Original Article

Serum osteocalcin is associated with dietary vitamin D, body weight and serum magnesium in postmenopausal women with and without significant coronary artery disease

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Osteoporosis and atherosclerosis often present atypically in postmenopausal women, making clinical recognition difficult. Prospective studies suggest independent associations between bone mass and vascular calcification through vitamin D deficiency as an established predictor of both conditions. We aimed to examine the relationship between serum osteocalcin and vitamin D status in postmenopausal women with and without angiographic evidence of coronary artery disease (CAD). One hundred and eighty postmenopausal women undergoing coronary angiography were selected sequentially from the Catheterization unit of King Abdulaziz University Hospital. Socio-demographic, anthropometric parameters and dietary habits were measured. Biochemical variables were estimated in blood samples. Half of the postmenopausal women did not have significant CAD, 24% had significant CAD in a single and/or double coronary vessels, 26% had significant CAD in three coronary vessels. Mean serum vitamin D concentrations showed that vitamin D deficiency was a common finding in the whole population. Vitamin D and calcium intakes were uniformly low in the study cohort. Serum osteocalcin was significantly correlated with dietary vitamin D in all subgroups (r=-0.172, $p<0.05$) and positively correlated among the patients (r=0.269, $p=0.01$). Serum magnesium, alkaline phosphatase, dietary vitamin D, and body weight were independent variables of serum osteocalcin level. In conclusion, elevated levels of serum C reactive protein and vitamin D were associated with low serum osteocalcin levels. Therefore, osteocalcin may be a potential cardiovascular risk marker. However, further studies are needed to clarify the pathophysiological processes underlying the relationship between serum osteocalcin level and atherosclerosis parameters.

Key Words: vitamin D, osteocalcin, weight, postmenopausal women, coronary artery disease

INTRODUCTION

Osteoporosis is associated with cardiovascular disease (CVD) and it is known to influence overall mortality among postmenopausal women.[1] Although both diseases are viewed as separate entities that increase in prevalence with aging, there is evidence that there are some common pathophysiological mechanisms that lead to both conditions, including vitamin D deficiency.[2] Epidemiological studies have reported that serum levels of 25-hydroxy vitamin D (25(OH)D) are inversely associated with hypertension, diabetes, carotid atherosclerosis, and myocardial infarction risk.[3,4] The link between osteoporosis and atherosclerosis could potentially influence the therapeutic approach to both disorders, with vitamin D supplementation playing an important role in the prevention of both conditions. C reactive protein (CRP) is a serum marker of low grade inflammation and is associated with CVD.[5] It is possible that there is a poorly understood more direct link between CVD and low bone mineral density.[6,7] Biochemical markers of bone turnover are currently used for predicting the rate of bone loss and for assessing the risk of fractures in postmenopausal women. These markers may either reflect bone formation or resorption.[8] Osteocalcin is one of the bone formation markers that has several hormonal features and is secreted by osteoblasts.[9] Previous studies have shown that bone-associated proteins, such as osteocalcin, are present in atherosclerotic arteries[10] suggesting that these proteins could be directly associated with vascular diseases, but there is lack of understanding of the biological significance of circulating osteocalcin in inflammatory conditions.

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Vitamin D is derived from exposure of the skin to sunlight, from food, and from dietary supplements. Nonetheless, vitamin D insufficiency or deficiency persists in many parts of the world possibly due to nutritional deficits and perhaps to avoidance of sunlight and the use of sunscreens.[11] Furthermore, the elderly, and especially postmenopausal women, may be at risk of inadequate vitamin D because of limited outdoor activity, lower dietary intake, and reduced capacity of the skin to produce vitamin D.[12]

Osteoporosis and CVD, in particular coronary heart disease (CHD), often present atypically in postmenopausal women, making clinical recognition difficult. Thus, there is a need for surrogate markers of both conditions among high risk individuals. Prospective studies suggest independent associations between bone mass and vascular calcification through vitamin D deficiency as an established predictor of atherosclerosis and osteoporosis.[1]

Several recent studies have shown a high prevalence of vitamin D deficiency in Saudi Arabia as well as other neighbouring countries.[13-15] Because the implications of vitamin D insufficiency, or deficiency, for cardiovascular health could be substantial, we aimed to examine the relationship between serum osteocalcin and vitamin D status in postmenopausal women with and without angiographic evidence of coronary artery disease (CAD).

METHODS

The subjects were selected sequentially from the Catheterization laboratory of the Department of Internal Medicine at King Abdulaziz University Hospital (KAUH). The primary exclusion criteria were the presence of an acute major cardiovascular event in the previous six months, diabetes mellitus, vascular diseases, chronic hepatic or renal diseases, thyroid dysfunction, recent or ongoing infection, cancer disease, established osteoporosis, endocrine disorders, or on any form of drug treatment with possible effect on bone metabolism, or treatment with statins, aspirin, antioxidants, anti-inflammatory drugs, vitamin D or Ca supplementations. All study participants gave their informed consent, and the ethics committee at the KAUH approved the study.

Postmenopausal status was defined as no natural menses for ≥1 year and serum follicle-stimulating hormone level >40 IU/L.[16] Angiography was performed for evaluation of CAD in all cases. CAD was classified as being clinically significant if luminal narrowing ≥50% was identified in a major coronary artery. The severity of angiographically defined disease was scored by a cardiologist as previously described.[17]

The study participants were asked about their age, age of menopause onset, marital status, socioeconomic status, family history of osteoporosis and CVD, smoking habits, physical activity level, and frequency of exposure to sunlight. Smoking habit was categorized as non-smoker, former smoker, and current smoker. Physical activity was self-graded by the participant according to the number of episodes of exercise undertaken per week and were categorized as active (≥3 times/week) or inactive (<3 times/week) according to the recommendations of the American Heart Association consensus statement on primary prevention of coronary diseases and from the USA Surgeon General's report.[18]

Dietary total energy and nutrients over the previous year were assessed using a previously validated semi-quantitative food frequency questionnaire (FFQ).[19] The nutrient database used was based on UK food composition tables together with food composition tables for use in East Asia and the United States handbook of food composition. The estimated dietary intake of all nutrients was calculated in terms of percentage recommended nutritional intake (%RNI for UK adults) for each individual, as there are no published data for a Saudi population. The most recent version of the UK dietary recommended values[20] was used to standardize the pattern of nutrient intake. Nutrient intakes were computed by multiplying the frequency of consumption of each unit of food from the FFQ by the nutrient content of the specified portion size. To control for total energy intake, all nutrients were adjusted for total energy intake by using the regression residual method.[21]

Height and weight were measured with the patient standing in light clothes and without shoes. Body mass index (BMI) was calculated as body weight divided by height squared (kg/m^2). Waist circumference (WC) was measured using a tape measure at the mid-point between the lower costal margin and the level of the anterior superior iliac crest to the nearest 0.1 cm. Hip circumference (HC) was measured at the level of the greater trochanters. Waist-to-hip ratio (WHR) was calculated as the ratio of waist and hip circumferences. Arterial blood pressure was measured using the right arm (average of 3 measurements having the patient seated and rested for 5 minutes).

Blood samples were drawn from each subject after fasting for at least 12 h and an overnight rest. All the samples were stored at -80°C until analytical measurements were performed. Serum calcium, phosphate, bone specific alkaline phosphatase (ALP) and albumin were measured using kits and reagents supplied by Ortho-Clinical Diagnostics, USA using Vitros 250 Chemistry System Autoanalyzer (Ortho-Clinical Diagnostics, Johnson & Johnson Co., USA). Serum 25(OH)D and intact parathyroid hormone (PTH) concentrations were measured by direct competitive and a direct sandwich chemiluminescence immunoassays using LIASON autoanalyzer, respectively (DiaSorin Inc, Stillwater, MN, USA). Subjects with serum circulating levels <50 nmol/L were considered as having 25(OH)D deficiency.[22] Deficiency was further defined as severe deficiency as <12.5 nmol/L, moderate deficiency as 12.5-25 nmol/L, and mild deficiency as 25-50 nmol/L. Serum-intact osteocalcin was measured using ECLIA Elecsys autoanalyzer (Roche Diagnostics GmbH, D-68298 Mannheim, Germany). Serum CRP was measured by a high sensitivity competitive immunoassay kit (Calbiochem, La Jolla, CA).

Descriptive statistics are presented as mean ± standard deviation (SD). In the case of variables that were not normally distributed, log transformation was performed prior to analysis. Comparisons between the 4 subgroups were carried out by ANOVA test for normal distributed variables and by Kruskal-Wallis test for nonparametric variables. A χ^2 test was used for comparison of categorical data. Relationships among variables were sought by Pearson's and Spearman's correlation coefficient. Step-

wise multiple regression model was performed to identify independent factors affecting dietary vitamin D level.

A p value <0.05, was considered statistically significant. All the statistical analyses were performed using the Statistical Package for Social Sciences (SPSS/Windows version 21.0, SPSS Inc., Chicago IL, USA).

RESULTS

In a total of 180 postmenopausal women undergoing coronary angiography, 50% did not have significant CAD (<50% luminal stenosis), 24% had significant CAD (≥50% luminal stenosis) in a single and/or double coronary vessels, 26% had significant CAD in three coronary vessels. The study participants were divided into 4 groups according to quartiles of serum osteocalcin level.

Socio-demographic characteristics of the study cohort are presented in Table 1. A sedentary lifestyle was reported by the majority of study participants. Most participants were poorly educated housewives, living in apartments, who were physically inactive, and had limited exposure to ultraviolet sunlight. In addition, obesity was highly prevalent among the whole study cohort as indicated by mean values of anthropometric measures (i.e.) BMI, WC, and WHR. Furthermore, WC and WHR values varied significantly across the groups (p<0.05).

Table 2 shows that serum magnesium and intact PTH levels tended to decline with serum osteocalcin values (p<0.01). Mean serum vitamin D concentrations showed that vitamin D deficiency was a very common finding in the whole population based on a threshold of <50 nmol/L. The levels of serum 25(OH)D <12.5 nmol/L, 12.5-25 nmol/L, and 25-50 nmol/L were designated as severe, mild, and moderate vitamin D deficiencies, respectively. However, the 4 groups did not differ significantly for serum vitamin D level (p>0.05) but the overall prevalence of hypovitaminosis D was 69%.

Although there was no significant difference in serum CRP levels across the osteocalcin subgroups, about half

Table 1. Socio-demographic characteristics in 180 postmenopausal women classified according to quartiles of serum osteocalcin level

	Serum osteocalcin quartiles				p
	1st Quartile	2nd Quartile	3rd Quartile	4th Quartile	
Age (years)	61.9±0.9	63.9±1.4	62.5±1.3	64.1±1.2	NS
Menopausal age (years)	50.8±0.8	51.2±0.8	49.6±0.8	51.6±0.8	NS
Marital status					
Single	0 (0)	0 (0)	0 (0)	1 (2)	
Married	25 (56)	26 (58)	23 (51)	18 (40)	
Widowed	20 (44)	18 (40)	20 (44)	22 (49)	NS
Divorced	0 (0)	1 (2)	2 (4)	4 (9)	
Education level					
Illiterate	22 (49)	29 (64)	30 (67)	28 (62)	
Intermediate	14 (31)	9 (20)	10 (22)	12 (27)	
High school	8 (18)	3 (7)	2 (4)	3 (7)	NS
University	1 (2)	4 (9)	3 (7)	2 (4)	
Occupation					
House wife	45 (100)	42 (93)	42 (93)	45 (100)	
Administrative	0 (0)	2 (4)	2 (4)	0 (0)	NS
Director/ physician	0 (0)	1 (2)	1 (2)	0 (0)	
Type of residency					
Traditional housing	6 (13)	16 (36)	13 (29)	12 (27)	
Apartment	34 (76)	24 (53)	30 (67)	30 (67)	NS
Villa	5 (11)	5(11)	2 (4)	3 (7)	
Frequency of sunlight exposure					
<1 time	32 (71)	34 (76)	34 (76)	35 (78)	
1-2 times	9 (20)	6 (13)	7 (16)	7 (16)	NS
≥3 times	4 (9)	5 (11)	4 (9)	3 (7)	
Physical activity					
<1 time	31 (69)	20 (44)	28 (62)	28 (62)	
1-2 times	5 (11)	7 (16)	3 (7)	7 (16)	NS
≥3 times	9 (20)	18 (40)	14 (31)	10 (22)	
Veiling types					
Covering hair only	13 (29)	18 (40)	12 (27)	15 (33)	
Eyes shown only	32 (71)	27 (60)	31 (69)	30 (67)	NS
Full cover	0 (0)	0 (0)	2 (4)	0 (0)	
Smoking status					
Non-smoker	42 (93)	43 (96)	39 (87)	43 (96)	
Former smoker	1 (2)	1 (2)	5 (11)	0 (0)	NS
Current smoker	2 (4)	1 (2)	1 (2)	2 (4)	
Soda consumption	16 (36)	22 (49)	16 (36)	18 (40)	NS
Coffee consumption	29 (64)	29 (64)	31 (69)	23 (51)	NS

Numeric data are presented as mean±SD and categorical data as number and percentage. Categorical data were compared by χ^2 test; continuous variables were compared by ANOVA or Kruskal-Wallis tests for non-normally distributed data. NS: not significant.

of the study participants in each quartile of serum osteocalcin were categorized as high coronary risk (>3 mg/L) according to the cut-off value of serum CRP level (Table 2).

The reported dietary intake of energy and nutrients between the subgroups is indicated in Table 3. The total energy intake and macronutrient profiles differed significantly across the serum osteocalcin quartiles (p<0.0001). Statistical significance remained even after the macronutrient intakes were adjusted for energy intake but disappeared following the calculation of the percentage of energy supplied by carbohydrates and total fat. Only protein intake expressed as the percent of energy intake retained statistical significance in between the serum osteocalcin subgroups (p<0.05). Patients in the lowest serum osteocalcin quartile had significantly lower intakes of energy, carbohydrates, total fat, protein, saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA) (crude and adjusted for total caloric level), cholesterol, vitamin D and calcium of all groups (p<0.01). Patients in the highest serum osteocalcin quartile had significantly higher intake of percentage of energy intake from protein than those in the first and second quartiles (p<0.01).

Vitamin D and calcium intakes were uniformly low in the study cohort (Table 3). All the participants had less than adequate levels (i.e.) less than the estimated average requirements (EAR) of vitamin D intake and half of them had <EAR value of dietary calcium intake. The number of subjects with a dietary intake of calcium <EAR value decreased with increasing quartile for serum osteocalcin (p<0.01).

Serum osteocalcin showed strong correlations with serum magnesium (r=0.373, p<0.0001), intact PTH (r=0.471, p<0.0001) and ALP (r=0.275, p<0.001) within the whole population (Table 4). Serum osteocalcin was inversely correlated with serum high-sensitivity C reactive protein (hsCRP) among the controls (r=-0.283, p<0.01) and was inversely correlated with serum vitamin D (r=-0.165, p<0.05), dietary vitamin D (r=-0.172, p<0.05), body weight (r=-0.168, p<0.05), BMI (r=-0.168, p<0.05), WC (r=-0.228, p<0.01) and WHR(r=-0.240, p<0.01) within the whole population. The increase in serum osteocalcin level with increasing levels of dietary vitamin D just reached statistical significance (Figure 1). Serum osteocalcin was significantly correlated with dietary vitamin D in all subgroups (r=-0.172, p<0.05) and positively correlated among the CAD patients (r=0.269,

Table 2. Clinical characteristics in 180 postmenopausal women classified according to quartiles of serum osteocalcin level

	Serum osteocalcin quartiles				p
	1st Quartile	2nd Quartile	3rd Quartile	4th Quartile	
Albumin (g/L)	40.4±0.5	40.8±0.4	40.6±0.4	39.6±0.6	NS
Calcium (mmol/L)	2.34±0.02	2.38±0.02	2.35±0.01	2.35±0.02	NS
Corrected-calcium	2.36±0.02	2.39±0.02	2.36±0.01	2.36±0.02	NS
Phosphate (mmol/L)	1.35±0.03	1.32±0.02	1.34±0.03	1.35±0.03	NS
Magnesium (mmol/L)	0.77±0.01	0.79±0.02	0.83±0.01[¥]	0.86±0.01[¶§]	<0.0001
Intact PTH (pmol/L)	4.42±0.4	5.36±0.3	6.45±0.5[¥]	8.22±0.6[¶§#]	<0.01
ALP (U/L)	94.2±5.1	94.7±4.0[*]	97.03±4.8	113.6±4.9	NS
hsCRP (mg/L)	8.2±1.1	7.0±1.0	6.15±0.9	5.73±1.3	NS
hsCRP subgroups					
<1mg/L	7 (16)	9 (20)	5 (11)	16 (36)	
1-3mg/L	9 (20)	9 (20)	14 (31)	11 (24)	<0.05
>3mg/L	29 (64)	27 (60)	26 (58)	18 (40)	
Serum vitamin D (nmol/L)	29.1 (16.5-48.5)	32.6 (18.4-42.2)	27.1 (16.7-41.4)	23.3 (12.1-36.9)	NS
Serum vitamin D subgroups					
Mild deficiency	16 (36)	20 (44)	15 (33)	16 (36)	
Moderate deficiency	13 (29)	14 (31)	15 (33)	13 (29)	
Severe deficiency	6 (13)	3 (7)	6 (13)	12 (27)	<0.05
Body weight (Kg)	79.4±2.7	75.3±2.4	75.3±1.9	72.6±2.0	NS
Body height (cm)	152.6±0.9	152.4±1.0	152.5±0.9	152.0±1.1	NS
BMI (Kg/m^2)	33.8±0.9	32.4±0.9	32.4±0.8	31.4±0.8	NS
BMI subgroups					
Normal	0 (0)	2 (4)	2 (4)	3 (7)	
Overweight	16 (36)	17 (38)	12 (27)	18 (40)	NS
Obese	29 (64)	26 (58)	31 (69)	24 (53)	
WC (cm)	103.7±1.9[¥¶]	102.5±2.8	97.6±1.2	96.6±1.4	<0.05
HC (cm)	109.7±1.9	106.7±2.5	106.9±1.6	106.4±1.5	NS
WHR	0.95±0.01[¥¶]	0.97±0.03[†§]	0.92±0.01	0.91±0.02	<0.01
SBP (mmHg)	143.4±3.3	138.5±3.3	139.4±2.8	144.3±3.9	NS
DBP (mmHg)	76.7±1.8	77.2±1.8	76.4±1.9	78.1±1.9	NS

Meric data are presented as mean±SD and as median (IQR). Categorical data as number and percentage. Categorical data were compared by χ^2 test; continuous variables were compared by ANOVA or Kruskal-Wallis tests for non-normally distributed data. ALP: alkaline phosphatase, BMI: body mass index, DBP: diastolic blood pressure, HC: hip circumference, hsCRP: high sensitivity C reactive protein, NS: not significant, PTH: parathyroid hormone, SBP: systolic blood pressure, WC: waist circumference, WHR: waist-to-hip ratio. [*] p<0.05 (first & second quartiles), [¥] p<0.05 (first & third quartiles), [¶] p<0.05 (first & forth quartiles), [†] p<0.05(second & third quartiles), [§] p<0.05 (second & forth quartiles), [#] p<0.05 (third & forth quartiles).

p=0.01).

Multiple linear regression analysis was performed for serum osteocalcin as a continuous variable for biological markers potentially linked to cardiovascular risk, including dietary components (Table 5). Serum magnesium, ALP, dietary vitamin D, and body weight were independent variables associated with serum osteocalcin level. About 29.8% of the variation in serum osteocalcin level could be explained by this model.

DISCUSSION

Osteoporosis and CVD are now recognized as a global epidemic. Many authors suggest that hypovitaminosis D may increase the risk of both conditions through common

Table 3. Dietary intake values in 180 postmenopausal women classified according to quartiles of serum osteocalcin level

	RNI	Serum osteocalcin quartiles				p
		1st Quartile	2nd Quartile	3rd Quartile	4th Quartile	
Energy (Kcal)	1900 (19-74yr) 1810 (75+yr)	1801±89.7	1821±98.5	2142±92.2$^{¥†\#}$	1846±99.9	<0.05
Carbohydrate (gm)		206±11.7	203±12.3	243±11.6$^{¥†\#}$	212±10.1	<0.05
Energy-adjusted carbohydrate (gm)		206±0.13	206±0.15	206±0.14$^{¥†\#}$	206±0.15	<0.05
% of energy	55 %	45.3±0.73	44.3±0.75	45.1±0.73	46.8±0.91	NS
Total fat (gm)		79.6±3.53	84.4±4.43	94.9±4.17$^{¥†\#}$	82.8±5.28	<0.05
Energy-adjusted fat (gm)		82.1±0.14	82.1±0.15	82.6±0.14$^{¥†\#}$	82.1±0.15	<0.05
% of energy	30 %	40.5±0.82	42.0±0.85	40.2±0.73	39.6±0.97	NS
Protein (gm)		65.2±4.16	62.4±3.78	78.9±4.01$^{¥†\#}$	63.5±4.34	<0.01
Energy-adjusted protein (gm)		63.1±0.14	63.2±0.15	63.6±0.14$^{¥†\#}$	63.2±0.15	<0.05
% of energy	15 %	14.1±0.30	13.7±0.30	14.7±0.31$^{†\#}$	13.6±0.29	<0.05
SFA (gm)		23.8±1.47	24.1±1.32	27.3±1.52	24.0±1.82	<0.05
Energy-adjusted SFA (gm)		22.4±0.14	22.4±0.15	22.9±0.14$^{¥†\#}$	22.5±0.15	<0.05
% of energy	10%	13.1±0.43	13.4±0.44	12.6±0.37	12.6±0.45	NS
MUFA (gm)		24.0±1.13	26.0±1.43	29.2±1.42$^{¥}$	25.6±1.74	<0.05
Energy-adjusted MUFA (gm)		25.1±0.14	25.1±0.15	25.6±0.14$^{¥†\#}$	25.1±0.15	<0.05
% of energy	10%	13.6±0.33	14.4±0.33	13.7±0.30	13.5±0.38	NS
PUFA (gm)		24.4±1.04	26.7±1.54	29.4±1.23$^{¥\#}$	25.3±1.43	<0.05
Energy-adjusted PUFA (gm)		25.4±0.14	25.4±0.15	25.9±0.14$^{¥†\#}$	25.5±0.15	<0.05
% of energy	10%	14.2±0.49	14.8±0.49	14.2±0.48	13.8±0.56	NS
Fibre (gm)	18	17.7±0.89	18.1±1.12	20.2±0.77$^{†\#}$	17.2±0.90	<0.05
Cholesterol (mg)	200	196±19.2	197±26.8	297±28.5$^{¥\#†}$	200±22.5	<0.05
Calcium (mg)	1000 (19-50yr) 1200 (50+yr)	776±47.9	756±45.3	967±53.3$^{¥\#†}$	787±58.4	<0.05
Calcium intake <EAR		26 (58)	24 (53)	14 (31)	26 (58)	NS
Vitamin D intake (µg)	10 (19-50 yr) 15 (50+ yr)	0.67±0.05	0.74±0.11	0.98±0.09$^{¥†}$	0.85±0.09	<0.05
Vitamin D intake <EAR		45 (100)	45 (100)	45 (100)	45 (100)	NS

Umeric data are presented as mean±SD and categorical data as number and percentage. Categorical data were compared by χ^2 test; continuous variables were compared by ANOVA or Kruskal-Wallis tests for non-normally distributed data. EAR: estimated average requirements, MUFA: monounsaturated fatty acid, PUFA: polyunsaturated fatty acid, RNI: recommended nutrient intake, SFA: saturated fatty acid. * p<0.05 (first & second quartiles), $^{¥}$ p<0.05 (first & third quartiles), ¶ p<0.05 (first & forth quartiles), † p<0.05 (second & third quartiles), § p<0.05 (second & forth quartiles), $^{\#}$ p<0.05 (third & forth quartiles).

Table 4. Correlation between serum osteocalcin level and clinical characteristics in 180 postmenopausal women undergoing coronary angiography

	Whole population of postmenopausal women (n=180)		Postmenopausal women without CAD (n=90)		Postmenopausal women with CAD (n=90)	
	r	p	r	p	r	p
Serum hsCRP (mg/L)	-0.202	0.006	-0.283	0.007	-0.120	0.258
Magnesium (mmol/L)	0.373	<0.0001	0.383	<0.0001	0.362	<0.0001
Intact PTH (pmol/L)	0.471	<0.0001	0.534	<0.0001	0.348	0.001
ALP (U/L)	0.275	0.001	0.248	0.018	0.259	0.014
Weight	-0.168	0.024	-0.112	0.295	-0.184	0.082
BMI	-0.168	0.032	-0.172	0.106	-0.161	0.129
WC	-0.228	0.002	-0.190	0.073	-0.266	0.011
WHR	-0.240	0.001	-0.227	0.032	-0.228	0.031
Serum vitamin D (nmol/L)	-0.165	0.027	-0.149	0.161	-0.129	0.225
Dietary vitamin D (µg)	0.172	0.021	0.080	0.455	0.269	0.010

ALP: alkaline phosphatase, BMI: body mass index, hsCRP: high sensitivity C reactive protein, PTH: parathyroid hormone, WC: waist circumference, WHR: waist hip ratio.

Table 5. Multiple regression analysis between serum osteocalcin level and independent variables in 180 postmenopausal women undergoing coronary angiography

Dependent variable	Independent variables	β	*p*	95% CI for β	
				Lower limit	Upper limit
Serum osteocalcin					
	Magnesium (mmol/L)	0.213	0.001	13.6	55.7
	ALP (U/L)	0.185	0.006	0.027	0.155
	Dietary vitamin D (μg)	0.170	0.009	1.13	7.71
	Weight (Kg)	-0.191	0.004	-0.333	-0.066
Total R^2 = 29.8					

ALP: alkaline phosphatase, ß=standardized regression coefficient, R^2=percent variance explained by each variable. Stepwise variable inclusion with $p<0.05$ and exclusion with $p>0.10$. 95% CI: confidence intervals.

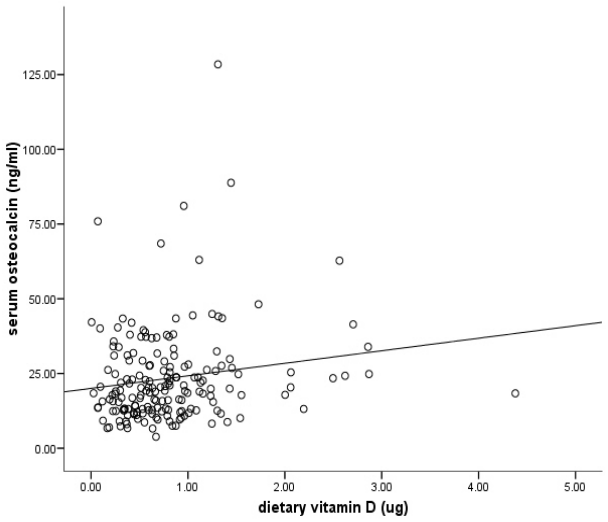

Figure 1. Scatter plot demonstrating correlation between circulating level of osteocalcin and dietary vitamin D level in 180 postmenopausal women undergoing coronary angiography (r=0.172, p=0.021).

pathophysiological mechanism especially among the elderly. Both ageing and obesity are characterized by a low-grade inflammatory state. The relationship between vitamin D and inflammation are equivocal.[23] Although, persons with lower serum vitamin D levels appear to be at increased cardiovascular risk,[12] it is unclear if 25(OH)D deficiency is related with prevalent cardiovascular diseases in Saudi Arabia.[24] The biological mechanisms by which vitamin D might protect against the development and progression of CVD has not been fully delineated. Among Saudi males there is a high prevalence of poor diet and high levels of markers of inflammation, suggesting that combined lifestyle risk factors are implicated in the pathogenesis of atherosclerosis.[25] Furthermore, confounding effects of dietary factors are yet unaccounted for in subjects without pre-existing CVD in clinical setting.[11] Not surprisingly, earlier studies that have documented poor vitamin D status, initially focused on the implications of this for bone health. Moreover, the increased PTH secretion that is consequent to low 25(OH)D, while it contributes importantly to low bone mineral density, may also have adverse consequences for insulin sensitivity, body composition, and vascular health.[26] Nevertheless, the estimated mean serum PTH level in the present cohort is reported to be within the reference range (1.59-6.89 pmol/L).

In this study, significantly lower serum magnesium, ALP, and intact PTH levels were found among postmenopausal women categorized in the lower osteocalcin quartiles compared with those in higher osteocalcin quartiles. After adjusting for potential confounders, only magnesium and ALP remained significantly correlated with serum osteocalcin levels. This suggests that the relationship of magnesium and ALP, as one of the bone turnover markers, with osteocalcin is independent of the other bone-related parameters. Total serum osteocalcin, which comprises both the carboxylated and the under carboxylated forms, has been utilized traditionally as a marker of bone formation or bone turnover. It has been reported that regulators of bone metabolism are also present in atherosclerotic arteries.[27] Thus, the vascular microenvironment possesses mechanisms similar to those in bone tissues to maintain mineral homeostasis. This direct relationship is yet to be elucidated, as the biological actions of osteocalcin are only partly understood.[28]

Low calcium intakes are known to increase the efficiency of calcium absorption.[29] However, this adaptive mechanism, because it is 1, 25-$(OH)_2$D dependent, may not be fully efficient when the 25-(OH)D levels are too low (below 50 nmol/L) to allow sufficient production of 1,25-$(OH)_2$D. The degree of serum vitamin D deficiency has been proposed to be very important in determining cardiovascular risk.[22] About two thirds of postmenopausal women in all quartiles of serum osteocalcin level were of mild and moderate vitamin D deficiency (Figure 1). However, mild hypovitaminosis D was not associated with incident hypertension or cardiovascular events in recent prospective studies.[4,30] Vitamin D deficiency is becoming increasingly prevalent in Saudi Arabia, a country with abundant year round sunlight, because of the concerns about skin cancer and extreme outdoor heat.[14] Adequate vitamin D status is subjected to skin pigmentation, lifestyle and environmental factors such as clothing habits, seasonal variation, and geographical latitude. In addition to limited physical activity, obesity and consumption of unbalanced meals are potential causes to develop CVD early in life. Increased adiposity has been consistently associated with reduced serum vitamin D concentrations and adverse cardiovascular outcomes, although the mechanism underlying the relation to serum 25(OH)D is not clear.[31] Our data are consistent with this finding demonstrated by high prevalence of obesity as indicated by anthropometrical indices (Table 2). However, only WC and WHR measures showed significant differ-

ence between CAD patients and their control counterparts ($p<0.05$). Fat distribution changes with age such that there is an increase in visceral fat, which is more marked in women than in men. BMI can either underestimate the degree of fatness in older people because of changes in body composition or overestimate it due to loss of height from vertebral compression and kyphosis. WC, which correlates highly with total fat and intra-abdominal fat, might better predict adverse health effects of obesity in the elderly.[32] However, it is not clear which measure of adiposity best predicts the impact of obesity on health outcomes in the elderly.

Despite the established implication of serum vitamin D in CVD, the importance of adequate vitamin D intake is often neglected.[11] Upon univariate analysis, serum osteocalcin was significantly and negatively correlated with serum hsCRP, serum vitamin D and dietary vitamin D among the whole population. The role of dietary vitamin D as independent predictor persisted after multivariate analysis, suggesting a possible atherogenic effect of hypovitaminosis D as reported by previous studies.[4] These findings also suggests that inflammation, assessed by biomarkers such as hsCRP, may be associated with the intake of specific dietary components.[33,34] Of many inflammatory markers, CRP is the one that is most consistently related to cardiovascular risk.[5] Hence, the results of current study might be partially mediated via the mechanism of chronic low-grade inflammation. Circulating levels of pro-inflammatory cytokines were found to be elevated in older subjects and have been linked to CVD and osteoporosis.[35] Inverse correlations between osteocalcin and several obesity measures, namely body weight, BMI, WC, and WHR, were also observed (Table 4). Nevertheless, only body weight was found to be significantly correlated with serum osteocalcin independent of other potential confounders (Table 5). Osteocalcin is an osteoblast-specific protein, and may represent a link between bone metabolism and glucose/fat metabolism.[36] Foresta et al.[37] found that osteocalcin can be produced in, and even secreted from, adipose tissue. Furthermore, several studies have confirmed inverse relationships between osteocalcin and WC, metabolic syndrome and CHD.[38,39]

During atherogenesis, bone matrix proteins, including osteocalcin, may have a regulatory role in the atherosclerotic calcification process.[27] Study subjects were found to have lower categories of hsCRP with increasing osteoclacin quartiles (Table 2). Our proposal that serum osteocalcin levels may be an independent cardiovascular risk factor, is supported by previous studies that have suggested that low osteocalcin level may play a causal role in the development of atherosclerosis.[40,41]

Dietary energy, macronutrients and calcium are known to markedly influence the inflammatory process.[42] After adjustment for confounding factor, multiple regression analysis only showed significant contribution by dietary vitamin D (β= 0.170, 95% CI: 0.027, 0.155), of all dietary components, independent of other atherosclerosis-related factors in our population of postmenopausal women.

The present study has some limitations inherent to all cross-sectional studies. The observational character of this study does not allow one to conclude cause-effect relations. Self reported dietary intake can be misleading because of reporting bias associated with a tendency to overestimate low intakes of healthy foods and underestimate high intakes of unhealthy foods. Nevertheless, this was not the case in the present study since vitamin D and calcium intake estimates would have been greater had a more health conscious way of reporting dietary intakes been followed. Furthermore, total osteocalcin concentrations, which include carboxylated and uncarboxylated forms, were estimated in our study when recent evidence has indicated that lower uncarboxylated osteocalcin concentrations may be associated with β-cell dysfunction in patients with prediabetes.[43]

In conclusion, elevated levels of serum hsCRP and vitamin D were associated with low serum osteocalcin levels. Therefore, it is plausible that osteocalcin as a potential cardiovascular risk marker. Serum magnesium, ALP, dietary vitamin D, and body weight were independent predictors of serum osteocalcin level among our cohort of postmenopausal women. However, further studies are needed to clarify the pathophysiological processes underlying the relationship between serum osteocalcin level and atherosclerosis parameters.

This study further found that vitamin D and calcium deficiency are highly prevalent in the Saudi population of post menopausal women, and supports the recommendation for dietary fortification or supplementation with vitamin D especially in postmenopausal women with no or insufficient calcium/dairy product intakes. Further studies are needed to define optimal serum vitamin D levels for Saudi people with adequate calcium intakes.

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AUTHOR DISCLOSURES

The authors declare the originality of the manuscript and report no conflict of interest.

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Original Article

Serum osteocalcin is associated with dietary vitamin D, body weight and serum magnesium in postmenopausal women with and without significant coronary artery disease

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有或无明显冠状动脉疾病的绝经后妇女的血清骨钙素与膳食维生素 D，体重和血清镁有关

骨质疏松和动脉粥样硬化在绝经后妇女中的表现往往不典型，使临床识别困难。前瞻性研究表明：维生素 D 缺乏是骨量和血管钙化之间相关性的预测指标。我们的研究目的是验证绝经后妇女的血清骨钙素和维生素 D 状态之间的关系，通过造影确诊这些妇女有无冠状动脉疾病(CAD)。从阿卜杜勒阿齐兹国王大学医院征集了 180 名接受冠状动脉造影的绝经后妇女。收集其社会人口学指标、人体测量指标和饮食习惯，测定血中的生化指标。有一半的绝经后妇女没有明显的 CAD，24%的妇女单侧和/或双侧冠状血管有明显的 CAD，26%的妇女三条冠状血管均有明显的 CAD。平均血清维生素 D 浓度表明维生素 D 缺乏在所有人群中很常见。在研究人群中，维生素 D 和钙的摄入量均低。在所有亚组中，血清骨钙素与膳食维生素 D 显著负相关(r=-0.172, p<0.05)，而在患者中成正相关(r=0.269, p=0.01)。血清镁、碱性磷酸酶、膳食维生素 D 和体重是血清骨钙素水平的独立变量。总之，血清 C 反应蛋白和维生素 D 水平的升高与血清骨钙素水平降低有关。因此，骨钙素可能是潜在的心血管风险的标志物。然而，澄清血清骨钙素水平和动脉粥样硬化参数之间关系可能的病例生理过程还需要进一步的研究。

关键词：维生素 D、骨钙素、体重、绝经后妇女、冠状动脉疾病