Free 25-hydroxyvitamin D is low in obesity, but there are no adverse associations with bone health\textsuperscript{1,3}

Jennifer S Walsh,\textsuperscript{4,*} Amy L Evans,\textsuperscript{4} Simon Bowles,\textsuperscript{4} Kim E Naylor,\textsuperscript{4} Kerry S Jones,\textsuperscript{6} Inez Schoenmakers,\textsuperscript{6} Richard M Jacques,\textsuperscript{5} and Richard Eastell\textsuperscript{4}

\textsuperscript{4}Academic Unit of Bone Metabolism and \textsuperscript{5}School of Health and Related Research, University of Sheffield, United Kingdom; and \textsuperscript{6}Medical Research Council Human Nutrition Research, Cambridge, United Kingdom

ABSTRACT

Background: The mechanism and clinical significance of low circulating 25-hydroxyvitamin D [25(OH)D] in obese people are unknown. Low total 25(OH)D may be due to low vitamin D–binding proteins (DBPs) or faster metabolic clearance. However, obese people have a higher bone mineral density (BMD), which suggests that low 25(OH)D may not be associated with adverse consequences for bone.

Objective: We sought to determine whether 1) vitamin D metabolism and 2) its association with bone health differ by body weight.

Design: We conducted a cross-sectional observational study of 223 normal-weight, overweight, and obese men and women aged 25–75 y in South Yorkshire, United Kingdom, in the fall and spring. A subgroup of 106 subjects was also assessed in the winter. We used novel techniques, including an immunoassay for free 25(OH)D, a stable isotope for the 25(OH)D3 half-life, and high-resolution quantitative tomography, to make a detailed assessment of vitamin D physiology and bone health.

Results: Serum total 25(OH)D was lower in obese and overweight subjects than in normal-weight subjects in the fall and spring (geometric means: 45.0 and 40.8 compared with 58.6 nmol/L, respectively; \( P < 0.001 \)) but not in the winter. Serum 25(OH)D was inversely correlated with body mass index (BMI) in the fall and spring and in the winter. Free 25(OH)D and 1,25-dihydroxyvitamin D [1,25(OH)\(_2\)D] were lower in obese subjects. DBP, the DBP genotype, and the 25(OH)D\(_3\) half-life did not differ between BMI groups. Bone turnover was lower, and bone density was higher, in obese people.

Conclusions: Total and free 25(OH)D and 1,25(OH)\(_2\)D are lower at higher BMI, which cannot be explained by lower DBP or the shorter half-life of 25(OH)D\(_3\). We speculate that low 25(OH)D in obesity is due to a greater pool of distribution. Lower 25(OH)D may not reflect at-risk skeletal health in obese people, and BMI should be considered when interpreting serum 25(OH)D as a marker of vitamin D status. \textit{Am J Clin Nutr} 2016;103:1465–71.

Keywords: bone density, bone turnover, half-life, obesity, vitamin D, vitamin D–binding protein

INTRODUCTION

Vitamin D is essential for the intestinal absorption of dietary calcium and skeletal mineralization. Vitamin D deficiency causes undermineralization, increased bone resorption, osteomalacia, and rickets. Vitamin D insufficiency is associated with increased risk of osteoporosis (1) and possibly poorer muscle function and other adverse health outcomes (2).

Serum total 25-hydroxyvitamin D [25(OH)D]\textsuperscript{1} is the most commonly used biomarker for vitamin D status; it has a long plasma half-life and reflects both skin synthesis and oral intake. The Institute of Medicine recommends that 50 nmol/L (20 ng/mL) is sufficient for most of the population (3). The Endocrine Society recommends that <50 nmol/L is deficient and that 50–75 nmol/L (20–30 ng/mL) is insufficient (4).

Serum total 25(OH)D is lower in obese people and inversely correlated with BMI. These findings have been reported in adults and children of different ethnic groups all over the world (5–13).

However, the causes and clinical significance of low 25(OH)D and, hence, the value of total 25(OH)D as a biomarker of vitamin D status in different body weights are not clear. Possible causes of low serum 25(OH)D in obesity are a lower vitamin D supply [less sunlight exposure (14) or lower dietary intake (15)], greater volume of distribution, reduced biological availability, or more rapid clearance.

Greater than 99% of circulating 25(OH)D and 1,25-dihydroxyvitamin D [1,25(OH)\(_2\)D] are bound to vitamin D–binding protein.
protein (DBP) and albumin, and the remaining free fraction is the most biologically available. Also, genetic polymorphism results in 3 DBP phenotypes with differing circulating DBP concentrations and affinities for 25(OH)D (16, 17). Lower concentrations of binding proteins would reduce total 25(OH)D measurements, but free 25(OH)D might be unchanged. It is not clear whether DBP concentrations differ by body weight (18, 19). Parathyroid hormone (PTH) may be increased in obesity (15, 20), and higher PTH could increase the metabolic clearance rate of 25(OH)D.

There is a paradox in body weight, vitamin D, and bone: low 25(OH)D would be expected to be associated with higher bone turnover and lower bone mineral density (BMD), but BMI and fat mass are positively correlated with BMD (21), and higher body weight is generally protective against fracture (22).

The aims of this study were to apply newly available techniques [including an immunoassay for free 25(OH)D and a stable-isotope method for the 25(0H)D3 half-life] as part of a comprehensive set of measurements to determine how the supply, availability, and metabolism of vitamin D are affected by body weight combined with a detailed assessment of bone [with the use of multiple biochemical markers of bone turnover, dual-energy X-ray absorptiometry (DXA) and high-resolution peripheral quantitative computed tomography (HR-pQCT)] to determine whether 25(OH)D is associated with bone health in obesity.

METHODS

We conducted a cross-sectional study of healthy Caucasian men and women (aged 25–40 and 55–75 y) from South Yorkshire, United Kingdom (latitude: 53°N). Participants were approached through poster advertisements, e-mails to hospital staff, mailings from general practice surgeries, and a database of volunteers. Participants were recruited in the following 3 BMI (in kg/m2) categories: normal weight (18.5–24.9), overweight (25–29.9), and obese (>30). Exclusion criteria were pregnancy or breast feeding within the past year, conditions (including diabetes) or use of a medication (including hormonal contraception) that is known to affect vitamin D or bone metabolism, immobilization, high alcohol intake, and being a competitive athlete. Older women were ≥5 y postmenopausal. There were no restrictions on supplement intake, and supplement use was included in the dietary calcium and vitamin D assessment. (For recruitment details, see Supplemental Table 1). The study was approved by South Yorkshire Research Ethics Committee and conducted according to the Declaration of Helsinki, and all subjects gave written informed consent.

All participants were assessed in the fall or spring (from 19 September 2012 to 31 October 2012 and from 2 April 2013 to 16 May 2013) when UVB is available. Fasting morning blood samples were taken for the measurement of serum total and free 25(OH)D, 1,25(OH)2D, DBP, albumin, PTH, biochemical markers of bone turnover, and DBP genotype. Statistical analyses were adjusted for the date of visit. Sunlight exposure, dietary vitamin D intake, and muscle function were also assessed.

A subgroup of 106 participants (normal weight: n = 34; overweight: n = 32; obese: n = 40) were also assessed in the winter (from 11 December 2012 to 1 April 2013) to assess vitamin D status when there is negligible UVB and to avoid the perturbation of the isotope tracer study by sunlight exposure. Fasting morning blood samples were taken for the measurement of 25(OH)D, and the 25(OH)D3 half-life was assessed with a stable isotope tracer.

Measurements

A short physical performance battery (SPPB) score (maximum score of 12) was calculated from narrow-walk and chair-stand tests (23). Grip strength was measured with the use of a digital dynamometer (Seahan Corp.).

A sunlight questionnaire was used to assess habitual sunlight exposure by the season and during holidays (5). A questionnaire assessment of sunlight exposure has been shown to be correlated with vitamin D status (24). Dietary vitamin D intake was assessed with the DIETQ questionnaire (Tinuviel Software), which is a semiquantitative habitual food-frequency intake questionnaire with a computerized analysis that is based on the United Kingdom nutrient database (25).

25(OH)D was measured at the Manchester Institute of Human Development, United Kingdom, with the use of liquid chromatography–tandem mass spectrometry. This laboratory participates in the Vitamin D External Quality Assessment Scheme, and the assay was calibrated against the National Institute of Standards and Technology standard. 25(OH)D2 was undetectable in most subjects. Free (unbound) 25(OH)D was determined with the use of an immunoassay (26) (inter assay CV at 13.2 pg/mL: 5.3%; Future Diagnostics). Free 25(OH)D can also be estimated by the calculation from total 25(OH)D, DBP, albumin, and their binding affinities, but this approach has limitations because of the genetic variation in DBP, and the direct measurement by an immunoassay is more closely correlated with serum PTH and calcium (27). 1,25(OH)2D was measured with the use of a manual immunoassay after immunoextraction (interassay CV at 6.0%, intra-assay CV: 2.6%; ImmunoDagnostic Systems). DBP was measured with the use of a Quantikine manual immunoassay (interassay CV: 3.3%; intra-assay CV: 3.9%; R&D Systems).

C-terminal telopeptide of type I collagen (CTX), which is a bone-resorption marker, procollagen type I N-terminal propeptide (PINP) and osteocalcin, both of which are bone formation markers, were measured with the use of an automated immunoassay (Cobas e411; Roche Diagnostics). Interassay CVs were as follows: CTX, 4.0%; PINP, 4.1%; and osteocalcin, 2.2%. Bone alkaline phosphatase (ALP), which is a bone formation marker, was measured with the use of an automated immunoassay (interassay CV: 4.5%; iSYS; ImmunoDagnostic Systems).

Albumin, creatinine, calcium, and PTH were measured with the use of an autoanalyzer (interassay precision <2.0% for all tests; Cobas c701; Roche Diagnostics).

DBP genotyping was done at Sheffield Children’s Hospital, United Kingdom. The pyrosequencing assay was developed with the use of PSQ software (version 1.0.6; Qiagen) to detect rs4588 and rs7041 polymorphisms.

The 25(OH)D half-life was measured with a 24-μg orally administered tracer stable isotope of 25(OH)D3 (3-2H-25-hydroxyvitamin D3 (6, 19, and 19-d3)). The tracer was given dissolved in olive oil with a standard breakfast. Venous blood was taken at 6 ± 1, 9 ± 2, 27 ± 2, and 30 ± 2 d after administration.
The 25(OH)D₃ half-life was calculated from the terminal slope of the disappearance of d3-25(OH)D₃ as \( t_{1/2} = \ln(2) \div k_B \), where \( k_B \) is the ln of the slope of the line of best fit from days 5 to 30 (28). Preparation of the tracer and the liquid chromatography-tandem mass spectrometry measurements (29) were performed at the Medical Research Council Human Nutrition Research, Cambridge, United Kingdom.

BMD and fat mass were assessed with the use of DXA and HR-pQCT. Whole-body, lumbar spine, and hip DXA were performed with a Discovery densitometer (Hologic Inc.). The weight and height were measured to the nearest 0.1 kg and 0.1 cm, respectively. HR-pQCT images of the distal radius and tibia (4% site, nondominant, and nonfractured) were obtained with the use of an XtremeCT device (Scanco Medical AG). Images were analyzed with Scanco software (version 6; Scanco Medical AG). The short-term precision of the BMD measurements was 0.2–5.5% (30).

**Statistics**

Normality was assessed with the use of histograms. Skewed variables were log10-transformed for the analysis. Variables that differed between the 3 BMI groups were identified with the use of an ANOVA. Effects of the age group and sex were tested with the use of an ANCOVA. Post hoc testing for differences between pairs of BMI groups was adjusted for multiple comparisons with the use of Tukey’s method. Relations between variables and BMI (as a continuous variable) were examined with the use of univariate linear models. Multiple linear regression models were used to adjust for age (as a continuous variable) and sex. Correlations between variables were calculated with the use of the Spearman rank test, and 95% CIs were calculated with the use of bootstrapping. Statistical analyses were performed with SPSS software (IBM, version 21) and R software (GNU project, version 3.2.1).

The fall and spring study \( (n = 223) \) had 90% power at 5% 2-sided significance to detect a 0.22 correlation coefficient between BMI and 25(OH)D. For the ANOVA, 65 participants/BMI group had 90% power to detect a standardized effect size of 0.26 at 5% 2-sided significance. The winter study \( (n = 106) \) had 90% power at 5% 2-sided significance to detect a 0.30 correlation coefficient between BMI and 25(OH)D. For the ANOVA, 32 participants/BMI group had 90% power to detect a standardized effect size of 0.37 at 5% 2-sided significance. For a report of missing data, see Supplemental Table 2.

**RESULTS**

Characteristics of study participants are shown in Table 1. Dietary calcium intake did not differ between BMI groups [means: normal weight, 1072 mg/d (95% CI: 1002, 1145 mg/d); overweight, 1074 mg/d (95% CI: 998, 1158 mg/d); and obese, 1055 mg/d (95% CI: 1001, 1112 mg/d)]. The subset who were also assessed in the winter were representative of the whole group \( (n = 106) \); normal BMI, \( n = 34 \); overweight, \( n = 32 \); obese, \( n = 40 \); younger, \( n = 46 \); older, \( n = 60 \); men, \( n = 50 \); women, \( n = 56 \).

Total 25(OH)D₃ was lower in obese and overweight people than in normal-weight people in the fall and spring but not in the winter (Figure 1). Geometric means of total 25(OH)D₃ in each BMI group in the fall and spring were as follows: normal weight, 58.6 nmol/L (95% CI: 52.2, 65.8 nmol/L); overweight, 40.8 nmol/L (95% CI: 36.2, 46.0 nmol/L); and obese, 45.0 nmol/L (95% CI: 40.4, 50.0 nmol/L). In the winter, geometric means of total 25(OH)D₃ were as follows: normal weight, 40.8 nmol/L (95% CI: 33.9, 50.1 nmol/L); overweight, 39.8 nmol/L (95% CI: 33.1, 47.9 nmol/L); and obese, 33.9 nmol/L (95% CI: 28.8, 40.8 nmol/L).

In the fall and spring, 56% of overweight and obese people compared with 37% of normal-weight people had 25(OH)D₃ concentrations <50 nmol/L. In the winter, 75% of overweight and obese people compared with 62% of normal-weight people had 25(OH)D₃ concentrations <50 nmol/L.

Total 25(OH)D₃ in the fall and spring was inversely correlated with BMI (adjusted for the date of visit, age, and sex; model-adjusted \( R^2 = 0.339, P < 0.001 \)). For every 5-U increase in BMI, total 25(OH)D₃ decreased by 10.0% (95% CI: 5.7%, 14.0%; \( P < 0.001 \)). After the same adjustments, total 25(OH)D₃ was also negatively correlated with whole-body fat mass (model-adjusted \( R^2 = 0.334, P < 0.001 \)). For every 10-kg increase in fat mass, total 25(OH)D₃ decreased by 11% (95% CI: 6%, 15%; \( P < 0.001 \)). Although total 25(OH)D₃ did not differ by BMI group in the winter, 25(OH)D₃ was negatively correlated with BMI (adjusted for age and sex; model-adjusted \( R^2 = 0.172, P < 0.001 \)). For every 5-U increase in BMI, 25(OH)D₃ decreased by 8.2% (95% CI: 0.5%, 15.3%; \( P = 0.038 \)). Dietary vitamin D and sunlight exposure did not differ by BMI group (Table 2). The mean number of hours of sunlight (irradiance measurement >120 W/m²) in Sheffield during the period of the study measurements was 4.6 h in the fall and spring and 1.9 h in the winter (data provided by Weston Park Weather Station).

DBP and albumin did not differ by BMI group, and adjustment for age and sex did not change this result (Table 2). The DBP-genotype distribution (Gc1-1: 47%; Gc2-1: 42%; and Gc2-2: 11%) was similar to that of other reported white European populations (16). The genotype distribution did not differ by BMI group, and BMI did not differ by genotype. Total 25(OH)D₃ concentrations did differ by genotype (means: Gc1-1, 52.2 nmol/L (95% CI: 47.2, 57.6 nmol/L); Gc2-1, 45.3 nmol/L (95% CI: 40.9, 50.3 nmol/L); and Gc2-2, 39.4 nmol/L (95% CI: 32.1, 48.3 nmol/L); \( P = 0.024 \)).

The 25(OH)D₃ half-life did not differ by BMI group (Table 2). Free 25(OH)D₃ was lower in the obese and overweight groups than in the normal-weight group in the fall and spring. BMI was negatively correlated with free 25(OH)D₃ (adjusted for the date of visit, age, and sex).

**TABLE 1**

<table>
<thead>
<tr>
<th>Participant characteristics by BMI group</th>
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<tbody>
<tr>
<td>BMI group</td>
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<tr>
<td>--------------------------</td>
</tr>
<tr>
<td>Normal (18.5–24.9 kg/m²)</td>
</tr>
<tr>
<td>Overweight (25.0–29.9 kg/m²)</td>
</tr>
<tr>
<td>Obese (&gt;30.0 kg/m²)</td>
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</table>

¹Mean ± SD (all such values).
of visit, age and sex; model-adjusted $R^2 = 0.296$, $P < 0.001$). For every 5-U increase in BMI, free 25(OH)D decreased by 12.3% (95% CI: 7.7%, 16.6%; $P < 0.001$). When total 25(OH)D was added to the model, the relation between free 25(OH)D and BMI was no longer significant ($R^2 = 0.619$, $P = 0.16$). Total 1,25(OH)_2D was also lower in the obese and overweight groups than in the normal-weight group in the fall and spring (Table 3).

PTH did not differ by BMI group (Table 3) and was not correlated with BMI. Adjustment for age and sex did not change this result. In the regression of PTH on BMI, age, sex, and 25(OH)D, there was a negative correlation of PTH with 25(OH)D but no interaction of BMI and 25(OH)D, and thus, BMI did not predictably modulate the relation between 25(OH)D and PTH.

CTX and osteocalcin were lower in the obese group than in the normal-weight and overweight groups. Bone ALP and PINP did not differ between BMI groups (Table 3).

BMD, which was assessed with the use of DXA of the whole body, lumbar spine, and hip and with the use of HR-pQCT at the distal radius and tibia, was higher in the overweight and obese groups than in the normal-weight group (Table 3). Grip strength did not differ by BMI group. Adjustment for age and sex did not change this result. The SPPB score was lower in the overweight and obese groups than in the normal-weight group ($P < 0.001$). However, the SPPB score was not correlated with 25(OH)D (Spearman $\rho$: $-0.122$; 95% CI: $-0.261$, 0.014; $P = 0.073$).

**DISCUSSION**

To our knowledge, this is the first study to use the free 25(OH)D assay and stable-isotope half-life method to investigate the effect of body weight on vitamin D metabolism. We have applied a comprehensive set of measurements to assess the supply, availability, and metabolism of 25(OH)D and musculoskeletal health across a wide range of body weights. As expected, serum total 25(OH)D was lower at a higher body weight; it was lower in obese people than in normal-weight people in the fall and spring and was negatively correlated with BMI in the fall and spring and in the winter.

**TABLE 2**

Possible contributors to low vitamin D in obesity

<table>
<thead>
<tr>
<th>BMI group</th>
<th>Normal (n = 77)</th>
<th>Overweight (n = 63)</th>
<th>Obese (n = 83)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dietary vitamin D intake,$^2$ μg</td>
<td>3.61 (3.01, 4.34)</td>
<td>3.05 (2.50, 3.72)</td>
<td>2.72 (2.24, 3.31)</td>
</tr>
<tr>
<td>Annual sunlight-exposure score$^2$</td>
<td>90.48 (82.44, 98.53)</td>
<td>96.34 (86.69, 105.98)</td>
<td>92.33 (84.54, 100.13)</td>
</tr>
<tr>
<td>Summer sunlight-exposure score$^2$</td>
<td>48.45 (43.96, 53.74)</td>
<td>51.15 (45.37, 56.94)</td>
<td>47.55 (42.65, 52.46)</td>
</tr>
<tr>
<td>Vitamin D-binding protein,$^3$ μg/mL</td>
<td>136.0 (124.9, 147.0)</td>
<td>124.9 (112.3, 137.6)</td>
<td>130.5 (120.7, 140.4)</td>
</tr>
<tr>
<td>Albumin,$^3$ g/L</td>
<td>46.0 (45.3, 46.8)</td>
<td>45.7 (45.0, 46.4)</td>
<td>45.1 (44.2, 45.9)</td>
</tr>
<tr>
<td>25(OH)D$_3$ half-life,$^4$ d</td>
<td>17.8 (16.6, 19.1)</td>
<td>17.0 (15.8, 18.2)</td>
<td>18.2 (17.0, 19.1)</td>
</tr>
</tbody>
</table>

$^1$All $P > 0.05$ (ANOVA).

$^2$All values are geometric means; 95% CIs in parentheses.

$^3$All values are means; 95% CIs in parentheses.

$^4$ 25(OH)D$_3$, 25-hydroxyvitamin D$_3$. 

**FIGURE 1** Geometric means and 95% CIs of total 25(OH)D$_3$ (assessed with the use of liquid chromatography–tandem mass spectrometry) by BMI group in the fall and spring (n = 223) (A) and in the winter (n = 106) (B). Results were ANCOVA-adjusted for the date of visit (April and May compared with September and October), age group, and sex. 25(OH)D$_3$, 25-hydroxyvitamin D$_3$. 

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We investigated several possible mechanisms for the effects of body weight on vitamin D status. Dietary vitamin D intake and sunlight exposure were similar across BMI groups. A previous United Kingdom study also showed that sunlight exposure did not vary with BMI (31). Lower total 25(OH)D in obesity was not due to differences in protein binding; free 25(OH)D was also lower, and serum albumin, DBP, and the DBP genotype did not differ by BMI group. The 25(OH)D half-life did not differ by BMI group, and serum albumin, DBP, and the DBP genotype did not differ by BMI group. The 25(OH)D half-life did not differ by BMI group, and thus, lower 25(OH)D in obesity was not due to a more rapid metabolic clearance.

After cutaneous synthesis and absorption, vitamin D is distributed into fat, muscle, and other tissues (32), and when the volume of distribution is greater, less vitamin D may be available for 25-hydroxylation. 25(OH)D is also distributed into fat and muscle and into serum (33), and all of these compartments are increased in obesity. Consistent with this, other investigators have reported that the summer rise in circulating 25(OH)D is blunted in obesity (31, 34). When exposed to UVB, normal-weight and obese people have a similar cutaneous synthesis of vitamin D (49), but the serum 25(OH)D rise is attenuated in obese people (18), which is consistent with our observation that the 25(OH)D difference between normal-weight and obese people was greater in the fall and spring than in the winter. This theory is supported by evidence that the serum 25(OH)D response to oral vitamin D dosing is BMI dependent (35, 36).

Because of the greater volume of distribution, if whole-body tissue stores of vitamin D and 25(OH)D were similar in obese and normal-weight people, measured serum concentrations would be lower in obese people [and conversely, people with low BMI may have relatively high serum 25(OH)D but lower whole body stores]. We also identified that the biologically available free serum 25(OH)D and active hormone 1,25(OH)₂D were lower in obesity. However, PTH was similar across BMI groups. PTH is thought to be a useful indicator of the biological significance of low vitamin D. If vitamin D is low enough for gut calcium absorption to be reduced, PTH should rise, and other studies have described a positive association of PTH with fat mass (15, 20, 37–39). However, the relation between 25(OH)D and PTH may be altered in obesity; in some studies, body weight was a strong predictor of PTH, but 25(OH)D had little or no relation with PTH (40, 41). Calcium-clamp studies have suggested that the calcium-PTH curve is altered in obesity (42). PTH suppresses at a 25(OH)D concentration of 11.1 ng/mL in obese women compared with 21.7 ng/mL in normal-weight women (43), which suggests that PTH may be less responsive to lower serum 25(OH)D in obesity. In our regression analysis, BMI did not predictably modulate the relation between 25(OH)D and PTH.

If the low vitamin D in obesity was negatively affecting bone health, we would have expected that bone-turnover markers would have been increased, and BMD would have been decreased. However, bone-resorption markers were lower in the obese subjects than in normal-weight subjects, formation markers were similar across BMI groups, and BMD, which was assessed with the use of DXA and HR-pQCT, was higher in obese people at all measured sites. It has previously been shown that the bone microarchitecture is generally more favorable for bone strength in obese adults, with a greater cortical thickness and trabecular number (44–47), although a positive relation between BMI and cortical volumetric BMD has not been shown in all studies (45–47). Although higher BMI is generally protective against fracture, there is an excess of some limb fractures in obesity, which suggests that the changes in bone density and structure may not be adequate for the increase in body weight (48). It is possible that the lower serum 25(OH)D in obesity does reflect true vitamin D deficiency, but that adverse skeletal

### TABLE 3

Bone health and muscle function

<table>
<thead>
<tr>
<th>BMI group</th>
<th>Normal (n = 77)</th>
<th>Overweight (n = 63)</th>
<th>Obese (n = 83)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free 25(OH)D, ² pmol/L</td>
<td>10.6 (9.4, 12.0)</td>
<td>7.5 (6.5, 8.6)</td>
<td>7.8 (6.9, 8.8)</td>
</tr>
<tr>
<td>Total 1,25(OH)₂D, ² pmol/L</td>
<td>95.0 (87.1, 103.7)</td>
<td>79.4 (72.3, 87.1)</td>
<td>78.5 (72.3, 85.3)</td>
</tr>
<tr>
<td>PTH, ng/L</td>
<td>41.4 (38.4, 44.7)</td>
<td>41.4 (37.6, 45.5)</td>
<td>43.5 (40.5, 46.7)</td>
</tr>
<tr>
<td>CTX, ³ ng/L</td>
<td>0.45 (0.40, 0.50)</td>
<td>0.47 (0.43, 0.51)</td>
<td>0.38 (0.33, 0.42)</td>
</tr>
<tr>
<td>Osteocalcin, ³ ng/mL</td>
<td>23.0 (21.3, 24.8)</td>
<td>22.0 (20.5, 23.6)</td>
<td>19.1 (18.0, 20.4)</td>
</tr>
<tr>
<td>PINP, ng/mL</td>
<td>40.8 (36.9, 45.2)</td>
<td>41.4 (38.3, 44.8)</td>
<td>37.8 (34.8, 41.0)</td>
</tr>
<tr>
<td>Bone ALP, ng/mL</td>
<td>12.8 (11.7, 13.9)</td>
<td>12.9 (11.8, 14.0)</td>
<td>12.7 (11.8, 13.7)</td>
</tr>
<tr>
<td>Whole body DXA BMD, ² g/cm²</td>
<td>1.07 (1.05, 1.09)</td>
<td>1.14 (1.11, 1.16)</td>
<td>1.16 (1.13, 1.18)</td>
</tr>
<tr>
<td>Lumbar spine DXA BMD, ² g/cm²</td>
<td>0.95 (0.91, 0.98)</td>
<td>1.04 (1.01, 1.08)</td>
<td>1.09 (1.06, 1.13)</td>
</tr>
<tr>
<td>Total hip DXA BMD, ² g/cm²</td>
<td>0.88 (0.85, 0.91)</td>
<td>1.00 (0.97, 1.03)</td>
<td>1.06 (1.03, 1.09)</td>
</tr>
<tr>
<td>Distal radius HR-pQCT BMD, ² mgHA/cm³</td>
<td>272.0 (258.6, 286.0)</td>
<td>303.0 (290.6, 315.9)</td>
<td>315.0 (303.9, 326.5)</td>
</tr>
<tr>
<td>Distal tibia HR-pQCT BMD, ² mgHA/cm³</td>
<td>280.0 (269.5, 290.8)</td>
<td>312.2 (298.3, 326.7)</td>
<td>327.6 (316.8, 338.8)</td>
</tr>
<tr>
<td>Grip strength, kg</td>
<td>22.1 (20.3, 23.9)</td>
<td>24.1 (21.6, 26.6)</td>
<td>23.1 (21.0, 25.1)</td>
</tr>
<tr>
<td>Short physical performance battery score</td>
<td>9.5 (9.1, 9.9)</td>
<td>9.1 (8.7, 9.4)</td>
<td>8.3 (8.0, 8.7)</td>
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All values are geometric means; 95% CIs in parentheses. Measurements were taken in the fall and spring. Results were ANCOVA adjusted for age and sex (and date of visit for biochemistry). Means that do not share a common superscript letter were significantly different at P < 0.05 on the basis of post hoc testing that was adjusted for multiple comparisons with the use of Tukey’s method. ALP, alkaline phosphatase; BMD, bone mineral density; CTX, C-terminal telopeptide of type I collagen; DXA, dual-energy X-ray absorptiometry; HR-pQCT, high-resolution peripheral quantitative computed tomography; PINP, procollagen type I N-terminal propeptide; PTH, parathyroid hormone; 1,25(OH)₂D, 1,25-dihydroxy-vitamin D; 25(OH)D, 25-hydroxyvitamin D.

²p < 0.001.
³p < 0.01.
effects are countered by positive skeletal effects of obesity such as increased loading, estrogen synthesis from adipocyte aromatase, or adipocyte hormones such as leptin.

The physical function score was poorer in obese people but was not correlated with 25(OH)D. Vitamin D and calcium supplementation may improve physical functioning in older people, but there has been less evidence for a benefit in young adults (34–36). Other factors, such as less physical activity and fat infiltration of muscle, might contribute to poorer function. It is possible that vitamin D maintains muscle integrity in older adults by preventing intramuscular fat accumulation (49), which might be relevant to muscle function in obesity.

There are some limitations to this study. The study was powered on the basis of the expected effect size of BMI on serum 25(OH)D. Therefore, the power for multiple outcomes and the secondary analyses, such as genotype and muscle function, may have been limited. Dietary and sunlight-exposure habits differ by geography and culture, and it is very possible that lower dietary D and sunlight exposure contribute to low 25(OH)D in obese people elsewhere. We did not measure volume of distribution directly, which would have required an intravenous isotope, of which there are none available for human use. We did not measure intestinal calcium absorption. We used the DBP assay from R&D Systems; other DBP assays may have given different results because the influence of the DBP genotype varies by assay (50), and the assay may not recognize all the posttranslational modifications of DBP. However, all participants were Caucasian, which minimized the genotype variation (the genotype distribution varies by ethnic group), and the DBP genotype distribution did not differ between BMI groups. In the Rotterdam study of 6181 older adults, height and weight did not differ by rs4588 and rs7041 haplotypes (51). We also excluded effects of protein binding by the direct measurement of free 25(OH)D. In addition, the relation between obesity and bone may differ between children and adults, and thus, these results cannot be extrapolated to children (52).

We did not assess the effects of low 25(OH)D beyond the musculoskeletal system. Vitamin D deficiency has been associated with diseases such as cancer and metabolic syndrome, for which obesity is also a risk factor. However, there is not yet evidence for a causative role of vitamin D deficiency (53).

In conclusion, it is well recognized that serum total 25(OH)D is low in obesity, but we have shown that biologically available free serum 25(OH)D and the active hormone 1,25(OH)2D are also lower at higher body weight. The likely cause of lower 25(OH)D in obesity is the greater volume of distribution. The lower 25(OH)D in obesity is not associated with higher PTH or bone turnover, lower bone density, or poorer physical function. BMI affects the relation between serum 25(OH)D and bone health, and lower serum 25(OH)D at higher body weight may not indicate at-risk skeletal health.

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