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C-REACTIVE PROTEIN AND FIBRINOGEN OF BEDRIDDEN OLDER PATIENTS IN A SIX-MONTH VITAMIN D SUPPLEMENTATION TRIAL

M.P. BJORKMAN[1], A.J. SORVA[2], R.S. TILVIS[1]

1. Clinics of Internal Medicine and Geriatrics, Helsinki University Central Hospital, Helsinki, Finland; 2. Department of Long-Term Care, Helsinki Health Center, Helsinki, Finland.
Correspondence: Mikko Björkman, Tel. +358 50 341 4022, Fax: +358 9 4717 4693, Email: mikko.bjorkman@helsinki.fi

Abstract: *Objective:* To elucidate the association between vitamin D status, C-reactive protein (CRP) and fibrinogen. *Design:* Secondary analysis of a randomised double-blind placebo controlled trial. *Setting:* Four long-term care hospitals (1215 beds) in Helsinki, Finland. *Participants:* 218 long-term inpatients aged over 65 years. Intervention: Eligible patients (n = 218) were randomized to receive 0 IU/d, 400 IU/d, or 1200 IU/d cholecalciferol for six months. *Methods:* Plasma 25-hydroxyvitamin D (25-OHD), parathyroid hormone (PTH), high sensitive CRP, fibrinogen, amino-terminal propeptide of type I procollagen (PINP), and carboxy-terminal telopeptide of type I collagen (ICTP) were measured. *Results:* The patients were aged (84.5 ± 7.5 years), vitamin D deficient (25-OHD = 23 ± 10 nmol/l), chronically bedridden and in stable general condition. The mean baseline CRP and fibrinogen were 10.86 mg/l (0.12 mg/l - 125.00 mg/l) and 4,7 g/l (2.3 g/l - 8.6 g/l), respectively. CRP correlated with ICTP (r = 0.217, p = 0.001), but not with vitamin D status. Supplementation significantly increased 25-OHD concentrations, but the changes in CRP and fibrinogen were insignificant and inconsistent. The post-trial CRP concentrations (0.23 mg/l -138.00 mg/l) correlated with ICTP (r = 0.156, p < 0.001), but no association was found with vitamin D status. The baseline and post-trial fibrinogen correlated with CRP, only. *Conclusions:* CRP concentrations are associated with bone turnover, but not with vitamin D status, and vitamin D supplementation has no major effect on CRP or fibrinogen concentrations in bedridden older patients.

Key words: Vitamin D, C-reactive protein, fibrinogen, acute phase response, aged.

Introduction

Experimental and clinical evidence is accumulating on the various nonskeletal effects of vitamin D deficiency (1). Low 25-hydroxyvitamin D (25-OHD) concentrations are inversely associated with several diseases with known or putative inflammatory etiology, such as rheumatoid arthritis, metabolic syndrome, type II diabetes, cardiovascular diseases, and even with some cancers (1). Hypovitaminosis D has been suggested to cause mild acute phase response resulting in elevated concentrations of C-reactive protein (CRP), several haemostatic factors and different proinflammatory cytokines (1-4). In several cross-sectional studies on selected patient groups and healthy subjects CRP has been inversely associated with 1,25-hydroxyvitamin D (1,25-OHD) (5-9) as well as with serum 25-hydroxyvitamin D (25-OHD) (10-12). Similar associations have also been reported for fibrinogen (11, 13). Vitamin D deficiency, particularly common in the elderly, is also known to result in elevated parathyroid hormone (PTH) concentrations. Interestingly, it has been proposed that elevated PTH concentrations, promote the acute phase response (3). Thus, vitamin D supplementation tempering harmful increase in parathyroid activity could attenuate the chronic inflammation and thus be useful in prevention of related diseases. Improving vitamin D status definitely decreases parathyroid over-activity (1), but the effects of vitamin D supplementation on the acute phase response are scarcely documented (12, 14-18).

We studied the effects of vitamin D supplementation on CRP and fibrinogen in a secondary analysis of a randomised controlled double-blind vitamin D supplementation trial on frail hospitalized older patients. In addition, we evaluated correlates of these proteins with serum vitamin D concentrations.

Subjects and methods

Patients

The inpatients (n = 1215) of four municipal long-term care hospitals in Helsinki were screened in 2005 to participated in a randomized double blind controlled vitamin D supplementation trial (19). Briefly, the inclusion criteria were age over 65 years, chronically impaired mobility, stable general condition, and no known present or recent disease (other than osteoporosis) or medication affecting calcium or bone metabolism. After baseline laboratory analyses patients with markedly elevated creatinine (> 125 µmol/l), hypercalcaemia (ionised calcium > 1.32 mmol/l), hypo- (thyrotropin > 5.3 mU/l) or hyperthyroidism (thyrotropin < 0.2 mU/l) were also excluded.

Intervention

Eligible patients (n = 218) were successfully randomised into three groups with similar baseline characteristics. These treatments groups I (n = 68), II (n = 77) , and III (n = 73) were supplemented with cholecalciferol (Vigantol®, Merck KGaA, Darmstadt, Germany 20 000 IU/ml in Migliol® oil) in doses of 0 ug, 140 µg, or 420 µg (groups I,II,III) every two weeks,

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equivalent with average daily intakes of 0 IU, 400 IU, or 1200 IU, respectively. Identical diluted volumes were used. Both the patients and ward nurses were blinded to the content of the bottles as well as the group labels (I, II, III). Before the start of the intervention, the use of dairy products was roughly evaluated to be insufficient among 40 patients, who received a daily calcium carbonate substitution of 500 mg during the intervention. Three other patients also received a previous daily medication of 500 mg calcium carbonate at baseline, which they continued to receive through the intervention. The study protocol was approved by the local ethics committee and all participants provided an informed consent before the start of the trial. If the patient was severely cognitively impaired, the informed consent was obtained from the participant's surrogate or health care power of attorney.

Measurements

In addition to routine laboratory analyses, plasma intact PTH was determined at baseline and after six months. Plasma 25-OHD, amino-terminal propeptide of type I procollagen (PINP), carboxyl-terminal telopeptide of type I collagen (ICTP) concentrations were measured in addition to high sensitivity CRP from deep-frozen (-20˚C) EDTA-plasma samples collected at baseline and after six months. Fibrinogen was measured also from deep-frozen EDTA-plasma samples at the same time points in a subgroup of 108 random patients (fibrinogen subgroup). Vacuum tubes were used to draw venous blood from patients in a supine position the morning after an overnight fast.

High performance liquid chromatography was used to measure plasma 25-OHD concentrations (20). The method's limit of quantification, defined as the lowest concentration with a signal-to-noise ratio of 10:1, was 10 nmol/l. In 12 patients (6%), 25-OHD was under this measurement threshold (= 10 nmol/l); they received a value of 9 nmol/l. The within-assay CV was 5.6% at 21.6 nmol/l (n = 14) and 3.7% at 138 nmol/l (n = 15). The total CV was 7.3% at 16.4 nmol/l (n = 12) and 5.7% at 167 nmol/l (n = 15). The PTH concentrations were determined with a solid-phase, two-site chemiluminescent enzyme-labeled immunometric assay (immulite 2000 Intact PTH). The intra-assay coefficient of variation (CV) (n = 20) was 5.7% at 72 ng/l and 4.3% at 258 ng/l (21). The inter-assay CV (n = 10) was 6.3% at 54 ng/l and 8.8% at 387 ng/l.

The high sensitivity CRP was measured by particle-enhanced immunoturbidimetric assay (Ultrasensitive CRP Kit, Orion Diagnostica, Espoo, Finland) on the Hitachi 917 or Modular (Hitachi Ltd, Tokyo, Japan) automatic analyzers (reference range: men 0.05 - 2.50 mg/l, women 0.05 - 3.00 mg/l). The intra-assay coefficients of variation (CV) were 1.1 % at 0.70 mg/l and 0.7 % at 5.9 mg/l and the inter-assay coefficients of variation were 6.3 % at 0.70 mg/l (n=22) and 2.2 % at 6.6 mg/l (n= 27). The method is accreditated by FINAS Accreditation (SFS-EN ISO/IEC 17025). Fibrinogen concentrations were analyzed by a modification of the Clauss method (Multifibren U trademark of Dade Behring Marburg GmbH, reference range 1.7 - 4.0 g/l).

Plasma ICTP concentrations were determined by radioimmonuassay (RIA) (22). The method yielded CVs between 3% and 8% for a wide range of concentrations. The concentrations of PINP were also analysed using RIA (23). The inter- and intra-assay CVs ranged from 3.1% to 9.3% (n = 191) for values within the reference intervals (mean ± 2SD) for intact PINP in serum, which ranged from 19 to 84 µg/l for women and from 20 to 76 µg/l for men.

Estimated glomerular filtration rate (eGFR) was calculated by Cockcroft-Gault equation (24).

Statistical analysis

The data were analysed using Windows SPSS, release 15.0 (SPSS for Windows, Chicago: SPSS Inc.). Patients were categorized into three groups by CRP (< 3 mg/l, 3-10 mg/l, and > 10 mg/l). CRP values > 3 mg/l are frequently used to indicate an increased risk of adverse outcomes (25) while values > 10 mg/l indicate clinically relevant inflammation (26). The frequencies and their levels of significance for dichotomous variables were determined by the Chi-Square test. The analysis of variance was carried out by the One-Way ANOVA procedure. In calculating the Pearson's bivariate correlation coefficients and their level of significance, a natural logarithmic transformation of the variable was used if the distribution was not normal. A series of multivariate linear models were created, into which each predictor was entered one by one.

Results

Characteristics of patients

The patients (n = 218) were aged (mean age = 84.5 ± 7.5 years, range 65-104 years), vitamin D deficient (mean 25-OHD = 23 ± 10 nmol/l, range from 9 nmol/l to 60 nmol/l), and chronically bedridden. However, despite low 25-OHD levels the upper laboratory reference value (73 ng/l) of PTH (mean PTH = 61 ± 36 ng/l, range from 12 ng/l to 213 ng/l) was exceeded in 23% of patients, only. CRP and fibrinogen concentrations varied from 0.12 mg/l to 125.00 mg/l and from 2.3 g/l to 8.6 g/l, the mean values being 10.86 mg/l and 4.7 g/l, respectively. Up to 72.5% (168/218) and 48.1% (56/108) of patients had CRP levels below 10 mg/l and fibrinogen levels below 4.0 g/l, respectively. The ICTP concentrations appeared to increase and those of albumin to decrease with increasing CRP (Table 1). When patients were stratified by median fibrinogen concentration (4,7 g/l) the characteristics were similar with one exception, eGFR was higher (71 ml/min vs. 59 ml/min, p = 0.030) in subjects with elevated fibrinogen.

Baseline correlations of CRP and fibrinogen

Neither CRP nor fibrinogen correlated with concentrations of 25-OHD or PTH at baseline and fibrinogen correlated significantly with CRP (r = 0.632, p < 0.001), only (Table 2). CRP correlated also with albumin (r = -0.271, p < 0.001) and ICTP (r = 0.217, p = 0.001).

Table 1
Characteristics of patients by categories of high sensitive C-reactive protein (CRP)

Variables, mean ± SD	CRP < 3 mg/l	CRP 3-10 mg/l	CRP > 10 mg/l	P-value
Number	71	87	60	
Female, %	81.7	85.1	76.7	0.434
Age, years	84.0 ± 7.7	84.6 ± 6.6	85.2 ± 8.3	0.667
Movement in bed*, %	25.4	28.7	40.0	0.170
25-hydroxyvitamin D, nmol/l	23.4 ± 10.6	22.3 ± 9.5	22.8 ± 10.3	0.803
Parathyroid hormone, ng/l	62.7 ± 34.4	62.2 ± 38.2	57.0 ± 33.6	0.610
ICTP[2], g/l	7.18 ± 2.54	8.07 ± 3.43	8.83 ± 3.69	0.015
PINP[3], g/l	57.21 ± 26.74	52.53 ± 20.59	49.04 ± 18.02	0.108
Ionised calcium, mmol/l	1.23 ± 0.04	1.23 ± 0.04	1.21 ± 0.04	0.074
Total calcium, mmol/l	2.32 ± 0.09	2.34 ± 0.10	2.31 ± 0.10	0.260
Phosphate, mmol/l	1.01 ± 0.14	1.01 ± 0.12	1.00 ± 0.15	0.832
Creatinine, mol/l	59.4 ± 19.9	59.8 ± 18.9	64.2 ± 20.3	0.310
GFR[4], ml/min	64.0 ± 24.3	66.2 ± 28.3	62.7 ± 25.9	0.729
Albumin, g/l	34.2 ± 3.4	33.6 ± 3.2	32.1 ± 3.2	0.001
Fibrinogen[1], g/l	4.1 ± 0.8	4.5 ± 0.9	5.5 ± 0.9	<0.001

* Patients able to change position in bed with the physical assistance of one person; 1. Subgroup of 108 patients (group 1 n = 33, group II n = 34, group III n = 41); 2. Carboxyl-terminal telopeptide of type I collagen (ICTP); 3. Amino-terminal propeptide of type I procollagen (PINP); 4. Glomerular filtration rate estimated by Cockcroft-Gault equation

Table 2
Correlations of high sensitive C-reactive protein (CRP) and fibrinogen

	Baseline (N = 218)		Post trial (N = 181*)	
	CRP[1], mg/l	Fibrinogen, g/l	CRP[1], mg/l	Fibrinogen, g/l
CRP[1]	1.000[2]	0.632[2]	1.000[2]	0.533[2]
25-hydroxyvitamin D, nmol/l	-0.061	-0.079	-0.043	-0.113
Parathyroid hormone[1], ng/l	-0.052	-0.118	0.025	0.063
ICTP, g/l	0.217[2]	-0.036	0.156§	-0.074
PINP, g/l	-0.075	0.063	-0.136	0.103
Ionized calcium, mmol/l	-0.114	-0.008	-0.230[2]	-0.061
Albumin, g/l	-0.271[2]	-0.056	-0.235[2]	0.017

* One patient with +11 SD increase in CRP was excluded from post supplementation analyses; 1. Natural logarithmic transformation of the variable was used; 2. Correlation is significant at the 0.01 level (2-tailed); § Correlation is significant at the 0.05 level (2-tailed).

Effects of vitamin D supplementation

The randomization of patients succeeded well and the six-month vitamin D supplementation was successfully completed by 83.5% (182/218) of patients (Table 3). The completion rate was similar among the three groups ($p = 0.262$) and all dropouts died during the six-month intervention. The supplementation significantly increased 25-OHD in dose-dependent fashion ($p < 0.001$) and decreased also the concentrations of PTH ($p = 0.021$). The mean changes in CRP (+33% group I, -13% group II, +50%, $p = 0.523$) and fibrinogen (+6.7% group I, -3.8% group II, +6.9% group III, $p = 0.208$) were insignificant and inconsistent among the three intervention groups.

Correlations of CRP and fibrinogen at six months

One patient with a marked increase in CRP concentration that was widely outside (+11 SD) the range of all other patients was excluded before calculating the correlations coefficients. Again, neither CRP nor fibrinogen correlated with 25-OHD or PTH (Table 2). The post trial CRP (mean 10.42 ± 15.41, range 0.23-138.00 mg/l) correlated with ICTP ($r = 0.156$, $p < 0.001$), albumin ($r = -0.299$, $p < 0.001$) and ionized calcium ($r = -0.230$, $p = 0.002$). No new correlates were found for fibrinogen after the intervention.

Consistency of the observations

Subgroup analyses showed that the effects of vitamin D supplementation on CRP and fibrinogen concentrations were insignificant and inconsistent in all CRP categories as well as in patients with varying fibrinogen concentrations. Furthermore, 25-OHD concentration did not emerge as a significant predictor of CRP after controlling for age, sex, BMI, ICTP, albumin, and fibrinogen in the multiple regression analysis when baseline and six-month data were combined. However, the significance of the baseline and six-month correlations between CRP, ICTP, albumin and ionized calcium was lost after exclusion of patients with clinically relevant inflammation (CRP > 10 mg/l).

Discussion

In this secondary analysis of a randomized double-blind placebo controlled trial in frail hospitalized older patients vitamin D status was associated neither with CRP nor with fibrinogen, neither at baseline nor after six-month vitamin D supplementation. Nor did the supplementation markedly affect the plasma concentrations of these indicators of inflammation.

The baseline as well as the six-month cross-sectional data does not support the results of the earlier studies, in which 25-OHD has been inversely associated with CRP (10-12) and fibrinogen (11, 13) in selected patient groups. The concentrations of 1,25-OHD were not determined in the present study, because the 1,25-OHD concentrations are known to be a poor indicator of vitamin D status (1, 27). Thus, earlier reports on the inverse association between CRP and 1,25-OHD in stead of 25-OHD (5-9) are likely to reflect involvement of other diseases not related to vitamin D status.

To the best of our knowledge this is the first large scale randomized placebo controlled trial on aged patients in stable general condition that has addressed the effects of vitamin D supplementation in different doses on CRP and fibrinogen. The results are also supported by a previous and smaller randomized controlled trial on younger patients (n = 123, age 50-63 years) with severe congestive heart failure, in which the changes in CRP concentrations were also minor and insignificant after nine-month single dose (2000 IU/d) cholecalciferol (15). Furthermore, in three earlier uncontrolled trials on postmenopausal women, CRP or fibrinogen concentrations were not decreased by up to two-year vitamin D supplementation in daily doses of 250 IU to 800 IU (16-18). It should be also noted that the positive effects of vitamin D supplementation on CRP and fibrinogen concentrations have been characteristically demonstrated in small and selected

Table 3
Baseline concentrations and changes in 25-hydroxyvitamin D (25-OHD), parathyroid hormone (PTH), high sensitive C-reactive protein (CRP) and fibrinogen in subjects completing the six-month vitamin D supplementation trial

Variables, mean (range)	Group I Placebo	II 400 IU/d	III 1200 IU/d	P-value
All patients (N = 182):				
Number	59	60	63	
Baseline 25-OHD, nmol/l	23.8 (9-59)	21.1 (9-60)	23.5 (9-60)	0.258
Change in 25-OHD, nmol/l	1.9 (-34-33)	26.5 (-8-67)	49.1 (5-85)	<0.001
Baseline PTH, ng/l	61.6 (12-162)	59.1 (16-202)	56.1 (17-213)	0.644
Change in PTH, ng/l	5.2 (-54-63)	-4.2 (-48-80)	-4.7 (-58-77)	0.021
Baseline CRP, mg/l	8.71 (0.53-45.47)	10.2 (0.47-125.00)	11.35 (0.12-112.91)	0.685
High baseline CRP*, %	30.5	23.3	27.0	0.678
Change in CRP, mg/l	2.93 (-29.92-132.71)	-1.60 (-121.32-58.59)	5.80 (-96.75-395.62)	0.523
Fibrinogen subgroup (N = 86):				
Number	28	24	34	
Baseline 25-OHD, nmol/l	29.1 (9-59)	26.9 (12-60)	27.3 (9-48)	0.685
Change in 25-OHD, nmol/l	-2.0 (-34-17)	20.4 (-8-45)	42.7 (5-85)	<0.001
Baseline PTH, ng/l	53.4 (12-130)	51.0 (16-98)	52.4 (17-131)	0.952
Change in PTH, ng/l	1.5 (-54-45)	-2.0 (-32-29)	-2.7 (-53-60)	0.709
Baseline fibrinogen[1], g/l	4.6 (2.6-7.4)	4.8 (3.7-8.6)	4.7 (2.3-7.6)	0.762
High baseline fibrinogen[1], %	50.0	50.0	52.9	0.965
Change in fibrinogen, g/l	0.4 (-1.9-1.3)	-0.2 (-3.4-1.1)	0.3 (-3.3-4.6)	0.184

* CRP > 10 mg/l; 1. Fibrinogen > 4.0 g/l

patient groups. A significant 23% decrease in CRP concentrations (from 6.12 mg/l to 4.71 mg/l) was shown in an uncontrolled one-year cholecalciferol supplementation trial on 24 healthy British vitamin D deficient adults of Bangladeshi origin, in which 25-OHD concentration increased from 21 nmol/l to 35 nmol/l and PTH decreased from 44.3 ng/l to 37.8 ng/l (12). The same study also reported a tendency for fibrinogen concentrations to fall in the high-dose supplementation group (three monthly injections of 50000 IU cholecalciferol), but detailed data was not provided. Furthermore, a significantly more pronounced decrease in initially elevated CRP concentrations (mean baseline CRP 174 mg/l) in dose-dependent fashion has been observed in 22 critically ill emergency ward patients (age 36-73 years) during the first week, when supplemented with either 200 IU/d or 500 IU/d and compared to age-, gender-, and BMI-matched controls (14).

Given the proposed mechanism for the adverse effects of vitamin D deficiency (3), it can also be discussed whether and to what extent the lacking effect of vitamin D supplementation on CRP and fibrinogen is resulted by the minor decrease in PTH concentrations observed in the present study. However, no association was found between PTH and these proteins at baseline or even after six-month vitamin D supplementation, suggesting a minor involvement of PTH. In fact, the concentrations of CRP and fibrinogen tended to increase in patients with decreased in PTH concentration after vitamin D supplementation (data not shown). However, the results of the present study do not rule out the possibility that vitamin D supplementation may increase the production of anti-inflammatory cytokines and suppress other markers of inflammation (15) or thrombogenicity not measured in this study (12, 28).

The design and sample size are major strengths of this study despite the fact that the power calculations were not originally made for markers of inflammation, because interest was laid on PTH and bone turnover. However, the consistent lack of association between 25-OHD and CRP concentrations at baseline as well as after vitamin D supplementation together with the inconsistent changes, suggest that the sample size is not likely to cause any major bias to the results in terms of CRP concentrations. However, fibrinogen was measured in relatively small number of patients and larger sample size may be required.

This study also showed a significant positive correlation between CRP and ICTP concentrations at baseline as well as after six-month vitamin D supplementation. However, this significance was lost after exclusion of patients with elevated CRP concentrations (> 10 mg/l), suggesting that increased bone resorption is characteristic for clinically relevant inflammation. In fact, elevated CRP and ICTP concentrations in early rheumatoid arthritis have been shown to predict more progressive course of disease (29). Furthermore, elevated ICTP and CRP concentrations have also been associated with myocardial ischemia in patients with chronic heart failure (30).

In conclusion, CRP concentrations are associated with bone turnover, but not with vitamin D status, and vitamin D supplementation has no major effect on CRP or fibrinogen concentrations in bedridden older patients.

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References

1. Holick MF. Vitamin D deficiency. N Engl J Med 2007;357:266-81.
2. Canning MO, Grotenhuis K, de Wit H, Ruwhof C, Drexhage HA. 1-alpha,25-Dihydroxyvitamin D3 (1,25(OH)(2)D(3)) hampers the maturation of fully active immature dendritic cells from monocytes. Eur J Endocrinol 2001;145:351–7.
3. 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;64:1022-6.
4. Zhu Y, Mahon BD, Froicu M, Cantorna MT. Calcium and 1alpha,25-dihydroxyvitamin D3 target the TNF-alpha pathway to suppress experimental inflammatory bowel disease. Eur J Immunol 2005;35:217–24.
5. Oelzner P, Müller A, Deschner F, et al. Relationship between disease activity and serum levels of vitamin D metabolites and PTH in rheumatoid arthritis. Calcif Tissue Int 1998;62:193-8.
6. Oelzner P, Franke S, Müller A, et al. Relationship between soluble markers of immune activation and bone turnover in post-menopausal women with rheumatoid arthritis. Rheumatology (Oxford) 1999;38:841-7.
7. Lange U, Teichmann J, Strunk J, Müller-Ladner U, Schmidt KL. Association of 1.25 vitamin D3 deficiency, disease activity and low bone mass in ankylosing spondylitis. Osteoporos Int. 2005;16:1999-2004.
8. Oelzner P, Lehmann G, Eidner T, et al. Hypercalcemia in rheumatoid arthritis: relationship with disease activity and bone metabolism. Rheumatol Int 2006;26:908-15.
9. Jorde R, Haug E, Figenschau Y, Hansen JB. Serum levels of vitamin D and haemostatic factors in healthy subjects: the Tromsø study. Acta Haematol 2007;117:91-97.
10. Patel S, Farragher T, Berry J, Bunn D, Silman A, Symmons D. Association between serum vitamin D metabolite levels and disease activity in patients with early inflammatory polyarthritis. Arthritis Rheum 2007;56:2143-9.
11. Targher G, Bertolini L, Padovani R, et al. Serum 25-hydroxyvitamin D3 concentrations and carotid artery intima-media thickness among type 2 diabetic patients. Clin Endocrinol (Oxf) 2006;65:593-7.
12. 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.
13. Landin-Wilhelmsen K, Wilhelmsen L, Wilske J, et al. Sunlight increases serum 25(OH) vitamin D concentration whereas 1,25(OH)2D3 is unaffected. Results from a general population study in Göteborg, Sweden (The WHO MONICA Project). Eur J Clin Nutr 1995;49:400-7.
14. Van den Berghe G, Van Roosbroeck D, Vanhove P, Wouters PJ, De Pourcq L, Bouillon R. Bone turnover in prolonged critical illness: effect of vitamin D. J Clin Endocrinol Metab 2003;88:4623-32.
15. 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.
16. Gannagé-Yared MH, Azoury M, Mansour I, Baddoura R, Halaby G, Naaman R. Effects of a short-term calcium and vitamin D treatment on serum cytokines, bone markers, insulin and lipid concentrations in healthy post-menopausal women. J Endocrinol Invest 2003;26:748-53.
17. McClung MR, Siris E, Cummings S, et al. Prevention of bone loss in postmenopausal women treated with lasofoxifene compared with raloxifene. Menopause 2006;13:377-86.
18. Atteritano M, Marini H, Minutoli L, et al. Effects of the phytoestrogen genistein on some predictors of cardiovascular risk in osteopenic, postmenopausal women: a two-year randomized, double-blind, placebo-controlled study. J Clin Endocrinol Metab 2007;92:3068-75.
19. Björkman M, Sorva A, Risteli J, Tilvis R. Vitamin D supplementation has minor effects on parathyroid hormone and bone turnover markers in vitamin D deficient bedridden older patients. Age Ageing 2008;37:25-31.
20. Turpeinen U, Hohenthal U, Stenman U-H. Determination of 25-Hydroxyvitamin D in Serum by HPLC and Immunoassay. Clin Chem 2003;49:1521-4.
21. Kao PC, Grant CS, Klee GG, Khosla S. Clinical performance of parathyroid hormone immunometric assays. Mayo Clin Proc 1992;67:637-45.
22. Risteli J, Elomaa I, Niemi S, Novamo A, Risteli L. Radioimmunoassay for the pyridinoline cross-linked carboxy-terminal telopeptide of type I collagen: a new serum marker of bone collagen degradation. Clin Chem 1993;39:635-40.
23. Melkko J, Kauppila S, Niemi S, et al. Immunoassay for the intact aminoterminal propeptide of human type I procollagen (PINP). Clin Chem 1996;42:947-54.
24. Levey AS, Coresh J, Balk E, et al. National Kidney Foundation. National Kidney Foundation practice guidelines for chronic kidney disease: evaluation, classification, and stratification. Ann Intern Med 2003;139:137-47.
25. Ridker PM, Cook N. Clinical usefulness of very high and very low levels of C-reactive protein across the full range of Framingham Risk Scores. Circulation 2004;109:1955–9.
26. Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. N Engl J Med 1999;340:448–54.
27. Holick MF. Vitamin D. The underappreciated D-lightful hormone that is important for skeletal and cellular health. Curr Opin Endocrinol Diabetes 2002;9:87–98.
28. Aihara K, Azuma H, Akaike M, et al. Disruption of nuclear vitamin D receptor gene causes enhanced thrombogenicity in mice. J Biol Chem 2004;279:35798-802.
29. Aman S, Paimela L, Leirisalo-Repo M, et al. Prediction of disease progression in early rheumatoid arthritis by ICTP, RF and CRP. A comparative 3-year follow-up study. Rheumatology (Oxford) 2000;39:1009-13.
30. Nishio Y, Sato Y, Taniguchi R, et al. Cardiac troponin T vs other biochemical markers in patients with congestive heart failure. Circ J 2007;71:631-5.