Higher Serum Osteocalcin Is Associated With Lower Abdominal Aortic Calcification Progression and Longer 10-Year Survival in Elderly Men of the MINOS Cohort

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Context: Abdominal aortic calcification (AAC) is an indicator of cardiovascular risk, especially in the diseases characterized by insulin resistance such as type 2 diabetes. Osteocalcin is a bone-secreted hormone that favors insulin sensitivity and insulin secretion.

Objectives: We investigated whether total serum osteocalcin level at baseline is associated with AAC progression and 10-year all-cause mortality in elderly men.

Design and Participants: We assessed 774 men aged 51–85 years from the MINOS cohort who had osteocalcin measurement and lumbar spine radiographs at baseline. They were followed-up prospectively for 10 years. Among them, 615 patients had a follow-up radiograph at 3.5 or 7 years.

Main Outcome Measures: Serum total osteocalcin was measured with an immunoradiometric assay on morning fasting serum collected at baseline. Kauppila's AAC score was assessed from lumbar spine radiographs. AAC progression rate was calculated as the difference between AAC on the last available radiograph and AAC at baseline divided by the follow-up time. Death status was collected over 10 years.

Results: In multivariate analysis, higher baseline total osteocalcin was associated with lower AAC progression rate (odds ratio = 0.74 [0.57–0.97] per 10 ng/mL variation; $P = 0.029$). At the 10-year follow-up, there were 599 men alive (77%), 181 dead (23%), and 2 lost to follow-up. Higher osteocalcin was associated with lower 10-year all-cause mortality (hazard ratio = 0.62 [0.44–0.86] per 10 ng/mL variation; $P = 0.005$).

Conclusion: Higher baseline total osteocalcin concentrations were associated with lower AAC progression rate and lower mortality. These data suggest that osteocalcin level might be an independent indicator of cardiovascular risk and global health in elderly Caucasian men. ***(J Clin Endocrinol Metab* 98: 1084–1092, 2013)**

Diseases characterized by insulin resistance, such as type 2 diabetes mellitus and metabolic syndrome, are associated with elevated cardiovascular morbidity and mortality (1–4). Severe abdominal aortic calcification (AAC) is predictive of cardiovascular morbidity and mortality in type 2 diabetes and end-stage renal disease (5–7) as well as in the general population (8, 9).

Several factors have been implicated in the vascular calcification process such as hypertension, tobacco smoking, insulin resistance, lipid abnormalities, or hyperphos-

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Abbreviations: AAC, abdominal aortic calcification; CV, coefficient of variation; HDL, high-density lipoprotein; HR, hazard ratio; MGP, matrix gla-protein; 25OHD, 25-hydroxycholecalciferol; OR, odds ratio; Q1, quartile 1; VSMC, vascular smooth muscle cell.

phatemia (10). Locally, the transdifferentiation of the media vascular smooth muscle cells into osteoblast-like cells (demonstrated by the up-regulation of bone-specific genes such as *Runx2* and osteopontin) contributes to the development of vascular calcifications (11, 12). In addition, the combination of decreased insulin secretion, increased insulin resistance, elevated fatty acid serum level, and low circulating adiponectin level probably also play a critical role (10).

Recent studies show that bone is an endocrine organ that can regulate glucose homeostasis through the secretion of osteocalcin. Osteocalcin is a hormone that induces insulin secretion and promotes proliferation of pancreatic β-cells (13). Osteocalcin also acts on adipocytes to promote adiponectin secretion that reduces insulin resistance (14). Even when on a regular diet, the osteocalcin-deficient mouse phenotype, characterized by impaired glucose tolerance, increased visceral fat mass, elevated circulating fatty acids, and low circulating adiponectin (15), recapitulates characteristics of the current pathophysiological model of vascular calcification (10). Therefore, we hypothesized that there may be a link between serum osteocalcin and vascular calcifications.

The aim of this study was to assess the association of the baseline serum total osteocalcin level with the progression rate of AAC and with the all-cause 10-year mortality in elderly men.

Materials and Methods

Description of the cohort

The MINOS study is a single-center prospective cohort study of osteoporosis and its determinants in men (16). It is a collaboration between Institut National de la Santé et de la Recherche Médicale (INSERM) and Société de Secours Minière de Bourgogne (SSMB). SSMB is one of the largest local health insurance companies dedicated to insure mineworkers and their families in Montceau-les-Mines, a French city of 35 000 inhabitants. There were no major differences between the SSMB population and the general French population in terms of way of life and habits, morbidity, or hospitalization (unpublished data). The study was accepted by the local ethics committee and performed in accordance with the Helsinki declaration revised in 1983.

Enrollment, nonresponse bias survey, and follow-up of the cohort

Letters inviting participation in the study were sent in 1995–96 to a randomly selected sample of 3400 men aged 51–85 years and covered by the SSMB. Among 841 men who responded to the invitation (response rate of 25%) and provided informed consent, 782 men underwent diagnostic tests and 59 men refused blood and urinary collection (Figure 1). All the men willing to participate in the study were accepted. The participants had lateral spine radiographs at baseline and after 3 or 7.5 years of follow-up to detect vertebral fracture. Dates of death were obtained from the SSMB over 10 years of follow-up. In 1996, we carried out a nonresponse bias survey to ensure that participants were not different from the whole randomly invited sample. A questionnaire was sent to 120 randomly selected men who had been invited to participate in the study but declined. Responders and nonresponders did not differ in terms of education level, smoking, calcium and alcohol intake, former professional and current leisure physical activity, personal and family history of the fragility fracture, health status, and medication use (17).

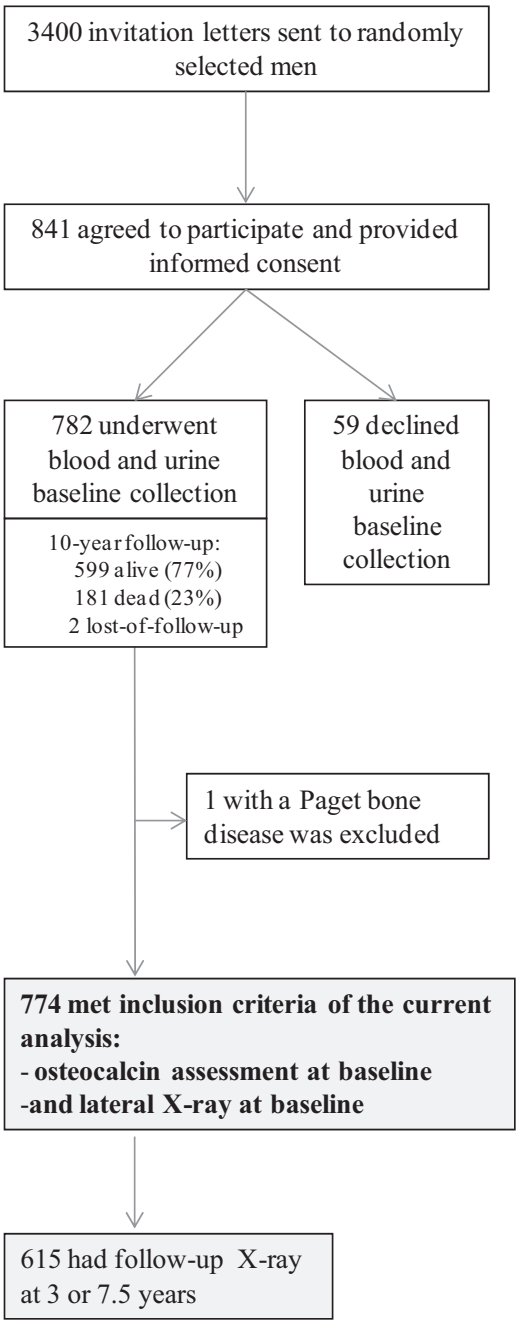

Figure 1. Flow chart.

Inclusion criteria in the current analysis

The current analysis was conducted in the participants who had both an osteocalcin measurement and a lateral spine radiograph at baseline. One patient with a Paget bone disease was excluded (Figure 1).

Clinical data

At enrollment, patients filled out an interviewer-administered epidemiological questionnaire to assess lifestyle and health status. Cigarette smoking was self-reported and classified as smoker vs nonsmoker. Alcohol intake was quantified as the average quantity of alcoholic beverages drunk weekly. Physical activity (leisure sport activity, gardening, and walking) was calculated on the basis of the overall amount of time (hours per month). Comorbidities (diabetes, hypertension, and ischemic heart disease) and current medication including vitamin K antagonists were self-reported and dichotomized as yes/no. Whenever possible, we verified information on medical prescriptions and previous hospitalization reports. Body weight, height, and waist were measured according to a standardized procedure.

Biochemical measurements

Fasting serum samples were collected at baseline. All the samples were immediately centrifuged and then frozen. No measurements were performed using fresh blood samples. The samples were stored at −80°C until assessment at 18 to 20 months. Serum calcium, phosphate, and creatinine were assessed using standard laboratory methods. Serum total osteocalcin was measured with a human-specific 2-site immunoradiometric assay (ELSA-OSTEO; CIS Bio International, Bagnols sur Cèze, France). Intra- and interassay coefficients of variation (CV) were ≤4% and ≤6%, respectively, and the detection limit was 0.08 ng/mL. Serum 25-hydroxycholecalciferol (25OHD) was measured by RIA, which excludes any interference with lipids (Incstar Corp, Stillwater, Minnesota). Triglycerides were measured by a colorimetric test (modular analyzer; Roche Diagnostics Ltd, Rotkreuz, Switzerland) with a detection limit of 4 mg/dl and interassay CV of 1.5%. High-density lipoprotein (HDL)-cholesterol was measured by a homogeneous enzymatic colorimetric test (modular analyzer; Roche) with a detection limit of 3 mg/dl and interassay CV of 0.6% to 0.95%. Details about each method have been published previously (17, 18).

Vascular calcification assessment

AAC was assessed from lateral radiographs of lumbar spine using the semiquantitative method described by Kauppila et al (19). Briefly, the calcifications of the anterior and posterior wall of the aorta, adjacent to the first 4 lumbar vertebrae, are evaluated. Boundaries are defined by the midpoint of the intervertebral space. Semiquantitative severity scores (0–3) for each segment are added to yield the global AAC score, ranging from 0 to 24 (Figure 2). The reproducibility was assessed using 30 radiographs. Intraobserver reproducibility assessed by intraclass correlation coefficient was 0.92 to 0.95. For interobserver reproducibility (two independent readers), intraclass correlation coefficient was 0.91. AAC score was assessed using the same method on the baseline and follow-up radiographs. AAC score was assessed by 1 reader (P.S.) who evaluated side by side the baseline and the follow-up radiographs and who knew the order of the radiographs.

The AAC progression for each patient was calculated as the difference between AAC score on the last available follow-up x-ray (3 or 7.5 years) and AAC score at baseline divided by duration of the follow-up. AAC progression rate was expressed as a yearly increase rate (points per year).

Statistical analysis

All calculations were performed using SAS version 9.3 software version (SAS Institute Inc, Cary, North Carolina). All *P* values were two-tailed, and values <.05 were considered significant. Data are presented as mean ± SD for continuous variables and number (percentage) for categorical variables. AAC score is presented as median (interquartile range).

Cross-sectional AAC analysis

We split the AAC score according to quartiles and considered it as an ordinal variable. This unbiased approach for the analysis of ordinal response data allowed us to use a cumulative logit

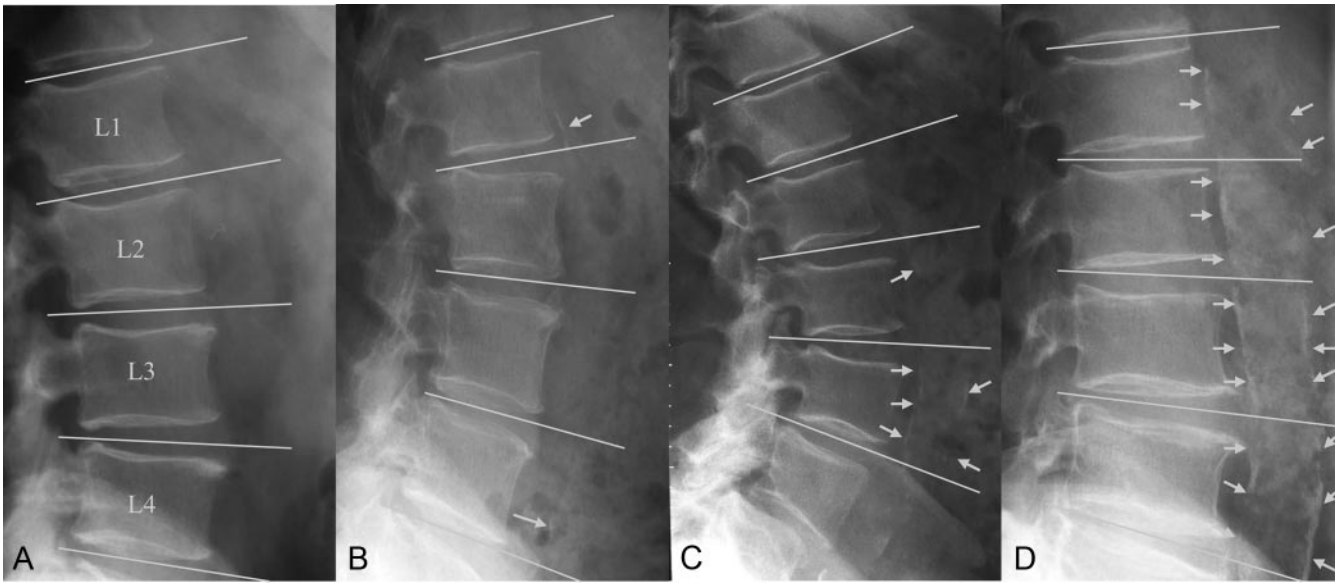

Figure 2. AAC was assessed using the semiquantitative score of Kauppila (from 0–24). A, AAC score = 0, ie, no aortic calcification (first quartile). B, AAC score = 2, ie, small calcification at the posterior aortic wall at the level of first and fourth lumbar vertebrae (second quartile). C, AAC score = 6, ie, calcification at the posterior wall at the level of the third and fourth lumbar vertebrae as well as at the anterior wall of fourth lumbar vertebrae (third quartile). D, AAC score = 20, ie, severe calcification on most the abdominal aorta (fourth quartile).

model. Cumulative logit model is a type of ordinal logistic model that involves all levels of the response and dichotomizes the response scale. The proportional odds method used here, assumes that the odds of response below a given response level are constant regardless of which level you pick. This model allows separate intercepts for the cumulative logit but restricts the parameter sets for the predictors to be the same across all logits. Results are expressed as odds ratio (OR) as the effect of a 1-U change in the independent variable on a success of a dependent variable. Either continuous or categorical variables can be used in this model to explain the independent outcome ordinal variable. Thus, we analyzed the association between quartiles of AAC score and osteocalcin (continuous) after adjustment for known confounders: age, 25OHD, serum phosphate, HDL-cholesterol, triglycerides, creatinine, physical activity (hours per month), hypertension (yes/no), diabetes (yes/no), vitamin K antagonist use (yes/no), alcohol intake (quartiles of the average weekly intake), and smoking status (yes/no). Functional form of all continuous predictors was checked. Proportional-odds assumption and the deviance and Pearson goodness-of-fit statistics were also checked.

Association of serum total osteocalcin with AAC progression rate

Using the same methodological approach as the cross-sectional analysis, we split the AAC progression rate according to quartiles and considered it as an ordinal variable. Then the association between quartiles of AAC progression rate and osteocalcin was assessed using cumulative logit models after adjustment for the same confounders as in the cross-sectional analysis. In addition, we adjusted the model to AAC score at baseline to estimate the relationship between osteocalcin and AAC progression rate independently of the initial AAC score.

The 10-year overall mortality

Cox proportional hazard regression was used to estimate the association of osteocalcin with all-cause mortality at 10 years. We used the variables associated with mortality in the literature (age, diabetes, hip to waist ratio, hypertension, smoking status, alcohol intake, serum phosphate, low HDL-cholesterol, AAC score, physical activity, and 25OHD) to build a multivariate Cox model. We also included in this multivariate model the variables used in the AAC analysis that were associated with survival in univariate analysis with a significance level of $P < .15$. The assumption of proportional hazards was tested using analysis of Martingale's residuals and time-interaction tests.

Results

Baseline characteristics

At baseline, 774 men had osteocalcin measurement and lateral spine radiograph. Their average age was 65.3 years, their average body mass index was 28.0 kg/m^2, and their hip to waist ratio was 1.01 (Table 1). Nearly 25% self-reported hypertension, 15% ischemic heart disease, and 7% diabetes. More than two thirds of the patients were current (12%) or former (56%) smokers. The median AAC score was 2 of 24.

Table 1. Baseline Characteristics of the 774 Men From the MINOS Cohort[a]

Clinical Parameters	Data
Age, y	65 ± 7
Weight, kg	80 ± 13
Height, cm	169 ± 6
BMI, kg/m^2	28 ± 4
Hip-waist ratio	1.01 ± 0.08
Ischemic heart disease	118 (15.2)
Hypertension	192 (24.8)
Diabetes	55 (7.1)
Smoker	525 (67.8)
Alcohol intake	
No	183 (23.6)
Occasional	201 (26.0)
<3 IU/d	236 (30.5)
≥3 IU/d	154 (19.9)
Physical activity, h/mo	21.9 (12.7)
Vitamin K antagonist use	31 (4.0)
Bone biology	
Serum calcium, mmol/L	2.42 ± 0,10
Serum phosphate, mmol/L	1.06 ± 0,15
25 OHD, nmol/L	71 ± 30
Serum creatinine, μmol/L	100 ± 17
Osteocalcin, ng/mL	19.1 ± 6.6
Metabolic biology	
Blood glucose, mmol/L	6.1 ± 1.4
Total cholesterol, mmol/L	5.9 ± 1.0
HDL-cholesterol, mmol/L	1.3 ± 0.4
Triglycerides, mmol/L	1.9 ± 1.1
AAC score	2 (0–6)

Abbreviation: BMI, body mass index.

[a] Data are presented as mean ± SD, median (Q1–Q3), or n (%).

Association of serum total osteocalcin with AAC score at baseline

AAC quartiles at baseline according to Kauppila score (range from 0–24) corresponded to no calcification (quartile 1 [Q1]: AAC score = 0, n = 221), limited calcifications (Q2: AAC score 1–2, n = 179), moderate amount of calcifications (Q3: AAC score 3–6, n = 188), and severe calcifications (Q4: AAC score > 6, n = 186). In univariate analysis, higher age, serum phosphate, triglycerides, creatinine, and alcohol intake as well as presence of hypertension, diabetes, smoking, or vitamin K antagonist use were each associated with greater AAC severity. In contrast, higher 25OHD, HDL-cholesterol, physical activity, and osteocalcin were associated with a lower AAC severity. In multivariate analysis using the cumulative logit model adjusted for all the previous confounders, age, hypertension, diabetes, smoking, triglycerides, alcohol intake, and serum phosphate were positively associated with AAC quartiles at baseline. Higher HDL-cholesterol and higher osteocalcin tended to be protective for AAC (with odds ratio [95% confidence interval] of 0.66 per 1 mmol/L [0.43–1.02] and OR of 0.98 per 1 ng/mL [0.96–1.01], respectively) (Table 2).

Table 2. Crude and Adjusted ORs for Predictors of AAC Score at Baseline[a]

	Univariate		Multivariate	
	P	OR (95% CI)	*P*	OR (95% CI)
Age, y	<.001	1.10 (1.08–1.12)	<.001	1.11 (1.09–1.14)
25 OHD, nmol/L	<.001	0.99 (0.99–1.00)	.44	1.00 (0.99–1.00)
Serum phosphate, mmol/L	<.001	4.72 (1.93–11.53)	<.001	5.74 (2.15–15.33)
HDL-cholesterol, mmol/L	.003	0.58 (0.40–0.83)	.061	0.66 (0.43–1.02)
Triglycerides, mmol/L	<.001	1.35 (1.18–1.55)	.012	1.23 (1.05–1.44)
Serum creatinine, μmol/L	.001	1.01 (1.01–1.02)	.494	1.00 (0.99–1.01)
Hypertension	<.001	2.48 (1.81–3.41)	.010	1.56 (1.11–2.20)
Diabetes	<.001	3.78 (2.22–6.44)	<.001	2.78 (1.57–4.94)
Vitamin K antagonist use	.008	2.56 (1.28–5.12)	.083	1.94 (0.92–4.08)
Alcohol intake				
≥3 IU/d vs no	.105	1.53 (1.02–2.32)	.013	1.60 (1.03–2.48)
<3 IU/d vs no		1.47 (1.02–2.14)		0.95 (0.64–1.41)
Occasional vs no		1.16 (0.80–1.70)		0.78 (0.52–1.17)
Physical activity, h/mo	<.001	0.98 (0.97–0.99)	.231	0.99 (0.98–1.01)
Tobacco smoking	<.001	2.90 (2.15–3.91)	<.001	3.15 (2.29–4.32)
Osteocalcin, ng/mL	.040	0.98 (0.96–1.00)	.119	0.98 (0.96–1.01)

Abbreviation: CI, confidence interval.

[a] The cumulative logit model (n = 681 complete cases) uses the quartile of AAC score as the dependent variable.

Association of serum total osteocalcin with AAC progression rate

A total of 615 men had at least 1 follow-up radiograph. The median increase in the AAC score was 0.27 (interquartile range, 0.13–0.67) points/year. Median AAC progression rate for the 3-year interval was 0.33 points/year (interquartile range, 0.0–0.67) and for the 7.5-year interval was 0.23 points/year (0.0–0.45) ($P = .23$). The men who did not have the follow-up radiographs were on average 5 years older ($P < .001$), had higher AAC score at baseline (median, 5 vs 2, $P < .001$) and had 2.7 cm greater waist circumference ($P = .003$) and lower 25OHD concentration ($P < .001$). They self-reported more often ischemic heart disease (20% vs 14%, $P = .044$), hypertension (34% vs 23%, $P = .004$), and diabetes (11% vs 6%, $P = .048$). After adjustment for potential confounders, average osteocalcin levels were 2.7% (0.22 SD) lower in men who had no AAC follow-up ($P < .05$).

Quartiles of AAC progression rate corresponded to no progression (Q1 = 0 point per year, n = 228), slow progression (Q2 = 0.25 point per year on average, n = 84), moderate progression (Q3 = 0.5 point per year on average, n = 191), and fast progression (Q4 = 1.0 point per year on average, n = 114), meaning that patients with a slow AAC progression rate increased their Kauppila score of 1 calcification every 4 years, whereas patients with moderate and fast progression rate got 1 more calcification every 2 years and every year, respectively.

In univariate analysis, only age, 25OHD, serum phosphate, alcohol intake, and the presence of hypertension, diabetes, and smoking were significantly positively associated with AAC progression rate, whereas higher osteocalcin was associated with a lower AAC progression rate. In multivariate analysis, AAC progression rate remained positively associated with age (OR = 1.07 per year [1.04–1.09], $P < .001$), serum phosphate (OR = 3.16 per 1 mmol/L [1.13–8.82], $P = .028$), and smoking (OR = 1.64 [1.17–2.30], $P = .004$). After adjustment for all the confounders, higher osteocalcin remained associated with a lower AAC progression rate (OR = 0.97 per 1 ng/mL [0.95–0.997], $P = .029$) (Supplemental Table 1, published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org).

Furthermore, AAC score at baseline was also significantly associated with AAC progression rate. When included in the multivariate analysis (data not shown), higher AAC score at baseline was predictive of faster progression rate (OR = 1.14 [1.09–1.20], $P < .001$). The OR of osteocalcin remained unchanged (OR = 0.97 [0.95–1.00], $P = .05$).

Association of serum total osteocalcin with 10-year all-cause mortality

Except 2 men who were lost to follow-up, there was no loss of mortality follow-up at 10 years. During this period, 181 men (23% of the cohort) died. The median time to death was 2139 days (interquartile range, 1270–2866). In univariate analysis, among variables used in AAC progression model, only vitamin K antagonist use ($P = .11$) was retained for the multivariate analysis, whereas triglycerides and serum creatinine were not (Supplemental Table 2).

We found in multivariate Cox analysis that age was associated with a higher 10-year mortality (hazard ratio [HR], 1.05 per 1 year [95% confidence interval = 1.01–

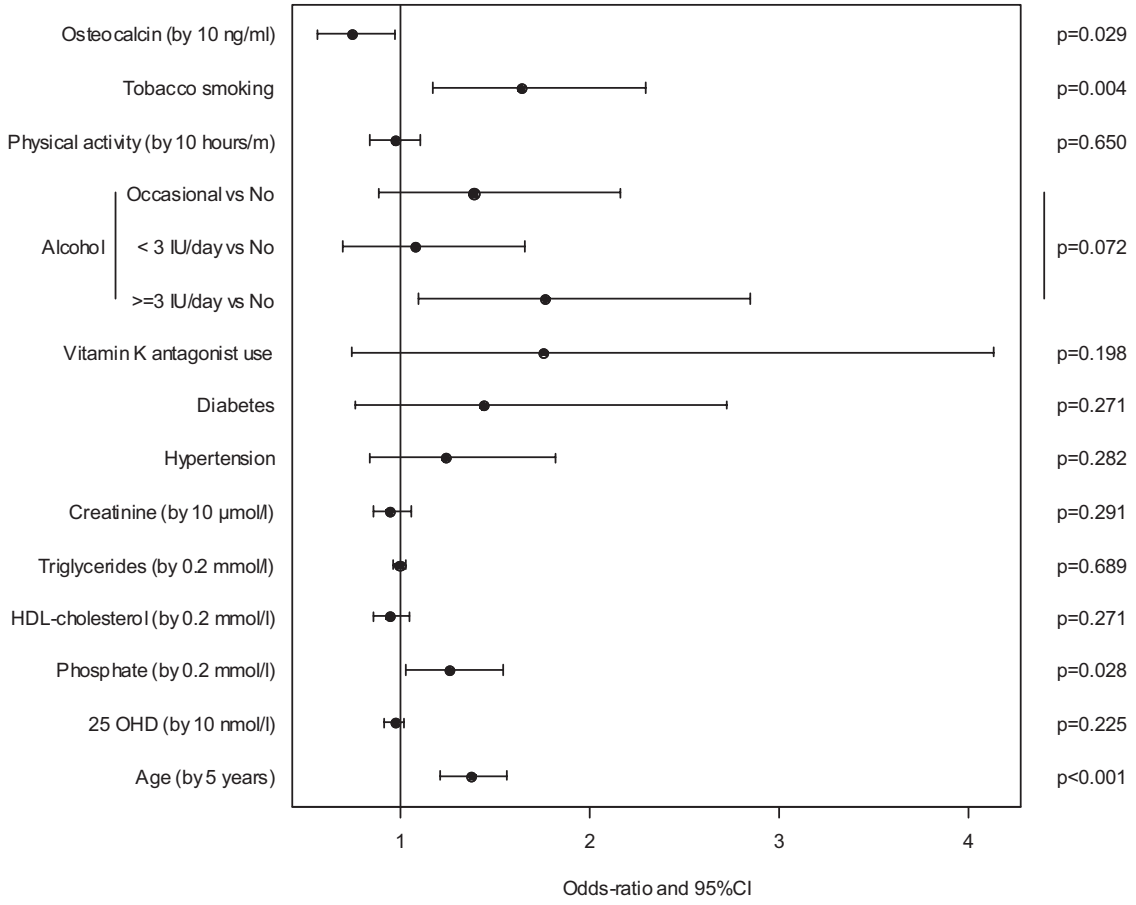

Figure 3. Model with clinically relevant variations for predictors of AAC progression rate (adjusted ORs) with the cumulative logit model using the quartile of AAC progression as the dependent variable.

1.08], $P = .009$). By contrast, higher osteocalcin level was the only factor to be associated with a lower 10-year mortality (HR = 0.95 per 1 ng/mL [0.92–0.99]; $P = .005$). A trend was observed with the hip to waist ratio ($P = .052$). Other variables were not significantly associated with the overall survival.

Statistical approach for clinical relevance

The conservative statistical approach that we chose used continuous variables. We found that higher osteocalcin was associated with a lower AAC progression rate and lower mortality. Nevertheless, in this analysis, OR corresponded to the variation of 1 U of the considered variable. Biologically, variation of 1 ng/mL of osteocalcin is not relevant, whereas variation of 1 mmol/L of serum phosphate is a major variation. Based on the significant results we previously observed, in an attempt to be clinically relevant, we propose a graphical presentation of the results of the cumulative logit model using clinically relevant variations. We used a 10 ng/mL variation for osteocalcin, 0.2 mmol/L for serum phosphate and triglycerides, 10 μmol/L for creatinine, 10 nmol/L for 25OHD, 0.1 for hip to waist ratio, 10 h/mo for physical activity, and 5 years for age. Thus, we observed (Figures 3 and 4) that an increase of 10 ng/mL of total osteocalcin was associated with a reduced risk of AAC progression rate of OR = 0.74 (0.57–0.97) and of all-cause mortality of HR = 0.62 (0.44–0.86).

Discussion

In this cohort of elderly men, we found that higher serum total osteocalcin concentrations at baseline were associated with lower AAC progression rate and lower 10-year overall mortality.

To our knowledge, this is the first report of a prospectively assessed negative link between osteocalcin and AAC progression that was investigated specifically in community-dwelling elderly men. Previously, in 92 patients with essential hypertension, higher undercarboxylated osteocalcin was correlated with increased calcification in the common carotid artery assessed by ultrasound (20). However, the study design did not allow the inclusion of multiple variables for adjustment in the regression model. In a group of community-living women aged ≥65 (Study of

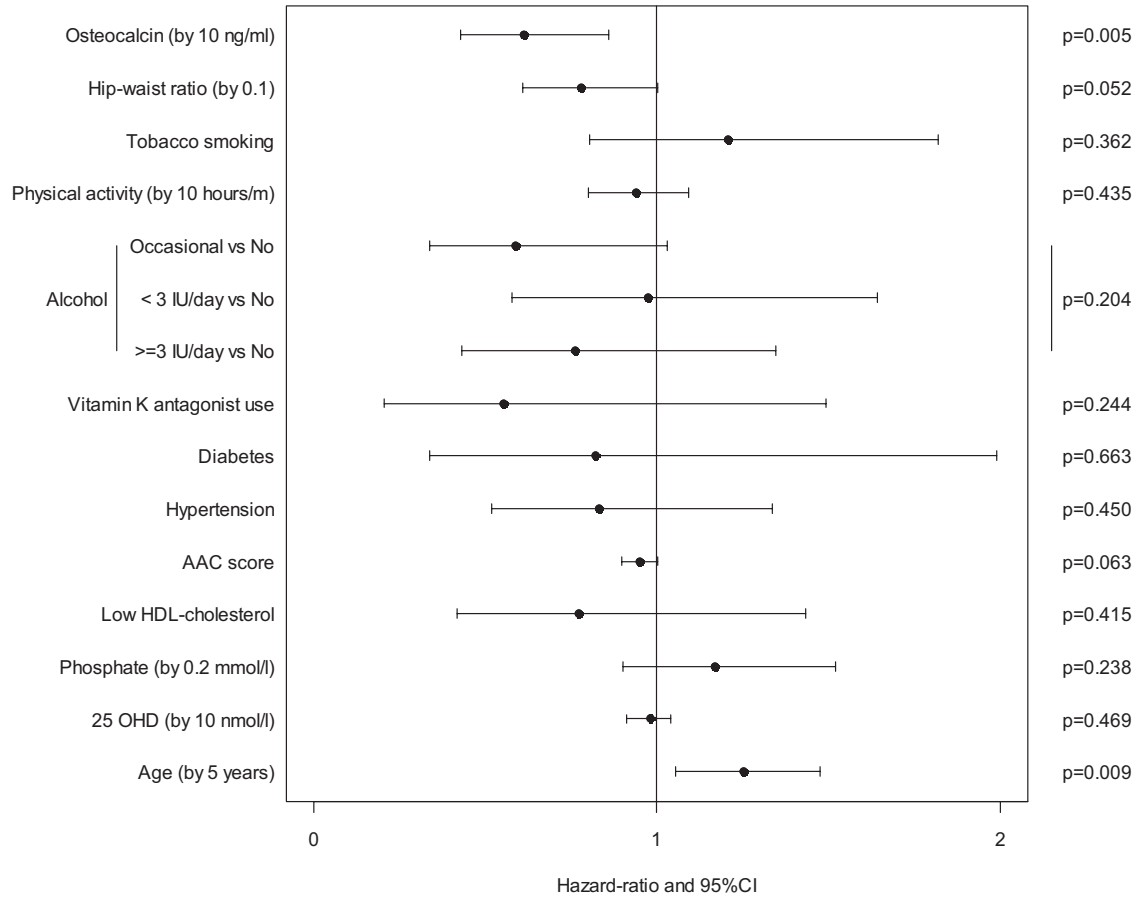

Figure 4. Model with clinically relevant variations for adjusted mortality HRs during 10 years follow-up. Estimations were obtained by Cox models.

Osteoporotic Fractures cohort), no association between osteocalcin and AAC severity was found; however, bone markers were not measured in fasting morning samples, which impaired accuracy of the results (21). Furthermore, this difference may also be due to the gender, because in rodents, the metabolic phenotype in female is less pronounced. Indeed, it has been recently demonstrated that osteocalcin acts on Leydig cells to regulate testosterone and fertility in males. By contrast, osteocalcin-deficient females do not have any fertility abnormality (22). Interestingly, a significant negative correlation between osteocalcin level and AAC severity was found in a small group of men and women with familial hypercholesterolemia (23). Recently, in an observational study of the relationship between low 25OHD level and the risk of AAC progression, Naves-Diaz et al. (24) also reported a negative association between osteocalcin and AAC progression. However, in this study, both genders were analyzed jointly, and the statistical model was not adjusted for hypertension, serum phosphate, vitamin K antagonist use, or blood glucose.

Osteocalcin may be involved in the aortic calcification process indirectly by its action on insulin and insulin resistance. More and more epidemiological studies support the role of osteocalcin in the regulation of energy metabolism in humans through the effect on the glucose and lipid metabolism (25–30). We have recently shown that after surgical resection of osteoid osteoma in young men, serum osteocalcin level decreased, whereas blood glucose increased, providing the first direct evidence of the action of osteocalcin in humans (31). Osteocalcin may act through its effect on adiponectin as reported in mice (15) and chronic kidney disease patients (32). Adiponectin is an anti-inflammatory adipokine secreted by adipocytes. In the arterial media, adiponectin prevents the transdifferentiation of vascular smooth muscle cells (VSMCs) into osteoblast-like cells (33). Currently, a direct action of osteocalcin as a secreted hormone on the aortic calcification process is unknown, and additional experiments are needed. Osteocalcin may also have a local action through its expression by osteoblast-like cells from VSMC transdifferentiation. Recent studies suggest that osteocalcin may locally induce metabolic changes in chondrocytes and VSMCs through hypoxia-inducible factor 1-α. Nevertheless, these results did not include balance with local calcification inhibitor such as matrix gla-protein (MGP) (34). Results of our multivariate analysis on AAC progression show an independent effect of osteocalcin from diabetes.

This supports the physiopathological hypothesis of a direct action of osteocalcin on AAC in addition to its role on insulin sensitivity.

The association between osteocalcin level and overall survival is of particular interest in this homogeneous population of elderly men. Our results are consistent with the recent findings of the LUdwigshafen RIsk and Cardiovascular health study (LURIC) study and the Australian Health in Men Study showing that total osteocalcin was associated with cardiovascular mortality (35, 36). In a previous analysis, we showed that poor bone health reflected by low bone mineral density, major osteoporotic fracture history, and high bone turnover was associated with higher mortality. In this context of mechanical analysis, we failed to demonstrate an association between quartiles of osteocalcin and mortality. The current analysis considering the hormonal function of osteocalcin on energy metabolism allowed us to demonstrate the association between osteocalcin and mortality (37). Altogether, human studies and preclinical models suggest that bone may be an important organ to integrate lifestyle parameters of the body (physical activity, food intake, alcohol, and stress) and act on energy metabolism.

Our study has limitations. The cohort includes predominantly lower- and middle-class Caucasian men, and the data cannot be extrapolated to women or men from other ethnic groups. They were recruited in a small town, and its population may not be perfectly representative of the French general population. Because of the ribs, we assessed calcification only at the abdominal aorta. It is not sure to what extent these results can be extrapolated to other large arteries (38). Kauppila's score enables the assessment of the AAC extension, but not the actual arterial calcium content. Lifestyle factors and comorbidities were self-reported but confirmed, when available, by the medical prescriptions and previous hospitalization reports. In our cohort, 68% of the men were smokers including 56% former and 12% current smokers. These results are essentially similar to those observed in the French male general population of the same age (http://www.inpes.sante.fr/10000/themes/tabac/consommation/profils-fumeurs.asp). Date of death was transmitted by the SSMB, which has no access to the cause of death, by law. Thus, only all-cause mortality could be assessed. In particular, we could not assess separately deaths of cardiovascular origin. The food frequency questionnaire used in our study did not allow assessment of the dietary vitamin K intake. We did not measure undercarboxylated osteocalcin, insulin, and adiponectin because our serum samples were collected more than 15 years ago and all the samples from the initial blood collection were thawed at least once. In addition, we did not measure levels of other proteins known to regulate calcification process such as MGP (39). Nevertheless, because vitamin K antagonists prevent MGP γ-carboxylation and impair its function, we have included vitamin K inhibitor treatment in our multivariate analysis.

In conclusion, in this community-dwelling cohort of elderly men, higher osteocalcin levels were associated with lower AAC progression rate and lower 10-year all-cause mortality. Our data suggest that total serum osteocalcin level may be an independent indicator of cardiovascular risk and of global health in elderly men.

Acknowledgments

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References

1. **Hu G, Qiao Q, Tuomilehto J, Balkau B, Borch-Johnsen K, Pyorala K.** Prevalence of the metabolic syndrome and its relation to all-cause and cardiovascular mortality in nondiabetic European men and women. *Arch Intern Med*. 2004;164:1066–1076.
2. **Isomaa B, Almgren P, Tuomi T, et al.** Cardiovascular morbidity and mortality associated with the metabolic syndrome. *Diabetes Care*. 2001;24:683–689.
3. **Lakka HM, Laaksonen DE, Lakka TA, et al.** The metabolic syndrome and total and cardiovascular disease mortality in middle-aged men. *JAMA*. 2002;288:2709–2716.
4. **Malik S, Wong ND, Franklin SS, et al.** Impact of the metabolic syndrome on mortality from coronary heart disease, cardiovascular disease, and all causes in United States adults. *Circulation*. 2004; 110:1245–1250.
5. **Lehto S, Niskanen L, Suhonen M, Ronnemaa T, Laakso M.** Medial artery calcification. A neglected harbinger of cardiovascular complications in non-insulin-dependent diabetes mellitus. *Arterioscler Thromb Vasc Biol*. 1996;16:978–983.
6. **London GM, Guerin AP, Marchais SJ, Metivier F, Pannier B, Adda H.** Arterial media calcification in end-stage renal disease: impact on all-cause and cardiovascular mortality. *Nephrol Dial Transplant*. 2003;18:1731–1740.
7. **Marchais SJ, Metivier F, Guerin AP, London GM.** Association of hyperphosphataemia with haemodynamic disturbances in end-stage renal disease. *Nephrol Dial Transplant*. 1999;14:2178–2183.
8. **Bastos Goncalves F, Voute MT, Hoeks SE, et al.** Calcification of the abdominal aorta as an independent predictor of cardiovascular events: a meta-analysis. *Heart*. 2012;98:988–994.
9. **Wilson PW, Kauppila LI, O'Donnell CJ, et al.** Abdominal aortic calcific deposits are an important predictor of vascular morbidity and mortality. *Circulation*. 2001;103:1529–1534.

10. **Giachelli CM.** Vascular calcification mechanisms. *J Am Soc Nephrol*. 2004;15:2959–2964.
11. **Giachelli CM.** Vascular calcification: in vitro evidence for the role of inorganic phosphate. *J Am Soc Nephrol*. 2003;14:S300–S304.
12. **Jono S, McKee MD, Murry CE, et al.** Phosphate regulation of vascular smooth muscle cell calcification. *Circ Res*. 2000;87:E10–E17.
13. **Karsenty G, Ferron M.** The contribution of bone to whole-organism physiology. *Nature*. 2012;481:314–320.
14. **Confavreux CB.** Bone: from a reservoir of minerals to a regulator of energy metabolism. *Kidney Int Suppl*. 2011;121:S14–S19.
15. **Lee NK, Sowa H, Hinoi E, et al.** Endocrine regulation of energy metabolism by the skeleton. *Cell*. 2007;130:456–469.
16. **Szulc P, Marchand F, Duboeuf F, Delmas PD.** Cross-sectional assessment of age-related bone loss in men: the MINOS study. *Bone*. 2000;26:123–129.
17. **Szulc P, Claustrat B, Delmas PD.** Serum concentrations of 17β-E2 and 25-hydroxycholecalciferol (25OHD) in relation to all-cause mortality in older men: the MINOS study. *Clin Endocrinol (Oxf)*. 2009;71:594–602.
18. **Szulc P, Garnero P, Munoz F, Marchand F, Delmas PD.** Cross-sectional evaluation of bone metabolism in men. *J Bone Miner Res*. 2001;16:1642–1650.
19. **Kauppila LI, Polak JF, Cupples LA, Hannan MT, Kiel DP, Wilson PW.** New indices to classify location, severity and progression of calcific lesions in the abdominal aorta: a 25-year follow-up study. *Atherosclerosis*. 1997;132:245–250.
20. **Okura T, Kurata M, Enomoto D, Jotoku M, Nagao T, Desilva VR, Irita J, Miyoshi K, Higaki J.** Undercarboxylated osteocalcin is a biomarker of carotid calcification in patients with essential hypertension. *Kidney Blood Press Res*. 2010;33:66–71.
21. **Parker BD, Bauer DC, Ensrud KE, Ix JH.** Association of osteocalcin and abdominal aortic calcification in older women: the study of osteoporotic fractures. *Calcif Tissue Int*. 2010;86:185–191.
22. **Oury F, Sumara G, Sumara O, et al.** Endocrine regulation of male fertility by the skeleton. *Cell*. 2011;144:796–809.
23. **Awan Z, Alwaili K, Alshahrani A, Langsetmo L, Goltzman D, Genest J.** Calcium homeostasis and skeletal integrity in individuals with familial hypercholesterolemia and aortic calcification. *Clin Chem*. 2010;56:1599–1607.
24. **Naves-Diaz M, Cabezas-Rodriguez I, Barrio-Vazquez S, Fernandez E, Diaz-Lopez JB, Cannata-Andia JB.** Low calcidiol levels and risk of progression of aortic calcification. *Osteoporos Int*. 2012;23:1177–1182.
25. **Bao Y, Ma X, Yang R, et al.** Inverse relationship between serum osteocalcin levels and visceral fat area in Chinese men. *J Clin Endocrinol Metab*. 2012;98:345–351
26. **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;23:761–770.
27. **Kanazawa I, Yamaguchi T, Tada Y, Yamauchi M, Yano S, Sugimoto T.** Serum osteocalcin level is positively associated with insulin sensitivity and secretion in patients with type 2 diabetes. *Bone*. 2011;48:720–725.
28. **Kanazawa I, Yamaguchi T, Yamamoto M, et al.** Serum osteocalcin level is associated with glucose metabolism and atherosclerosis parameters in type 2 diabetes mellitus. *J Clin Endocrinol Metab*. 2009;94:45–49.
29. **Kindblom JM, Ohlsson C, Ljunggren O, et al.** Plasma osteocalcin is inversely related to fat mass and plasma glucose in elderly Swedish men. *J Bone Miner Res*. 2009;24:785–791.
30. **Saleem U, Mosley TH Jr, Kullo IJ.** Serum osteocalcin is associated with measures of insulin resistance, adipokine levels, and the presence of metabolic syndrome. *Arterioscler Thromb Vasc Biol*. 2010;30:1474–1478.
31. **Confavreux CB, Borel O, Lee F, et al.** Osteoid osteoma is an osteocalcinoma affecting glucose metabolism. *Osteoporos Int*. 2012;23:1645–1650.
32. **Bacchetta J, Boutroy S, Guebre-Egziabher F, et al.** The relationship between adipokines, osteocalcin and bone quality in chronic kidney disease. *Nephrol Dial Transplant*. 2009;24:3120–3125.
33. **Luo XH, Zhao LL, Yuan LQ, Wang M, Xie H, Liao EY.** Development of arterial calcification in adiponectin-deficient mice: adiponectin regulates arterial calcification. *J Bone Miner Res*. 2009;24:1461–1468.
34. **Idelevich A, Rais Y, Monsonego-Ornan E.** Bone Gla protein increases HIF-1α-dependent glucose metabolism and induces cartilage and vascular calcification. *Arterioscler Thromb Vasc Biol*. 2011;31:e55–e71.
35. **Lerchbaum E, Schwetz V, Pilz S, et al.** Association of bone turnover markers with mortality in men referred to coronary angiography [published online ahead of print July 10, 2012]. *Osteoporos Int*. doi: 10.1007/s00198-012-2076-9.
36. **Yeap BB, Chubb SA, Flicker L, et al.** Association of total osteocalcin with all-cause mortality in older men: the Health In Men Study. *Osteoporos Int*. 2012;23:599–606.
37. **Szulc P, Maurice C, Marchand F, Delmas PD.** Increased bone resorption is associated with higher mortality in community-dwelling men $\geq$50 years of age: the MINOS study. *J Bone Miner Res*. 2009;24:1116–1124.
38. **Pennisi P, Signorelli SS, Riccobene S, et al.** Low bone density and abnormal bone turnover in patients with atherosclerosis of peripheral vessels. *Osteoporos Int*. 2004;15:389–395.
39. **Murshed M, Schinke T, McKee MD, Karsenty G.** Extracellular matrix mineralization is regulated locally; different roles of two gla-containing proteins. *J Cell Biol*. 2004;165:625–630.