

Plasma osteocalcin levels as a predictor of cardiovascular disease in older men and women: a population-based cohort study

Kristin Holvik^{1,2}, Natasja M van Schoor², Elisabeth M W Eekhoff³,
Martin den Heijer³, Dorly J H Deeg², Paul Lips³ and Renate de Jongh³

¹Division of Epidemiology, Norwegian Institute of Public Health, PO Box 4404 Nydalen, N-0403 Oslo, Norway, ²Department of Epidemiology and Biostatistics, EMGO Institute for Health and Care Research and ³Endocrine Section, Department of Internal Medicine, VU University Medical Center, Amsterdam, The Netherlands

Correspondence should be addressed to K Holvik
Email
kristin.holvik@fhi.no

Abstract

Objective: The role of osteocalcin (OC) in cardiovascular disease (CVD) is unresolved. We aimed to study the association between plasma OC concentrations and the risk of non-fatal and fatal CVDs. We also aimed to investigate whether such an association, if present, would be mediated by established metabolic risk factors.

Design: A population-based longitudinal cohort study.

Methods: In 1995/1996, OC was determined in blood samples drawn from 1319 subjects aged 65–88 years participating in the Longitudinal Aging Study Amsterdam in 1995/1996. The self-reported CVD events were collected every 3 years until 2005/2006, and CVD deaths until 1st January 2007. Cox proportional hazards regression was performed, considering potential confounders (smoking, physical activity, and BMI) and mediators (blood pressure, plasma triglycerides, total and HDL cholesterol, fructosamine, and aortic calcification).

Results: During the median 4.1 years follow-up, 709 subjects (53.8%) suffered a CVD event. There was no overall association between OC and CVD: hazard ratio (HR) was 0.97 (95% CI 0.90–1.04) per nmol/l higher plasma OC, adjusted for age and sex. There was a statistical interaction between plasma OC, age, and sex on CVD ($P=0.014$). In those subjects aged ≥ 75 years, age-adjusted HRs (95% CI) were 0.86 (0.75–0.99) in men and 1.16 (1.03–1.31) in women per nmol/l higher plasma OC. Adjustment for covariates only slightly attenuated the association in older-old men, but did not affect the association in older-old women.

Conclusion: A higher plasma OC concentration was associated with a reduced risk of CVD in older-old men and with an increased risk of CVD in older-old women. We found no evidence that this was mediated by arterial calcification or metabolic risk factors.

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Introduction

Osteocalcin (OC (BGLAP)) is a non-collagenous protein expressed by the bone-forming osteoblasts (1), thought to be central in bone mineralization. Elevated OC levels have been shown to be associated with osteoporosis (2). OC is also expressed in calcified atherosclerotic lesions, where it is believed to be a mediator in the calcification process (1, 3, 4, 5). Vascular calcification appears to be

an organized process corresponding to bone mineralization, regulated by several bone-associated proteins including OC.

However, bone cells also participate in glucose metabolism via OC secretion, and this may have an impact on the risk of cardiovascular disease (CVD) (6). In animal studies, OC seems to act on pancreatic β -cells and

adipocytes to regulate insulin secretion and sensitivity and fat mass (7, 8). Knockout mice lacking *Oc* showed decreased β -cell proliferation, glucose intolerance, and insulin resistance (7). This notion is supported by recent human studies, which have consistently reported inverse associations between circulating OC and components of the metabolic syndrome in community-dwelling populations (9, 10, 11, 12, 13, 14, 15). A low plasma OC concentration was strongly associated with a higher prevalence of metabolic syndrome in the Longitudinal Aging Study Amsterdam (LASA) (16). This was mediated through an association with blood levels of triglycerides and HDL cholesterol, waist circumference, and hypertension.

The role of OC in CVD remains to be clarified. While some patient studies have demonstrated inverse associations between circulating OC and CVD (17, 18, 19), a large cohort study in community-dwelling older men aged 70–89 years found the highest CVD mortality at a high serum OC concentration (20).

We aimed to investigate whether plasma OC levels were independently associated with the risk of non-fatal and fatal CVDs during up to 11 years of follow-up. We also aimed to investigate whether such an association, if present, would be mediated by atherosclerosis as defined by aortic calcification scores (ACs) or by cardiovascular risk factors including blood pressure, glucose tolerance status, and blood lipids.

Subjects and methods

Data were collected as part of LASA, an ongoing population-based cohort study in older people in The Netherlands. The cohort has been described in detail previously (21). Briefly, a sample of older men and women (aged 55–85 years at baseline), stratified by age, sex, urbanization grade, and expected 5-year mortality, was drawn from the population registers of 11 municipalities in three regions in The Netherlands, being a representative sample of the Dutch population. At baseline (1992/1993) and every third year thereafter, subjects participated in home interviews performed by trained nurses.

Study subjects

The current study included a subgroup participating in the second medical interview of LASA (1995/1996), aged ≥ 65 years, and living in Amsterdam, Zwolle, and Oss and surroundings ($n=1509$). After the medical interview at home, participants were invited to the VU University Medical Center (VUmc) or to hospitals in Zwolle (East) and Oss (South) or a health care center near their homes in the

morning after a light (dairy-free) breakfast, where blood and urine samples were collected ($n=1319$, constituting 87.4%). Participants were interviewed in three consecutive cycles (1998/1999, 2001/2002, and 2005/2006) about cardiovascular events that had occurred since their previous interview.

Biochemical assays

Urine and blood samples were centrifuged and stored at -20 or -70 °C until determination in 1997/1998 at the Endocrine Laboratory in VUmc.

Plasma intact OC was determined using an IRMA (Biosource Diagnostics, now DIASource ImmunoAssays, Louvain-la-Neuve, Belgium) (22). The interassay coefficient of variation (CV) values were 8% at 3.5 nmol/l and 5% at 0.7 nmol/l.

Overnight urinary excretion of the bone resorption marker deoxypyridinoline (DPD) was determined by a competitive immunoassay on an automated ACS 180 System (Chiron Diagnostics, Emeryville, CA, USA). The values were corrected for creatinine (Cr) concentration in the same urine sample. The interassay CV value was 5% (23).

In 2005, blood lipids (total and HDL cholesterol, triglycerides) and fructosamine were determined in EDTA plasma samples stored at -80 °C (24). Cholesterol and triglycerides were determined by an enzymatic colorimetric test (Roche Diagnostics). The interassay CV values were 1.7% for total cholesterol at low and high levels, 6.3% for HDL cholesterol, 2.2% for triglycerides at lower levels (1.0 mmol/l), and 1.8% at higher levels (1.9 mmol/l). LDL cholesterol was calculated by the following formula (25): $LDL = \text{total cholesterol} - \text{HDL cholesterol} - (0.45 \times \text{triglycerides})$.

CVD outcome

Data on incident non-fatal CVD were based on self-reported events collected through interview conducted in three consecutive cycles: 1998/1999, 2001/2002, and 2005/2006. CVD was identified by three predefined questions about whether the subject had experienced myocardial infarction or other heart diseases, including cardiac arrhythmia, congestive heart failure, and angina pectoris, cerebral stroke, or diseases or abnormalities of the arteries, respectively, since their previous interview. The time of event was defined as the mid-date in the interval between the interview where CVD was first reported and the previous cycle ((26)). Cardiovascular deaths occurring until 1st January 2007 were identified

using the cause of death data coded by the Dutch Central Bureau of Statistics (The Netherlands). Cardiovascular deaths were defined by the International Classification of Disease (ICD-10) codes I20–I79 as a primary cause of death (26). The time of event was defined as the date of death.

These outcomes were combined to study the time to first CVD event during follow-up, whether non-fatal or fatal. For censored participants, the end of follow-up was set as the date of death for those deceased before 1st January 2007, or final participation date in a LASA interview for those alive. Maximum observation time was 11.1 years.

Variables collected at baseline

Weight, height, and waist circumference were measured as described previously (16, 23). Participants were asked about smoking and categorized as current, former, or never smokers. They were also asked to present their current medications, which were coded according to the Anatomical Therapeutic Chemical Classification (ATC) system, including hormone replacement therapy (HRT), bisphosphonates, corticosteroids, lipid-lowering medications, anti-hypertensive medications, and anti-diabetic medications. Blood pressure in the arm was measured in the sitting position using a standard mercury sphygmomanometer. As an indicator of socioeconomic status, participants were asked to specify the highest level of education completed, in nine predefined levels ranging from not completed elementary school to university education. This was categorized into years of education ranging from 5 to 18 years. Physical activity was estimated using the validated LASA Physical Activity Questionnaire (LAPAQ) (27), covering minutes spent on household activities, sports, walking outdoors, and bicycling during the previous 2 weeks. The resulting variable was divided into tertiles due to a right-skewed distribution. The presence of aortic calcification was assessed in a subsample of participants living in Amsterdam and surroundings ($n=502$), with lateral-view conventional X-rays of the thoracic and lumbar spine (T4–L5), according to the EVOS protocol (28).

Statistical analyses

Statistical analyses were performed using IBM SPSS 20 and R. We performed the Cox proportional hazards regression model to investigate the relationship between plasma OC levels and incident CVD (29). Initial analysis with penalized splines of OC (30) demonstrated a linear relationship with CVD. Plasma OC was therefore treated as a continuous variable. All analyses were adjusted for age and sex.

We considered BMI, smoking, and physical activity as potential confounders. In addition, we examined potentially mediating factors related to insulin resistance and the risk of CVD, including blood pressure, blood lipids, and fructosamine, in a second series of analyses. Waist circumference was initially examined as a measure of abdominal adiposity (31) and a potential mediator between OC and CVD (11, 14, 15, 16), but was replaced by BMI in the resulting model. The two were highly correlated (Spearman's ρ , 0.87 in men and 0.83 in women), and BMI was collected in a standardized way across study centers and available for almost all subjects (99.5 vs 95.6% for waist circumference). Additional analyses were restricted to those not reporting prevalent CVD at baseline, including 832 subjects (63% of the total sample). Additional analyses were also performed examining non-fatal or fatal CVD respectively. Possible interaction effects of plasma OC with sex and age on CVD were considered by including a three-way interaction term. The significance level, α , was set at 0.05, except for tests of interaction terms where an α value of 0.10 was accepted. After detecting interactions, analyses were performed stratified by sex and age divided at 75 years, corresponding to the median age. Tests and plots of Schoenfeld residuals showed no evidence of time-dependent effects of OC, sex, age, or any other included covariates on CVD, indicating that the data met the assumption of non-proportional hazards.

The cross-sectional association between plasma OC and ACSs at baseline was investigated using logistic regression. Based on an observed increased risk of CVD at an ACS of 4 and above in the data, ACSs were dichotomized into ACS ≥ 4 (indicating calcified lesions of 5 cm or more) compared with ACS ≤ 3 (lesions < 5 cm). In addition, ACSs were examined as a mediator in the Cox regression model in a complete case analysis ($n=502$).

Ethical approval

This study was approved by the Medical Ethics Committee of the VUmc in Amsterdam, The Netherlands, and written informed consent was obtained from all respondents.

Results

During the median 4.1 (interquartile range 1.4–8.1) years of follow-up, 709 (53.8%) of the 1319 participants suffered an incident non-fatal or fatal CVD event. Of these, men experienced 366 events (cumulative incidence 56.8%) and women 343 events (50.8%). Among the 588 subjects reporting a CVD event during follow-up, 268 reported heart

disease only, 76 reported cerebral stroke or cerebral hemorrhage only, and 67 reported diseases or abnormalities of the arteries only, while 177 subjects reported at least two of the above-mentioned events. Among the 219 fatal CVD events during follow-up, 151 (69.0%) were attributed to ischemic heart disease, pulmonary heart disease, and other forms of heart diseases (ICD-10 codes, I20–I52), 53 (24.2%) were attributed to cerebrovascular diseases (ICD-10 codes, I60–I69), and 15 (6.8%) were attributed to diseases of arteries, arterioles, and capillaries (ICD-10 codes, I70–I79).

Baseline characteristics

Mean (range) age at baseline was 75 (65–88) years and 51% were women. There was a positive relationship between age and plasma OC, and OC concentration was higher in women than in men. Characteristics according to age and sex are presented in Table 1. When comparing the baseline characteristics of men and women regardless of age, women had a lower smoking prevalence (12 vs 25%), a lower educational level (mean 8.1 vs 9.7 years), and a higher BMI (mean 27.6 vs 26.0 kg/m²), and they reported more time spent on physical activity than men (mean 173 vs 123 min/day); $P < 0.001$ for all comparisons. Blood pressure and plasma lipids also differed between the age and sex strata. The characteristics that varied significantly across the quartiles of plasma OC are shown in Table 2. The proportion of never smokers was higher at higher plasma OC levels, explained by a higher proportion of never smokers among women. There was a trend of decreasing BMI and waist circumference across increasing plasma OC concentrations. As expected, urinary DPD/Cr increased through increasing plasma OC concentration (Table 2). Use of HRT in women was not significantly associated with plasma OC (not shown). Only one subject, having plasma OC in the highest quartile, reported use of bisphosphonates (not shown).

Plasma OC concentration and incident CVD

We did not observe an overall association between plasma OC and non-fatal or fatal CVD in the Cox regression model: the hazard ratio (HR) was 0.97 (95% CI 0.90–1.04) per nmol/l higher plasma OC, adjusted for age and sex. However, an interaction term between sex and plasma OC was statistically significant (age-adjusted, $P = 0.006$). Sex-stratified analyses revealed an inverse relationship between plasma OC and CVD in men, with a reduced risk at a higher plasma OC concentration (Fig. 1). In men, the age-adjusted HR for CVD was 0.88 (0.79–0.99) per nmol/l

higher plasma OC. In women, there was a tendency for a positive association, which was not statistically significant: the age-adjusted HR was 1.06 (95% CI 0.96–1.16) per nmol/l.

In the overall sample, we observed a statistically significant three-way interaction between plasma OC, sex, and age on the risk of CVD ($P = 0.014$). There was also a statistically significant two-way interaction between plasma OC and sex ($P = 0.024$), while it was less pronounced for plasma OC and age ($P = 0.104$). When stratifying both sexes into two age groups, no association was found between plasma OC and CVD in the younger-old participants (65–74 years) in either sex. However, in the older-old men (≥ 75 years), there was an inverse linear association between plasma OC and CVD, while there was a positive linear association in the older-old women (Fig. 2). Older-old men had a 14% reduced risk of CVD per nmol/l higher plasma OC, while the older-old women had a corresponding 16% increased risk (Table 3). The associations persisted with adjustment for potential confounders and cardiovascular mediators respectively. When including adjustment for bone turnover (DPD/Cr), the positive relation in the older-old women persisted, while the inverse association in older-old men was slightly attenuated (Table 3).

Additional analyses

Analyses restricted to those who did not report CVD at baseline yielded similar associations as in the total sample: the HR was 1.01 (95% CI 0.90–1.13) per nmol/l, with statistically significant interaction terms: $P = 0.004$ for a three-way interaction between plasma OC, age, and sex; $P = 0.004$ for plasma OC and sex; and $P = 0.054$ for plasma OC and age. In older-old women (≥ 75 years), the positive linear association between plasma OC and CVD was even stronger (Fig. 2 and Table 3).

Additional analysis considering non-fatal CVD showed the same patterns but with less precision. The association between plasma OC and non-fatal CVD was statistically significant only in the older-old women, with an age-adjusted HR of 1.16 (95% CI 1.01–1.33) per nmol/l higher plasma OC. The same was found when studying fatal CVD only, with an age-adjusted HR of 1.20 (95% CI 1.00–1.44) per nmol/l for the older-old women, and no statistically significant findings in the other age and sex strata.

Analysis with urinary DPD/Cr as exposure revealed a similar relationship with CVD as plasma OC. There was a statistical interaction between sex and urinary DPD/Cr on CVD ($P = 0.005$), but not between age and urinary DPD/Cr.

Table 1 Baseline characteristics and cardiovascular events during the follow-up in sex and age strata of participants aged 65 years and older, who provided blood samples in the LASA C-cycle 1995/1996 ($n=1319$)^a.

	Overall	Men 65–74 ($n=309$)	Men 75–88 ($n=335$)	Women 65–74 ($n=339$)	Women 75–88 ($n=336$)	P value ^b
Osteocalcin and bone turnover						
Plasma OC (nmol/l), range	0.05–9.48	0.43–5.85	0.37–9.48	0.05–8.54	0.10–7.87	–
Plasma OC (nmol/l), mean (s.d.)	2.19 (1.11)	1.82 (0.75)	2.20 (1.27)	2.22 (1.04)	2.50 (1.16)	<0.001
Urinary DPD/Cr (nmol/mmol), mean (s.d.)	5.6 (2.4)	4.6 (1.9)	5.4 (2.4)	5.7 (2.4)	6.7 (2.6)	<0.001
Potential confounders						
Age, mean (s.d.)	75.6 (6.6)	69.8 (2.7)	81.2 (3.8)	69.9 (2.8)	81.2 (3.8)	<0.001
Current smoker, n (%)	241 (18.3)	83 (26.9)	78 (23.3)	61 (18.0)	19 (5.7)	
Former smoker, n (%)	609 (46.2)	199 (64.4)	219 (65.4)	116 (34.2)	75 (22.3)	
Never smoker, n (%)	469 (35.6)	27 (8.7)	38 (11.3)	162 (47.8)	242 (72.0)	<0.001
Use of corticosteroids, n (%)	106 (8.0)	26 (8.4)	39 (11.6)	21 (6.2)	20 (6.0)	0.024
Hormone replacement therapy (women), n (%)	13 (1.9)	–	–	5 (1.5)	8 (2.4)	0.39
Use of anti-hypertensive medications, n (%)	528 (40.0)	110 (35.6)	131 (39.1)	111 (32.7)	176 (52.4)	<0.001
Use of lipid-lowering medications, n (%)	64 (4.0)	15 (4.9)	8 (2.4)	31 (9.1)	10 (3.0)	<0.001
Use of anti-diabetic medications, n (%)	76 (5.8)	13 (4.2)	21 (6.3)	17 (5.0)	25 (7.4)	0.31
Educational level attained (years), mean (s.d.)	8.9 (3.3)	10.0 (3.3)	9.5 (3.5)	8.2 (2.9)	8.0 (3.2)	<0.001
BMI (kg/m^2), mean (s.d.)	26.9 (4.2)	26.3 (3.3)	25.8 (3.5)	27.5 (4.4)	27.8 (5.1)	<0.001
Physical activity (total minutes per day), mean (s.d.)	149 (97)	133 (93)	114 (88)	192 (99)	153 (91)	<0.001
Potential mediators (mean (s.d.))						
Systolic blood pressure (mmHg)	152.7 (26.1)	151.4 (24.2)	153.8 (26.0)	149.3 (25.0)	156.4 (28.5)	0.004
Diastolic blood pressure (mmHg)	83.2 (13.6)	85.9 (14.3)	81.1 (13.5)	83.6 (11.9)	82.5 (14.1)	<0.001
Total plasma estradiol (pmol/l)	54.2 (30.9)	76.3 (24.7)	78.2 (25.7)	31.4 (13.9)	32.9 (18.3)	<0.001
Plasma fructosamine (mmol/l)	235 (38)	229 (31)	236 (40)	235 (37)	241 (42)	0.001
Plasma triglycerides (mmol/l)	1.52 (0.78)	1.57 (0.84)	1.41 (0.63)	1.54 (0.85)	1.57 (0.78)	0.024
Plasma total cholesterol (mmol/l)	5.67 (1.03)	5.58 (0.95)	5.28 (1.00)	6.06 (0.99)	5.77 (1.03)	<0.001
Plasma HDL cholesterol (mmol/l)	1.33 (0.43)	1.22 (0.41)	1.25 (0.40)	1.47 (0.43)	1.39 (0.42)	<0.001
Plasma LDL cholesterol (mmol/l)	3.66 (0.95)	3.65 (0.85)	3.40 (0.96)	3.91 (0.96)	3.69 (0.96)	<0.001
Waist circumference (cm)	95.7 (11.4)	99.3 (10.1)	99.0 (10.3)	91.4 (11.3)	93.4 (11.6)	<0.001
CVD events during follow-up (n (%))						
Incident self-reported CVD	588 (44.6)	153 (49.5)	150 (44.8)	129 (38.1)	156 (46.4)	0.025
Incident fatal CVD	219 (16.6)	32 (10.4)	79 (23.6)	31 (9.1)	77 (22.9)	<0.001
Total incident CVD events (non-fatal or fatal)	709 (53.8)	173 (56.0)	193 (57.6)	148 (43.7)	195 (58.0)	<0.001

^aThe number with valid data varies from $n=1319$ (age, sex, plasma OC, smoking, medications, and CVD follow-up) down to $n=1261$ (waist circumference).

^bOne-way ANOVA for comparison of continuous variables or the χ^2 test for comparison of percentages across all four strata.

Urinary DPD/Cr was not associated with CVD overall or in men, but positively associated with CVD in women: the age-adjusted HR per nmol/mmol of higher urinary DPD/Cr was 1.05 (95% CI 1.01–1.10), and this was not affected by adjustment for plasma OC or by BMI.

Arterial calcification

No relationship was found between plasma OC and odds of ACS ≥ 4 . In women, we observed reduced estimates at a higher plasma OC concentration, although they did not

reach statistical significance: the age-adjusted OR was 0.69 (95% CI 0.45–1.02) for ACS ≥ 4 /nmol/l higher plasma OC in older-old women. Additional analysis showed a significantly reduced odds ratio for detectable AC (ACS ≥ 2) compared with no AC (ACS=1) at a higher plasma OC concentration in all women: the OR was 0.76 (95% CI 0.58–0.99) per nmol/l higher plasma OC in age-adjusted analysis, but no association in men. Including ACSs as a potential mediator in a secondary analysis of the association between plasma OC and CVD did not attenuate the associations (not shown).

Table 2 Baseline characteristics that varied significantly across quartiles of plasma OC in men and women aged 65 years and older, who provided blood samples in the LASA C-cycle 1995/1996 (all men and women combined).

	<i>n</i>	Overall	Q1 (<i>n</i> =334)	Q2 (<i>n</i> =329)	Q3 (<i>n</i> =326)	Q4 (<i>n</i> =330)	<i>P</i> value ^a
Osteocalcin and bone turnover							
Plasma OC (nmol/l), range	1319	0.05–9.48	0.05–1.44	1.45–2.01	2.02–2.63	2.64–9.48	–
Plasma OC (nmol/l), mean (s.d.)	1319	2.19 (1.11)	1.10 (0.28)	1.75 (0.16)	2.31 (0.19)	3.61 (1.14)	–
Urinary DPD/Cr (nmol/mmol), mean (s.d.)	1276	5.6 (2.4)	4.8 (1.9)	5.1 (2.1)	5.6 (2.3)	6.9 (2.9)	<0.001
Potential confounders							
Age, mean (s.d.)	1319	75.6 (6.6)	74.6 (6.4)	74.8 (6.4)	75.6 (6.5)	77.4 (6.6)	<0.001
Sex (women), <i>n</i> (%)	1319	675 (51.2)	130 (38.9)	144 (43.8)	195 (59.8)	206 (62.4)	<0.001
Current smoker, <i>n</i> (%)	1319	241 (18.3)	60 (18.0)	62 (18.8)	53 (16.3)	66 (20.0)	
Former smoker, <i>n</i> (%)		609 (46.2)	167 (50.0)	169 (51.4)	145 (44.5)	128 (38.8)	
Never smoker, <i>n</i> (%)		469 (35.6)	107 (32.0)	98 (29.8)	128 (39.3)	136 (41.2)	0.010
Use of corticosteroids, <i>n</i> (%)	1319	106 (8.0)	33 (9.9)	36 (10.9)	22 (6.7)	15 (4.5)	0.009
Use of anti-hypertensive medications, <i>n</i> (%)	1319	528 (40.0)	161 (48.2)	120 (36.5)	119 (36.5)	128 (38.8)	0.005
BMI (kg/m ²), mean (s.d.)	1313	26.9 (4.2)	27.9 (4.1)	27.1 (4.1)	26.6 (4.3)	25.8 (4.2)	<0.001
Potential mediators (mean (s.d.))							
Plasma fructosamine (mmol/l)	1295	235 (38)	240 (43)	233 (41)	235 (35)	233 (31)	0.047
Plasma triglycerides (mmol/l)	1293	1.52 (0.78)	1.74 (0.94)	1.55 (0.76)	1.44 (0.70)	1.34 (0.64)	<0.001
Plasma HDL cholesterol (mmol/l)	1292	1.33 (0.43)	1.24 (0.41)	1.30 (0.42)	1.39 (0.43)	1.42 (0.43)	<0.001
Waist circumference (cm)	1261	95.7 (11.4)	99.6 (10.7)	97.2 (10.5)	93.6 (11.4)	92.2 (11.5)	<0.001
Total plasma estradiol (men; pmol/l)	635	77.3 (25.2)	82.5 (25.7)	75.6 (22.9)	76.4 (24.6)	72.2 (26.9)	0.002
Total plasma estradiol (women; pmol/l)	664	32.1 (16.3)	38.7 (24.9)	30.6 (11.7)	32.1 (14.8)	29.0 (11.6)	<0.001

^aOne-way ANOVA for comparison of continuous variables or the χ^2 test for comparison of percentages across quartiles.

Discussion

In this population-based longitudinal cohort study, we found that higher plasma OC concentrations were associated with a reduced risk of CVD in men aged 75 years and older, but with an increased risk of CVD in women aged 75 years and older. We did not find any evidence that associations between plasma OC and CVD were mediated by established metabolic risk factors or by aortic calcification. Plasma OC was unrelated to the risk of CVD in the younger-old subjects.

Despite the growing literature on mechanisms linking OC to the atherosclerotic process, very few studies have investigated the influence of circulating OC on the risk of CVD in community-dwelling older subjects, and the findings are contradictory. In a cohort of 3542 older men followed for a median of 5.2 years with 198 fatal CVD events, a U-shaped association between plasma OC and fatal CVD was found (20). While CVD mortality was lowest in the second OC quintile, a significant 30% increased risk was observed in the first quintile and a 69% increased risk in the fifth quintile. As observed in our study, plasma total OC at baseline was the exposure measured, and the Cox regression model was adjusted for conventional cardiovascular risk factors. In our study with 111 CVD-related deaths in men and 108 in women, the results suggested a linear positive association between

plasma OC and fatal CVD in women only, with an increased risk of CVD at high OC levels, while we could not find any evidence of a non-linear effect. A positive association was also found between serum OC and prevalence of coronary heart disease (CHD) in a cross-sectional study of 78 patients with type 2 diabetes mellitus (32). By contrast, inverse associations showing an increased risk of CVD events at low serum OC levels were found in hemodialysis patients (19) and in young survivors of myocardial infarction (18). A cross-sectional study in patients who underwent coronary angiography found a linear negative relationship between serum OC and CHD prevalence (17).

These contradictory results imply that OC measured in plasma may be a correlate of different unmeasured influences among different population subgroups and that causality cannot be inferred at this stage. To what extent OC measured in blood samples reflects OC produced by osteoblasts in bone or in other tissues, such as the arterial walls, is unresolved. Taking cardiovascular risk factors into account did not substantially influence the observed associations between plasma OC and the risk of CVD in our study. However, when taking a marker of bone resorption (urinary DPD/Cr) into account, the association was attenuated and non-significant in older-old men, while it was still significant in older-old women. In women, both OC and DPD/Cr

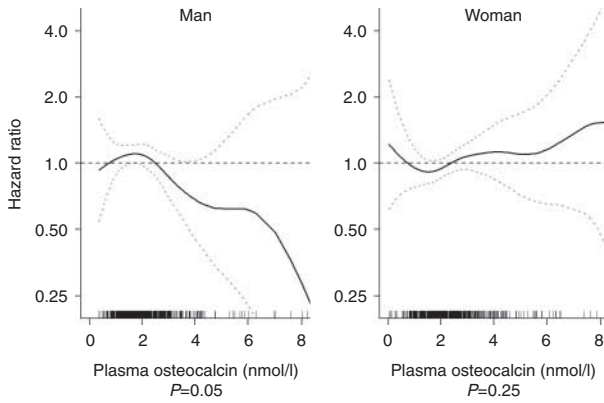


Figure 1

Age-adjusted hazard ratios with 95% CI of non-fatal or fatal cardiovascular disease across the distribution of plasma OC in men and women. Men: mean (s.d.) plasma OC concentration was 2.01 (1.07) nmol/l, 366 events in 644 participants. Women: mean (s.d.) plasma OC concentration was 2.36 (1.11) nmol/l, 343 events in 675 participants. Rug plots along the horizontal axes represent individual plasma OC concentrations. *P* values for the linear association between plasma OC and CVD are given.

showed independent positive associations with the risk of CVD. It is recognized that osteoporosis and CVD are age-related conditions that seem to share common characteristics and potentially common underlying pathophysiological mechanisms. Inflammatory cytokines, endogenous sex hormones, lipid oxidation, vitamin K deficiency, and vitamin D deficiency have been implicated as possible mediators in the progression of both conditions (33).

The inverse association observed in men is plausible in light of the growing body of evidence from studies in community-dwelling populations (9, 10, 11, 12, 13, 14, 15), including our own study (16), that consistently confirm a relationship between low circulating OC levels and established predictors of CVD. These include central adiposity, hypertension, and unfavorable lipid composition. In the large study in Australian men, where a U-shaped relationship between plasma OC and fatal CVD was found (20), an inverse association between plasma OC and metabolic syndrome was also demonstrated (11). This seemed to be mediated by waist circumference, hyperglycemia, and hypertriglyceridemia. These factors were strongly related to the risk of CVD in men in LASA, but could not provide a link for the negative association between plasma OC and CVD in older-old men in our data. Larger cohorts and inclusion of measurements of

undercarboxylated OC are required to clarify the relationship between OC and the risk of CVD in men.

The degree of aortic calcification detectable on lateral radiographs of the spine is strongly predictive of CVD events (34). This was true also for our data. Positive associations between circulating OC and parameters of atherosclerosis were found in a cross-sectional study in 78 participants with type 2 diabetes mellitus (32), in a 6-month follow-up study in 50 participants with type 2 diabetes mellitus (35), and in a study in 67 patients on peritoneal dialysis (36). In the Study of Osteoporotic Fractures, no cross-sectional association was detected between serum OC and abdominal aortic calcification in 363 older women (37). Correspondingly, there was no

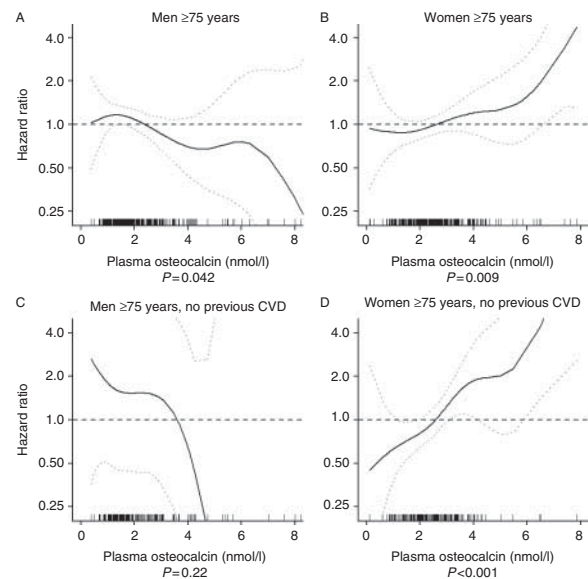


Figure 2

Age-adjusted hazard ratios with 95% confidence bands for non-fatal or fatal cardiovascular disease across the distribution of plasma OC in men and women aged 75 years and older. (A) Men: mean (s.d.) plasma OC concentration was 2.20 (1.27) nmol/l, 193 events in 335 participants. (B) Women: mean (s.d.) plasma OC concentration was 2.50 (1.16) nmol/l, 195 events in 336 participants. (C) Men, restricted to those not reporting CVD at baseline: mean (s.d.) plasma OC concentration was 2.27 (1.43) nmol/l, 57 events in 174 participants. (D) Women, restricted to those not reporting CVD at baseline: mean (s.d.) plasma OC concentration was 2.45 (1.05) nmol/l, 82 events in 200 participants. Rug plots along the horizontal axes represent individual plasma OC concentrations. *P* values for the linear association between plasma OC and CVD are given.

Table 3 Sex- and age-specific hazard ratios for incident non-fatal or fatal cardiovascular disease per nmol/l higher plasma OC at baseline.

Sex	Age at baseline (years)	n	CVD events	HR ^a	95% CI ^a	HR ^b	95% CI ^b	HR ^c	95% CI ^c	HR ^d	95% CI ^d
All participants (n=1319)											
Men	65–74	309	173	0.95	0.77–1.16	1.04	0.84–1.31	1.08	0.86–1.35	1.15	0.91–1.46
	75–88	335	193	0.86	0.75–0.99	0.86	0.75–1.00	0.84	0.72–0.98	0.89	0.76–1.03
Women	65–74	339	148	0.93	0.79–1.09	0.96	0.82–1.14	0.97	0.81–1.16	0.91	0.76–1.08
	75–88	336	195	1.16	1.03–1.31	1.16	1.01–1.32	1.16	1.01–1.33	1.15	1.00–1.32
No previous CVD (n=832)											
Men	65–74	191	76	1.07	0.79–1.45	1.21	0.84–1.74	1.26	0.86–1.84	1.39	0.97–1.98
	75–88	174	57	0.75	0.56–1.00	0.71	0.52–0.98	0.74	0.53–1.01	0.79	0.58–1.08
Women	65–74	267	84	0.90	0.73–1.12	0.88	0.71–1.10	0.92	0.72–1.17	0.86	0.67–1.10
	75–88	200	82	1.47	1.21–1.79	1.49	1.21–1.83	1.49	1.17–1.89	1.38	1.10–1.73

^aAdjusted for age.^bAdjusted for age and potential confounders: BMI, ever smoking, and physical activity.^cAdjusted for age, BMI, and possible cardiovascular mediators: systolic BP, plasma triglycerides, total and HDL cholesterol, and fructosamine.^dAdjusted for age, BMI, and bone resorption (urinary DPD:creatinine ratio).

positive association between plasma OC and ACSs in our data. Thus, our findings did not support a mediating role of aortic calcifications in the relationship between plasma OC and CVD. If anything, there was a suggested inverse association between plasma OC and aortic calcification in older-old women, with a higher degree of aortic calcification at lower plasma OC levels. This corresponds with the results of relatively large studies carried out in community-dwelling populations demonstrating inverse relationships between circulating OC and progression of atherosclerosis (17, 38, 39, 40). The authors attribute this apparent protective effect of OC to a plausible indirect effect via its regulation of glucose and fat metabolism or other unidentified pathways. One author (39) noted that the effect of OC could be sex specific. Murine data have suggested that OC regulates testosterone levels in males to modulate fertility, while *Oc*-deficient females do not have any fertility abnormality (41).

A high bone turnover measured by urinary DPD/Cr predicted CVD, but did not account for the effect of OC in women. Estrogen deficiency causes a dramatic increase in bone resorption after menopause. In LASA, both men and women in the lowest quartile of bioavailable estradiol (E_2) measured in plasma had a higher bone turnover (plasma OC and urinary DPD/Cr) compared with those in the highest E_2 quartile (23). In *post-hoc* analyses, our observed associations between plasma OC and the risk of CVD could not be attributed to plasma E_2 . By contrast, with adjustment for plasma E_2 , the positive association between plasma OC and CVD in the older-old women persisted, and the inverse association between plasma OC and

CVD in the oldest men was strengthened (not shown). Moreover, statistical adjustment for plasma testosterone (23) did not influence the association between plasma OC and CVD within the older-old men. Thus, the observed sex differences were not explained by differing sex hormone levels.

Differences in metabolic factors or physical activity did not explain the sex differences (not shown). It may also be speculated whether there may be sex differences in renal function impairment explaining the observed estimates. Men had a higher urinary Cr concentration than women. However, plasma OC was not associated with urinary Cr within the sexes, and including urinary Cr in a *post-hoc* analysis did not influence the observed associations.

In summary, the reasons for the contradictory findings in men and women could not be identified in this study. We may speculate that the associations are attributable to unidentified factors correlated with circulating OC, such as adiponectin (42) or vitamin K deficiency (33).

A limitation of our study is that information on the proportion of undercarboxylated OC was not available. Therefore, it could not be determined whether risk of CVD was associated with the degree of carboxylation. Experimental studies have suggested that fat and glucose metabolism is regulated by the undercarboxylated isoform (7). Nevertheless, measures of carboxylated or total OC concentrations have been associated with CVD and metabolic risk factors in several studies (9, 11, 12, 14, 20, 32, 40), including LASA (16). The use of mid-date between

interviews due to the lack of exact event dates for self-reported CVD is also a limitation with the potential to dilute the true associations. Another limitation is that only a subsample of 502 participants, all living in Amsterdam and surroundings, had ACS measurements. However, the age and sex distribution and the risk of CVD in the subsample were similar to that in the overall sample, indicating that they were representative of LASA.

Conclusion

A higher plasma OC concentration was associated with a reduced risk of CVD in older-old men, but with an increased risk in older-old women. The positive association observed in older-old women was more robust. This was not mediated by the established metabolic risk factors for CVD or by aortic calcification. The underlying mechanisms for the observed sex differences represent a challenge for future studies.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

K Holvik prepared the data, performed the statistical analyses and drafted the manuscript, and takes responsibility for the integrity of the data analysis. N M van Schoor, P Lips, and R de Jongh provided scientific advice and contributed to the methods and the design of the manuscript. All coauthors have critically discussed the results, revised the manuscript, and accepted the final version.

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