Higher Serum Undercarboxylated Osteocalcin and Other Bone Turnover Markers Are Associated With Reduced Diabetes Risk and Lower Estradiol Concentrations in Older Men

Bu B. Yeap, Helman Alfonso, S. A. Paul Chubb, Richard Gauci, Elizabeth Byrnes, John P. Beilby, Peter R. Ebeling, David J. Handelsman, Carolyn A. Allan, Mathis Grossmann, Paul E. Norman, and Leon Flicker

Schools of Medicine and Pharmacology (B.B.Y., S.A.P.C., L.F.) and Western Australian Centre for Health and Ageing (L.F.), Centre for Medical Research, University of Western Australia, Perth, Western Australia 6009, Australia; Department of Endocrinology and Diabetes (B.B.Y., R.G.), Fremantle and Fiona Stanley Hospitals, Perth, Western Australia 6160, Australia; School of Public Health (H.A.), Curtin University, Perth, Western Australia 6102, Australia; PathWest Laboratory Medicine (S.A.P.C., J.P.B.), Fremantle, Royal Perth and Sir Charles Gardiner Hospitals, Perth, Western Australia 6160, Australia; Department of Medicine (P.R.E.), School for Clinical Sciences, Monash University, Melbourne, Victoria 3800, Australia; ANZAC Research Institute (D.J.H.), University of Sydney, Sydney, New South Wales 2139, Australia; Monash Institute of Medical Research (C.A.A.), Prince Henry’s Research Institute, Melbourne, Victoria 3168, Australia; and Department of Medicine (M.G.), Austin Health, University of Melbourne, Melbourne, Victoria, 3084 Australia

Context: In mice, undercarboxylated osteocalcin (ucOC) modulates insulin secretion and sensitivity and increases testosterone (T) secretion from Leydig cells, but human data are lacking. We hypothesized that ucOC is associated with diabetes risk and modulates sex hormone concentrations in older men, distinct from other bone turnover markers.

Participants: Participants were community-dwelling men aged 70 to 89 years resident in Perth, Western Australia.

Main Outcome Measures: Serum total osteocalcin (TOC), N-terminal propeptide of type I collagen (P1NP), and collagen type I C-terminal cross-linked telopeptide (CTX) were measured by immunoassay, and ucOC by hydroxyapatite binding. Plasma total T, DHT, and estradiol (E2) were assayed by mass spectrometry.

Results: Excluding men with osteoporosis or conditions affecting sex hormones or on bisphosphonates, glucocorticoids, or warfarin, 2966 men were included. In multivariate analyses, higher ucOC was associated with reduced diabetes risk (odds ratio [OR] per 1 SD increase = 0.55, P < .001). Similar results were seen for TOC (OR = 0.60, P < .001), P1NP (OR = 0.64, P < .001), and CTX (OR = 0.60, P < .001) but not ucOC/TOC. When all 4 markers were included in the fully adjusted model, higher ucOC (OR = 0.56, P < .001) and CTX (OR = 0.76, P = .008) remained associated with reduced diabetes risk. E2 was inversely associated with ucOC (coefficient −0.04, P = .031), TOC (−0.05, P = .001) and CTX (−0.04, P = .016); and positively with ucOC/TOC (0.05, P = .002). DHT was inversely associated with ucOC/TOC (−0.04, P = .040). T was not associated with bone turnover.

Conclusions: Higher bone remodeling rates are associated with reduced diabetes risk in older men. Higher ucOC is both a marker of bone remodeling and an independent predictor of reduced diabetes risk. E2 is inversely associated with bone turnover markers. We found no evidence ucOC modulates circulating T in older men. (J Clin Endocrinol Metab 100: 63–71, 2015)

Abbreviations: BMI, body mass index; cOC, γ-carboxylated osteocalcin; CTX, collagen type I C-terminal cross-linked telopeptide; CV, coefficient of variation; E2, estradiol; HIMS, Health In Men Study; HOMA-IR, homeostasis model assessment of insulin resistance; LC-MS/MS, liquid chromatography-tandem mass spectrometry; OR, odds ratio; P1NP, N-terminal propeptide of type I collagen; T, testosterone; TOC, total osteocalcin; ucOC, undercarboxylated osteocalcin; W1, wave 1; WHR, waist to hip ratio.
Osteocalcin, an osteoblast secreted protein that constitutes a major component of bone matrix, functions as an endocrine regulator of glucose metabolism in mice (1). Mice with a targeted deletion of the osteocalcin gene (Ocn−/−) develop glucose intolerance and obesity in later life, with reduced pancreatic β-cell mass and insulin content. In the circulation total osteocalcin (TOC), a marker of bone turnover, comprises both γ-carboxylated osteocalcin (cOC) and undercarboxylated osteocalcin (ucOC) lacking γ-carboxylation at 1 or more sites (2). The γ-carboxylation increases binding in vitro to hydroxyapatite; thus, osteocalcin in bone is predominantly carboxylated, whereas ucOC comprises a large proportion of osteocalcin in the circulation and is considered to be the principal fraction regulating glucose metabolism. Consistent with this, administration of ucOC in vitro and in wild-type mice increased insulin secretion and sensitivity, identifying it as the metabolically active form (3, 4). The receptor for ucOC is a G protein-coupled receptor, GPRC6A, expressed in murine Leydig cells (5). In mice, ucOC regulated production of testosterone (T) in a parallel pathway to LH, illuminating a complex relationship between bone turnover and androgen secretion (5).

Translation of these findings from the experimental context to humans has been problematic. Although several epidemiological studies have associated lower levels of TOC with adiposity, metabolic syndrome, or diabetes (6–10), it is not known whether these observations might reflect differences in underlying bone turnover rather than a specific influence of osteocalcin. Studies that have reported ucOC data in relation to measures of glucose metabolism showed contrasting results or no clear differentiation between associations of cOC vs ucOC with outcomes (11–15). This is due to the small size of previous studies and the use of immunoassays to measure ucOC without any precipitation step to remove cOC, which have been shown to overestimate ucOC (2). However, Levinger et al (16), using a euglycemic-hyperinsulinemic clamp, recently showed that ucOC was associated with whole-body insulin sensitivity both at rest and after exercise in obese men.

Data examining whether ucOC regulates T levels in men are even more limited. In a study of 69 men with type 2 diabetes, Kanazawa et al (17) reported a positive association of ucOC with calculated free T and a negative association of ucOC with LH. However, free T was measured using an invalid RIA, the results of which correspond poorly to the reference method of equilibrium dialysis (18). In addition, the relationship of ucOC to the T-derived sex steroids DHT and estradiol (E2), and to SHBG, which also modulates bone loss and fracture risk in older men (19–22) remains unclear.

In the present study, we tested the hypothesis that ucOC is 1) independently associated with diabetes risk and 2) modulates circulating T levels in older men. To overcome limitations of previous studies, we examined the associations of T, DHT, E2, SHBG, and LH with TOC, ucOC, and the ratio of ucOC to TOC and compared these with N-terminal propeptide of type I collagen (P1NP) and collagen type I C-terminal cross-linked telopeptide (CTX) as distinct markers of bone formation and resorption, respectively (23), in a large population-based cohort of older men.

Subjects and Methods

Study population

The Health In Men Study (HIMS) is a cohort study of community-dwelling older men from Perth, Western Australia, which has been described previously (24). Briefly, men aged 65 years or more were randomly selected from the electoral roll (voting being compulsory for Australian citizens) and invited to participate in the study, from which 12,203 men completed a questionnaire and attended for physical examination in wave 1 (W1, 1996–1999), and 4,248 men attended for reassessment and venesection in wave 2 (W2, 2001–2004). Approximately 95% of the men were of Caucasian ethnic origin. The University of Western Australia Human Research Ethics Committee approved the study, and all men gave written informed consent.

Assessment of medical comorbidities

Medical data collected at W2 were used to identify men with a history of prostate cancer, osteoporosis or bone fracture, and Paget’s disease. Medications data were analyzed to identify men receiving androgens or antiandrogen therapy, bisphosphonates, or glucocorticoids. The list of medications included then available oral and parenteral bisphosphonates (alendronate, risedronate, and zoledronic acid) and the range of glucocorticoid preparations (cortisone, hydrocortisone, dexamethasone, and prednisolone). Because γ-carboxylation is a vitamin K-dependent process, we also identified men who were receiving warfarin. Men were considered to have hypertension if they reported this diagnosis at W1 or W2 or used antihypertensive medication or had blood pressure ≥140/90 mm Hg at W2. Dyslipidemia was defined as having fasting high-density lipoprotein <0.9 mmol/L, low-density lipoprotein ≥3.4 mmol/L, triglycerides ≥1.8 mmol/L, or total cholesterol ≥5.5 mmol/L or receiving lipid-lowering therapy at W2. Men diagnosed with diabetes, reporting use of glucose-lowering medication, or with fasting or nonfasting glucose at W2 of ≥7 mmol/L or ≥11.1 mmol/L, respectively, were considered to have (predominantly type 2) diabetes. Further assessment of morbidity was performed via the Western Australian Data Linkage System, which provides electronic linkage to records from death, hospital, and cancer registries and captures admissions to all public and private hospitals in Western Australia (25). We used the Charlson score to determine the presence of significant medical comorbidity (26). Medical diagnoses are weighted for severity and summed to provide a weighted index of medical comorbidity. Data were included from 1990 to the time of blood sampling, providing a measure of recent comorbidity.
Laboratory assays

Blood samples were collected between 8:00 AM and 10:30 AM at W2. Aliquots of plasma and serum were prepared immediately after phlebotomy and stored at −80°C until assayed. Serum TOC, P1NP, and CTX were measured by electrochemiluminescence immunoassay using a Modular E170 analyzer (Roche Diagnostics, Australia). The coefficients of variation (CV) were 3.7% and 2.9% at 18 and 89 μg/L TOC, 4.0% and 5.7% at 28 and 191 μg/L P1NP, and 4.1% and 3.8% at 0.31 and 0.71 μg/L CTX. Serum samples were incubated with hydroxyapatite (5 mg/mL), mixed, and centrifuged to separate out ucOC as previously described (2). The ucOC in the supernatant was measured using the same assay as for TOC and was reported as a concentration and as a fraction of the total. For a reference osteocalcin standard with expected fractional hydroxyapatite binding of 0.80, kindly supplied by Professor Caren Gundberg (Yale School of Medicine, New Haven, CT), mean fractional hydroxyapatite-binding osteocalcin was 0.77, and between-run precision was 6.0%. Plasma total T, DHT, and E2 were quantified using liquid chromatography-tandem mass spectrometry (LC-MS/MS) without derivatization using atmospheric pressure photo-ionization chromatography-tandem mass spectrometry (LC-MS/MS) with CV of <7% for both. Free T was calculated using an empirical formula that provides closer concordance with free T measured by equilibrium dialysis compared with calculations based on equilibrium binding equations (28). Insulin resistance was estimated using homeostasis model assessment of insulin resistance (HOMA-IR), as previously described (29). Vitamin D was measured using a chemiluminescent immunoassay, as previously reported (30).

Statistical analysis

The statistical package Stata version 12.1 (StataCorp) was used. Baseline descriptive data are shown as mean and SD or percentages. Comparisons of means were performed using 2-sample t tests with equal variances, which are robust for parametric and modestly skewed distributions with sufficiently large sample sizes (31). Linear regression analyses were used to examine the associations of each bone turnover marker and the ratio of ucOC to TOC with diabetes, insulin resistance, and sex hormones. Models were adjusted for age, smoking, body mass index (BMI), waist to hip ratio (WHR), hypertension, dyslipidemia, creatinine, vitamin D, and medical comorbidity (Charlson index). Trimmed analyses were performed excluding the lowest and the highest 1% of values to ensure the analyses were not biased by low or high outliers. The fully adjusted models for DHT and E2 were further explored by incorporation of SHBG. A 2-tailed P value <.05 was considered significant.

Results

Baseline characteristics of the study population

Of the 4248 men who participated at W2, 4233 had sex hormone measurements, 4010 had measurements of bone turnover markers, and 3992 had both sex hormones and bone turnover assessed. From these men, we excluded 71 receiving testosterone or antiandrogen therapy, 420 with prostate cancer or orchidectomy, and 23 men with Paget’s disease of bone. We excluded another 277 men with a history of osteoporosis, fracture, or bisphosphonate use and finally 53 men using glucocorticoids and 182 using warfarin (see Supplemental Figure 1). This left 2966 men who were included in the analysis. Baseline characteristics of men included and excluded from the analysis are shown (Table 1). Men who were excluded from the analysis were older, more likely to have smoked, had more medical comorbidity, and had higher ucOC, ucOC to TOC ratio, P1NP, and CTX levels.

Associations of ucOC and other bone turnover markers with prevalent diabetes

There were 445 men with diabetes (15.0%). Men with diabetes had lower levels of TOC, ucOC, P1NP, and CTX and a higher ratio of ucOC to TOC ratio compared with men without diabetes (Table 2). In analyses adjusting for age, smoking, BMI, WHR, hypertension, dyslipidemia, creatinine, vitamin D, and medical comorbidity, a 1-SD increase in ucOC was associated with an odds ratio (OR) of 0.55 for having diabetes (P < .001) (Table 3). There were comparable results with TOC (OR = 0.60, P <
Table 2. Bone Turnover Markers in Men Without and With Diabetesa Who Were Included in the Analysis (n = 2966)

<table>
<thead>
<tr>
<th>Variable</th>
<th>No Diabetes, n = 2521</th>
<th>Diabetes, n = 445</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOC, µg/L</td>
<td>21.21 ± 10.85</td>
<td>18.56 ± 19.47</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>ucOC, µg/L</td>
<td>11.22 ± 4.73</td>
<td>9.58 ± 6.29</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>ucOC/TOC</td>
<td>0.54 ± 0.09</td>
<td>0.56 ± 0.10</td>
<td>.005</td>
</tr>
<tr>
<td>P1NP, µg/L</td>
<td>42.96 ± 25.09</td>
<td>37.45 ± 29.61</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>CTX, µg/L</td>
<td>0.32 ± 0.17</td>
<td>0.26 ± 0.18</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

a Men reporting use of glucose-lowering medication or with fasting or nonfasting glucose at W2 of ≥7 mmol/L or ≥11.1 mmol/L, respectively, were considered to have diabetes.

The fully adjusted trimmed analyses were repeated, with inclusion of SHBG and ucOC to TOC ratio in the models containing T, DHT, and E2 (Table 6). In the combined model, TOC and ucOC, P1NP, and CTX were included in the multivariate model simultaneously, higher ucOC (OR = 0.56, P < .001) and CTX (OR = 0.76, P = .008) remained associated with reduced diabetes risk (Table 3).

Table 3. Odds Ratio of Prevalent Diabetes According to a 1-SD Increase in ucOC and Other Bone Turnover Markers in 2966 Older Mena

<table>
<thead>
<tr>
<th>Variable</th>
<th>Unadjusted</th>
<th></th>
<th>Adjusted</th>
<th></th>
<th>Combined Model, Adjusted</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>TOC</td>
<td>0.66 (0.55–0.79)</td>
<td>&lt;.001</td>
<td>0.60 (0.50–0.72)</td>
<td>&lt;.001</td>
<td>1.18 (0.96–1.45)</td>
<td>.106</td>
</tr>
<tr>
<td>ucOC</td>
<td>0.58 (0.50–0.68)</td>
<td>&lt;.001</td>
<td>0.55 (0.47–0.64)</td>
<td>&lt;.001</td>
<td>0.56 (0.44–0.72)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>ucOC to TOC ratio</td>
<td>1.16 (1.05–1.28)</td>
<td>.005</td>
<td>1.11 (1.00–1.24)</td>
<td>.062</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>P1NP</td>
<td>0.72 (0.62–0.84)</td>
<td>&lt;.001</td>
<td>0.64 (0.54–0.76)</td>
<td>&lt;.001</td>
<td>1.05 (0.87–1.28)</td>
<td>.587</td>
</tr>
<tr>
<td>CTX</td>
<td>0.64 (0.56–0.73)</td>
<td>&lt;.001</td>
<td>0.60 (0.52–0.69)</td>
<td>&lt;.001</td>
<td>0.76 (0.62–0.93)</td>
<td>.008</td>
</tr>
</tbody>
</table>

a Men diagnosed with diabetes, reporting use of glucose-lowering medication, or with fasting glucose of ≥7 mmol/L or nonfasting glucose ≥11.1 mmol/L were considered to have diabetes. For the individual models, each bone turnover marker and the ratio of ucOC to TOC was analyzed separately; for the combined model, TOC, ucOC, P1NP, and CTX were included in the model simultaneously. The ratio of ucOC to TOC was excluded from the combined model. Adjustment was for age, smoking, BMI, WHR, hypertension, dyslipidemia, creatinine, vitamin D, and medical comorbidity (Charison index).

The adjusted analyses were repeated after excluding men with hormone levels in the lowest and highest 1% of values to ensure that the results were not biased by low or high outliers. Associations of LH with bone turnover markers and ucOC to TOC ratio were no longer significant (Table 5). DHT remained inversely, and E2 positively, associated with ucOC to TOC ratio. E2 remained inversely associated with TOC, and SHBG positively, associated with TOC, ucOC, P1NP, and CTX.

Bone turnover and sex hormones: trimmed analyses

The adjusted analyses were repeated after excluding men with hormone levels in the lowest and highest 1% of values to ensure that the results were not biased by low or high outliers. Associations of LH with bone turnover markers and ucOC to TOC ratio were no longer significant (Table 5). DHT remained inversely, and E2 positively, associated with ucOC to TOC ratio. E2 remained inversely associated with TOC, and SHBG positively, associated with TOC, ucOC, P1NP, and CTX.

Combined models with T and SHBG, DHT and SHBG, and E2 and SHBG

The fully adjusted trimmed analyses were repeated, with inclusion of SHBG in the models containing T, DHT, and E2 (Table 6). In the combined model, T was not associated with any bone turnover marker or with ucOC to TOC ratio. DHT was inversely associated with ucOC and TOC. E2 remained inversely associated with TOC and ucOC. E2 remained inversely associated with TOC, ucOC, and CTX and positively associated with ucOC to TOC ratio.

Discussion

In this large cross-sectional analysis of older men, we showed that ucOC and other markers of bone turnover are associated with lower risk of predominantly type 2 diabetes. This association of higher bone remodeling with...
reduced diabetes risk was not accounted for by age, BMI, or other risk factors or comorbidities. Although higher ucOC may reflect rates of bone remodeling, it remains independently associated with lower risk of having diabetes. E2, but not T, was inversely associated with bone turnover. There was no evidence of a specific association of ucOC with T; however, ucOC was inversely associated with circulating DHT, the more potent androgenic metabolite of T.

These results extend previous cohort studies in older men where TOC concentrations have been lower in the presence of diabetes, inversely associated with BMI and fat mass (6), and inversely associated with fasting insulin, plasma glucose, and HOMA-IR (8, 9). However, those studies did not measure ucOC. We found that although older men with diabetes had lower ucOC concentrations compared with nondiabetic men, comparable associations were present for TOC and for P1NP and CTX.

Previous studies of ucOC have been smaller in size with results differing between studies. Both ucOC and cOC were associated inversely with fasting and 2-hour postchallenge glucose concentrations and HOMA-IR in a study of 199 men with a mean age of 47 years (11). In a study of 348 nondiabetic men and women aged on average 68 years, ucOC was not associated with HOMA-IR at baseline or after 3 years (12). In a prospective, nested case-control study of 153 cases with newly diagnosed diabetes and 306 matched controls, both ucOC and cOC were lower in cases compared with controls at baseline, and cOC, but not ucOC, was inversely associated with HOMA-IR in nondiabetic controls (15). However, differences in assays for ucOC may have affected these findings.

Although these studies did not support a role for ucOC as a modulator of glucose metabolism as proposed by Lee et al (1), other studies supported a specific role for ucOC in diabetes risk. In 289 adults with type 2 diabetes, ucOC correlated negatively with hemoglobin A1c and fasting plasma glucose in men but not in postmenopausal women (13). In 79 older men (aged 55–80 years), increases in ucOC over a 2-year follow-up were associated with decreases in HOMA-IR (14). Both these studies used immunoassays for ucOC. Levinger et al (16), using a hydroxypatite-binding assay, showed that ucOC was associated with whole-body insulin sensitivity both at rest and after exercise in obese men. However, in a study of 129 adults with type 2 diabetes, ucOC did not correlate with insulin

### Table 4.

<table>
<thead>
<tr>
<th>Variable</th>
<th>TOC Coefficient</th>
<th>P Value</th>
<th>UcOC Coefficient</th>
<th>P Value</th>
<th>ucOC to TOC Ratio Coefficient</th>
<th>P Value</th>
<th>P1NP Coefficient</th>
<th>P Value</th>
<th>CTX Coefficient</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>−0.005</td>
<td>0.772</td>
<td>−0.002</td>
<td>0.928</td>
<td>−0.029</td>
<td>0.121</td>
<td>0.009</td>
<td>0.651</td>
<td>0.008</td>
<td>0.678</td>
</tr>
<tr>
<td>Free T</td>
<td>−0.027</td>
<td>0.121</td>
<td>−0.025</td>
<td>0.177</td>
<td>−0.012</td>
<td>0.523</td>
<td>−0.013</td>
<td>0.487</td>
<td>−0.016</td>
<td>0.397</td>
</tr>
<tr>
<td>DHT</td>
<td>−0.009</td>
<td>0.577</td>
<td>−0.014</td>
<td>0.400</td>
<td>−0.038</td>
<td>0.040</td>
<td>−0.001</td>
<td>0.949</td>
<td>−0.009</td>
<td>0.609</td>
</tr>
<tr>
<td>E2</td>
<td>−0.052</td>
<td>0.001</td>
<td>−0.037</td>
<td>0.031</td>
<td>0.055</td>
<td>0.002</td>
<td>−0.009</td>
<td>0.630</td>
<td>−0.043</td>
<td>0.016</td>
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<tr>
<td>SHBG</td>
<td>0.058</td>
<td>0.001</td>
<td>0.072</td>
<td>&lt;.001</td>
<td>−0.051</td>
<td>0.008</td>
<td>0.056</td>
<td>0.004</td>
<td>0.069</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>LH</td>
<td>0.057</td>
<td>0.001</td>
<td>0.051</td>
<td>0.004</td>
<td>−0.055</td>
<td>0.003</td>
<td>0.037</td>
<td>0.043</td>
<td>0.061</td>
<td>0.001</td>
</tr>
</tbody>
</table>

aData are shown as standardized regression coefficients with corresponding P values.

### Table 5.

<table>
<thead>
<tr>
<th>Variable</th>
<th>TOC Coefficient</th>
<th>P Value</th>
<th>UcOC Coefficient</th>
<th>P Value</th>
<th>ucOC to TOC Ratio Coefficient</th>
<th>variable</th>
<th>P1NP Coefficient</th>
<th>P Value</th>
<th>CTX Coefficient</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>0.015</td>
<td>.454</td>
<td>0.023</td>
<td>.289</td>
<td>−0.036</td>
<td>0.13</td>
<td>0.011</td>
<td>.642</td>
<td>0.036</td>
<td>.119</td>
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<tr>
<td>Free T</td>
<td>−0.004</td>
<td>.851</td>
<td>0.002</td>
<td>.946</td>
<td>−0.024</td>
<td>0.318</td>
<td>−0.008</td>
<td>.725</td>
<td>0.016</td>
<td>.495</td>
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<tr>
<td>DHT</td>
<td>−0.010</td>
<td>.608</td>
<td>−0.019</td>
<td>.374</td>
<td>−0.049</td>
<td>0.035</td>
<td>0.001</td>
<td>.972</td>
<td>−0.001</td>
<td>.969</td>
</tr>
<tr>
<td>E2</td>
<td>−0.051</td>
<td>.005</td>
<td>−0.030</td>
<td>.115</td>
<td>0.066</td>
<td>0.002</td>
<td>−0.018</td>
<td>.388</td>
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<tr>
<td>SHBG</td>
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<td>.007</td>
<td>0.066</td>
<td>.002</td>
<td>−0.046</td>
<td>0.051</td>
<td>0.053</td>
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<tr>
<td>LH</td>
<td>0.042</td>
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<td>.316</td>
<td>−0.044</td>
<td>0.108</td>
<td>0.031</td>
<td>.247</td>
<td>0.040</td>
<td>.141</td>
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</table>
Table 6. Multivariable Analysis of Associations Between the T and Its Metabolites DHT and E2, With TOC, ucOC, Ratio of ucOC to TOC, P1NP, and CTX in 2643 Community-dwelling Older Men

<table>
<thead>
<tr>
<th>Variable</th>
<th>TOC</th>
<th>UCOC</th>
<th>ucOC to TOC Ratio</th>
<th>P1NP</th>
<th>CTX</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>-0.037</td>
<td>-0.036</td>
<td>-0.009</td>
<td>-0.099</td>
<td>-0.020</td>
</tr>
<tr>
<td>SHBG</td>
<td>0.080</td>
<td>0.091</td>
<td>0.026</td>
<td>0.763</td>
<td>0.520</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DHT</td>
<td>-0.047</td>
<td>-0.064</td>
<td>-0.035</td>
<td>-0.029</td>
<td>-0.045</td>
</tr>
<tr>
<td>SHBG</td>
<td>0.078</td>
<td>0.098</td>
<td>0.080</td>
<td>0.278</td>
<td>0.085</td>
</tr>
<tr>
<td>C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E2</td>
<td>-0.065</td>
<td>-0.045</td>
<td>0.078</td>
<td>0.029</td>
<td>-0.056</td>
</tr>
<tr>
<td>SHBG</td>
<td>0.071</td>
<td>0.077</td>
<td>0.001</td>
<td>0.061</td>
<td>0.008</td>
</tr>
</tbody>
</table>

* With both T and SHBG included in the model (A), DHT and SHBG included in the model (B), and E2 and SHBG included in the model (C). Analyses were adjusted for age, smoking, BMI, WHR, hypertension, dyslipidemia, creatinine, vitamin D, and medical comorbidity and excluded men with hormone levels in the lowest or highest 1% of values.

resistance assessed using a euglycemic-hyperinsulinemic clamp (32). In a Japanese study of 1597 men aged 65 years and above (33), both TOC and ucOC, and the ratio of ucOC to TOC were lower in men with diabetes, but the association of ucOC with diabetes remained after adjustment for TOC in that study.

By contrast, our results from a larger cohort of older men showed that ucOC (measured using a hydroxyapatite-binding step) and higher bone remodeling rates in general were associated with lower risk of diabetes, almost entirely type 2. Furthermore, in nondiabetic older men TOC, ucOC, and CTX were associated inversely with HOMA-IR in unadjusted analyses but not after adjustment for covariates. An association of lower ucOC but not TOC, P1NP, or CTX with diabetes would have supported the Karsenty hypothesis (1). However, finding that higher ucOC, TOC, P1NP, and CTX are all associated with lower diabetes risk suggested that ucOC may behave as a marker of bone remodeling without exhibiting a specific association with glucose metabolism. The combined model indicates that ucOC and CTX are robust predictors that contribute independently of the other bone turnover markers. Although the difference between mean ucOC concentrations in the groups of men with and without diabetes is small (−2 μg/L), its significance as a biomarker is appreciable with a 1-SD increase in ucOC (−5 μg/L) corresponding to a 45% reduction in the odds of having diabetes. Interestingly, recent work by De Toni et al (34) showed ucOC increased vitamin D production from murine Leydig cells. In our study, the association of ucOC with diabetes risk was independent of vitamin D concentrations.

Oury et al (5) described a mechanism by which ucOC acting via the G protein-coupled receptor GPRC6A expressed in testes regulated production of T in a parallel pathway to LH in mice. However, the relevance of this mechanism to humans had been unclear. Kanazawa et al (17) reported a study of 69 men with type 2 diabetes in which ucOC and ucOC to TOC ratio correlated positively with free T concentrations. UcOC and ucOC to TOC ratio correlated inversely with LH, and neither TOC nor urinary N-telopeptide concentrations were associated (17). In that study, ucOC was measured using an immunoassay without a hydroxyapatite-binding step, and free T was measured by direct RIA that does not correspond to the reference method, making those results difficult to interpret. However, other studies have shown correlations of ucOC to TOC ratio with total T in overweight middle-aged men and age-matched controls (34) and of TOC with total T and more favorable metabolic parameters in predominantly middle-aged men (35).

In a study of 159 young adult men attending fertility clinics for assessment with their partners, cOC was separated from ucOC by hydroxyapatite binding, and total T was measured by immunoassay (36). In that study, after adjustment for age, and for BMI or WHR, none of TOC, cOC, and ucOC were associated with total T. In our large cohort study of older men with sex hormones assayed using LC-MS/MS, there was no association of total or calculated free T with ucOC or any other bone turnover marker. Higher SHBG was associated with TOC, ucOC, P1NP, and CTX. In fully adjusted models including SHBG, DHT was associated inversely with TOC and ucOC and E2 was associated inversely with TOC, ucOC, and CTX, and positively with ucOC to TOC ratio. Therefore, although we found no evidence that ucOC modulates circulating T in older men, its association with the more potent circulating androgen DHT indicates that the relationship of bone remodeling with overall androgen status needs to be further clarified. The association of DHT with TOC and ucOC requires further exploration, because this...
was specific for osteocalcin and was not seen with P1NP or CTX. Although lower circulating T has been identified as a risk predictor for bone loss and fracture in older men (19, 20), our results with E2 are consistent with previous reports identifying E2 as being the major sex hormone acting on bone in man and a determinant of osteoporosis and fracture risk in aging men (21, 22, 37–39).

Strengths of our study include the size of the cohort, which provides ample statistical power to detect associations of ucOC and other bone turnover markers with outcomes of interest, robust measurement of ucOC using hydroxyapatite binding, inclusion of P1NP and CTX for comparison, and accurate measurement of T, DHT, and E2 using LC-MS/MS. In our regression analyses, we adjusted systematically for potential confounders, and we performed a trimmed analysis to ensure results were not biased by high or low outliers. The Western Australian Data Linkage System links individual men to statewide and national databases covering hospital admissions and related morbidity as well as mortality (25). Because outcomes for the entire state of Western Australia are captured and because few men of this age emigrate, the resulting dataset is comprehensive with minimal loss to cross-boundary flows. We acknowledge several limitations of our study, including its observational and cross-sectional nature, which limits our ability to infer causation. The definition of diabetes encompassing elevated glucose concentrations differs from clinical definitions that require symptomatic hyperglycemia (40). We were not able to adjust for vitamin K intake, which may have influenced ucOC concentrations. Men participating in HIMS were drawn from a larger group seen earlier; hence, a healthy survivor effect may be present. We did not have serial blood samples for repeat assays of either bone turnover markers or hormones. Nevertheless, men were venepucted early in the morning, which would reduce confounding from circadian variation, and a single sample provides a reasonable estimate of hormone levels (41). Men in HIMS were almost entirely of Caucasian ethnicity; therefore, our results may not apply to younger men, men from different ethnic backgrounds, or women. The exclusion of men with prostate cancer or osteoporosis, as well as those receiving hormonal therapy or bisphosphonates, enhances the internal validity of our results but limits their wider generalizability.

Older men are the expanding demographic group with the highest prevalence of type 2 diabetes (42) and also the greatest risk of osteoporosis and its related morbidity (37). Higher bone remodeling rates in older men are considered markers for osteoporosis risk, yet may be associated with metabolically favorable outcomes. It is conceivable that although higher bone remodeling rates result in increased concentrations of all bone turnover markers including ucOC, ucOC also exerts metabolically favorable effects. In older men, ucOC behaves as a marker of bone remodeling and of reduced diabetes risk, whereas a lower E2 concentration is strongly associated with increased bone remodeling. γ-Carboxylation of osteocalcin is a vitamin K-dependent phenomenon, and higher TOC has been associated with reduced progression of abdominal aortic calcification (43). Additional studies would be warranted to determine whether ucOC is a biomarker for other health outcomes distinct from TOC, P1NP, or CTX.

In conclusion, higher bone remodeling rates are associated with reduced diabetes risk in older men. Although higher ucOC was associated with reduced diabetes risk, higher TOC, P1NP, and CTX were similarly associated. When all 4 markers were combined in the same model, higher ucOC remained associated, indicating its role as a marker of bone remodeling and an independent predictor of reduced diabetes risk. Older men with diabetes have an increased risk of fracture compared with nondiabetic men, despite relatively preserved bone mineral density (44). The association of diabetes with reduced bone remodeling rates illuminates another point of difference with nondiabetic older men, which should be considered when investigating bone metabolism. E2 and to a lesser extent DHT were inversely associated with bone remodeling rate. Although we found no evidence that ucOC modulates circulating T in men, its association with circulating DHT and therefore net androgen status require further investigation.

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Address all correspondence and requests for reprints to: Bu Beng Yeap MBBS, PhD, School of Medicine and Pharmacology, Level 2, T Block, Fremantle Hospital, Alma Street, Fremantle, WA 6160, Australia. E-mail: byeap@cyllene.uwa.edu.au.

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