Vitamin D: A Regulator of Metabolism and Inflammation

Evan P. Cherniack[1]*, Kenneth Seldeen[2] and Bruce R. Troen[2]

[1]*Division of Gerontology and Geriatric Medicine, University of Miami Miller School of Medicine, Bruce W. Carter Veterans Affairs Medical Center, Miami FL 33125, USA;* [2]*Division of Geriatrics and Palliative Medicine, University at Buffalo - State University of New York and Research Service, Buffalo Veterans Affairs Medical Center, Buffalo, NY 14215, USA*

Abstract: Vitamin D, a calciotropic hormone, is gaining attention for many aspects of health, particularly metabolic regulation. As societal trends towards increasing caloric intake become more prominent, the concern for diseases stemming from chronic inflammation grows. Excess caloric intake results in increased lipid and fatty acid storage in adipose tissue leading to adipocyte hypertrophy and hyperplasia. Adipocytes produce cytokines and adipose-related hormones and can promote a systematic chronic proinflammatory state with the development of the metabolic syndrome and increasing risk of cardiovascular disease. Proinflammatory cytokines can signal immunocytes to injure the pancreas and endothelium. This, in turn can result in a reduction in insulin secretion and increasing insulin resistance. Vitamin D may possess therapeutic potential for metabolism associated diseases, as a result of its role in metabolic regulation and its action on adipocytes and myocytes. Vitamin D inhibits adipocyte maturation and differentiation, and reduces the production of proinflammatory cytokines and adipose- related hormones by adipocytes. The purpose of this review is to elucidate the current literature on the therapeutic potential of vitamin D repletion in the treatment and prevention of metabolic and cardiovascular diseases. Although associations exist between vitamin D insufficiency and obesity, diabetes, hyperlipidemia, cardiovascular and all- cause mortality, there have not been prospective trials that have demonstrated the benefit of vitamin D in alleviating manifestations of disease. Nevertheless, correction of vitamin D insufficiency would be expected to confer many health benefits. A greater understanding of the interaction of vitamin D with not only adipose tissue but other components of the human metabolic regulatory system, such as the hypothalamus, pancreas, and bone, may help tailor interventions including vitamin D supplementations that address cardiovascular disease and obesity.

Keywords: Adipocyte, energy, vitamin D, proinflammatory cytokines.

INTRODUCTION: HISTORY AND EFFECT OF EXCESS ENERGY INTAKE

Synthesis of Vitamin D

Vitamin D is a steroid hormone which has multiple functions, including preserving skeletal integrity, neuromuscular function, immunity, and safeguarding against cardiovascular diseases and cancer [1]. Vitamin D can be acquired through diet or naturally produced through UVB exposure to the skin leading to the conversion of 7-dehydrocholesterol into cholecalciferol. Cholecalciferol has little bioactivity, and the canonical pathway for its activation begins in the liver where an initial hydroxylation creates 25-hydroxycholecalciferol (25-OH vitamin D or calcidiol) and subsequent hydroxylation in the kidney creates the active form of vitamin D: 1,25-dihydroxycholecalciferol ($1,25\text{-}(OH)_2$ vitamin D or calcitriol). The latter hydroxylase is also present in many other tissues including the colon, pancreas, brain, and skin [2]. Vitamin D status is typically assessed using serum 25-OH vitamin D concentrations, however therapeutic strategies normally involve supplementation with cholecalciferol, $1,25\text{-}(OH)_2$ vitamin D, or analogues such as paricalcitol and alfacalcidol.

*Address correspondence to this author at the Miami VA Medical Center, Room 1D200, 1201 NW 16th Street, Miami, FL 33125, USA; Tel: 305-575-7101; Fax: 305-575-7218; E-mail: evan.cherniack@va.gov

EVOLUTIONARY PERSPECTIVE

Despite the availability of vitamin D from both diet and sunlight, many individuals worldwide have subnormal 25-OH vitamin D concentrations (insufficiency $\leq$ 75nmol/L; deficiency $\leq$ 25nmol/L) [3]. An evolutionary perspective may provide insight on potential confounding factors to the vitamin D insufficiency pandemic. As the availability of sunlight and natural production of vitamin D during the course of human evolution, through persistence hunting thousands of years ago [4], gave way to man's migration to colder climates, it became necessary to cover his skin imposing a barrier to vitamin D production. The new environment also expanded into northern latitudes in which daylight times and sunlight exposure were more varied, further diminishing vitamin D synthesis. The potential for vitamin D deficiency and insufficiency were also significantly increased through industrialization as more time was spent indoors. In addition to the impacts on the availability of natural vitamin D sources, the changes may have increased the prevalence of metabolic diseases as life in regions with lower temperatures, where food is cyclically available, required physiologic adaptation in adipose tissue to generate more body heat in the winters and sustain health with less frequent meals [5].

Now, however, these adaptations may predispose individuals to metabolic diseases. Typical western diets contain increasing amounts of energy as a result of advances in food-

2212-3881/14 $58.00+.00

production technology. For most of the modern industrialized world, over consumption has become a greater concern than starvation. While the average caloric intake for a resident of the United States is 3770 calories a day [6], the maximum recommended caloric intake for men is 1000-2400 calories a day and for women is 1000- 2000 calories a day, depending upon one's age, body mass, and activity level [7]. Perhaps, in combination with decreased activity levels compared to prehistoric societies (although the activity levels of such societies cannot be known with certainty) [8], this has led to an epidemic in obesity and the attendant increase in morbidity and mortality from obesity-related disease [9]. The effects of excess caloric intake, possibly augmented by prevalent vitamin D insufficiency, include the components of the "metabolic syndrome", which affects 34.5% of all Americans and includes obesity, dyslipidemia, hypertension, impaired regulation of blood glucose, and sarcopenia [10].

ADIPOCYTE ENERGY REGULATION AND INFLAMMATION

Surplus dietary energy is generally stored in adipocytes making adipogenesis and adipocyte function attractive targets for strategies to prevent and/or treat obesity and sequelae. An overabundance of energy results in adipocyte hyperplasia and hypertrophy [11]. Receptors on adipocyte precursor cells detect nutrient excess and stimulate gene activation by the target of rapamycin (TOR) gene pathway [12, 13]. The TOR pathway in turn up regulates the expression of peroxisome proliferator–activated receptor-γ (PPARγ), a nuclear receptor that is a transcription factor for genes important to the maturation of adipocytes [12, 13]. PPARγ binds with additional nuclear receptors and transcription factors such as retinoid X receptor (RXR) and CCAAT/enhancer-binding protein (CEBP) to promote adipocyte differentiation [14].

Adipocytes do more than store fat; they influence many systemic effects through the production of cytokines and adipose-related hormones leading to an alteration of the growth and structure of the vasculature and regulation of appetite [15-17]. Adipose tissue directly releases numerous proinflammatory cytokines, including TNFα, IL-6, IL-12, and C-reactive protein (CRP), macrophage-chemoattractant pro-tein-1 (MCP-1), colony stimulating factor (CSF), and nitrous oxide synthetase (NOS). In addition proinflammatory cytok-ines are released by adipose tissue resident macrophages, leukocytes, and fibroblasts under adipocyte regulation [15, 16]. Local release of these factors recruit immunocytes, such as macrophages, leading to further production of cytokines, augmenting the proinflammatory signals [15]. Adipose tissue also releases angiogenic cytokines, such as vascular endothelial growth factors (VEGF), VEGF-C, VEGF-D, soluble VEGF receptor-2 (sVEGFr2), angiogenin, angio-poietin-2, hepatocyte growth factor (HGF), and anti-angiogenic cytokines, such as endostatin [17].

Additionally, adipose tissue secretes leptin, which typically suppresses appetite, although obesity is typically coupled with leptin resistance [18]. Leptin also causes macrophages to produce the highly inflammatory cytokine tumor necrosis factor (TNF-α) and induces the creation of reactive oxygen species (ROS) in endothelial tissue [15]. Adipocytes also produce the hormone resistin, which stimulates macrophages to release TNF-α, interleukin-12 (IL-12), and IL-6, and also induces insulin resistance [15]. Furthermore, adipocytes secrete another inflammatory hormone, retinal binding protein-4, which also causes insulin resistance [16, 19-21]. These inflammatory cytokines not only promote increased adipogenesis and atherosclerosis but lead to an increased risk of the development of vascular diseases.

VITAMIN D ACTION IN ENERGY REGULATION AND ITS INFLUENCE IN HUMAN DISEASE

The notion of using vitamin D as a therapeutic agent for metabolic diseases is supported by the fact that vitamin D receptors are present on adipocytes. Additionally several studies have investigated the localization of vitamin D receptors and vitamin D-activating enzymes in adipose tissue including one in which rats and humans were given radiolabeled vitamin D followed by measurement of body stores; adipose tissue sequestered greater quantities of cholecalciferol than did other organs and tissues, although adipose tissue does not store more vitamin D in obesity [22, 23]. Human preadipocytes can hydroxylate 25-OH vitamin D to its active form 1,25-$(OH)_2$ vitamin D [24].

Vitamin D regulates the size and location of stores of adipose tissue [25]. It has been theorized that seasonal variation in vitamin D intake in more polar latitudes with colder temperatures acts as a signaling mechanism to help animals store calories for thermogenesis [5]. Although it is unclear whether such mechanisms exist in humans, there is significant support suggesting that vitamin D does play a role in weight regulation. Several epidemiological studies reported inverse relationships between vitamin D and measures of adiposity, including overweight and obese individuals. In the Longitudinal Aging Study Amsterdam (LASA) several adiposity indices including body mass index, sum of skin folds, and waist circumference were inversely associated with serum 25-OH vitamin D concentrations (-0.136, -0.140, and -0.137, respectively, $p<0.05$ for these indices) in 453 subjects at least 65 years old [26]. Percentage body fat content was inversely correlated (-0.13, p=013) with 25-OH vitamin D concentrations in a population of 410 US women ages twenty to eighty [27]. There was an association between prevalence of 25-OH vitamin D insufficiency (<75 nmol/L) and obesity in 316 patients in Spanish obesity clinics [28]. In sixty morbidly obese patients awaiting bariatric surgery, there was a significant inverse correlation between body mass index and 25-OH serum vitamin D concentrations [29]. Several other series comparing obese subjects and matched controls have found significantly lower serum 25-OH vitamin D concentrations in obese individuals [30-33]. Overweight women who successfully lowered their body weight by at least 10% for two years had a corresponding 7.5 nmol/L increase in serum 25-OH vitamin D concentration (p=0.014) [34]. Those who lost less weight did not have a significant increase in 25-OH vitamin D concentration. In a recent genetic analysis using Mendelian randomization of 42,024 subjects, an increased BMI was associated with a reduced 25-OH vitamin concentration [35].

Obese individuals may have lower serum concentrations of vitamin D because the lipid-soluble vitamin is distributed

in a larger amount of adipose tissue [32, 36]. Furthermore, obese individuals have lower adipose concentrations of vitamin D-metabolizing enzymes. Subcutaneous adipose tissue samples from obese women had significantly less 25- hydroxylase (CYP2J2) and 1α-hydroxylase (CYP27B1) than those from lean women [37]. Alternatively, obese persons may, because of a higher incidence of disability spend less time outdoors, or a tendency to cover a greater percentage of their skin surface with clothing, receive less sunlight [38]. In contrast Pramyothin *et al*. report that obese subjects who lost weight did not increase serum vitamin D 25-OH concentrations despite alterations in adipose tissue vitamin D concentrations [39]. There is also an association between vitamin D receptor (VDR) polymorphisms and body weight, although not body mass index [40, 41].

Vitamin D may influence the impact of dieting in overweight individuals. Thirty-eight obese men had their serum 25-OH and 1,25-$(OH)_2$ vitamin D concentrations measured before and after dieting for eleven weeks [42]. Those with higher baseline 25-OH and 1,25-$(OH)_2$ vitamin D concentrations lost more weight ($r=0.52$, $p<0.01$ and $r=0.44$, $p=0.006$, respectively) [42]. However, participants in a study of 318 overweight persons who took vitamin D 20000-40000 IU cholecalciferol weekly for a year did not lose more weight or increase insulin sensitivity more than those who received a placebo, although obese subjects (BMI $\geq$ 35) were more likely than the other subjects (BMI 28) to have lower baseline 25-OH vitamin D concentrations [43].

Potential molecular mechanisms for vitamin D action, through the vitamin D receptor, include its reduction of PPARγ-regulation of adipocyte differentiation. Mouse mesenchymal stem cells grown in media with 1nM 1,25-$(OH)_2$ vitamin D express less PPARγ than those grown without vitamin D, and fail to differentiate into adipocytes [44]. When activated vitamin D binds to its receptor, the liganded VDR in turn binds to RXR [14, 45] reducing the availability of RXR to bind PPARγ [14]. In mouse pre-adipocytes, vitamin D upregulates the production of VDR [46], and correspondingly vitamin D does not affect adipocyte maturation in VDR knockout mice [47]. Interestingly, an increased density of VDRs will prevent pre-adipocyte maturation even in the absence of vitamin D [47]. This is consistent with the possibility that even unliganded VDRs compete with PPARγ for RXR [14].

Vitamin D also reduces the number of adipocytes through apoptosis. 1,25-$(OH)_2$ vitamin D causes a rapid non-genomic calcium influx [48] that activates the enzymes calpase and caspase-12 to activate an apoptotic pathway in mouse adipocytes [48]. In addition, vitamin D downregulates uncoupling protein 2 (UCP2), a molecule on the inner membrane of mitochondria that inhibits apoptosis [25] and this role is further supported in VDR knockout mice, where vitamin D fails to prevent UCP production [25]. Furthermore, 1,25-$(OH)_2$ vitamin D stimulates ATP production and apoptosis at high doses ($\geq$ 100 nmol/L) in both human and mouse adipocytes [25, 49]. 1,25-$(OH)_2$ vitamin D (>100 nmol/L) increases [Ca^{++}] within the mitochondria, which undergo pro-apoptotic changes [25]. At lower doses, vitamin D has a more complex effect, suppressing apoptosis though down-regulation of the caspase-1 and -3 and STC2 genes, increasing expression of the STC1 gene that induces apoptosis, and lowering mitochondrial [Ca^{++}], but also stimulating the pro-apoptotic gene BCL-2 [25].

VITAMIN D AND CYTOKINES

There are associations between vitamin D and serum cytokine concentrations in humans. A population study confirmed the relationship between vitamin D and proinflammatory cytokine levels [50]. In 206 healthy Norwegians higher quartiles of serum 25-OH vitamin D levels were associated with lower concentrations of plasminogen activator inhibitor 1 (PAI-1) and of tPA antigen ($p<0.05$ for trend across quartiles). There were also associations between high serum 1,25-$(OH)_2$ vitamin D levels, lower concentrations tPA antigen, and high-sensitivity C-reactive protein (CRP) [50]. In one hundred twenty-three patients with congestive heart failure, those who received a daily supplement of 2000 IU cholecalciferol for nine months exhibited achieved significantly higher levels of the anti- inflammatory cytokine IL-10 and lower levels of the pro-inflammatory cytokine TNF-α than did control subjects [51]. In 91 African-Americans with end-stage renal disease, serum concentrations of matrix metallopeptidase-9 (MMP-9) were inversely associated with 25-OH vitamin D concentrations [52]. In a study of twelve men examined a week after surgery, serum 25-OH inversely correlated with interferon-γ concentrations [53].

Vitamin D status also impacts important cytokines involved with adipocyte regulation of myocytes, immunocytes, and keratinocytes. 1,25-$(OH)_2$ vitamin D increases the concentration of C-X-C chemokine receptor 4 *in vitro* in human eosinophils [54]. 1, 25-$(OH)_2$ vitamin D increases concentrations of macrophage inflammatory protein (MIP), macrophage colony-stimulating factor, interleukin-6, interleukin-8, insulin growth factor-1, insulin growth factor-1 receptor, VEGF and *c-fos* induced growth factor in these cell types [25, 55, 56]. 1, 25-$(OH)_2$ vitamin D reduces the release of adipocyte IL-6, macrophage chemoattractant protein-1, and IL-1β by reducing nuclear factor κ B (NF- κB) and increasing glucose uptake in adipocytes and preadipocytes [57-59].

The effects of vitamin D on metabolism may also be mediated by changes in adipose-regulatory hormone levels. The addition of 1,25-$(OH)_2$ vitamin D to human adipose tissue culture *in vitro* reduces leptin secretion [60]. Twenty-three vitamin D deficient subjects (serum 25-OH vitamin D < 25 nmol/L) who received 300,000 IU of cholecalciferol through intramuscular injections monthly for three months had lower serum leptin levels and greater brachial artery mediated flow dilation (an indication of improved endothelial function) at the conclusion of treatment than at the onset [61]. The systemic significance of vitamin D on leptin secretion has not been determined.

VITAMIN D AND THE METABOLIC SYNDROME

Investigations have often identified an association between the metabolic syndrome and vitamin D concentrations. Data from 8,421 people who participated in the Third National Health and Nutrition Examination Survey (NHANES III) revealed a correlation between the lowest quintile of serum 25OH-vitamin D level and greater risk of metabolic

syndrome (adjusted OR for highest [> 96.nmol/L] relative to the lowest [< 48.4 nmol/L] was 0.38) [62]. In the 1868 participants of the Canadian Health Measures Survey, there was an inverse relationship between serum 25-OH vitamin D concentration and incidence of metabolic syndrome (OR=0.50 between individuals in the highest quartile of serum 25-OH vitamin D concentration as compared to the lowest quartile) [63]. In the Australian Diabetes, Obesity, and Lifestyle Study of 11,247 individuals, the risk of developing metabolic syndrome over five years was significantly higher in those participants in the two lowest quintiles (<45nmol/L, and 45-75.5nmol; OR 1.41 [95% CI 1.82-1.95] and 1.74 [95% CI 1.28-2.37] respectively) [64]. However, in 39,876 subjects of the Women's Health Survey, there was no association between vitamin D status and metabolic syndrome, although there was a positive association between dietary calcium intake and metabolic syndrome. Furthermore in 5559 South Koreans, investigators did not find an association between serum 25-OH vitamin D levels and metabolic syndrome risk [65].

VITAMIN D AND DIABETES

Higher serum 25-OH vitamin D concentrations have been associated with a lower risk of developing diabetes. Vitamin D was associated with the incidence of type 2 diabetes in 2656 persons between the ages of 41-71 [66]. In this study, there was a direct relationship between waste circumference and vitamin D concentration. Among the 2,039 participants in the Diabetes Prevention Program, those individuals with the highest tertile of 25-OH vitamin D concentration had a lower risk of developing diabetes than others [67]. In a birth cohort study that followed 10,366 children, those who took 2000 IU daily of vitamin D reduced their relative of risk of developing diabetes (odds ratio=0.22, 95% CI 0.05-0.89) [68]. In an investigation of 3155 children who participated in the EURODIAB trial, subjects who had received vitamin D had a lower chance of becoming diabetic (odds ratio=0.67, 95% CI 0.53-0.86) [69].

Inhibition of certain cytokines by vitamin D, such as interferon-γ, interleukin-2, and interleukin-12, may prevent the development of diabetes, although randomized-controlled trials have yet to be performed [70, 71]. 1,25-$(OH)_2$ vitamin D suppresses the release of these cytokines by T cells and dendritic cells *in vitro* [71, 72]. Furthermore, 1,25-$(OH)_2$ vitamin D inhibits antigen presentation by dendritic cells *in vitro*, suggesting that vitamin D might reduce autoimmune damage in type I diabetes by preventing dendritic cells in the pancreas from presenting antigens [70, 72].

Yet *in vivo* studies have not demonstrated a role for vitamin D supplementation in ameliorating diabetes. Treatment of seventy diabetic children with 25 μg 1,25-$(OH)_2$ vitamin D every other day for one year did not improve glucose control [73]. In subjects with type 2 diabetes but normal fasting glucose, adding alfacalcidiol (1-OH cholecalciferol) increases insulin release in response to a glucose challenge, however, those with abnormal fasting glucose did not benefit [74, 75]. In addition, a single injection of 300,000 IU ergocalciferol resulted in a higher glycosylated hemoglobin and insulin resistance in three vitamin D insufficient Asian subjects with type 2 diabetes [76]. Thus, prospective studies of vitamin D supplementation in the prevention of diabetes by modifying diabetes risk factors, such as reduction of inflammatory cytokines, are warranted.

Vitamin D, including proteins involved in its metabolic pathway, may have additional roles in the pathogenesis of type 2 diabetes [70, 74]. Vitamin D binding protein (DBP), which acts to transport vitamin D and its metabolites systemically, may have genetically based alterations in structure in certain populations of individuals related to type 2 diabetes. Polymorphisms in genes encoding for DBP are associated with variations in insulin secretion and sensitivity in some ethnic populations, but in other populations no correlation was found between DBP variants and type 2 diabetes [74]. Vitamin D may be important in the synthesis and metabolism of insulin [77-81]. In animal models, vitamin D is necessary for insulin secretion by the pancreas and the regulation of glucose [74]. Rats that are deprived of vitamin D have lowered levels of insulin release, exaggerated response to injected insulin, and reduction in insulin sensitivity [74, 82]. 1,25-$(OH)_2$ vitamin D stimulates pancreatic islet cells to release insulin-regulatory proteins [74]. Vitamin D binding to calcium channels on the membrane of the β-cell allows calcium entry which stimulates endopeptidase production that acts to synthesize insulin formation [74]. An association (p=0.0011) existed between 25-OH vitamin D concentration and insulin sensitivity index in 128 healthy young subjects on a hyperglycemic clamp [83]. Vitamin D-deficient individuals (1,25-$(OH)_2$ vitamin D $\leq$ 30 pg/ml) exhibited increased insulin release after supplementation with 2000 IU cholecalciferol a day for six months [84]. However, glucagon release was not altered [84, 85]. Diabetic patients who were treated with 1332 cholecalciferol once a day for a month had a 34.3% increase in first-phase insulin secretion [86]. Therefore, vitamin D appears to exert beneficial effects on insulin regulation.

There are several associations between vitamin D and the pathogenesis of diabetes [38]. VDRs are located in the beta cells of the pancreas, and VDR polymorphisms are associated with insulin-dependent diabetes and non-insulin dependent diabetes risk [70, 74, 87-90]. An association was found between the VDR receptor gene initiation codon and type I diabetes risk [88]. A relationship has been described between type 2 diabetes and the ApaI, TaqI, FokI, and BsmI VDR polymorphisms [74, 91-94], although not all studies agree on their significance. A systematic review of the literature analyzing this relationship in East Asia found a significant relationship between diabetes for the FokI polymorphism and type 2 diabetes, and for Apa1 and FokI polymorphisms and type 1 diabetes [94]. Epidemiologic studies have given further evidence to a correlation between vitamin D levels and glucose regulation [95]. A genome-wide association study noted enrichment of VDR binding site intervals associated with type 1 diabetes (and other autoimmune associated genes) [96]. There was a correlation between serum 25-OH vitamin D concentration and fasting serum glucose in 753 postmenopausal women (ages 35-94) in Australia (r=-0.15, p<0.001) [95].

VITAMIN D AND CARDIOVASCULAR DISEASE AND ALL-CAUSE MORTALITY

A number of large investigations have delineated the relationship between vitamin D and cardiovascular disease [97-100]. In the Framingham Offspring study, an inverse correlation was observed between 25-OH vitamin D serum concentration and one of several endpoints: ischemia, heart failure, stroke or MI [97]. In a retrospective analysis of a cohort of 10,899 individuals, there was a correlation between vitamin D insufficiency (<75 nmol/L 25-OH vitamin D) and risk of diabetes, hypertension, coronary artery disease, cardiomyopathy, cardiovascular mortality, and all-cause mortality [98]. Individuals who consumed vitamin D supplements had a lower chance of death ($p<0.0001$). When serum 25-OH vitamin D concentrations from forty-four patients residing in an inpatient stroke unit were measured every two months for a year and compared with 96 healthy ambulatory subjects, stroke patients had statistically significantly lower 25-OH vitamin D level (seasonally adjusted Z score of stroke patients -1.4 SD units versus controls, $p<0.00001$) [99]. One-hundred seventy-nine patients who had a myocardial infarction were matched with age- and sex-matched control subjects in several cohorts whose 25-OH vitamin D concentrations were obtained during different seasons of the year [101]. Subjects whose serum 25-OH vitamin D concentration was ≥ 43 nmol/L had a lower risk of MI than those whose levels was < 25nmol/L (relative risk = 0.30, 95% CI 0.215-0.61) [101]. Concentrations of 25-OH vitamin D were significantly lower in 179 patients admitted to the hospital with an MI (32.0 nmol/L) than controls (35.5 nmol/L) [101]. Comparison of hospitalized patients to ambulatory subjects, however, may subject the results to reverse confounding.

Many additional investigations, including meta-analyses, have concluded that serum 25-OH vitamin D concentrations are inversely associated with overall mortality and cardiovascular disease-related mortality [102]. One was conducted on the relationship between vitamin D and mortality involving 57,311 subjects who received various vitamin D supplements [102]. Subjects who took between 200-10,000 IU daily had a lower relative risk of dying than those who did not (relative risk = 0.93, 95% CI=0.87-0.99) [102]. Mean serum 25-OH vitamin D concentrations were 1.4-5.2 fold higher in the intervention than in the control groups. In a second meta-analysis, which included another twelve studies of 32,142 individuals, a 20 nmol/l higher 25-OH vitamin D level correlated with an 8% decreased mortality [103]. The Mini-Finland Health Survey, which followed a cohort of 6,219 persons of at least thirty years of age who did not have cardiovascular disease, found that subjects in the highest quintile of vitamin 25-OH vitamin D concentrations had a lower relative risk for dying from cardiovascular disease (relative risk = 0.76, 95% CI=0.60-0.95) [104]. Interestingly, the risk was primarily attributed to reduction in cerebrovascular disease death; there was not an association between vitamin D concentration and coronary artery disease death [104]. In pooled results from two large epidemiologic trials in 9,146 persons, a higher 25-OH vitamin D concentration was associated with lower all-cause mortality (odds ratio=0.95, p=0.005), although not specifically with ischemic heart disease and stroke [105]. Another meta-analysis of 65,594 subjects revealed a relative risk of cardiovascular disease of 1.03 for every 25 nmol/L decrement below 60 nmol/L [106]. Among 388 people admitted with chest pain secondary to coronary artery disease, low 25-OH vitamin D serum concentrations predicted an increased risk of cardiac death (HR=0.37 for the highest versus lowest quartile of vitamin D concentration, p=0.004) [107]. In 1259 individuals who were admitted for a myocardial infarction, lower quartiles of serum 25-OH vitamin D concentrations were associated with adverse events (subsequent hospitalization for heart failure and subsequent myocardial infarction, but not death) [108]. In 1801 patients following coronary angiography, those with serum 25-OH vitamin D concentrations of at least 75 nmol/L were less likely to die from all causes (HR=0.25) or cardiovascular disease (HR=0.25) [109]. Among 4418 patients undergoing cardiac surgery, those with serum 25-OH vitamin D concentrations of < 30 nmol/L or 30-40.9 nmol/L were more likely to develop post-operative complications (OR = 2.23 and 1.73 respectively) than those whose serum levels were at least 50 nmol/L [110].

Some of the more recent investigations reveal a complex relationship between vitamin D and cardiovascular disease. As noted above in the study of 9,146 participants, baseline serum 25-OH vitamin D concentrations were associated with all-cause mortality over a mean follow-up of 10 years, but there was no relationship between vitamin D concentration and stroke or ischemic heart disease [105]. In 10,001 statin-treated patients with coronary heart disease, serum 25-OH vitamin D concentrations were not associated with cardiovascular events or mortality over a mean follow-up of 14.4 years [111]. There was no relationship between dietary vitamin D intake and cardiovascular events or mortality in 2338 Scottish participants of a epidemiologic study of cardiovascular disease [112]. A study of records of 1,282,822 subjects from Israel who were at least 45 years old provides a more finely nuanced insight into the association between serum vitamin D concentration and cardiovascular disease. Those whose serum 25-OH vitamin D concentrations were both below 50 nmol/L and above 90 nmol/L had a higher probability of developing acute coronary syndromes than did those with 25-OH vitamin D levels between 50 and 90 nmol/L (HR 1.25 $p<0.5$ for 25-50 nmol/L and HR 1.13. for >90 nmol/L, both $p<0.05$) [113].

There is an association between vitamin D concentration and mortality that persists into old age. In the Women's Health and Aging Surveys I and II, in 714 women living in the community between ages seventy and seventy-nine, those participants whose serum level was in the lowest quintile of 25-OH vitamin D concentrations (<38.2 nmol/L) had a greater chance of dying (hazard ratio 2.45, 95% CI 1.12-5.36) than did those in the highest quintile (> 67.4 nmol/L) [114]. Among 3408 persons over sixty-five who were part of NHANES III, subjects whose serum 25-OH vitamin D concentrations were < 25.0nmol/L had a higher relative risk of mortality over a mean follow-up of 7.3 years than those whose serum was > 100 n mol/L (1.83, 95% CI 1.24-2.94) [115]. The relative risk for cardiovascular disease alone was 2.36 (95% CI 1.17-4.75) [115]. Such observations may be explained in part by apparent relationships between vitamin D concentrations and dyslipidemia [38]. Human macrophages from subjects with diabetes cultured with 1,25-$(OH)_2$ vitamin D take up less cholesterol and are less likely to dif-

ferentiate into foam cells [116]. Several epidemiologic studies have described an association between vitamin D concentrations and lipid levels. Concentrations of 25-OH vitamin D were correlated with fasting serum concentrations of apolipoprotein A-1 although not other lipids in a population of 170 healthy persons in Britain of South Asian origin [117]. In 301 Koreans sixty years or older, 25-OH vitamin D concentrations of less than 50nM were associated with hypertriglyceridemia and insulin resistance [118]. An inverse relationship between triglycerides, VLDL cholesterol, and 25-OH vitamin D levels was also observed in 6784 people between the ages of 30 and 60 [119].

THE USE OF VITAMIN D TO TREAT METABOLIC SYNDROME AND RELATED DISEASES AND FUTURE DIRECTIONS

As we have seen, there are associations between vitamin D insufficiency and manifestations of caloric excess. Recent investigations are starting to delineate the potential utility of vitamin D to treat diseases in prospective trials. Such studies have yielded mixed results on the ability of vitamin D to induce changes in adiposity. In one investigation, 110 young (ages 20-40) and elderly ($\geq$ 64) ingested 600 IU of cholecalciferol or a placebo for 5.5 months. In older but not younger subjects, BMI, waist circumference, and fat-free mass were negatively associated with 25-OH vitamin D concentration [120]. In another trial, 171 overweight adults, aged 28-65, consumed orange juice containing 300 IU of cholecalciferol and 1050 mg calcium or a placebo for four months [121]. Although vitamin D supplementation did not induce total weight loss, the subjects who ingested vitamin D lost 13% of their visceral adipose tissue. Individuals supplemented with vitamin D failed to lose weight or body fat despite increases in mean serum 25-OH vitamin D concentration from 58 to more than 100 nmol/L. In addition, the subjects did not have any better glucose tolerance or lipid profile [122]. In another trial in Norway in which 445 overweight participants (BMI 28-47) ages 21 to 70 took 20,000 IU cholecalciferol biweekly, weekly, or consumed a placebo for 12 months, vitamin D supplementation did not improve cardiovascular risk factors [123].

The lack of demonstrated ability of vitamin D thus far in prospective trials to improve parameters of metabolic regulation does not mean the effect of vitamin D on metabolism is unimportant. In 171 vitamin D deficient (< 27.5 nmol/L) subjects ages 35 to 65 who took either 500 IU or 50,000 IU cholecalciferol every three months for one year, significant reductions in serum concentrations of the inflammatory cytokines, MMP9 (68%), CRP (23%) and tissue inhibitor of metalloproteinase-1 (TIMP 1) (38%) occurred [124]. The influence of vitamin D on bone metabolism can be influenced by the initial vitamin D levels of the subjects, dose given to subjects, and potential relationship to co-nutrients, such as vitamin A [125]. This may also be true for other effects of vitamin D, such as metabolic regulation. Future investigations need to control for such possible confounding influences. If the initial levels are too low, for example, a low supplementation level may be inadequate to achieve a measurable effect. Furthermore, given the pathophysiology of cardiovascular disease, chronic rather than acute supplementation of vitamin D earlier, rather than later in the course of the disorder, might be necessary for benefit.

The role of vitamin D as a regulatory agent for energy metabolism is one example of our rapidly evolving view of "whole-organ" human energy regulation [126]. This model is quite complex and beyond the scope of this manuscript, but involves a bidirectional positive reinforcing feedback loop between secretion by the osteoblasts in the bone of osteocalcin (which is subsequently decarboxylated into its active form) and insulin secretion by the pancreas [128-130]. Leptin, produced by adipocytes, induces the CNS to augment sympathetic activity, which reduces concentrations of decarboxylated osteocalcin secreted by the bone [128]. Vitamin D co-cultured with osteoblasts, glucose, and insulin augments osteocalcin release [131]. Further research that increases our understanding of how vitamin D regulates energy metabolism in concert with other hormones should be undertaken in order to more completely elucidate the role of vitamin D in the prevention and treatment of metabolic syndrome and cardiovascular diseases.

CONCLUSION

The optimal vitamin D status needed to alleviate the complications of a chronic proinflammatory state associated with or induced by caloric excess may differ from current recommendations for cholecalciferol supplementation needed in most persons to optimize bone health, prevent fractures and reduce falls. Further prospective investigation of vitamin D supplementation targeted to improve diseases reflecting the complications of chronic inflammation will help establish more definitively the importance of vitamin D in the prevention and treatment of metabolic syndrome and cardiovascular diseases.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

Declared none.

REFERENCES

[1] Cherniack, EP, Florez H, Roos BA, Troen BR, Levis S. Hypovitaminosis D in the elderly: from bone to brain. J Nutr Health Aging 2008; 12: 366-73.

[2] Zehnder D, Bland R, Williams MC, *et al.* Extrarenal expression of 25-hydroxyvitamin d(3)-1 alpha-hydroxylase. J Clin Endocrinol Metab 2001; 86: 888-94.

[3] Cherniack EP, Levis S, Troen BR. Hypovitaminosis D: a widespread epidemic. Geriatrics 2008; 63: 24-30.

[4] Lienbenberg L. The relevance of persistence hunting to human evolution. J Hum Evol 2008; 55: 1156-9.

[5] Foss YJ. Vitamin D deficiency is the cause of common obesity. Med Hypotheses 2009; 72: 314-21.

[6] Food and Agriculture Organization of the United Nations. FAO Statistical Yearbook. Country profiles. United States of America. 2005. ftp://ftp.fao.org/docrep/fao/008/a0205m/United-States-of-America.pdf. Accessed on August 24, 2009.

[7] U.S. Department of Agriculture. Mypyramid.gov. Inside the pyramid. How many discrentionary calories can I have? 2008. Accessed on August 24, 2009. [cited; Available from: http://www.mypyramid.gov/pyramid/discretionary_calories_amount_print.html

[8] Pontzer H, Raichlen DA, Wood BM, Mabulla AZ, Racette SB, Marlowe FW. Hunter-gatherer energetics and human obesity. PLoS One 2012; 7: e40503.
[9] Vernochet C, Peres SB, Farmer SR. Mechanisms of obesity and related pathologies: transcriptional control of adipose tissue development. FEBS J 2009; 276: 5729-37.
[10] Ford ES. Prevalence of the metabolic syndrome defined by the International Diabetes Federation among adults in the U.S. Diabetes Care 2005; 28: 2745-9.
[11] Liang J, Fu M, Ciociola E, Chandalia M, Abate N. Role of ENPP1 on adipocyte maturation. PLoS One 2007; 2: e882.
[12] Kim JE, Chen J. regulation of peroxisome proliferator-activated receptor-gamma activity by mammalian target of rapamycin and amino acids in adipogenesis. Diabetes 2004; 53: 2748-56.
[13] Blagosklonny MV. Aging and immortality: quasi-programmed senescence and its pharmacologic inhibition. Cell Cycle 2006; 5: 2087-102.
[14] Wood RJ. Vitamin D and adipogenesis: new molecular insights. Nutr Rev 2008; 66: 40-6.
[15] Vachharajani V, Granger DN. Adipose tissue: a motor for the inflammation associated with obesity. IUBMB Life 2009; 61: 424-30.
[16] Galic S, Oakhill JS, Steinberg GR. Adipose tissue as an endocrine organ. Mol Cell Endocrinol 2009; 285: 115-22.
[17] Silha JV, Krsek M, Sucharda P, Murphy LJ. Angiogenic factors are elevated in overweight and obese individuals. Int J Obes (Lond) 2005; 29:1308-14.
[18] Jung CH, Kim MS. Molecular mechanisms of central leptin resistance in obesity. Arch Pharm Res 2013; 36: 201-7.
[19] Yang Q, Graham TE, Mody N, *et al*. Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes. Nature 2005; 436: 356-62.
[20] Kovacs P, Geyer M, Berndt J, *et al.* Effects of genetic variation in the human retinol binding protein-4 gene (RBP4) on insulin resistance and fat depot-specific mRNA expression. Diabetes 2007; 56: 3095-100.
[21] Graham TE, Yang Q, Bluher M, *et al.* Retinol-binding protein 4 and insulin resistance in lean, obese, and diabetic subjects. N Engl J Med 2006; 354: 2552-63.
[22] Rosenstreich SJ, Rich C, Volwiler W. Deposition in and release of vitamin D3 from body fat: evidence for a storage site in the rat. J Clin Invest 1971; 50: 679-87.
[23] Mawer EB, Backhouse J, Holman CA, Lumb GA, Stanbury SW. The distribution and storage of vitamin D and its metabolites in human tissues. Clin Sci 1972; 43: 413-31.
[24] Nimitphong H, Holick MF, Fried SK, Lee MJ. 25-hydroxyvitamin d(3) and 1,25-dihydroxyvitamin d(3) promote the differentiation of human subcutaneous preadipocytes. PLoS One 2012; 7: e52171.
[25] Zemel MB, Sun X. Calcitriol and energy metabolism. Nutr Rev 2008; 66: S139-46.
[26] Snijder MB, van Dam RM, Visser M, *et al.* Adiposity in relation to vitamin D status and parathyroid hormone levels: a population-based study in older men and women. J Clin Endocrinol Metab 2005; 90: 4119-23.
[27] Arunabh S, Pollack S, Yeh J, Aloia JF. Body fat content and 25-hydroxyvitamin D levels in healthy women. J Clin Endocrinol Metab 2003; 88: 157-61.
[28] Guasch A, Bullo M, Rabassa A, *et al.* Plasma vitamin D and parathormone are associated with obesity and atherogenic dyslipidemia: a cross-sectional study. Cardiovasc Diabetol 2012; 11: 149.
[29] Buffington C, Walker B, Cowan GS, Jr. Scruggs D. Vitamin D deficiency in the morbidly obese. Obes Surg 1993; 3: 421-4.
[30] Compston JE, Vedi S, Ledger JE, Webb A, Gazet JC, Pilkington TR. Vitamin D status and bone histomorphometry in gross obesity. Am J Clin Nutr 1981; 34: 2359-63.
[31] Bell NH, Epstein S, Greene A, Shary J, Oexmann MJ, Shaw S. Evidence for alteration of the vitamin D-endocrine system in obese subjects. J Clin Invest 1985; 76: 370-3.
[32] Liel Y, Ulmer E, Shary J, Hollis BW, Bell NH. Low circulating vitamin D in obesity. Calcif Tissue Int 1988; 43: 199-201.
[33] Jorde R, Sneve M, Emaus N, Figenschau Y, Grimnes G. Cross-sectional and longitudinal relation between serum 25-hydroxyvitamin D and body mass index: the Tromso study. Eur J Nutr 2010; 49: 401-7.
[34] Rock CL, Emond JA, Flatt SW, *et al.* Weight loss is associated with increased serum 25-hydroxyvitamin D in overweight or obese women. Obesity (Silver Spring) 2012; 20: 2296-301.
[35] Vimaleswaran KS, Berry DJ, Lu C, *et al.* Causal relationship between obesity and vitamin D status: bi-directional Mendelian randomization analysis of multiple cohorts. PLoS Med 2013; 10: e1001383.
[36] Wortsman J, Matsuoka LY, Chen TC, Lu Z, Holick MF. Decreased bioavailability of vitamin D in obesity. Am J Clin Nutr 2000; 72: 690-3.
[37] Wamberg L, Christiansen T, Paulsen SK, *et al*. Expression of vitamin D-metabolizing enzymes in human adipose tissue-the effect of obesity and diet-induced weight loss. Int J Obes (Lond) 2013; 37: 651-7.
[38] Martini LA, Wood RJ. Vitamin D status and the metabolic syndrome. Nutr Rev 2006; 64: 479-86.
[39] Pramyothin P, Biancuzzo RM, Lu Z, Hess DT, Apovian CM, Holick MF. Vitamin D in adipose tissue and serum 25-hydroxyvitamin D after roux-en-Y gastric bypass. Obesity (Silver Spring) 2011; 19: 2228-34.
[40] Barger-Lux MJ, Heaney RP, Hayes J, DeLuca HF, Johnson ML, Gong G. Vitamin D receptor gene polymorphism, bone mass, body size, and vitamin D receptor density. Calcif Tissue Int 1995; 57:161-2.
[41] Vimaleswaran KS, Cavadino A, Berry DJ, *et al.* Genetic association analysis of vitamin D pathway with obesity traits. Int J Obes (Lond) 2013 Feb 5. (In Press).
[42] Sibley S, Turner M, Earthman C. Plasma vitamin D: a predictor of subsequent weight loss success. Paper presented at Endo 09: The Endocrine Society, 91st Annual Meeting. Washington, DC, June 11, 2009.
[43] Kamycheva E, Berg V, Jorde R. Insulin-like growth factor I, growth hormone, and insulin sensitivity: the effects of a one-year cholecalciferol supplementation in middle-aged overweight and obese subjects. Endocrine 2012; 43: 412-8.
[44] Backesjo CM, Li Y, Lindgren U, Haldosen LA. Activation of Sirt1 decreases adipocyte formation during osteoblast differentiation of mesenchymal stem cells. Cells Tissues Organs 2009; 189: 93-7.
[45] Zhuang H, Lin Y, Yang G. Effects of 1,25-dihydroxyvitamin D3 on proliferation and differentiation of porcine preadipocyte *in vitro*. Chem Biol Interact 2007; 170: 114-23.
[46] Kamei Y, Kawada T, Kazuki R, Ono T, Kato S, Sugimoto E. Vitamin D receptor gene expression is up-regulated by 1, 25-dihydroxyvitamin D3 in 3T3-L1 preadipocytes. Biochem Biophys Res Commun 1993; 193: 948-55.
[47] Kong J, Li YC. Molecular mechanism of 1,25-dihydroxyvitamin D3 inhibition of adipogenesis in 3T3-L1 cells. Am J Physiol Endocrinol Metab 2006; 290: E916-24.
[48] Sergeev IN. 1,25-Dihydroxyvitamin D3 induces Ca2+-mediated apoptosis in adipocytes *via* activation of calpain and caspase-12. Biochem Biophys Res Commun 2009; 384: 18-21.
[49] Narvaez CJ, Byrne BM, Romu S, Valrance M, Welsh J. Induction of apoptosis by 1,25-dihydroxyvitamin D3 in MCF-7 Vitamin D3-resistant variant can be sensitized by TPA. J Steroid Biochem Mol Biol 2003; 84: 199-209.
[50] Jorde R, Haug E, Figenschau Y, Hansen JB. Serum levels of vitamin D and haemostatic factors in healthy subjects: the Tromso Study. Acta Haematol 2007; 117: 91-7.
[51] Schleithoff SS, Zittermann A, Tenderich G, Berthold HK, Stehle P, Koerfer R. Vitamin D supplementation improves cytokine profiles in patients with congestive heart failure: a double-blind, randomized, placebo-controlled trial. Am J Clin Nutr 2006; 83: 754-9.
[52] Wasse H, Cardarelli F, De Staercke C, Hooper C, Veledar E, Guessous I. 25-hydroxyvitamin D concentration is inversely associated with serum MMP-9 in a cross-sectional study of African American ESRD patients. BMC Nephrology 2011; 12: 24.
[53] Interleukin 8/Chemokines. Genzyme:112.
[54] Hiraguchi Y, Tanida H, Sugimoto M, *et al.* 1,25-Dihydroxyvitamin D3 upregulates functional C-x-C chemokine receptor type 4 expression in human eosinophils. Int Arch Allergy Immunol 2012; 158: 51-7.
[55] Sun X, Zemel MB. Calcitriol and calcium regulate cytokine production and adipocyte-macrophage cross-talk. J Nutr Biochem 2008; 19: 392-9.
[56] Gurlek A, Pittelkow MR, Kumar R. Modulation of growth factor/cytokine synthesis and signaling by 1alpha,25-dihydroxyvitamin D(3): implications in cell growth and differentiation. Endocr Rev 2002; 23: 763-86.

[57] Mutt SJ, Karhu T, Lehtonen S, *et al.* Inhibition of cytokine secretion from adipocytes by 1,25-dihydroxyvitamin D(3) *via* the NF-kappaB pathway. FASEB J 2012; 26: 4400-7.

[58] Marcotorchino J, Gouranton E, Romier B, *et al.* Vitamin D reduces the inflammatory response and restores glucose uptake in adipocytes. Mol Nutr Food Res 2012; 56:1771-82.

[59] Gao D, Trayhurn P, Bing C. 1,25-Dihydroxyvitamin D(3) inhibits the cytokine-induced secretion of MCP-1 and reduces monocyte recruitment by human preadipocytes. Int J Obes (Lond) 2012; 37: 357-65.

[60] Menendez C, Lage M, Peino R, *et al.* Retinoic acid and vitamin D(3) powerfully inhibit *in vitro* leptin secretion by human adipose tissue. J Endocrinol 2001; 170: 425-31.

[61] Tarcin O, Yavuz DG, Ozben B, *et al.* Effect of vitamin D deficiency and replacement on endothelial function in asymptomatic subjects. J Clin Endocrinol Metab 2009; 94: 4023-30.

[62] Ford ES, Ajani UA, McGuire LC, Liu S. Concentrations of serum vitamin D and the metabolic syndrome among U.S. adults. Diabetes Care 2005; 28: 1228-30.

[63] Brenner DR, Arora P, Garcia-Bailo B, *et al.* Plasma vitamin D levels and risk of metabolic syndrome in Canadians. Clin Invest Med 2011; 34: E377.

[64] Gagnon C, Lu ZX, Magliano DJ, *et al.* Low serum 25-hydroxyvitamin D is associated with increased risk of the development of the metabolic syndrome at five years: results from a national, population-based prospective study (The Australian Diabetes, Obesity and Lifestyle Study: AusDiab). J Clin Endocrinol Metab 2012; 97: 1953-61.

[65] Kim S, Lim J, Kye S, Joung H. Association between vitamin D status and metabolic syndrome risk among Korean population: based on the Korean National Health and Nutrition Examination Survey IV-2, 2008. Diabetes Res Clin Pract 2012; 96: 230-6.

[66] Husemoen LL, Skaaby T, Thuesen BH, Jorgensen T, Fenger RV, Linneberg A. Serum 25(OH)D and incident type 2 diabetes: a cohort study. Eur J Clin Nutr 2012; 66: 1309-14.

[67] Pittas AG, Nelson J, Mitri J, *et al.* Plasma 25-hydroxyvitamin D and progression to diabetes in patients at risk for diabetes: an ancillary analysis in the Diabetes Prevention Program. Diabetes Care 2012; 35: 565-73.

[68] Hypponen E, Laara E, Reunanen A, Jarvelin MR, Virtanen SM. Intake of vitamin D and risk of type 1 diabetes: a birth-cohort study. Lancet 2001; 358:1500-3.

[69] Dahlquist G. Vitamin D supplement in early childhood and risk for Type I (insulin-dependent) diabetes mellitus. The EURODIAB Substudy 2 Study Group. Diabetologia 1999; 42: 51-4.

[70] Alfonso B, Liao E, Busta A, Poretsky L. Vitamin D in diabetes mellitus-a new field of knowledge poised for D-velopment. Diabetes Metab Res Rev 2009; 25: 417-9.

[71] Lemire JM. Immunomodulatory actions of 1,25-dihydroxyvitamin D3. J Steroid Biochem Mol Biol 1995; 53: 599-602.

[72] Piemonti L, Monti P, Sironi M, *et al*. Vitamin D3 affects differentiation, maturation, and function of human monocyte-derived dendritic cells. J Immunol 2000; 164: 4443-51.

[73] Pitocco D, Crino A, Di Stasio E, *et al*. The effects of calcitriol and nicotinamide on residual pancreatic beta-cell function in patients with recent-onset Type 1 diabetes (IMDIAB XI). Diabet Med 2006; 23: 920-3.

[74] Palomer X, Gonzalez-Clemente JM, Blanco-Vaca F, Mauricio D. Role of vitamin D in the pathogenesis of type 2 diabetes mellitus. Diabetes Obes Metab 2008; 10: 185-97.

[75] Inomata S, Kadowaki S, Yamatani T, Fukase M, Fujita T. Effect of 1 alpha (OH)-vitamin D3 on insulin secretion in diabetes mellitus. Bone Miner 1986; 1: 187-92.

[76] Taylor AV, Wise PH. Vitamin D replacement in Asians with diabetes may increase insulin resistance. Postgrad Med J 1998; 74: 365-6.

[77] Christakos S, Norman AW. Studies on the mode of action of calciferol. XVIII. Evidence for a specific high affinity binding protein for 1,25 dihydroxyvitamin D3 in chick kidney and pancreas. Biochem Biophys Res Commun 1979; 89: 56-63.

[78] de Sousa Santos R, Vianna LM. Effect of cholecalciferol supplementation on blood glucose in an experimental model of type 2 diabetes mellitus in spontaneously hypertensive rats and Wistar rats. Clin Chim Acta 2005; 358: 146-50.

[79] Bourlon PM, Billaudel B, Faure-Dussert A. Influence of vitamin D3 deficiency and 1,25 dihydroxyvitamin D3 on de novo insulin biosynthesis in the islets of the rat endocrine pancreas. J Endocrinol 1999; 160: 87-95.

[80] Ayesha I, Bala TS, Reddy CV, Raghuramulu N. Vitamin D deficiency reduces insulin secretion and turnover in rats. Diabetes Nutr Metab 2001; 14: 78-84.

[81] Chiu KC, Chuang LM, Yoon C. The vitamin D receptor polymorphism in the translation initiation codon is a risk factor for insulin resistance in glucose tolerant Caucasians. BMC Med Genet 2001; 2: 2.

[82] Ismail A, Namala R. Impaired glucose tolerance in vitamin D deficiency can be corrected by calcium. J Nutr Biochem 2000; 11: 170-5.

[83] Chiu KC, Chu A, Go VL, Saad MF. Hypovitaminosis D is associated with insulin resistance and beta cell dysfunction. Am J Clin Nutr 2004; 79: 820-5.

[84] Gedik O, Akalin S. Effects of vitamin D deficiency and repletion on insulin and glucagon secretion in man. Diabetologia 1986; 29: 142-5.

[85] Orwoll E, Riddle M, Prince M. Effects of vitamin D on insulin and glucagon secretion in non-insulin-dependent diabetes mellitus. Am J Clin Nutr 1994; 59: 1083-7.

[86] Borissova AM, Tankova T, Kirilov G, Dakovska L, Kovacheva R. The effect of vitamin D3 on insulin secretion and peripheral insulin sensitivity in type 2 diabetic patients. Int J Clin Pract 2003; 57: 258-61.

[87] McDermott MF, Ramachandran A, Ogunkolade BW, *et al.* Allelic variation in the vitamin D receptor influences susceptibility to IDDM in Indian Asians. Diabetologia 1997; 40: 971-5.

[88] Ban Y, Taniyama M, Yanagawa T, Yamada S, Maruyama T, Kasuga A. Vitamin D receptor initiation codon polymorphism influences genetic susceptibility to type 1 diabetes mellitus in the Japanese population. BMC Med Genet 2001; 2: 7.

[89] Pani MA, Knapp M, Donner H, *et al.* Vitamin D receptor allele combinations influence genetic susceptibility to type 1 diabetes in Germans. Diabetes 2000; 49: 504-7.

[90] Chang TJ, Lei HH, Yeh JI, *et al.* Vitamin D receptor gene polymorphisms influence susceptibility to type 1 diabetes mellitus in the Taiwanese population. Clin Endocrinol (Oxf) 2000; 52: 575-80.

[91] Hitman GA, Mannan N, McDermott MF, *et al*. Vitamin D receptor gene polymorphisms influence insulin secretion in Bangladeshi Asians. Diabetes 1998; 47: 688-90.

[92] Ogunkolade BW, Boucher BJ, Prahl JM, *et al*. Vitamin D receptor (VDR) mRNA and VDR protein levels in relation to vitamin D status, insulin secretory capacity, and VDR genotype in Bangladeshi Asians. Diabetes 2002; 51: 2294-300.

[93] Speer G, Cseh K, Winkler G, *et al.* Vitamin D and estrogen receptor gene polymorphisms in type 2 diabetes mellitus and in android type obesity. Eur J Endocrinol 2001; 144: 385-9.

[94] Wang Q, Xi B, Reilly KH, Liu M, Fu M. Quantitative assessment of the associations between four polymorphisms (FokI, ApaI, BsmI, TaqI) of vitamin D receptor gene and risk of diabetes mellitus. Mol Biol Rep 2012; 39: 9405-14.

[95] Need AG, O'Loughlin PD, Horowitz M, Nordin BE. Relationship between fasting serum glucose, age, body mass index and serum 25 hydroxyvitamin D in postmenopausal women. Clin Endocrinol (Oxf) 2005; 62: 738-41.

[96] Ramagopalan SV, Heger A, Berlanga AJ, *et al.* A ChIP-seq defined genome-wide map of vitamin D receptor binding: associations with disease and evolution. Genome Res 2010; 20: 1352-60.

[97] Wang TJ, Pencina MJ, Booth SL, *et al.* Vitamin D deficiency and risk of cardiovascular disease. Circulation 2008; 117: 503-11.

[98] Vacek JL, Vanga SR, Good M, Lai SM, Lakkireddy D, Howard PA. Vitamin D deficiency and supplementation and relation to cardiovascular health. Am J Cardiol 2012; 109: 359-63.

[99] Poole KE, Loveridge N, Barker PJ, *et al.* Reduced vitamin D in acute stroke. Stroke 2006; 37: 243-5.

[100] Zittermann A, Schleithoff SS, Tenderich G, Berthold HK, Korfer R, Stehle P. Low vitamin D status: a contributing factor in the pathogenesis of congestive heart failure? J Am Coll Cardiol 2003; 41: 105-12.

[101] Scragg R, Jackson R, Holdaway IM, Lim T, Beaglehole R. Myocardial infarction is inversely associated with plasma 25-hydroxyvitamin D3 levels: a community-based study. Int J Epidemiol 1990; 19: 559-63.

[102] Autier P, Gandini S. Vitamin D supplementation and total mortality: a meta-analysis of randomized controlled trials. Arch Intern Med 2007; 167: 1730-7.

[103] Schottker B, Ball D, Gellert C, Brenner H. Serum 25-hydroxyvitamin D levels and overall mortality. A systematic review and meta-analysis of prospective cohort studies. Ageing Res Rev 2013; 12: 708-18.

[104] Kilkkinen A, Knekt P, Aro A, *et al*. Vitamin D status and the risk of cardiovascular disease death. Am J Epidemiol 2009; 170: 1032-9.

[105] Skaaby T, Husemoen LL, Pisinger C, *et al*. Vitamin D status and incident cardiovascular disease and all-cause mortality: a general population study. Endocrine 2013; 43: 618-25.

[106] Wang L, Song Y, Manson JE, *et al.* Circulating 25-hydroxyvitamin D and risk of cardiovascular disease: a meta-analysis of prospective studies. Circ Cardiovasc Qual Outcomes 2012; 5: 819-29.

[107] Naesgaard PA, Leon De La Fuente RA, Nilsen ST, *et al*. Serum 25(OH)D is a 2-year predictor of all-cause mortality, cardiac death and sudden cardiac death in chest pain patients from Northern Argentina. PLoS One 2012; 7: e43228.

[108] Ng LL, Sandhu JK, Squire IB, Davies JE, Jones DJ. Vitamin D and prognosis in acute myocardial infarction. Int J Cardiol 2013 Feb 13.

[109] Thomas GN, o Hartaigh B, Bosch JA, *et al.* Vitamin D levels predict all-cause and cardiovascular disease mortality in subjects with the metabolic syndrome: the Ludwigshafen Risk and Cardiovascular Health (LURIC) Study. Diabetes Care 2012; 35: 1158-64.

[110] Zittermann A, Kuhn J, Dreier J, Knabbe C, Gummert JF, Borgermann J. Vitamin D status and the risk of major adverse cardiac and cerebrovascular events in cardiac surgery. Eur Heart J 2013; 34: 1358-64.

[111] Bittner V, Wenger NK, Waters DD, DeMicco DA, Messig M, LaRosa JC. Vitamin D levels do not predict cardiovascular events in statin-treated patients with stable coronary disease. Am Heart J 2012; 164: 387-93.

[112] Welsh P, Doolin O, McConnachie A, *et al.* Circulating 25OHD, dietary vitamin D, PTH, and calcium associations with incident cardiovascular disease and mortality: the MIDSPAN Family Study. J Clin Endocrinol Metab 2012; 97: 4578-87.

[113] Dror Y, Giveon S, Hoshen M, Feldhamer I, Balicer R, Feldman B. Vitamin D levels for preventing acute coronary syndrome and mortality: evidence of a Non-linear association. J Clin Endocrinol Metab 2013; 98: 2160-7.

[114] Semba RD, Houston DK, Ferrucci L, *et al.* Low serum 25-hydroxyvitamin D concentrations are associated with greater all-cause mortality in older community-dwelling women. Nutr Res 2009; 29: 525-30.

[115] Ginde AA, Scragg R, Schwartz RS, Camargo CA, Jr. Prospective study of serum 25-Hydroxyvitamin D Level, cardiovascular disease mortality, and all-cause mortality in older U.S. adults. J Am Geriatr Soc 2009; 57: 1595-603.

[116] Oh J, Weng S, Felton SK, *et al*. 1,25(OH)2 vitamin D inhibits foam cell formation and suppresses macrophage cholesterol uptake in patients with type 2 diabetes mellitus. Circulation 200; 120: 687-98

[117] John WG, Noonan K, Mannan N, Boucher BJ. Hypovitaminosis D is associated with reductions in serum apolipoprotein A-I but not with fasting lipids in British Bangladeshis. Am J Clin Nutr 2005; 82: 517-22.

[118] Park HY, Lim YH, Kim JH, Bae S, Oh SY, Hong YC. Association of serum 25-hydroxyvitamin D levels with markers for metabolic syndrome in the elderly: a repeated measure analysis. J Korean Med Sci 2012; 27: 653-60.

[119] Skaaby T, Husemoen LL, Pisinger C, *et al*. Vitamin D status and changes in cardiovascular risk factors: a prospective study of a general population. Cardiology 2012; 123: 62-70.

[120] Forsythe LK, Livingstone MB, Barnes MS, *et al.* Effect of adiposity on vitamin D status and the 25-hydroxycholecalciferol response to supplementation in healthy young and older Irish adults. Br J Nutr 2011; 107: 126-34.

[121] Rosenblum JL, Castro VM, Moore CE, Kaplan LM. Calcium and vitamin D supplementation is associated with decreased abdominal visceral adipose tissue in overweight and obese adults. Am J Clin Nutr 2012; 95: 101-8.

[122] Jorde R, Sneve M, Torjesen P, Figenschau Y. No improvement in cardiovascular risk factors in overweight and obese subjects after supplementation with vitamin D3 for 1 year. J Intern Med 2010; 267: 462-72.

[123] Sneve M, Figenschau Y, Jorde R. Supplementation with cholecalciferol does not result in weight reduction in overweight and obese subjects. Eur J Endocrinol 2008; 159: 675-84.

[124] Timms PM, Mannan N, Hitman GA, *et al*. Circulating MMP9, vitamin D and variation in the TIMP-1 response with VDR genotype: mechanisms for inflammatory damage in chronic disorders? QJM 2002; 95: 787-96.

[125] Lappe JM, Heaney RP. Why randomized controlled trials of calcium and vitamin D sometimes fail. Dermatoendocrinol 2012; 4: 95-100.

[126] Karsenty G, Ferron M. The contribution of bone to whole-organism physiology. Nature 2012; 481: 314-20.

[127] Iki M, Tamaki J, Fujita Y, *et al*. Serum undercarboxylated osteocalcin levels are inversely associated with glycemic status and insulin resistance in an elderly Japanese male population: Fujiwara-kyo Osteoporosis Risk in Men (FORMEN) Study. Osteoporos Int 2012; 23: 761-70.

[128] Ducy P. The role of osteocalcin in the endocrine cross-talk between bone remodelling and energy metabolism. Diabetologia 2011; 54: 1291-7.

[129] Motyl KJ, McCabe LR, Schwartz AV. Bone and glucose metabolism: a two-way street. Arch Biochem Biophys 2010; 503: 2-10.

[130] Hwang YC, Jeong IK, Ahn KJ, Chung HY. Circulating osteocalcin level is associated with improved glucose tolerance, insulin secretion and sensitivity independent of the plasma adiponectin level. Osteoporos Int 2012; 23: 1337-42.

[131] Wu YY, Yu T, Zhang XH, *et al.* 1,25(OH)2D3 inhibits the deleterious effects induced by high glucose on osteoblasts through undercarboxylated osteocalcin and insulin signaling. J Steroid Biochem Mol Biol 2012; 132: 112-9.

Received: August 05, 2012 Revised: July 16, 2013 Accepted: July 20, 2013