Correlation between serum 25 hydroxy vitamin D3 and laboratory risk markers of cardiovascular diseases in type 2 diabetic patients

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ABSTRACT

الأهداف: تحديد العلاقة بين نقص فيتامين D وعلامات الخطر للجهاز القلبي الوعائي بين المرضى المصابين بداء السكري.

الطريقة: أجريت دراسة على شريحة عرضية شملت 119 مريضاً مصاباً بداء السكري من النوع الثاني بمدينة مشهد بإيران، خلال الفترة ما بين ديسمبر 2007م وحتى مارس 2008م. تم تأكيد أمراض الشريان التاجي، أمراض الجهاز القلبي الوعائي وأمراض الأوعية المحيطية. كما تم تحديد القياسات بما فيها علامات الخطر المخبرية للإصابة بأمراض الجهاز القلبي الوعائي. قيس مصل 25(OH) خلال الشتاء. كما تم أيضاً تحديد الصلة بين نقص فيتامين D وانتشاء أمراض الجهاز القلبي الوعائي وكذلك المتغيرات المخبرية.

النتائج: بلغ متوسط عمر المرضى 11.2±55.3 عاماً، بلغ متوسط تركيز مصل 25OH 32.4±21.6ng/ml. بلغت نسبة انتشار نقص فيتامين D 26.1% بين المرضى المصابين بداء السكري. لم يكن الفرق مع مجموعة التحكم ملحوظاً (p=0.12). بشكل عام، 36 (30.3%) من المرضى كانت حالتهم موجبة بالإصابة بالأمراض الوعائية التاجية (CVD). لم تكن العلاقة بين نقص فيتامين D والأمراض الوعائية التاجية (CVD) ملحوظة (p=0.11). كان لدى المرضى المصابين بنقص فيتامين D فروقات ملحوظة في كتلة الجسم المدخلة (p=0.003)، متلازمة الاستقلاب (p=0.05)، الحساسية العالية (p=0.009) (CRP)، البيلة الزلالية الدقيقة (p=0.04) ومعدل الترشيح (p=0.02) مقارنة مع المرضى الذين لديهم اكتفاء بفيتامين D . ليس لدى (HbAlC)، (FBS)، ملفات الدهون، الهوموسيستين، حمض اليوريك ومقاومة الأنسولين علاقة مع نقص فيتامين D.

خاتمة: أظهرت النتائج أن العلاقة بين نقص فيتامين D والعلامات الالتهابية قد تكون مساهمة في أمراض الجهاز القلبي الوعائي، لذلك فإن فيتامين D قد يكون مهماً في صحة الجهاز القلبي الوعائي.

Objectives: To determine the association between vitamin D deficiency and cardiovascular risk markers among diabetic patients.

Methods: This was a cross-sectional study conducted in Ghaem Hospital, Mashhad, Iran, from December 2007 to March 2008 in 119 type 2 diabetic patients. Coronary, cerebrovascular, and peripheral vascular diseases were confirmed. Blood biochemical parameters including laboratory risk markers of cardiovascular disease were determined. Serum 25 hydoxy (OH) D was measured during winter. The correlation between vitamin D deficiency and cardiovascular prevalence, and also laboratory variables was determined.

Results: The mean age of patients was 55.3 ± 11.2 years. The mean 25(OH) D concentration was 32.4 ± 21.6ng/ml. The prevalence of hypovitaminous D was 26.1% among the diabetic patients. The difference with the control group was not significant (p=0.12). Overall, 36 (30.3%) patients were positive for coronary vascular disease (CVD). The correlation between hypovitaminous D and CVD was not significant (p=0.11). Patients with vitamin D deficiency had significant differences in body mass index (p=0.003), metabolic syndrome (p=0.05), high sensitive C-reactive protein (p=0.009), microalbuminuria (p=0.04), and glumerular filtration rate (p=0.02), compared to patients with sufficient vitamin D. The fasting blood sugar, glycosylated hemoglobin, lipid profiles, homocysteine, uric acid, and insulin resistance were not related to vitamin D deficiency.

Conclusion: There is an association between hypovitaminous D and inflammatory markers that contributed to CVD, so vitamin D may be important in maintaining cardiovascular health.

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Cardiovascular disease (CVD) is a major cause of mortality and morbidity in type 2 diabetes mellitus (DM), and DM is equivalent to coronary ischemic disease.[1] Diabetes mellitus is a concomitant occurrence of many classical risk factors including hyperglycemia, hypertension, hyperlipidemia, insulin resistance, obesity, coagulation disease, and many others. Several epidemiological and clinical studies suggests that there is an excess risk of type 2 DM,[2-4] metabolic syndrome (MS),[5,6] insulin resistance,[7] hypertension,[8] hyperlipidemia,[9] and CVD[9,10] among persons with vitamin D deficiency. Vitamin D plays an important role in glucose and insulin metabolism and it affects the pancreatic islet cell through vitamin D receptor (VDR) and vitamin D dependent calcium binding proteins that increase insulin secretion. On the other hand, increased parathyroid hormone (PTH) level in vitamin D deficiency can decrease insulin sensitivity. The increase in the inflammatory markers including C-reactive protein (CRP), tumor necrosis factor, plasminogen activator inhibitor -1, and IL-6 may precede the development of type 2 DM. Vitamin D has an anti-inflammatory and immunomedulatory effect, and may also decrease insulin resistance and increase insulin secretion by modulating the immune system.[3,4] Several mechanisms may explain the relation between vitamin D deficiency and CVD, firstly; the relation between serum levels of 1, 25 hydroxy (OH) D3 and plasma renin activity. Vitamin D signaling is required for maintaining proper levels of renin production.[11-13] Thus, vitamin D appears to play a key role in the homeostasis of the renocardiovascular system by a negative endocrine regulator of the renin angiotensin aldosterone system. Secondly, the effect of vitamin D level on CVD is putative vascular effects including modulation of smooth muscle cell proliferation, inflammation, and thrombosis. Vitamin D has an anti-inflammatory, anti-athergenic property.[14,15] It seems that the immunomedulatory effects of vitamin D are mediated by the VDR that is expressed in most immune cells. Vitamin D inhibits antigen presenting cell maturation,[16] as well as angiogenesis and smooth muscle cell proliferation.[17] Inflammation is now regarded as a key factor for atherogenicity. Vitamin D causes down regulation of nuclear factor -KB activity, increased IL-10 production, and decrease of IL-6, IL-12, IFN-gamma production that lead to a cytokine profile, which favors less inflammation.[18,19] In addition, vitamin D has a possible role in the modulation of expression of tissue matrix metalloproteinase's (MMP's) that are involved in the remodeling of the vascular wall and myocytes. It seems that vitamin D status is an independent determinant of CRP and MMP-9 levels. The CRP was demonstrated in human studies to predict CVD, and its long-term outcome.[20] Vitamin D treatment in vitamin D deficient patients causes a significant decrease in CRP level.[19] Thus, vitamin D may be important in both the prevention of inflammation and stimulation of endothelial progenitor cells that was associated with a number of key biologic processes in cardiovascular health.[21] Thirdly, vitamin D deficiency triggers secondary hyperparathyroidism that PTH in turn causes myocytes hypertrophy, and probably has a proinflammatory effect that stimulates the release of cytokines by vascular smooth muscle cells.[22] The aim of this study was to evaluate the relationship between serum 25 (OH) D levels and the prevalent laboratory risk markers of CVD in type 2 DM patients.

Methods. Using a convenient sampling technique, 119 type 2 DM outpatients from one diabetes unit of Ghaem Hospital, Mashhad, Iran, were included in this study. Patients with recent acute illness, history of chronic liver or renal disease, and those who were taking medications that alter vitamin D metabolism and status, patients with history of hypercortisolism, malabsorption, lactation, smoking, pregnancy, and alcoholism were excluded from this study. Biochemical blood measurement was determined by the standard laboratory procedures. Serum concentration of 25 (OH) D was measured by radioimmunoassay method (Biosource Europe, Nivelles, Belgium). Fasting plasma glucose was measured by the glucose oxidase method (Human, Wiesbaden, Germany). Total cholesterol (TC), triglyceride (TG), and high density lipoprotein (HDL) were measured by enzymatic method (Parsazmon, Karaj, Iran). Low density lipoprotein (LDL) was calculated according to Friedwall formula: LDL=TC (HDL+[TG/5]). Glycosylated hemoglobin (HbA1C) was assessed by Column chromatography (Biosource Kit, Barcelona, Spain). Urine albumin in spot urine, and serum high sensitive C-reactive protein (hsCRP) concentration was measured by Immunoturbidimetry assay (Parsazmon, Karaj, Iran). Urine creatinine was measured by enzymatic colorimetric assay, and the urine albumin to creatinine ratio was used. Homocysteine was determined by enzyme linked immunosorbent assay (IBL Inc., Hamburg, Germany). The metabolic syndrome was defined according to the adult treatment panel III criteria. On the basis of patient history, medical history, examination, and vascular assessment, patients were divided in 2 subgroups. Group I consisted of patients with coronary problems (myocardial infarction, angina, history of revascularization or coronary artery bypass graft, or cerebrovascular problems [ischemic stroke, recurrent transient ischemic attack or carotid endarterectomy]), or peripheral vascular disease, and group II, the absence of all of the above mentioned coronary problems. Insulin resistance was estimated with the homeostasis model assessment-insulin resistance (HOMA-IR) index (fasting blood sugar x insulin/22.5),

in patients without a history of insulin treatment. The HOMA-insulin secretion (ISCR) was estimated by HOMA calculator version 2.2. Hypovitaminous D was defined on the basis of a population based study in Iran.[23] In this study, vitamin D deficiency was defined according to 25 (OH) D <16.6 ng/ml in females, and <14.5 ng/ml in males. The study protocol was approved by the research ethics committee of Mashhad University of Medical Sciences, and written informed consent was obtained from all patients.

Data was expressed as mean ± SD. Variants that fail the normality test were calculated by Mann Whitney test. Other variables were tested by either one-way ANOVA or student t test. Categorical variables were compared by Chi-square test. Pearson correlation coefficients and Spearman were compared to quantify the correlation of vitamin D and other variants. A *p*-value <0.05 was considered significant.

Results. The clinical and biochemical characteristics of type 2 DM patients are shown in **Table 1**. From the total number of patients, 56% were female. Overall, 31 (26.1%) of the 119 patients were positive for vitamin D deficiency. Patients with hypovitaminous D were mostly female (76.7%) than male (23.3%) (p=0.10). Age, duration of diabetes, HbA1C, homocysteine, calcium, and phosphorous were all similar among patients, with or without hypovitaminous D, but patients with vitamin D deficiency had increased prevalence of higher BMI, hsCRP, urine albumin to creatinine ratio, and lower glumerolar filtration rate compared to vitamin D sufficient patients. The proportion of patients with MS was significantly higher among patients with hypovitaminous D. Patients taking angiotensin converting enzyme (ACE) inhibitor drugs were significantly lower in the hypovitaminous D group. Patients with vitamin D deficiency had greater

Table 1 - Baseline characteristics of diabetic patients.

Characteristics	Mean ± SD
Age, years	55.3 ± 11.2
Diabetes duration, years	8.2 ± 6.8
Body mass index, kg/m^2	27.3 ± 4.2
Waist circumference, cm	97.4 ± 12.3
Systolic blood pressure, mm Hg	127.5 ± 26.1
Diastolic blood pressure, mm Hg	79.4 ± 13.8
Fasting blood sugar, mg/dl	184.1 ± 60.3
Glycosylated hemoglobin, %	8.1 ± 1.6
Insulin, µ/l	13.4 ± 9.5
Total cholesterol, mg/dl	198.9 ± 37.2
Triglyceride, mg/dl	184.8 ± 76.3
HDL cholesterol, mg/dl	47.0 ± 11.1
LDL cholesterol, mg/dl	103.6 ± 23.3
Homocysteine, µmol/l	11.4 ± 6.6
hsCRP, mg/l	4.7 ± 8.7
Urine albumin creatinine ratio	67.2 ± 141.9
Creatinine, mg/dl	0.9 ± 0.2
Calcium, mg/dl	9.9 ± 0.5
Phosphorous, mg/dl	3.7 ± 0.4
25 (OH) D, ng/ml	32.4 ± 21.6
HOMA-IR	2.1 ± 1.4
HOMA-SCR	72.5 ± 44.6

HDL - high density lipoprotein, LDL - low density lipoprotein, hsCRP - high sensitive C-reactive protein, 25 (OH) D - 25 hydroxy vitamin D, HOMA-IR - homeostatic model assessment-insulin resistance, HOMA-ISCR - homeostatic model assessment-insulin secretion

Table 2 - Baseline characteristics according to vitamin D status (N=119).

Characteristics	Normal vitamin D n=88	Vitamin D deficient n=31	*P*-value
Age, years	50.9 ± 9.0	56.4 ± 11.4	0.24
BMI, kg/m^2	26.6 ± 4.0	29.3 ± 3.8	0.003
Duration of diabetes, years	8.38 ± 6.9	7.23 ± 6.2	0.43
Systolic BP, mm Hg	128.5 ± 25.9	126.9 ± 28.1	0.77
Diastolic BP, mm Hg	79.8 ± 14.2	78.4 ± 13.2	0.63
FBS, mg/dl	176.9 ± 45.7	187.4 ± 63.0	0.41
HbA1C, %	7.7 ± 1.2	8.2 ± 1.6	0.16
Insulin, µ/l	12.9 ± 8.9	13.5 ± 10.4	0.78
Cholesterol, mg/dl	202.5 ± 37.8	192.5 ± 33.0	0.19
Triglyceride, mg/dl	181.4 ± 82.6	188.0 ± 57.2	0.68
HDL, mg/dl	47.5 ± 10.9	45.6 ± 11.7	0.43
LDL, mg/dl	106.7 ± 24.2	100.1 ± 20.2	0.17
Homocysteine, µmol/l	11.4 ± 5.9	10.9 ± 8.2	0.72
hsCRP, mg/l	3.8 ± 6.5	7.4 ± 12.5	0.009
Albumin excretion rate	45.9 ± 112.2	89.9 ± 192.4	0.04
Creatinine, mg/dl	0.9 ± 0.2	0.9 ± 0.1	0.12
Calcium	9.9 ± 0.5	9.8 ± 0.4	0.26
HOMA-IR	2.1 ± 1.3	2.0 ±1.4	0.09
HOMA-SECR	73.1 ± 43.1	71.3 ± 44.9	0.86
Uric acid	4.8 ± 1.5	4.2 ± 1.2	0.16
GFR, ml/min	107.2 ± 22.5	90.5 ± 30.0	0.02
ACEI, %	70	30	0.03
Metabolic syndrome, %	75.4	93	0.05

BMI - body mass index, BP - blood pressure, FBS - fasting blood sugar, HbA1C - glycated hemoglobin, HDL - high density lipoprotein, LDL - low density lipoprotein, hsCRP - high sensitive C-reactive protein, HOMA-IR - homeostatic model assessment-insulin resistance, HOMA-ISCR - homeostatic model assessment-insulin secretion, GFR - glumerular filtration rate, ACEI - angiotensin converting enzyme inhibitors. Data were expressed as mean ± SD, unless otherwise indicated. Differences were assessed by the unpaired t test (for normally distributed variables), and by the χ^2 test (for categorical variables)

prevalence of retinopathy compared with vitamin D sufficient group (38.6% versus 16.3%, [p=0.02]). The characteristics of patients that were grouped according to vitamin D status is listed in **Table 2**. In total, 36 (30.3%) patients were positive for CVD. From these, 26 patients had coronary heart disease (CHD), and 10 patients had cerebrovascular disease. In 5 patients with CHD, history of peripheral vascular disease was positive. No significant correlation was found between CVD and vitamin D levels (p=0.11). A significant correlation was found between vitamin D level and age (p=0.01, r=-0.21), body mass index (BMI) (p=0.01, r=-0.25), hsCRP (p=0.05, r=-0.06), and ACE inhibitor usage (p=0.03, r=-0.2). The correlation of vitamin D level with other parameters was not significant.

Discussion. The mean serum levels of 25 (OH) D in our patients was greater than the other studies in the diabetic and normal population.[23-26] The discrepancy of our results from other studies may be related to the improvement in nutrition, due to the fortification with vitamin D of some foodstuffs in Iran that started 3 years ago. The results of the present study showed a significant correlation between hypovitaminous D, and high BMI and MS. Patients with hypovitaminous D had higher weight and more prevalence of MS. Other studies have shown a correlation between vitamin D deficiency, obesity, and MS.[27,28] Ford et al[5] showed that the mean concentration of 25(OH)D among those with MS was lower than those without MS. Reis et al[28] demonstrated that an inverse association of 25(OH)D with MS, independent of confounding factors includes calcium intake, and PTH level. In another study, Buffington et al[29] showed that serum vitamin D3 was inversely related to BMI and weight. Reis et al[30] in another study reported an increased risk of MS with elevated PTH levels in older men, but no significant correlation was found with vitamin D levels.

In the current study, we found a correlation between vitamin D deficiency and elevation in hsCRP and albuminuria. The hsCRP was demonstrated in human studies to be a predictor of CVD and its long-term outcomes. Timms[18] showed that CRP levels was inversely related to vitamin D status, and when patients with vitamin D deficiency were treated with cholecalciferol, the mean CRP levels significantly decrease after a year. Cigolini[25] demonstrated that hypovitaminous D is related to an elevation in plasma inflammatory markers including CRP and fibrinogen levels. A significant inverse relationship between serum 25 (OH) D and tumor necrosis factor-α concentrations, but not in CRP level was shown by Peterson et al.[31]

In this study, we showed a relation between vitamin D deficiency and elevated urine albumin excretion rate (UAER). de Boer et al[32] demonstrated a linear increase in the prevalence of albuminuria with decreasing quartiles of vitamin D concentration. An elevated UAER is associated with an increased risk of CVD and mortality.[33,34] It seems that elevated UAER reflects a process, predisposing a patient to atherothrombosis. Atherothrombosis is a low grade inflammatory disease, and probably there is an association between CRP levels and microalbuminuria.[35] In our study, the relation between vitamin D level and other parameters including FBS, lipid profile, HbA1C, homocysteine and insulin resistance beta cell function was not significant, but John et al[36] showed a significant positive correlation between serum concentration of vitamin D and lipid profiles, especially HDL and apolipoprotein A-1.

Several studies[7,37] have demonstrated a direct relation between hypovitaminous D and elevated insulin resistance, and it seems that vitamin D affects either insulin sensitivity and beta cell function, or both. We did not observe a significant relation between vitamin D level, HOMA-IR, and HOMA-B. Other studies have shown a correlation between vitamin D deficiency and CVD among healthy and diabetic patients,[38,39] but in our study after dividing patients into 2 groups, with and without overt CVD, a significant correlation was not found. Our findings suggest that low serum levels of 25 (OH) D impacts plasma inflammatory factors (HSCRP, microalbuminuria), and it is correlated with elevated BMI and MS. The combination of these factors is associated with increased risk of CVD.[20] We showed a significant difference between the usage of ACE inhibitors in the 2 vitamin D groups. In patients using ACE inhibitors, the prevalence of vitamin D deficiency was lower than sufficient vitamin D patients. This finding was opposite to another study by Pérez-Castrillón et al,[40] which demonstrated that the ACE inhibitor usage in combination with the presence of the ACE polymorphism (DD genotype) can decrease the level of 1,25 (OH) D2. This difference may be related to the difference in genotyping. In our results, a significant association was found between hypovitaminous D and the presence of retinopathy. It is previously reported that higher serum vitamin D level is inversely associated with prevalent early age-related macular degeneration, and with soft drusen, specifically in the American population, aged 40 years and older,[41] however, the association between vitamin D deficiency and diabetic retinopathy is not clear.

This study has some limitations; the sample size was small, and PTH and 1, 25 (OH) D levels were not measured in the present study. Further clinical and experimental studies may be warranted to validate our findings.

In conclusion, the lower 25 (OH) D levels appear to be associated with hsCRP elevation, high albumin excretion rate, and high BMI in diabetic patients that

these markers predict cardiovascular risk and outcomes. The impact of vitamin D insufficiency on cardiovascular system is an important issue in diabetes, and further investigations should be carried out in this regard.

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References

1. Bell DS. Diabetes: a cardiac condition manifesting as hyperglycemia. ***Endocr Pract*** 2008; 14: 924-932.
2. 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: 820-825.
3. 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: 258-261.
4. Scragg R, Sowers M, Bell C, Third National Health and Nutrition Examination Survey. Serum 25-hydroxyvitamin D, diabetes, and ethnicity in the Third National Health and Nutrition Examination Survey. ***Diabetes Care*** 2004; 27: 2813-2818.
5. Ford ES, Ajani UA, McGuire L, Liu S. Concentration of serum vitamin D and the metabolic syndrome among U.S. adults. ***Diabetes Care*** 2005; 28: 1228-1230.
6. McGill AT, Stewart JM, Lithander FE, Strik CM, Poppitt SD. Relationships of low serum vitamin D3 with anthropometry and markers of the metabolic syndrome and diabetes in overweight and obesity. ***Nutr J*** 2008; 7: 4.
7. Danesco LG, Levy S, Levy J. Vitamin D and diabetes mellitus. ***Endocrine*** 2009; 35: 11-17.
8. Forman JP, Curhan GC, Taylor EN. Plasma 25-hydroxyvitamin D levels and risk of incident hypertension among young women. ***Hypertension*** 2008; 52: 828-832.
9. Luong KV, Nguyen LT. Vitamin D and cardiovascular disease. ***Curr Med Chem*** 2006; 13: 2443-2447.
10. Achinger SG, Ayus JC. The role of vitamin D in left ventricular hypertrophy and cardiac function. ***Kidney Int Suppl*** 2005; Suppl 95: S37-S42.
11. Carbone LD, Rosenberg EW, Tolley EA, Holick MF, Hughes TA, Watsky MA, et al. 25-Hydroxyvitamin D, cholesterol, and ultraviolet irradiation. ***Metabolism*** 2008; 57: 741-748.
12. Grundy SM. Obesity, metabolic syndrome, and cardiovascular disease. ***J Clin Endocrinol Metab*** 2004; 89: 2595-2600.
13. Li YC, Kong J, Wei M, Chen ZF, Liu SQ, Cao LP. 1,25-Dihydroxyvitamin D(3) is a negative endocrine regulator of the renin-angiotensin system. ***J Clin Invest*** 2002; 110: 229-238.
14. Mathieu C, Adorini L. The coming of age of 1,25-dihydroxyvitamin D(3) analogs as immunomodulatory agents. ***Trends Mol Med*** 2002; 8: 174-179.
15. Xing N, Maldonado ML, Bachman LA, McKean DJ, Kumar R, Griffin MD. Distinctive dendritic cell modulation by vitamin D(3) and glucocorticoid pathways. ***Biochem Biophys Res Commun*** 2002; 297: 645-652.
16. Griffin MD, Lutz W, Phan VA, Bachman LA, McKean DJ, Kumar R. Dendritic cell modulation by 1alpha, 25 dihydroxyvitamin D3 and its analogs: a vitamin D receptor-dependent pathway that promotes a persistent state of immaturity in vitro and in vivo. ***Proc Natl Acad Sci U S A*** 2001; 98: 6800-6805.
17. O'Connell TD, Berry JE, Jarvis AK, Somerman MJ, Simpson RU. 1,25-Dihydroxyvitamin D3 regulation of cardiac myocyte proliferation and hypertrophy. ***Am J Physiol*** 1997; 272: H1751-H1758.
18. Timms PM, Mannan N, Hitman GA, Noonan K, Mills PG, Syndercombe-Court D, 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: 787-796.
19. Pearson TA, Mensah GA, Alexander RW, Anderson JL, Cannon RO 3rd, Criqui M, et al. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: A statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. ***Circulation*** 2003; 107: 499-511.
20. Szmitko PE, Wang CH, Weisel RD, Jeffries GA, Anderson TJ, Verma S. Biomarkers of vascular disease linking inflammation to endothelial activation: Part II. ***Circulation*** 2003; 108: 2041-2048.
21. Jono S, McKee MD, Murry CE, Shioi A, Nishizawa Y, Mori K, et al. Phosphate regulation of vascular smooth muscle cell calcification. ***Circ Res*** 2000; 87: E10-E17.
22. McCarty MF. Secondary hyperparathyroidism promotes the acute phase response - a rationale for supplemental vitamin D in prevention of vascular events in the elderly. ***Med Hypotheses*** 2005; 64: 1022-1026.
23. Moradzadeh K, Larijani B, Keshtkar AA, Hossein-Nezhad A, Rajabian R, Nabipour I, et al. Normative values of vitamin D among Iranian population: a population based study. ***International Journal of Osteoporosis and Metabolic Disorders*** 2008; 1: 8-15.
24. Di Cesar DJ, Ploutz-Snyder R, Weinstock RS, Moses AM. Vitamin D deficiency is more common in type 2 than in type 1 diabetes. ***Diabetes Care*** 2006; 29: 174.
25. Cigolini M, Iagulli MP, Miconi V, Galiotto M, Lombardi S, Targher G. Serum 25-hydroxyvitamin D3 concentrations and prevalence of cardiovascular disease among type 2 diabetic patients. ***Diabetes Care*** 2006; 29: 722-724.
26. Scragg R, Holdaway I, Singh V, Metcalf P, Baker J, Dryson E. Serum 25-hydroxyvitamin D3 levels decreased in impaired glucose tolerance and diabetes mellitus. ***Diabetes Res Clin Pract*** 1995; 27: 181-188.
27. 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: 738-741.
28. Reis JP, von Mühlen D, Miller ER 3rd. Relation of 25-hydroxyvitamin D and parathyroid hormone levels with metabolic syndrome among US adults. ***Eur J Endocrinol*** 2008; 159: 41-48.
29. Buffington C, Walker B, Cowan GS Jr, Scruggs D. Vitamin D Deficiency in the Morbidly Obese. ***Obes Surg*** 1993; 3: 421-424.
30. Reis JP, von Mühlen D, Kritz-Silverstein D, Wingard DL, Barrett-Connor E. Vitamin D, parathyroid hormone levels, and the prevalence of metabolic syndrome in community-dwelling older adults. ***Diabetes Care*** 2007; 30: 1549-1555.
31. Peterson CA, Heffernan ME. Serum tumor necrosis factor-alpha concentrations are negatively correlated with serum 25(OH)D concentrations in healthy women. ***J Inflamm (Lond)*** 2008; 5: 10.
32. de Boer IH, Ioannou GN, Kestenbaum B, Brunzell JD, Weiss NS. 25-Hydroxyvitamin D levels and albuminuria in the Third National Health and Nutrition Examination Survey (NHANES III). ***Am J Kidney Dis*** 2007; 50: 69-77.

33. Jager A, Kostense PJ, Ruhé HG, Heine RJ, Nijpels G, Dekker JM, et al. Microalbuminuria and peripheral arterial disease are independent predictors of cardiovascular and all-cause mortality, especially among hypertensive subjects: five-year follow-up of the Hoorn Study. ***Arterioscler Thromb Vasc Biol*** 1999; 19: 617-624.
34. Diercks GF, Stroes ES, van Boven AJ, van Roon AM, Hillege HL, de Jong PE, et al. Urinary albumin excretion is related to cardiovascular risk indicators, not to flow-mediated vasodilation, in apparently healthy subjects. ***Atherosclerosis*** 2002; 163: 121-126.
35. Nakamura M, Onoda T, Itai K, Ohsawa M, Satou K, Sakai T, et al. Association between serum C-reactive protein levels and microalbuminuria: a population-based cross-sectional study in northern Iwate, Japan. ***Intern Med*** 2004; 43: 919-925.
36. 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: 517-522.
37. Baynes KC, Boucher BJ, Feskens EJ, Kromhout D. Vitamin D, glucose tolerance and insulinaemia in elderly men. ***Diabetologia*** 1997; 40: 344-347.
38. Wang TJ, Pencina MJ, Booth SL, Jacques PF, Ingelsson E, Lanier K, et al. Vitamin D deficiency and risk of cardiovascular disease. ***Circulation*** 2008; 117: 503-511.
39. Michos ED, Melamed ML. Vitamin D and cardiovascular disease risk. ***Curr Opin Clin Nutr Metab Care*** 2008; 11: 7-12.
40. Pérez-Castrillón JL, Justo I, Sanz A, De Luis D, Dueñas A. Effect of angiotensin converting enzyme inhibitors on 1.25-(OH)2 D levels of hypertensive patients. Relationship with ACE polymorphisms. ***Horm Metab Res*** 2006; 38: 812-816.
41. Parekh N, Chappell RJ, Millen AE, Albert DM, Mares JA. Association between vitamin D and age-related macular degeneration in the Third National Health and Nutrition Examination Survey, 1988 through 1994. ***Arch Ophthalmol*** 2007; 125: 661-669.

Statistics

Excerpts from the Uniform Requirements for Manuscripts Submitted to Biomedical Journals updated November 2003.
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Describe statistical methods with enough detail to enable a knowledgeable reader with access to the original data to verify the reported results. When possible, quantify findings and present them with appropriate indicators of measurement error or uncertainty (such as confidence intervals). Avoid relying solely on statistical hypothesis testing, such as the use of *P* values, which fails to convey important information about effect size. References for the design of the study and statistical methods should be to standard works when possible (with pages stated). Define statistical terms, abbreviations, and most symbols. Specify the computer software used.