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# Osteocalcin is associated with testosterone in the general population and selected patients with bone disorders

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## SUMMARY

Research in the last decade has revealed that bone is not only a target tissue for numerous circulating hormones but functions as an endocrine organ itself. As a recent study demonstrated a stimulatory effect of the osteoblast-derived hormone osteocalcin (OCN) on testosterone production in mice, we investigated whether such an association can be replicated in humans. We used data from 1338 men (25–86 years) in the population-based epidemiological Study of Health in Pomerania and from 110 male outpatients with bone disorders (18–85 years) for the study. We analysed cross-sectional associations between OCN and total testosterone serum concentrations (TT), as well as associations between further markers of bone turnover [bone-specific alkaline phosphatase (BAP), serum C-terminal telopeptides of Type I collagen (CTX), urinary deoxypyridinoline] and TT using ordinary least square (OLS) regression models. Multivariable OLS models revealed a positive association between OCN and TT in the population-based (β coefficients for a one standard deviation increase, 0.590; standard error (SE), 0.175; *p*-value, <0.01) and patient-based (β coefficient, 0.575; SE, 0.132; *p*-value, <0.01) samples even after adjustment for age and body mass index (both samples), and time of blood sampling (population-based sample only). Furthermore, we observed positive associations between BAP and TT (β coefficient, 0.403; SE, 0.170; *p*-value, 0.02) as well as between CTX and TT (β coefficient, 0.733; SE, 0.172; *p*-value, <0.01) in men from the general population. The present investigation shows that OCN is associated with TT in the general population and in patients with bone disorders, and may thus indicate general male health status. Additional longitudinal observational studies are warranted to confirm our findings and future experimental research is necessary to elucidate potential mechanisms underlying the observed associations.

## INTRODUCTION

Given the key role of the skeleton in supporting posture, protecting internal organs and serving as a reservoir of calcium and phosphate, it is not surprising that the regulation of bone mass is tightly controlled by cytokines, hormones and neuropeptides (Weiss *et al.*, 2010). While many of the important regulators involved in this process such as parathyroid hormone, growth hormone, corticosteroids or sex hormones are well-known, there is accumulating evidence suggesting mutual influence between bone metabolism and organ function (Fukumoto & Martin, 2009). At least two hormones produced by bone cells have been identified: fibroblast growth factor 23 (FGF23), a major regulator of phosphate homeostasis in mice and humans (Razzaque, 2009), and osteocalcin (OCN), which was reported to control energy homeostasis and male fertility in mice (Lee *et al.*, 2007; Oury *et al.*, 2011). OCN is considered a marker of bone formation mainly produced by osteoblasts, but also released from the bone matrix during the resorption phase (Ferron *et al.*, 2010a). The function of OCN as an endocrine hormone has only recently been suggested through the study of genetically modified mouse models (Lee *et al.*, 2007). OCN-deficient mice were found to display decreased insulin secretion and sensitivity, and mice lacking a tyrosine phosphatase encoded by the *Esp*-gene showed the opposite phenotype owing to increased serum levels of OCN (Lee *et al.*, 2007).

While these findings were important to introduce the concept of bone as an endocrine organ, it was surprising that a recent study reported a second function of OCN, namely the control of testosterone production in Leydig cells (Oury *et al.*, 2011). Male OCN-deficient mice display reduced fertility, testis size and markedly decreased testosterone levels, despite high serum concentrations of LH (Oury *et al.*, 2011). A similar phenotype was

reported in mice lacking the serpentine receptor Gprc6a in Leydig cells (Pi *et al.*, 2008), and molecular experiments suggested that the effects of OCN in testis are mediated through this receptor.

As these observations in mice may contribute to a pivotal change in our understanding of the homeostatic mechanisms regulating androgen biosynthesis in the testis, it is important to assess the functional role of the postulated bone-testis-axis in humans. To date, this question has only been addressed in males aged 20 years or less (Kirmani *et al.*, 2011; Valimaki *et al.*, 2004), where total OCN was shown to positively correlate with the total testosterone serum concentration (TT). However, as *Gprc6a* expression was found to be highest in adult mice (Oury *et al.*, 2011), it is of utmost relevance to investigate a possible influence of OCN on TT in adult men. Therefore, we used data from a large population-based epidemiological study and from male outpatients with bone disorders to comprehensively analyse whether TT is associated with OCN, and if so, how this correlation compares with other markers of bone formation or resorption.

## MATERIALS AND METHODS

### Study populations

The population-based Study of Health in Pomerania (SHIP) is an epidemiological study conducted in West Pomerania, a region in north-eastern Germany. Details regarding the design, recruitment and procedures of the SHIP study have been published previously (Volzke *et al.*, 2011). In brief, after written informed consent was obtained, 4308 (2116 men) participants (response rate 68.8%) attended the baseline examinations (SHIP-0) between 1997 and 2001. During the first 5-year follow-up (SHIP-1), 3300 (1589 men) participants were re-examined (response rate 83.6%) between 2002 and 2006. The study conformed to the principles of the Declaration of Helsinki and the protocol was approved by the Ethics Committee of the University of Greifswald. Of the 1589 male follow-up participants in SHIP-1, we excluded all participants (overlap exists) with missing values ($n$ = 32) or values below the measurement range of the assays ($n$ = 13) of TT, OCN, bone-specific alkaline phosphatase (BAP) or serum C-terminal telopeptides of Type I collagen (CTX), all participants with presence of or missing information on self-reported osteoporosis ($n$ = 61), testicular cancer or any malignancy diagnosed within the last 12 months ($n$ = 28), impaired renal function defined as estimated glomerular filtration rate according (eGFR) to MDRD formula <60mL/min/1.73m$^2$ ($n$ = 137), or missing data on body mass index (BMI) ($n$ = 6). Furthermore, on the basis of the anatomical, therapeutic and chemical (ATC) code, we excluded 15 men who were treated with bisphosphonates (M05BA, M05BB), antiandrogens (G03H, L02BB), Gonadotropin releasing hormone analogues (L02AE) or testosterone (G03BA, G03BB), yielding a final study population of 1,338 men from the general population.

The patient-based sample comprised 134 men attending the University outpatient clinic (University Medical Center Hamburg-Eppendorf, Germany) between July 2010 and November 2011 for the diagnosis or treatment of bone diseases, such as osteoporosis, osteomalacia, osteoporotic fractures or osteonecrosis. The institutional review board approved the study and a written informed consent was obtained from each patient. Exclusion criteria of this study were age under 18 years, impaired renal function, anti-androgen therapy, testosterone replacement therapy, hypogonadism, malignancies, testicular cancer, any other diseases or medication known to affect the hypothalamic–pituitary-testis axis and current bisphosphonate treatment. This resulted in a final study sample of 110 male patients.

### Measurements

In the SHIP, information about socio-demographic characteristics, medical history and medications were collected by computer-assisted personal interviews. Height and weight were measured for the calculation of BMI [weight (kg)/height (m)$^2$]. Non-fasting serum samples were drawn from the cubital vein in the supine position. Blood sampling was performed between 8:35 AM and 7:10 PM. with the majority of samples (90.1%) being taken until 2:30 PM. The samples were stored at −80 °C and measurement of total testosterone concentrations were carried out from April 2008 to May 2008 using competitive chemiluminescent enzyme immunoassays on an Immulite 2500 analyzer (Siemens Healthcare Medical Diagnostics, Bad Nauheim, Germany). The interassay coefficient of variation was 14.3% with a systematic deviation of −8.8% at the 4.1 nmol/L level, 10.5% with a systematic deviation of −4.4% at the 12.1 nmol/L level and 13.6 with a systematic deviation of −8.5% at the 29.4 nmol/L level. The concentration of free testosterone (free T) was calculated from TT and the concentration of sex hormone-binding globulin (SHBG) according to Vermeulen *et al.* (Vermeulen *et al.*, 1999). SHBG levels were measured on the Immulite 2500 analyzer (Siemens Healthcare Medical Diagnostics, Bad Nauheim, Germany). The interassay coefficient of variation was 4.5% with a systematic deviation of +1.1% at the 4.7 nmol/L level and 7.2% with a systematic deviation of +0.3% at the 73 nmol/L level.

Markers of bone formation and bone resorption including OCN, BAP and CTX were measured using an IDS-iSYS Multi-Discipline Automated Analyser (Immunodiagnostic Systems Limited, Frankfurt am Main, Germany). Three levels of control material were measured for each analyte by skilled technical personal to verify a decent working mode. OCN was measured with the IDS-iSYS N-Mid Osteocalcin assay (Immunodiagnostic Systems Limited). This assay detects the intact osteocalcin (amino acid 1-49) as well as the N-terminal-mid fragment (amino acids 1-43). The coefficients of variation (CVs) were 6.9% at low levels, 6.8% at medium levels and 5.1% at high levels of control material. CTX was measured with the IDS-iSYS CTX-I (CrossLaps) assay (Immunodiagnostic Systems Limited). The CVs were 12.2% at low levels, 10.4% at medium levels and 11.4% at high levels of control material. BAP was measured with the IDS-iSYS Ostase+ BAP assay (Immunodiagnostic Systems Limited). The CVs were 12.3% at low levels, 13.5% at medium levels and 13.2% at high levels of control material.

In the clinical sample, information including medical history, age, weight, height and current medications were recorded. Morning blood samples were obtained from antecubital veins (between 9:00 and 11:00 AM) and analysed for TT, OCN and BAP. Urine samples as first or second morning void were collected for quantitative measurement of deoxypyridinoline (DPD). TT was determined by a chemiluminescent competitive immunoassay (ADVIA Centaur, Siemens Healthcare Diagnostics Inc., Tarrytown, NY, USA; measuring range 0.35–52.1 nmol/L; CV of 7.6% at low levels, 5.8% at medium levels, and 2.7% at high levels). As

 

the focus of this study was to investigate whether the proposed bone-testis axis can be monitored by assessment of standard laboratory parameters like OCN and TT, SHBG measurement was not included in the patient sample. OCN and BAP were measured using chemiluminescent competitive immunoassays (LIAISON; DiaSorinInc, Stillwater, MN, USA) (OCN: measuring range 0.3–300 ng/mL, CV of 4% at low levels, 6% at medium levels and 4% at high levels; BAP: measuring range 1.50–120 μg/L, CV of 4.3% at low levels, 3.9% at medium levels, and 4.0% at high levels). DPD was measured using an enzyme-labelled chemiluminescent competitive immunoassay and analysed as nmol/mmol creatinine (Immulite 2000; Siemens Healthcare Diagnostics Inc., Llanberis, Gwynedd, UK; measuring range: 7–300 nmol, CV of 12% at low levels, 7.1% at medium levels, 4.3% at high levels).

### Statistical analysis

Characteristics of the two study samples were expressed as median ($p^{25}$–$p^{75}$). To investigate cross-sectional associations of OCN or further markers of bone turnover with TT, we used ordinary least square (OLS) regression models adjusted for age and BMI. In an additional analysis, we investigated the associations of the bone turnover markers with SHBG and free T in the population-based sample. As blood sampling was performed between 8:30 AM and 7:10 PM in SHIP, we further adjusted for time of blood sampling in the population-based study. Moreover, because of the well-known circadian rhythms in TT (Diver *et al.*, 2003; Brambilla *et al.*, 2009) as well as in OCN (Gundberg *et al.*, 1985), we performed sensitivity analyses in which we stratified the population-based sample in morning (blood sampling before 11:30 AM) and afternoon (blood sampling after 11:29 AM) samples. Effects were presented as β coefficient together with the corresponding standard error (SE) for a one standard deviation increase in the independent variable. We applied graphical and non-graphical methods to detect unusual and influential data, assure normality of residuals, homogeneity of variance of the residuals, linearity of the relationship and multicollinearity. We did not find any severe violations, which may have influenced the regression analyses and its results, verifying that our data have met the assumptions underlying OLS regression. Two-sided probability values <0.05 were considered statistically significant. All statistical analyses were performed using SAS 9.1 (SAS Institute Inc., Cary, NC, USA) and IBM SPSS Statistics Version 20.0 (SPSS Inc., Chicago, IL, USA).

## RESULTS

Table 1 presents the characteristics of the population-based and patient-based study samples.

Detailed information for the clinical sample is given in Table 2.

Scatterplots for the graphical interpretation of the relationship between OCN and TT depicted a positive linear trend in both study samples (Fig. 1).

Multivariable OLS models confirmed the positive association between OCN and TT in the population-based (β coefficient, 0.590; SE, 0.175; *p*-value, <0.01) and patient-based (β coefficient, 0.575; SE, 0.132; *p*-value, <0.01) sample after adjustment for age, BMI (both samples), and time of blood sampling (population-based sample) (Table 3). When the population-based sample was stratified in the morning ($n = 683$) and afternoon blood samples, ($n = 655$) the associations between OCN and TT remained statistically significant (morning samples fully adjusted: β coefficient, 0.594; SE, 0.260; *p*-value, 0.02; afternoon samples fully adjusted: β coefficient, 0.601; SE, 0.236; *p*-value 0.01).

We also observed positive associations between further markers of bone turnover and TT (Table 4).

**Table 1** Characteristics of the study populations

| Characteristics | Population-based sample ($N = 1338$) | Patient-based sample ($N = 110$) |
|---|---|---|
| Age, years | 53.5 (41.0–65.0) | 59.0 (44.0–68.0) [110] |
| BMI, kg/m$^2$ | 27.9 (25.4–30.7) | 25.7 (23.4–27.2) [98] |
| Total Testosterone, nmol/L | 16.6 (13.2–21.0) | 13.5 (9.7–16.9) [110] |
| OCN, ng/mL | 15.4 (12.1–19.6) | 11.5 (8.9–13.9) [110] |
| BAP, μg/L | 14.1 (11.5–17.2) | 11.3 (8.4–13.6) [103] |
| CTX, ng/mL | 0.28 (0.19–0.42) | – |
| DPD, nmol/mmol | – | 5.0 (4.0–6.0) [98] |

BAP, bone-specific alkaline phosphatase; BMI, body mass index; CTX, C-terminal telopeptides of type 1 collagen; DPD, urinary deoxypyridinoline; OCN, osteocalcin. Data are presented as median ($p^{25}$–$p^{75}$). Numbers in square brackets represent the maximum number of individuals in the patient-based sample for each variable.

**Table 2** Characteristics of the patient-based sample ($N = 110$)

| | Number (%) |
|---|---|
| Reason for visit | |
| Routine check-up | 18 (16.4) |
| Osteopenia | 39 (35.5) |
| Osteoporosis | 25 (22.7) |
| Stress fracture | 9 (8.2) |
| Bone oedema/necrosis | 10 (9.1) |
| Paget's disease of bone (inactive) | 3 (2.7) |
| Complex regional pain syndrome (CRPS) | 2 (1.8) |
| Others (FD; bone cyst; OI; OP) | 4 (3.6) |
| Patients with fractures | |
| Traumatic | 14 (12.7) |
| Atraumatic | 14 (12.7) |

FD, fibrous dysplasia; OI, osteogenesis imperfecta; OP, osteopetrosis.

**Figure 1** Scatterplots showing the relation between total testosterone and osteocalcin measurements in the population-based (A) and patient-based study sample (B). A trend line (solid line) with 95% confidence interval (dashed lines) was obtained from an unadjusted linear regression model.

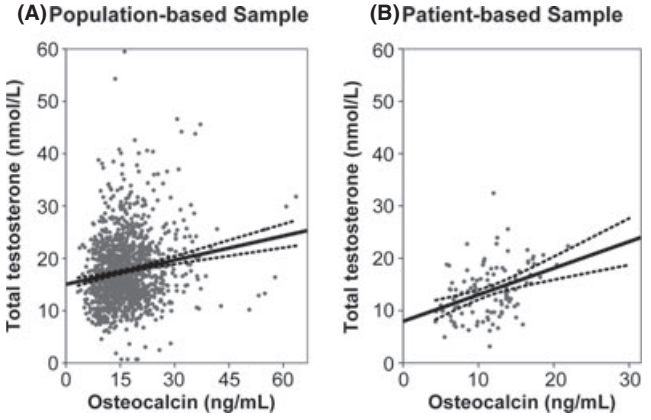

**Table 3** Association of osteocalcin (OCN) with total testosterone (TT) in a population-based sample of healthy men vs. a patient-based sample of men with bone disorders. A one standard deviation increase in OCN was modelled

| Model | Population-based sample ($N$ = 1338) | | | Patient-based sample ($N$ = 110) | | |
|---|---|---|---|---|---|---|
| | β coefficient | SE | *p*-value | β coefficient | SE | *p*-value |
| OCN | TT | | | TT | | |
| Age-adjusted | 0.885 | 0.176 | <0.01 | 0.574 | 0.129 | <0.01 |
| Fully adjusted* | 0.590 | 0.175 | <0.01 | 0.575 | 0.132 | <0.01 |

SE, standard error. *Fully adjusted linear regression models with total testosterone concentration as dependent and osteocalcin as independent variables were adjusted for age and body mass index. The population-based sample was further adjusted for time of blood sampling. Because of missing data on confounders, fully adjusted models comprise only 95 men in the patient-based sample. One standard deviation of OCN in the population-based sample: 6.71 ng/mL; in the patient-based sample: 3.70 ng/mL (whole sample of 110 men) and 3.80 ng/mL (95 men with information on BMI).

**Table 4** Associations of total testosterone with markers of bone remodelling in a population-based sample of healthy men vs. a patient-based sample of men with bone disorders. A one standard deviation increase in the markers of bone formation or resorption was modelled

| Linear regression models | Population-based sample | | | Patient-based sample | | |
|---|---|---|---|---|---|---|
| | β coefficient | SE | *p*-value | β coefficient | SE | *p*-value |
| Marker of bone formation | BAP ($N$ = 1338) | | | BAP ($N$ = 103) | | |
| Age-adjusted | 0.358 | 0.175 | 0.04 | 0.136 | 0.144 | 0.347 |
| Fully adjusted* | 0.403 | 0.170 | 0.02 | 0.132 | 0.146 | 0.368 |
| Marker of bone resorption | CTX ($N$ = 1338) | | | DPD ($N$ = 97) | | |
| Age-adjusted | 0.943 | 0.175 | <0.01 | 0.185 | 0.184 | 0.319 |
| Fully adjusted* | 0.733 | 0.172 | <0.01 | 0.200 | 0.187 | 0.291 |

BAP, bone-specific alkaline phosphatase; CTX, serum C-terminal telopeptides of Type I collagen; DPD, urinary deoxypyridinoline; SE, standard error. *Fully adjusted linear regression models with total testosterone concentration as dependent and bone remodelling markers as independent variables were adjusted for age and body mass index. The population-based sample was further adjusted for time of blood sampling. Because of missing data on confounders, fully adjusted models comprise only 95 men for BAP and 83 men for DPD in the patient-based sample. One standard deviation of BAP and CTX in the population-based sample: 5.26 μg/L and 0.19 ng/mL; one standard deviation in the patient-based sample: BAP 4.7 μg/L (whole sample of 103 men) and 5.0 μg/L (95 men with information on BMI) and DPD 1.9 ng/mL (whole sample of 97 men) and 1.7 ng/mL (83 men with information on BMI).

In the population-based sample, the bone formation marker BAP was positively associated with TT (β coefficient, 0.403; SE, 0.170; *p*-value, 0.02), and there was a positive and significant association between the bone resorption marker CTX (β coefficient, 0.733; SE, 0.172; *p*-value, <0.01) and TT. In contrast, we did not detect a significant association between BAP or the bone resorption marker DPD with TT in the patient-based sample (Table 4). An additional adjustment for diabetes mellitus, self-reported physical activity and eGFR in the population-based sample did not change the results (data not shown).

In the population-based sample, linear regression models revealed associations between OCN, BAP or CTX with SHBG in age- and fully adjusted models. Moreover, associations between OCN (age-adjusted model only) or CTX (age- and fully adjusted model) with free T were observed (Table S1). After exclusion of 12 men with either high (>50 nmol/L) or low (<5 nmol/L) TT concentrations, a borderline significant association between OCN and free T was detected (Table S2).

## DISCUSSION

There is growing evidence that testosterone, the most important testicular androgen, is a valuable biomarker of men's overall health status (Wu *et al.*, 2008). While conditions such as general ageing, obesity or smoking are known to alter serum TT concentrations (Wu *et al.*, 2008; Haring *et al.*, 2010), recent experimental findings (Oury *et al.*, 2011; Smith & Saunders, 2011;) suggested that bone-derived OCN is an important regulator of TT in mice. Also in healthy boys, aged 20 years or less, OCN was positively correlated with TT (Kirmani *et al.*, 2011). Given the high prevalence of skeletal disorders in the ageing population, it is important to assess the physiologic as well as pathologic importance of the postulated bone-testis axis in adult males. From a clinical point of view, bone disorders with abnormally high OCN (such as Paget's disease, high-turnover osteoporosis or hyperparathyroidism) or low OCN (such as low-turnover osteoporosis, glucocorticoid therapy or hypoparathyroidism) could therefore be associated with critical alterations in TT levels (Traish *et al.*, 2011).

In the present epidemiological study, we assessed the cross-talk between biochemical markers of bone remodelling and TT using two independent samples. The key finding of our study is the positive association between OCN and TT, which was consistently observed in the population-based as well as the patient-based sample. This finding is accompanied by results from the population-based sample that demonstrated an association between OCN and SHBG as well as a borderline significant association between OCN and free T after exclusion of 12 men with high or low TT.

Two previous cross-sectional studies (Valimaki *et al.*, 2004; Kirmani *et al.*, 2011) reported positive correlations between OCN and TT in 56 healthy boys 4–20 years of age (Kirmani *et al.*, 2011) and in 204 healthy young men 18–20 years of age (Valimaki *et al.*, 2004). Our results confirm and expand these previous results to healthy and diseased adult males. This supports the hypothesis of a role of bone metabolism in male reproduction (Oury *et al.*, 2011). Nevertheless, additional longitudinal studies are needed to address this issue and to evaluate the physiologic importance of the observed association. Moreover, the cross-sectional design of our study does not allow assessing the causality of the observed association. Therefore, we cannot rule out that the detected association is because of a stimulatory effect of TT on OCN secretion (Clarke & Khosla, 2009).

Our study further revealed a positive association between the bone formation marker BAP and TT in the population-based sample, but not in the patient-based sample. This may indicate that the association between OCN and TT is present in healthy and diseased adult males, while BAP is only associated with TT in healthy individuals, but not in those with accelerated (Kirmani *et al.*, 2011) or altered bone turnover (e.g. growth or bone disorders). Moreover, we detected a positive association between bone resorption (CTX) and TT in the population-based sample, supporting the notion that bone-resorbing osteoclasts

 

may indeed control the release of hormonally active OCN, as has been shown in mice (Ferron *et al.*, 2010a). In contrast, there was no association between the bone resorption marker DPD and TT in the patient-based sample. This difference might be explained by the smaller sample size of the patient cohort, or by the assessment of urinary collagen degradation products, exhibiting a higher biological variability than serum indices of bone resorption (Hannon *et al.*, 1998). Therefore, additional basic research and longitudinal studies are required to examine the effect of osteoclast activity on TT levels.

The relationship between androgens and markers of bone metabolism received increasing attention over the last years, with epidemiological, clinical and experimental research suggesting a link between the reproductive system and the skeleton. Yet, previous studies (Kasperk *et al.*, 1997; Clarke & Khosla, 2009; Krum, 2011) also produced conflicting results. Although androgen receptors are expressed in human osteoblasts (Kasperk *et al.*, 1997; Krum, 2011) and studies have suggested a stimulatory effect on osteoblast differentiation in vitro, TT and its derivatives were reported to have no effect or to result in a reduction in OCN expression (Clarke & Khosla, 2009), Previous epidemiological studies (Szulc *et al.*, 2003; Kuchuk *et al.*, 2007; Boonen *et al.*, 2011) further reported contradictory results regarding the associations of bone turnover markers with TT. A study among 1040 elderly men demonstrated that bone resorption markers (DPD and CTX) are increased in hypogonadal men compared to men with normal TT (Szulc *et al.*, 2003). However, in that study (Szulc *et al.*, 2003), the free testosterone index as well as the apparent free testosterone concentration, but not TT, were significant determinants of bone formation and bone resorption markers. Likewise, Kuchuk *et al.* (Kuchuk *et al.*, 2007) demonstrated that low serum levels of bioavailable testosterone are associated with high bone turnover. They found inverse associations between bioavailable testosterone with bone resorption (DPD) and bone formation (OCN) among 623 men aged 65–88 years from Amsterdam (Kuchuk *et al.*, 2007). In contrast, a cross-sectional survey among 3,120 middle-aged and elderly European men from the general population found no association between TT or free testosterone and bone formation (PINP) or bone resorption (CTX) in fully adjusted multivariable models (Boonen *et al.*, 2011). There were also no associations between testosterone serum levels (TT, free testosterone, free testosterone index) and bone resorption or formation markers (OCN, BAP, DPD, urinary CTX) in 40 middle-aged healthy men and 80 men with osteoporosis (Legrand *et al.*, 2001). The diverse nature of the studies, including healthy and diseased adult males of different ages and the different laboratory markers and methods applied to measure TT and the bone turnover markers, contribute to explain the observed differences of the study results.

In the present, large study, we were able to assess and replicate the population-based observations in a patient-based sample. Despite the consistent association between OCN and TT in the two samples, several shortcomings of this study have to be mentioned. Owing to the cross-sectional nature of the studies, caution needs to be taken with mechanistic interpretations of our findings. Thus, we cannot report causality between the TT and the bone markers. In addition, it was practically impossible to exclusively obtain morning serum samples in the population-based sample, given logistical concerns in a large-scale study like SHIP. Therefore, all laboratory measurements in SHIP were based on whole-day blood samples, which, however, reflect a clinical real life situation. Moreover, we investigated the association between OCN and TT in morning and afternoon samples and confirmed the results obtained in the analysis based on whole-day data. Another limitation of our study is, that we were not able to study the role of aromatization and oestrogens, which are also involved in the maintenance of male skeletal health, in bone remodelling (Callewaert *et al.*, 2010). Furthermore, the assay applied in this study is unable to discriminate between the different forms of OCN. Although bioactive OCN is generally considered to correspond to under-carboxylated OCN, experiments performed in mice have demonstrated that Glu-13 is the most critical residue within the OCN molecule determining its endocrine functions (Ferron *et al.*, 2010a,b). In humans, the carboxylation sites of OCN are different (Benton *et al.*, 1995). and have not been studied sufficiently regarding their relevance for bioactivity. However, total OCN has proven valuable in confirming results of animal studies in humans (Kindblom *et al.*, 2009; Kirmani *et al.*, 2011) and additionally offers the advantage of being readily assessable in everyday clinical practice. Finally, our results are not generally applicable to other ethnic groups or geographic regions, because they are based on measurements in Caucasians living in Germany.

In conclusion, using population-based as well as patient-based study samples, the present investigation shows that OCN, a marker of bone health, is associated with TT and may thus indicate general male health status. In addition, this study demonstrates a positive association between CTX and TT, warranting additional longitudinal studies to confirm these findings. Moreover, our observations emphasize the importance of developing an assay specifically detecting the active form of OCN in human serum, because only this will help to define the role of OCN as an endocrine regulator of glucose homeostasis and male fertility.

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## DISCLOSURES

The authors declare that they have no conflict of interests.

## AUTHOR CONTRIBUTION

Study design, study conduct, data collection, data analysis and data interpretation: AH, SB, HW, MN, SEB, FB, MA, TS, RH and JK. Drafting manuscript and revising manuscript content: AH, SB, TS, RH and JK. Approving final version of manuscript: AH, SB, HW, MN, SEB, FB, MA, TS, RH and JK. RH and JK take responsibility for the integrity of the data analysis.

## REFERENCES


Benton ME, Price PA & Suttie JW. (1995) Multi-site-specificity of the vitamin K-dependent carboxylase: in vitro carboxylation of des-gamma-carboxylated bone Gla protein and Des-gamma-carboxylated pro bone Gla protein. *Biochemistry* 34, 9541–9551.

Boonen S, Pye SR, O'Neill TW, Szulc P, Gielen E, Borghs H *et al.* (2011). Influence of bone remodelling rate on quantitative ultrasound parameters at the calcaneus and DXA BMDa of the hip and spine in middle-aged and elderly European men: the European Male Ageing Study (EMAS). *Eur J Endocrinol* 165, 977–986.

Brambilla DJ, Matsumoto AM, Araujo AB & McKinlay JB. (2009) The effect of diurnal variation on clinical measurement of serum testosterone and other sex hormone levels in men. J *Clin Endocrinol Metab* 94, 907–913.

Callewaert F, Boonen S & Vanderschueren D. (2010) Sex steroids and the male skeleton: a tale of two hormones. *Trends Endocrinol Metab* 21, 89–95.

Clarke BL & Khosla S. (2009) Androgens and bone. *Steroids* 74, 296–305.

Diver MJ, Imtiaz KE, Ahmad AM, Vora JP & Fraser WD. (2003) Diurnal rhythms of serum total, free and bioavailable testosterone and of SHBG in middle-aged men compared with those in young men. *Clin Endocrinol (Oxf)* 58, 710–717.

Ferron M, Wei J, Yoshizawa T, Del Fattore A, DePinho RA, Teti A *et al.* (2010a) Insulin signaling in osteoblasts integrates bone remodeling and energy metabolism. *Cell* 142, 296–308.

Ferron M, Wei J, Yoshizawa T, Ducy P & Karsenty G. (2010b) An ELISA-based method to quantify osteocalcin carboxylation in mice. *Biochem Biophys Res Commun* 397, 691–696.

Fukumoto S & Martin TJ. (2009) Bone as an endocrine organ. *Trends Endocrinol Metab* 20, 230–236.

Gundberg CM, Markowitz ME, Mizruchi M & Rosen JF. (1985) Osteocalcin in human serum: a circadian rhythm. *J Clin Endocrinol Metab* 60, 736–739.

Hannon R, Blumsohn A, Naylor K & Eastell R. (1998) Response of biochemical markers of bone turnover to hormone replacement therapy: impact of biological variability. *J Bone Miner Res* 13, 1124–1133.

Haring R, Ittermann T, Volzke H, Krebs A, Zygmunt M, Felix SB *et al.* (2010) Prevalence, incidence and risk factors of testosterone deficiency in a population-based cohort of men: results from the study of health in Pomerania. *Aging Male* 13, 247–257.

Kasperk C, Helmboldt A, Borcsok I, Heuthe S, Cloos O, Niethard F *et al.* (1997) Skeletal site-dependent expression of the androgen receptor in human osteoblastic cell populations. *Calcif Tissue Int* 61, 464–473.

Kindblom JM, Ohlsson C, Ljunggren O, Karlsson MK, Tivesten A, Smith U *et al.* (2009) Plasma osteocalcin is inversely related to fat mass and plasma glucose in elderly Swedish men. *J Bone Miner Res* 24, 785–791.

Kirmani S, Atkinson EJ, Melton LJ 3rd, Riggs BL, Amin S & Khosla S. (2011) Relationship of testosterone and osteocalcin levels during growth. *J Bone Miner Res* 26, 2212–2216.

Krum SA. (2011) Direct transcriptional targets of sex steroid hormones in bone. *J Cell Biochem* 112, 401–408.

Kuchuk NO, van Schoor NM, Pluijm SM, Smit JH, de Ronde W & Lips P. (2007) The association of sex hormone levels with quantitative ultrasound, bone mineral density, bone turnover and osteoporotic fractures in older men and women. *Clin Endocrinol (Oxf)* 67, 295–303.

Lee NK, Sowa H, Hinoi E, Ferron M, Ahn JD, Confavreux C *et al.* (2007) Endocrine regulation of energy metabolism by the skeleton. *Cell* 130, 456–469.

Legrand E, Hedde C, Gallois Y, Degasne I, Boux de Casson F, Mathieu E *et al.* (2001) Osteoporosis in men: a potential role for the sex hormone binding globulin. *Bone* 29, 90–95.

Oury F, Sumara G, Sumara O, Ferron M, Chang H, Smith CE *et al.* (2011) Endocrine regulation of male fertility by the skeleton. *Cell* 144, 796–809.

Pi M, Chen L, Huang MZ, Zhu W, Ringhofer B, Luo J *et al.* (2008) GPRC6A null mice exhibit osteopenia, feminization and metabolic syndrome. *PLoS ONE* 3, e3858.

Razzaque MS. (2009) The FGF23-Klotho axis: endocrine regulation of phosphate homeostasis. *Nat Rev Endocrinol* 5, 611–619.

Smith LB & Saunders PT. (2011) The skeleton: the new controller of male fertility? *Cell* 144, 642–643.

Szulc P, Claustrat B, Marchand F & Delmas PD. (2003) Increased risk of falls and increased bone resorption in elderly men with partial androgen deficiency: the MINOS study. *J Clin Endocrinol Metab* 88, 5240–5247.

Traish AM, Miner MM, Morgentaler A & Zitzmann M. (2011) Testosterone deficiency. *Am J Med* 124, 578–587.

Valimaki VV, Alfthan H, Ivaska KK, Loyttyniemi E, Pettersson K, Stenman UH *et al.* (2004) Serum estradiol, testosterone, and sex hormone-binding globulin as regulators of peak bone mass and bone turnover rate in young Finnish men. *J Clin Endocrinol Metab* 89, 3785–3789.

Vermeulen A, Verdonck L & Kaufman JM. (1999) A critical evaluation of simple methods for the estimation of free testosterone in serum. *J Clin Endocrinol Metab* 84, 3666–3672.

Volzke H, Alte D, Schmidt CO, Radke D, Lorbeer R, Friedrich N *et al.* (2011) Cohort profile: the study of health in Pomerania. *Int J Epidemiol* 40, 294–307.

Weiss AJ, Iqbal J, Zaidi N & Mechanick JI. (2010) The skeletal subsystem as an integrative physiology paradigm. *Curr Osteoporos Rep* 8, 168–177.

Wu FC, Tajar A, Pye SR, Silman AJ, Finn JD, O'Neill TW *et al.* (2008) Hypothalamic-pituitary-testicular axis disruptions in older men are differentially linked to age and modifiable risk factors: the European Male Aging Study. *J Clin Endocrinol Metab* 93, 2737–2745.


## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

**Table S1**. Associations of total testosterone, SHBG, and free testosterone with markers of bone remodeling in a population-based sample of healthy men ($n$ = 1338). A one standard deviation increase in the markers of bone formation or resorption was modeled.

**Table S2.** Associations of total testosterone, SHBG, and free testosterone with markers of bone remodeling after exclusion of 12 men with total T concentrations >50 nmol/L or <5 nmol/L in the population-based sample ($n$ = 1326). A one standard deviation increase in the markers of bone formation or resorption was modeled.