensible to  $1,25(\text{OH})_2\text{D}$ , having both genomic and rapid non-genomic effects. The effects of vitamin D on rat cardiac myocytes are mediated by mechanisms similar to VDR while there is not any effect of vitamin D when used the knockout cardiac myocyte [100].

In vitro studies with endothelial cells incubated with  $1,25(\text{OH})_2\text{D}$  showed a decreased action of advanced glycation end products on the endothelial nitric oxide synthase, and vitamin D decreased the elevated interleukin-6 mRNA [101].  $1,25(\text{OH})_2\text{D}$  increases vascular endothelial growth factor (VEGF) expression and release in vascular smooth muscle cells in vitro by binding of VDR to two response elements in the VEGF promoter. Thus, VDR activation acts as transcription factor to VEGF promoter [102].

$1\alpha$ -Hydroxylase which is responsible for the “on-site” conversion of vitamin D precursors into  $1,25(\text{OH})_2\text{D}$ , is expressed in human vascular smooth muscle cells and is upregulated by PTH and estrogens [103]. Vitamin D induces prostacyclin in vascular smooth muscle cells, which prevents thrombus formation, cell adhesion, antiplatelet aggregation, vasodilatation and smooth muscle cell proliferation [104].

The hormone  $1,25(\text{OH})_2\text{D}$  is also involved in the regulation of the renin–angiotensin system (RAS). It seems that  $1,25(\text{OH})_2\text{D}$  is an endocrine suppressor of renin synthesis since disruption of VDR induces overstimulation of the RAS, causing hypertension and cardiac hypertrophy. In VDR knockout mice left ventricular cardiomyocytes were markedly increased compared with wild-type mice due to activation of both the systemic and cardiac RAS [105].

In experimental conditions,  $1,25(\text{OH})_2\text{D}$  amplifies expression of natriuretic peptide type A receptors, which should be beneficial for cardiovascular health [98]. In VDR-null mice, VDR absence causes

hypertension and high renin expression and vitamin D treatment has a potent negative regulator effect on renin expression in vivo [99]. Vitamin D might also act as a negative regulator of the renin gene, and low vitamin D may increase the expression of the RAS [106].

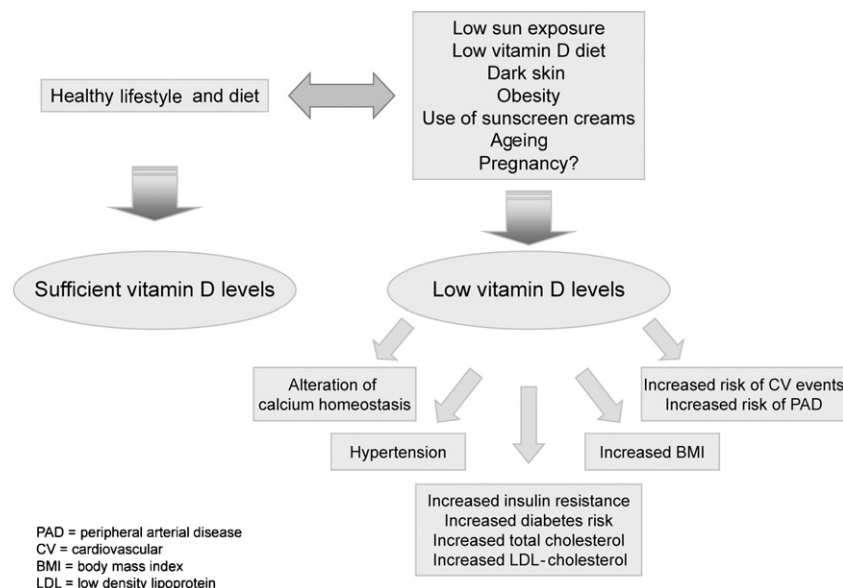
There is also evidence relating negative effects of vitamin D on the cardiovascular system. The relationships between vitamin D, calcium and other calcioregulatory hormones are complex. It cannot be omitted that in animals, toxic and hypercalcemic doses of vitamin D are proatherogenic [107,108]. Vitamin D also stimulates proliferation and migration of vascular smooth muscle cells which are involved in atherogenesis [109].

### 3.3. Obesity

Experimental studies with mice suggest that overeating, not the resulting obesity, is the actual cause of the metabolic syndrome [110]. The expression level of genes that regulate the adipogenic response to overnutrition influences the age of onset and severity of diet-induced type 2 diabetes and co-morbidities. The  $1\alpha$ -hydroxylase has been detected in cultured adipocytes, and did not differ with dietary level of calcium and vitamin D. Therefore, it seems likely that the  $1,25(\text{OH})_2\text{D}$  local production may have a role in adipocyte metabolism [111].

## 4. Vitamin D and cardiovascular disease

Low vitamin D levels may favour the metabolic syndrome, insulin resistance, glucose and lipoprotein alterations, hypertension, and endothelial dysfunction which are related to the cardiovascular disease (Fig. 1). Different geographic, epidemiological, and clinical classical studies have suggested that there are excess of cardiovascular risk factors in individuals with insufficient or low vitamin D serum levels [112–114]. The results from the WHI cohort suggest a lack of effect on coronary or cerebrovascular risk, as well as on the risk for cardiovascular calcifications, in generally healthy postmenopausal women using vitamin D supplements (400 IU/day) over a 7-year use period [115]. However, there might be some bias in regard to the dose of supplement, the use of supplements in the placebo group, and other already discussed methodological limitations. In addition the vitamin D dose used in the WHI cohort is insufficient to change circulating  $25(\text{OH})\text{D}$  levels,



**Fig. 1.** Effects of low vitamin D levels on different aspects related with cardiovascular risk in postmenopausal women.

**Table 5**  
Vitamin D levels and cardiovascular and related events.

Authors	Population	Outcome
Wang et al. [116]	Measurement of 25(OH)D in 1739 Framingham Offspring Study participants (55% women) without prior cardiovascular disease. 120 individuals developed a first cardiovascular event during a follow-up of 5.4 years.	Overall, 28% of individuals had levels < 15 ng/ml, and 9% had levels < 10 ng/ml. Individuals with 25(OH)D < 15 ng/ml had a multivariable-adjusted HR of 1.62 (95% CI 1.11–2.36, $P=0.01$ ) for incident cardiovascular events compared with those with 25(OH)D $\geq$ 15 ng/ml.
Melamed et al. [117]	Measurement of 25(OH)D levels and the prevalence of PAD in 4839 participants from the NHANES population.	Across quartiles of 25(OH)D, from lowest to highest, the prevalence of PAD was 8.1%, 5.4%, 4.9%, and 3.7% ( $P$ trend < 0.001). After multivariable adjustment for different factors, the prevalence ratio of PAD for the lowest, compared to the highest, 25(OH)D quartile (<17.8 and $\geq$ 29.2 ng/ml, respectively) was 1.80 (95% CI 1.19, 2.74). Multivariate-adjusted HRs for patients in the lower two 25(OH)D quartiles (median, 7.6 and 13.3 ng/ml) were higher for all-cause mortality (HR, 2.08; 95% CI, 1.60–2.70; and HR, 1.53; 95% CI, 1.17–2.01; respectively) and for cardiovascular mortality (HR, 2.22; 95% CI, 1.57–3.13; and HR, 1.82; 95% CI, 1.29–2.58; respectively) compared with patients in the highest of 25(OH)D quartile (median, 28.4 ng/ml). Similar results were obtained for patients in the lowest 1,25(OH) <sub>2</sub> D quartile. Low 25(OH)D levels were significantly correlated with variables of inflammation (CRP and interleukin 6 levels), oxidative burden, and cell adhesion. The HR (95% CI) for death due to heart failure and for SCD were 2.84 (1.20–6.74) and 5.05 (2.13–11.97), respectively. The statistical analyses give similar results with 25(OH)D and with 1,25(OH) <sub>2</sub> D.
Dobnig et al. [118]	Prospective cohort study of 25(OH)D and 1,25(OH) <sub>2</sub> D levels and all-cause and cardiovascular mortality in 3258 male and female patients scheduled for coronary angiography. During a median follow-up period of 7.7 years, 737 patients (22.6%) died, including 463 deaths from cardiovascular causes.	When compared with survivors, the OR (with 95% CIs) for fatal stroke were 0.58 (0.43–0.78; $P < 0.001$ ) per z-value of 25(OH)D and 0.62 (0.47–0.81; $P < 0.001$ ) per z-value of 1,25(OH) <sub>2</sub> D. After adjustment for several possible confounders, these OR remained significant for 25(OH)D at 0.67 (0.46–0.97; $P = 0.032$ ) and for 1,25(OH) <sub>2</sub> D at 0.72 (0.52–0.99; $P = 0.047$ ). z-Values of 25(OH)D and 1,25(OH) <sub>2</sub> D were also reduced in the 274 patients who had a history of previous cerebrovascular disease events at baseline. During a median 8.7 years of follow-up, there were 1806 deaths, including 777 from CVD. Compared with the highest quartile, being in the lowest quartile (25(OH)D levels <17.8 ng/ml) was associated with a 26% increased rate of all-cause mortality (mortality rate ratio, 1.26; 95% CI, 1.08–1.46) and a population attributable risk of 3.1%. The lowest quartile of 25(OH)D level (<17.8 ng/ml) is independently associated with all-cause mortality in the general population.
Pilz et al. [119]	Prospective cohort study of 25(OH)D and 1,25(OH) <sub>2</sub> D levels and all-cause and cardiovascular mortality in 3299 patients scheduled for coronary angiography. During a median follow-up time of 7.7 years, 116 patients died due to heart failure and 188 due to SCD. Serum 25(OH)D and 1,25(OH) <sub>2</sub> D levels were measured in 3315 study participants who were referred to coronary angiography and had a median follow-up time of 7.75 years. 769 patients died, including 42 fatal (ischemic and hemorrhagic) strokes.	
Pilz et al. [120]		
Melamed et al. [121]	The association of low 25(OH)D levels with all-cause, cancer, and CVD mortality in 13,331 nationally representative adults 20 years or older from the third NHANES linked mortality files.	
Rajasree et al. [122]	Serum 25(OH)D, cholesterol, triglyceride, calcium, magnesium and inorganic phosphate were determined. Prevalences of diabetes, hypertension, and IHD were noted.	Serum levels of 25(OH)D <sub>3</sub> above 222.5 nmol/l was observed in 59.4% of cases compared to 22.1% in controls ( $P < 0.001$ ; unadjusted OR: 5.17; 95% CI: 2.62–10.21. Using the multivariate logistic regression, the adjusted OR relating elevated serum 25(OH)D levels ( $\geq$ 222.5 nmol/l, $\geq$ 89 ng/ml) and IHD is 3.18 (95% CI: 1.31–7.73).

CRP = C-reactive protein; CVD = cardiovascular disease; HR = hazard ratio; IHD = ischemic heart disease; NHANES = National Health and Nutrition Examination; PAD = peripheral arterial disease; SCD = sudden cardiac death.

and is below the most recent recommendations concerning vitamin D supplements [3,7].

In the third NHANES cohort, more than half of all individuals surveyed had insufficient 25(OH)D levels by current standards (<30 ng/mL). When the results were classified by quartiles, the low vitamin D levels were associated to different cardiovascular risk factors [43]. The results concerning blood pressure and diabetes are perhaps the most noteworthy, since these are important contributors to cardiovascular disease. The 25(OH)D levels were lower in women, elderly persons ( $\geq$ 60 years), racial/ethnic minorities, and participants with obesity, hypertension, and diabetes mellitus. After the levels were distributed by quartile and adjustment for confounding factors, the prevalence of hypertension, diabetes mellitus, obesity and high serum triglyceride levels was significantly higher in the lower vitamin D level quartile as compared to the highest quartile (Table 2).

Table 5 presents studies that link vitamin D and cardiovascular disease and related conditions. Cardiovascular risk and 25(OH)D levels have been studied in individuals without prior cardiovascular disease from the Framingham Offspring Study [116]. Overall, 28% of individuals had vitamin D levels below 15 ng/ml, and 9% had levels below 10 ng/ml. Only 10% of the participants had levels above

30 ng/ml which are considered optimal for bone metabolism. During a mean follow-up of 5.4 years, 120 participants developed a first cardiovascular event including fatal and nonfatal coronary heart disease, 28 participants had fatal or nonfatal cerebrovascular events, such as non-hemorrhagic stroke, 19 participants were diagnosed with heart failure, and 8 had occurrences of claudication. Individuals with 25(OH)D below 15 ng/ml had a higher incidence of cardiovascular events compared with those whose levels were higher to 15 ng/ml. This effect was evident in participants with hypertension but not in those without hypertension. Thus, people with low vitamin D levels and high BP, had twice the risk of experiencing cardiovascular events compared to people with normal blood pressure and vitamin D levels. Vitamin D deficiency was not linked to cardiovascular problems in people who do not had hypertension. However, some arguments have been raised concerning the studied population, the presumed normal or even high initial endogenous vitamin D level, and the possible negative vascular effect of vitamin D and calcium supplements if the basal levels might already high before treatment.

Melamed et al. [117] analyzed data from 4839 participants of the NHANES to examine a possible relationship between 25(OH)D and peripheral arterial disease (PAD) defined as an ankle-brachial

index <0.9. This index measures blood flow to the legs. The 25(OH)D levels were divided in quartiles and, from lowest to highest, the prevalence of PAD was 8.1%, 5.4%, 4.9%, and 3.7% ( $P$  trend <0.001) (Table 5). Adjustments for age, sex, race and co-existing health problems were applied and it remained that those in the lowest vitamin D group had a 64% higher prevalence of PAD compared to those with the highest vitamin D levels. For each 10 ng/ml drop in vitamin D level, the risk for PAD increased by 29%. Although it seems that low serum 25(OH)D levels are associated with a higher prevalence of PAD, the cross-sectional design of this study does not allow to interpret a causal relationship or that vitamin D by itself has a protective effect on the circulatory system but possibly that higher vitamin D levels may be related to other health practices such as a healthy diet and being physically active. Other possibilities include some type relationship or association in the triangle formed by inflammation, PAD and vitamin D which has never been explored.

In a prospective cohort study of 3258 individuals aged 62 years ( $\pm 10$  years, S.D.) scheduled for coronary angiography, 25(OH)D and 1,25(OH)<sub>2</sub>D serum levels were classified by quartiles and related with all-cause and cardiovascular deaths during a median follow-up period of 7.7 years. Patients in the lower two 25(OH)D quartiles (median 7.6 and 13.3 ng/ml) had higher all-cause mortality (HR = 2.08; 95% CI 1.60–2.70; and HR = 1.53; 95% CI 1.17–2.01; respectively) and for cardiovascular mortality (HR = 2.22; 95% CI 1.57–3.13; and HR = 1.82; 95% CI 1.29–2.58; respectively) compared with patients in the highest 25(OH)D quartile (median, 28.4 ng/ml). Similar results were obtained for patients in the lowest 1,25(OH)<sub>2</sub>D quartile. These results were independent of confounding factors [118]. The association between 25(OH)D levels and sudden cardiac death were also studied in the same cohort during a median follow-up of 7.7 years. After adjustment for cardiovascular confounding factors, the hazard ratios for death due to heart failure and sudden death were 2.84 and 5.05, respectively, comparing subjects with severe vitamin D deficit (25(OH)D < 10 ng/ml) as compared to those with normal values (>30 ng/ml) [119]. In the same population referred for coronary angiography has been reported the influence of vitamin D levels on the fatal ischemic and hemorrhagic stroke cases ( $n = 769$ ) occurred over a median follow-up time of 7.75 years. The odds ratio for stroke survivors as compared to fatal stroke were 0.58 for 25(OH)D and 0.62 for 1,25(OH)<sub>2</sub>D higher levels [120]. Thus, it seems that low vitamin D levels are predictive independent markers for fatal strokes. It seems quite plausible that optimal vitamin D levels may protect myocardial calcium metabolism and contractility and cerebrovascular territory in these type of patients. However, associations do not preclude an etiological relevance for the general population.

The association of low 25(OH)D levels with all cause, cancer, and cardiovascular mortality has been reported in the third NHANES population. During a 9.7 years there were 1806 deaths, including 777 from cardiovascular disease. After adjustment for confounding factors, individuals with 25(OH)D in the lowest quartile 25(OH)D levels <17.8 ng/ml was associated with a 26% increased rate of all-cause mortality and a population attributable risk of 3.1%, while cardiovascular mortality had a higher risk that was not statistically significant [121].

Some experimental and clinical evidence point out a certain association between elevated vitamin D levels and cardiovascular disease which is contrary to the results already discussed. In an Indian case-control study including 143 patients with either angiographic evidence of coronary artery disease or patients with acute myocardial infarction and 70 controls 25(OH)D levels were measured. In 59.4% of cases and 22.1% of controls 25(OH)D levels were above 222.5 nmol/l (89 ng/ml). When results were submitted to the multivariate logistic regression considering confounding factors, the adjusted OR relating elevated serum 25(OH)D and ischemic heart disease is 3.18 (95% CI 1.31–7.73) [122].

Although the cross-sectional nature of some studies, the bulk of evidence suggest that adequate vitamin D levels might protect the cardiovascular system.

## 5. Final remarks

There is a general consensus among health researchers and practitioners held that the primary function of vitamin D is in helping the body to maintain a healthy level of calcium in the blood, primarily for proper bone health throughout an individual's lifetime. But research is showing that this important vitamin is playing a much larger role in the overall health of the body. Insufficiency of vitamin D has been associated with a large number of chronic conditions such as musculoskeletal disorders, cancer development, metabolic alterations, reproductive disorders, cognitive impairment, depressive symptoms, degenerative and autoimmune diseases. A growing body of evidence, as discussed here, suggests that low levels of vitamin D may adversely affect the cardiovascular system. The low 25(OH)D levels are associated with hypertension, obesity, glucose intolerance, and the metabolic syndrome (Fig. 1). In addition, the incidence of cardiovascular disease and complications are higher during winter and latitudes closer to the poles where sunlight effects are lower on the skin and vitamin D levels are low [123,124]. Several mechanisms have been invoked to support a potential vitamin D cardiovascular benefit. VDRs have a wide tissue distribution including heart, vascular smooth muscle and endothelium. Putative vascular effects of vitamin D are wide-ranging and include modulation of smooth muscle cell proliferation, inflammation, and thrombosis. Both hypertension and vitamin D deficiency may influence cardiac and vascular remodelling, and vitamin D deficiency directly promotes the development of hypertension. Supplementation with vitamin D may also reduce the serum concentration of inflammatory markers such as CRP, and some research has suggested an anticoagulant effect of vitamin D [85,125].

Presently, there is concern about the almost epidemic vitamin D insufficiency all over the world. Vitamin D deficiency is higher among postmenopausal women, elderly, and minorities as previously reported, being relevant in relation with osteoporosis and other health conditions [3,4,6,8]. Inadequate levels vitamin D have been related to dietetic causes, changes in lifestyle, use of sunscreens, and lack of sun exposure. Starting about 30 years ago, a cultural shift deepened our vitamin D deficit: public health campaigns to avoid the midday sun, cover up and apply sunscreen. They were justified attempts to save the skin from sun-induced aging and cancer, hence vitamin D deficiency has become commonplace, even in the tropics [3]. During the last decades there have been also changes in food consumption with negative consequences for vitamin D metabolism. Vitamin D supplementation is recommended to maintain bone health in the general population and in particular in patients with chronic kidney disease. The results reviewed here suggest that optimal circulating vitamin D levels could reduce the different conditions which are associated with cardiovascular risks (Fig. 1). It is likely that the negative effect of insufficient vitamin D levels inexorably act long before the clinical risks or complications could be detected. Therefore, it seems wise to stimulate an appropriate diet and leisure activities that may favour endogenous synthesis of vitamin D, and – if needed – specific vitamin D supplements without high amounts of calcium since normal vitamin D levels guarantee the regular calcium absorption from food. Although the evidence is limited, natural endogenous synthesis of vitamin D could be healthier than oral supplements [9]. Sun exposure guidelines that take into account geographic latitude, season, age, and factors such as race and clothing are needed. These recommendations should be balanced considering both the skin cancer and hypovitaminosis D risks. The international scientific

community and professional associations should give appropriate guidelines to prevent the epidemia of hypovitaminosis D.

The current dose of vitamin D in routine vitamin supplements is still insufficient to maintain adequate serum 25 (OH)D levels in a substantial portion of the general population. It has been recommended that those aged 51 through 70 should receive 400 IU daily, and that anyone over 70 get a net of 600 IU. Experts are increasingly pushing for higher daily recommended intakes of vitamin D, saying that while current amounts may prevent signs of deficiency, they are insufficient to provide a protective benefit against cancer and other chronic diseases [3,6,126]. On the other hand, vitamin D is now regarded as exceptionally safe; a person would have to consume at least 40,000 IU daily for months to develop any signs of toxicity [126]. However, high dose vitamin D supplements should not be used unless a deficiency is diagnosed.

The association between cardiovascular events and vitamin D levels remains controversial, with some research suggesting that high levels of vitamin D are protective against coronary heart disease and other research finding the opposite or neutral effect. The only way to disentangle the issue of indicator or predictor vs. causal factor is to design randomized trials for verifying the impact of vitamin D on all causes of cardiovascular disease, surrogate markers and on the incidence of other associated other conditions. The trials should test higher dosages of vitamin D that previously studied to guarantee sufficient 25(OH) circulating levels [3,5–7,84,85]. In addition, there is a need of well-designed studies to determine the separated effects of calcium and vitamin D on insulin resistance, hypertension, atherosclerotic disease and cardiovascular events in postmenopausal women. In the meantime people should be encouraged to achieve high levels of vitamin D within the normal and safe range.

### Conflict of interest

None.

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