Published in final edited form as:

Endocrine. 2015 December ; 50(3): 627–632. doi:10.1007/s12020-015-0618-6.

Osteocalcin carboxylation is not associated with body weight or percent fat changes during weight loss in post-menopausal women

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Abstract

Osteocalcin (OC) is a vitamin K-dependent bone protein used as a marker of bone formation. Mouse models have demonstrated a role for the uncarboxylated form of OC (ucOC) in energy metabolism, including energy expenditure and adiposity, but human data are equivocal. The purpose of this study was to determine the associations between changes in measures of OC and changes in body weight and percent body fat in obese, but otherwise healthy post-menopausal women undergoing a 20-week weight loss program. All participants received supplemental vitamins K and D and calcium. Body weight and body fat percentage (%BF) were assessed before and after the intervention. Serum OC [(total (tOC), ucOC, percent uncarboxylated (%ucOC)], and procollagen type 1N-terminal propeptide (P1NP; a measure of bone formation) were measured. Women lost an average of 10.9 ± 3.9 kg and 4 %BF. Serum concentrations of tOC, ucOC, %ucOC, and P1NP did not significantly change over the twenty-week intervention, nor were these measures associated with changes in weight (all $p > 0.27$) or %BF (all $p > 0.54$). Our data do not support an association between any serum measure of OC and weight or %BF loss in postmenopausal women supplemented with nutrients implicated in bone health.

Keywords

Osteocalcin; Vitamin K; Weight loss; Body fat

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Electronic supplementary material The online version of this article (doi:10.1007/s12020-015-0618-6) contains supplementary material, which is available to authorized users.

Conflict of interest The authors have no conflicts of interest to disclose.

Introduction

Osteocalcin (OC) is a vitamin K (VK)-dependent protein found primarily in bone. It is synthesized in osteoblasts [1] and undergoes a VK-dependent post-translational modification that results in carboxylation of specific glutamate residues. In its carboxylated (cOC) form, OC binds to hydroxyapatite in bone [2]. Serum concentrations of OC have been used to assess bone formation [3], whereas the percent of OC that is not carboxylated (%ucOC) is used as a measure of VK status [4]. Although the Adequate Intake for VK in the form of phylloquinone (vitamin K₁) has been established at 120 and 90 µg phylloquinone per day for men and women, respectively, intakes of 100 µg phylloquinone per day do not result in complete carboxylation of osteocalcin [5].

Based on studies in mice, OC, specifically the uncarboxylated (ucOC) form, regulates energy metabolism, including energy expenditure and measures of adiposity [6]. Subsequent studies corroborated the findings in mice and extended the hypothesis regarding the influence of ucOC to glucose metabolism, male fertility, and brain development [7–14]. Attempts to replicate these findings in humans have been inconclusive. Most analyses of the association between OC and metabolic outcomes in humans are limited by the reliance on the exclusive measurement of total OC (tOC) and not ucOC [2]. Furthermore, observed associations may be simply tracking a larger influence of changes in energy balance on bone turnover.

The purpose of this study was to determine the associations between measures of OC and weight loss or body fat changes in healthy overweight/obese post-menopausal women undergoing a weight loss program. All women received supplemental vitamins D and K and calcium, which would minimize the potential influence of inadequate or varied micronutrient intake on bone loss and OC carboxylation [15–18]. Multiple forms of OC were measured, along with an additional marker of bone formation to control for changes in bone turnover that might accompany weight loss or reduction in body fat.

Materials and methods

Data were obtained from the Diet, Exercise, and Metabolism for Older Women (DEMO) study, a 20-week randomized trial comparing the effects of caloric restriction (CR) with and without aerobic exercise on body composition changes and weight loss, as described elsewhere [19]. This study and protocol were approved by the Wake Forest University School of Medicine Institutional Review Board and the Tufts University School of Medicine Institutional Review Board, and all participants provided written informed consent that included language to inform participants that biological samples would be stored for future laboratory analyses, such as those measured in this study ([clinicaltrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT00664729) #NCT00664729).

Study participants

Women were recruited from the Forsyth County, NC area. Inclusion criteria were sedentary post-menopausal women between 50 and 70 years of age, self-reported not receiving hormone replacement therapy of any type, self-reported weight stable for 6 months prior to

enrollment, with a BMI between 25 and 40 kg/m² and waist circumference >88 cm. Exclusion criteria included those with systemic disorders, uncontrolled diabetes, and/or taking diabetic medications. Of the 1078 women initially screened, a total of 112 were randomly assigned to one of three interventions [19]. For this study, only women who had sufficient archived sera to conduct complete measurements from both time points were included ($n = 71$).

Anthropometrics

Absolute total, lean and fat body mass were measured pre-and post-intervention using dual-energy X-ray absorptiometry (DXA, Hologic Delphi QDR, Bedford, MA) and used to calculate percent body fat (%BF). Height was measured using a stadiometer and weight with a body weight scale. Height and weight were measured with the participant shoeless and wearing minimal clothing [19].

Intervention groups

The three intervention groups were controlled for an average 400 kcal/day (2800 kcal/week) deficit with similar diet composition between groups (25–30 % fat, 15–20 % protein, and 50–60 % carbohydrate). Participants received counseling on breakfast choices while food for lunch, dinner, and snacks were provided for the course of the study. The caloric restriction-only group (CR) had all caloric deficits through diet restriction alone. In addition to diet restriction, women in the moderate intensity exercise and vigorous intensity exercise groups were prescribed an exercise regimen equal in intensity to 45–50 % (moderate) or 70–75 % (vigorous) heart rate reserve (HRR) three times per week as described in detail in the parent paper [19]. All participants regardless of intervention were also given a daily dietary supplement (Viactiv®, Viactiv Lifestyle®) that provided 1000 mg/day calcium carbonate, 200 IU/day Vitamin D3, and 80 µg/day VK (phylloquinone).

Biochemical measures

Blood samples were collected from each participant pre-and post-intervention (0 and 20 weeks respectively) in commercial EDTA-containing evacuated tubes by venipuncture in the early morning after a 12-h fast. All samples were stored for less than 5 years at –70 °C without multiple freeze–thaw cycles. Samples were then shipped frozen to the Human Nutrition Research Center on Aging at Tufts University (Boston, MA) for biochemical analyses. The stability of tOC under these conditions has been tested out to one year with no significant difference between fresh and stored frozen sample [20].

Total and uncarboxylated plasma OC concentrations were determined by radioimmunoassay before (to determine tOC) and after (to determine ucOC) separation by hydroxyapatite binding, respectively, at the Human Nutrition Research Center on Aging at Tufts University (Boston, MA) as described in detail elsewhere [21]. The total (interassay plus intrassay) coefficient of variation (CV) for all ucOC and total OC measures conducted as part of this study was 24.7 %. Serum phylloquinone (vitamin K1) was determined using high-performance liquid chromatography (HPLC) [22]. Plasma vitamin D (25-(OH)D) was measured by radioimmunoassay (DiaSorin, Stillwater, MN) [23]. Plasma triglycerides were measured using standard methods as previously described [19]. Intact N-terminal propeptide

of type I procollagen (P1NP) was assessed by radioimmunoassay (Orion Diagnostica, Finland) [24]. According to the Orion website, the sensitivity of the P1NP assay is 2 µg/L with specificity of 2.7 % (<http://www.oriondiagnostica.com/>).

Statistical analysis

In the parent study, there were no significant differences in baseline weight or weight loss when compared among intervention groups ($p > 0.29$) [19]. Similarly, there were no statistical differences in measures of OC at either time point among the three groups (all $p > 0.13$, Supplementary Table 1). Therefore, the participants from all three groups were pooled for the current analysis. Baseline and follow-up characteristics were summarized using mean $\pm$ standard deviation (normally distributed data), medians $\pm$ interquartile range (skewed data) or proportions as appropriate. General linear models were used to determine if changes in serum concentrations of OC (ucOC, tOC, and %ucOC) predicted change in body weight or %BF. Covariates included age, race (non-Hispanic white or African- American), and treatment group. Similar methods were used to determine if changes in serum phyloquinone, 25-(OH)D and P1NP predicted change in either body weight or %BF. Since VK transport is dependent on triglyceride-rich lipoproteins [25], plasma triglycerides were included as a covariate in the VK the model while cholesterol was included in the 25-(OH)D model. Spearman correlations between tOC and ucOC (ng/mL) and tOC and %ucOC (%) were calculated. The statistical software SAS version 9.3 (SAS Institute Inc, Cary, NC) was used to analyze all data. Significance was set at $p < 0.05$.

Results

At baseline, women were 58.7 ± 5.4 years of age and had a mean BMI of 33.0 ± 3.8 kg/m² (Table 1). Women lost an average of 10.9 ± 3.9 kg (12.5 %) and 3.9 ± 2.0 %BF during the intervention. The median $\pm$ interquartile range for ucOC was 1.8 ± 3.0 ng/mL at baseline, and did not significantly change over the course of the intervention ($p = 0.09$). At baseline, fasting plasma phyloquinone concentrations were higher than observed in the general population [22, 26, 27], while 25-(OH)D concentrations were similar to those reported in the general population (47–65 nmol/L) [28, 29]. Plasma phyloquinone did not significantly change ($p = 0.17$) whereas 25-(OH)D concentrations increased ($p < 0.05$) over the course of the intervention.

Percent change in OC, independent of form, did not predict change in body weight or %BF (Table 2). Additionally, changes in phyloquinone, 25-(OH)D and P1NP did not predict changes in body weight or %BF (Table 2). Total OC and ucOCat baseline were significantly correlated ($r = 0.64$, $p < 0.001$). A similar correlation was observed post-intervention ($r = 0.65$, $p < 0.001$). In contrast, there was no significant correlation between tOC and %ucOC at baseline ($r = -0.13$, $p = 0.29$) or post-intervention ($r = -0.06$, $p = 0.60$), indicating that the absolute amount of ucOC may simply be tracking overall tOC.

Discussion

In this study of post-menopausal women enrolled in a weight loss intervention, changes in tOC, ucOC, and %ucOC were not significantly associated with weight loss or changes in %BF.

Prior human studies on the role of OC in energy metabolism have been equivocal. Some cross-sectional studies have reported higher serum concentrations of ucOC and tOC associated with lower body weight and measures of body fat [30–39]. Others have reported no associations [40, 41]. In the one longitudinal study, a three-month increase in ucOC was associated with a twelve-month decrease in body fat [42]. However, we demonstrated in our study that absolute ucOC in humans is correlated with tOC. Total OC is a robust marker of bone formation so significant findings using absolute ucOC concentrations may simply be tracking a larger influence of changes in energy metabolism on bone turnover, independent of any unique effect of ucOC.

To account for the high correlation between absolute ucOC and tOC concentrations, we calculated %ucOC to differentiate between carboxylation and total amount of circulating OC. In our study, measures of %ucOC were also not associated with weight loss or changes in %BF. Since both ucOC and %ucOC did not change but women still lost weight, it is unlikely that ucOC has a unique role in weight or %BF loss. This is supported by our previous work which found no form of OC associated with weight loss in older men and women [40]. Additionally, weight loss in a VK supplementation trial was not associated with ucOC [41]. However, neither of these studies was designed to specifically promote weight loss in a controlled manner, whereas the current study was intentionally designed to promote the weight loss through clearly defined interventions combining diet and exercise, which is one of the strengths of this study. Women achieved weight loss through caloric restriction and participation in an exercise regimen of moderate or vigorous intensity three times per week [19]. Similar to earlier reports finding no link between intensity of aerobic exercise and OC concentrations, we also found that no form of OC was associated with any intensity of aerobic exercise performed by post-menopausal women [43–47].

Another strength of our study is the inclusion of an additional independent bone formation marker to separate the putative OC effect from an overall bone effect. In our study neither ucOC nor P1NP was associated with body weight or %BF changes. This indicates no effect of bone turnover on weight loss, or conversely, no effect of the degree of weight loss on bone turnover. Additionally, intake of nutrients known to influence bone turnover was supplemented, which is likely to mitigate their influence [48, 49]. Since the majority of previously published studies did not take into account the status of nutrients involved in bone turnover, it is unclear how this difference in nutrient status may affect the reported results [31, 38, 42].

In the mouse model, only the ucOC form was responsible for weight loss [6]. In humans, the only known mechanism by which carboxylation of OC is modified is through manipulation of VK [50]. In the current study, the carboxylation of OC was kept constant in part because of low dose VK supplementation. However, participants still lost weight which suggests that

the carboxylation of OC is unrelated to weight loss in humans. In other studies using much higher doses of VK supplementation (500–1000 µg/day compared to the current 80 µg/day), there was also no observed association between ucOC and measures of body weight or body fat [40, 41].

Limitations of this study merit consideration. This was a secondary analysis and not the primary outcome of the overall study. Because this study was an observational analysis, we cannot establish causality. Our sample limits the generalizability of the results to postmenopausal women. Additionally, there is a high amount of bone turnover in the immediate time point following onset of menopause which subsides after about 5 years. Since there were some women who were less than 5 years postmenopausal, we conducted an analysis to determine if OC was different between these two groups and found no difference. Therefore, we included all women in the analysis. The stability of OC has not been published for greater than a one year timeframe, so, it is unknown if some degradation occurred from serum collection to analysis. Finally, since this study was conducted, the Institute of Medicine modified the Recommended Dietary Allowance for vitamin D from 400 to 600 IU/day [51]. Although at the time these women were considered vitamin D-replete, their intakes would fall below current recommendations. In contrast, our study was strengthened by the controlled feeding design because calorie and nutrient intake as well as weight change were closely monitored.

Conclusions

Our data do not support a role for the carboxylation of OC to affect body weight or fat loss in post-menopausal women undergoing a weight loss intervention with and without aerobic exercise of differing intensities in which nutrients known to affect bone turnover are supplemented. That PINP was also not associated with weight or %BF loss additionally suggests that bone formation is not associated with changes in body composition in nutrient-supplemented conditions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Supported by NIH/NIDDK Grant No. R01AGDK20583, Wake Forest University Claude D. Pepper Older Americans Independence Center (P30-AG21332), Wake Forest University General Clinical Research Center (M01-RR07122) and USDA Agricultural Research Service under Cooperative Agreement No. 58-1950-7-707.

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Table 1 Participant characteristics at baseline and 20-week follow-up ($n = 71$) (mean $\pm$ SD or median $\pm$ IQR)

	Baseline	20-week follow-up	p value
Age (years)	58.7 $\pm$ 5.4		
Years post-menopausal (years)	14.2 $\pm$ 10.3		
White, non-Hispanic (%)	72 %		
Weight (kg)	88.5 $\pm$ 11.3	77.6 $\pm$ 10.4	<0.001
BMI (kg/m ²)	33.0 $\pm$ 3.8	29.0 $\pm$ 3.5	<0.001
Body fat (%)	42.8 $\pm$ 3.3	38.8 $\pm$ 4.1	<0.001
Abdominal visceral fat (cm ³)	2210 $\pm$ 698	1639 $\pm$ 579	<0.001
Plasma phylloquinone (nmol/L)	1.3 $\pm$ 0.9 ^a	1.4 $\pm$ 1.3 ^a	0.17
Plasma 25-(OH)D (nmol/L)	52.4 $\pm$ 18.5	62.2 $\pm$ 18.2	<0.001
Triglycerides (mmol/L)	1.35 $\pm$ 0.85 ^a	1.12 $\pm$ 0.56 ^a	<0.001
Total osteocalcin (ng/mL)	5.9 $\pm$ 3.3	5.8 $\pm$ 3.4	0.85
Uncarboxylated osteocalcin (ng/mL)	1.8 $\pm$ 3.0 ^a	1.8 $\pm$ 2.3 ^a	0.09
Uncarboxylated osteocalcin (%)	39.5 $\pm$ 23.8	33.9 $\pm$ 20.1	0.06
PINP (ug/L)	53.3 $\pm$ 23.0 ^a	54.0 $\pm$ 25.6 ^a	0.54

^a Median $\pm$ interquartile range

Table 2
Predicted percent change in body fat percentage, body weight or BMI associated with 1 unit change in biomarker

Outcome					
% Change in %body fat		% Change in body weight (kg)		% Change in BMI (kg/m ²)	
Beta coefficient	p value	Beta coefficient	p value	Beta coefficient	p value
% Change in OC					
ucOC (ng/mL) ^a	0.005	0.42	0.31	-0.005	0.29
tOC (ng/mL) ^a	0.004	0.49	0.27	0.006	0.25
%ucOC (%) ^a	0.005	0.42	0.31	-0.005	0.29
% Change in phylloquinone (nmol/L) ^b	-0.006	0.26	0.14	-0.006	0.14
% Change in P1NP (ug/L) ^a	0.003	0.86	0.84	-0.003	0.84
% Change in 25-(OH)D (nmol/L) ^c	0.003	0.77	0.48	0.006	0.49

^a Adjusted for age, race, and treatment group

^b Adjusted for age, race, treatment group, and percent change in triglycerides

^c Adjusted for age, race, treatment group, and percent change in cholesterol