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Free 25-hydroxyvitamin D is low in obesity, but there are no adverse consequences for bone health.

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Abbreviations

25OHD	25-hydroxyvitamin D
$1,25(OH)_2D$	1,25-dihydroxyvitamin D
BMD	Bone mineral density
Bone ALP	Bone alkaline phosphatase
CTX	C-terminal telopeptide of type I collagen
DBP	Vitamin D binding protein
DXA	Dual-energy X-ray absorptiometry
HR-pQCT	High resolution peripheral quantitative tomography
LC-MS/MS	Liquid chromatography tandem mass spectrometry
PINP	procollagen type I N propeptide
PTH	Parathyroid hormone
SPPB	Short physical performance battery

Abstract

Background: The mechanism and clinical significance of low circulating 25-hydroxyvitamin D (25OHD) in obese people are unknown. Low total 25OHD may be due to low vitamin D binding proteins (DBP) or faster metabolic clearance. Obese people have higher bone mineral density (BMD), suggesting that the low total 25OHD may not have the expected adverse consequences for bone.

Objective: The aims of this study were 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 ages 25 to 75 in South Yorkshire, UK in fall/spring. A subgroup of 106 were also assessed in winter. We used novel techniques including an immunoassay for free 25OHD, stable isotope for $25OHD_3$ half-life, and high resolution quantitative tomography (HR-pQCT) to make a detailed assessment of vitamin D physiology and bone health.

Results: Total serum 25OHD was lower in obese and overweight than normal weight people in fall/spring (geometric means 45.0 and 40.8 vs 58.6 nmol/l, $p<0.001$), but not in winter. Serum 25OHD was inversely correlated with BMI in fall/spring and winter.

Free 25OHD measured by immunoassay or calculated from DBP and albumin was lower in obesity. DBP, DBP genotype, and $25OHD_3$ half-life did not differ between BMI groups. Bone turnover was lower and bone density was higher in obese people.

Conclusions: Total and free 25OHD and $1,25(OH)_2D$ are lower at higher body weight, and this can't be explained by lower DBP or shorter half-life of $25OHD_3$. However, obese people had lower bone turnover and higher bone density than normal weight.

24 We speculate that low 25OHD in obesity is due to greater pool of distribution. Lower

25 25OHD in obesity may not reflect at-risk skeletal health.

26

27 **Keywords:** vitamin D, obesity, vitamin D binding protein, half-life, bone density, bone
28 turnover

29 **Introduction**

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

34 Serum total 25-hydroxyvitamin D (25OHD) is the most commonly used biomarker for
35 vitamin D status; it has a long plasma half-life and reflects both skin synthesis and oral
36 intake. Recommended sufficiency levels are 50 to 75 nmol/l (20 to 30 ng/ml) (3), (4).

37 Serum total 25OHD is lower in obese people, and inversely correlated with BMI. This has
38 been reported in adults and children of different ethnic groups all over the world. (5-13).
39 However, the causes and clinical significance of the low 25OHD, and hence the value of total
40 25OHD as a biomarker of vitamin D status in different body weights is not clear.

41 Possible causes of low serum 25OHD in obesity are lower vitamin D supply (less sunlight
42 exposure (14) or lower dietary intake (15)), greater volume of distribution, reduced biological
43 availability or more rapid clearance.

44 More than 99% of circulating 25OHD and $1,25(OH)_2D$ are bound to vitamin D binding
45 protein (DBP) and albumin, and the remaining free fraction is the most biologically available.
46 Also, genetic polymorphism results in three DBP phenotypes, with differing circulating DBP
47 levels and affinity for 25OHD (16, 17). Lower concentrations of binding proteins would
48 reduce total 25OHD measurements, but free 25OHD might be unchanged. It is not clear
49 whether DBP levels differ by body weight (18, 19).

50 Parathyroid hormone (PTH) may be increased in obesity (15, 20), and higher PTH could
51 increase the metabolic clearance rate of 25OHD.

52 There is a paradox in body weight, vitamin D and bone; low 25OHD would be expected to be
53 associated with higher bone and lower BMD, but BMI and fat mass are positively correlated
54 with BMD (21), and higher body weight is generally protective against fracture (22).

55 The aims of this study were to apply newly available techniques (including an immunoassay
56 for free 25OHD and a stable isotope method for $25OHD_3$ half-life) to determine how vitamin
57 D metabolism is affected by body weight, and a detailed assessment of bone (with multiple
58 biochemical markers of bone turnover, dual energy X-ray absorptiometry (DXA) and high
59 resolution peripheral quantitative CT (HR-pQCT)) to determine whether lower 25OHD
60 affects bone health in obesity.

61

62 **Methods**

63 We conducted a cross-sectional study of healthy Caucasian men and women (ages 25 to 40
64 and 55 to 75) from South Yorkshire, UK (latitude 53° N).

65 Participants were approached through poster adverts, emails to hospital staff, mailing from
66 general practice surgeries and a database of volunteers. Participants were recruited in three
67 BMI categories: normal weight (BMI 18.5 to 24.9 kg/m^2), overweight (BMI 25 to 29.9
68 kg/m^2), and obese (BMI >30 kg/m^2). Exclusion criteria were: pregnancy or breast feeding
69 within the last year, conditions (including diabetes) or medication (including hormonal
70 contraception) known to affect vitamin D or bone metabolism, immobilisation, high alcohol
71 intake, and competitive athletes. Older women were at least five years postmenopausal. There
72 were no restrictions on supplement intake, and supplement use was included in the dietary
73 calcium and vitamin D assessment. (For recruitment detail see **Supplemental Table 1**).

74 The study was approved by South Yorkshire Research Ethics Committee, conducted
75 according to the Declaration of Helsinki, and all subjects gave written informed consent.

76 All participants were assessed in fall or spring (19 September to 31 October 2012, and 2
77 April to 16 May 2013) when UV-B is available. Fasting morning blood samples were taken
78 for measurement of serum total and free 25OHD, 1,25(OH)$_2$D, DBP, albumin, PTH,
79 biochemical markers of bone turnover and DBP genotype. Statistical analyses were adjusted
80 for date of visit. Sunlight exposure, dietary vitamin D intake and muscle function were also
81 assessed.

82 A subgroup of 106 participants were also assessed in winter (11 December 2012 to 1 April
83 2013), to assess vitamin D status when there is negligible UV-B and avoid perturbation of the
84 isotope tracer study by sunlight exposure. Fasting morning blood samples were taken for
85 measurement of 25OHD, and 25OHD$_3$ half-life was assessed with an isotope tracer.

86 *Measurements*

87 Short physical performance battery (SPPB) score (maximum score 12) was calculated from
88 narrow walk and chair stand tests (23). Grip strength was measured using a digital
89 dynamometer (Seahan Corp., Masan).

90 The sunlight questionnaire was supplied by Prof Lanham-New, University of Surrey, UK (5).
91 It assesses habitual sunlight exposure by season and during holidays. Questionnaire
92 assessment of sunlight exposure has been shown to correlate with vitamin D status (24).

93 Dietary vitamin D intake was assessed with DIETQ (Tinuviel Software, UK). This is a semi-
94 quantitative habitual food frequency intake questionnaire with computerised analysis based
95 on the UK nutrient database (25).

96 25OHD was measured in the Manchester Institute of Human Development, UK by liquid
97 chromatography tandem mass spectrometry (LC-MS/MS). This laboratory participates in
98 DEQAS and the assay is calibrated against the NIST standard. 25OHD$_2$ was undetectable in
99 most subjects.

100 Free (unbound) 25OHD was determined by immunoassay (26) (Future Diagnostics,
101 Netherlands, inter-assay CV at 13.2pg/ml 5.3%). Free 25OHD can also be estimated by
102 calculation from total 25OHD, DBP, albumin and their binding affinities, but this approach
103 has limitations due to genetic variation in DBP, and the direct measurement by immunoassay
104 is more closely correlated with serum PTH and calcium (27).

105 1,25$(OH)_2$D was measured by manual immunoassay after immunoextraction
106 (ImmunoDiagnostic Systems, UK, inter-assay CV 6.0%, intra-assay CV 2.6%).

107 DBP was measured by Quantikine manual immunoassay (R&D Systems, UK, inter-assay CV
108 3.3%, intra-assay CV 3.9%).

109 C-terminal telopeptide of type I collagen (CTX, bone resorption marker), procollagen type I
110 N propeptide (PINP) and osteocalcin (bone formation markers) were measured by automated
111 immunoassay (Cobas e411, Roche Diagnostics, Germany). Inter-assay CVs were: CTX 4.0%,
112 PINP 4.1%, osteocalcin 2.2%. Bone alkaline phosphatase (bone ALP, bone formation
113 marker) was measured by automated immunoassay (iSYS, ImmunoDiagnostic Systems,
114 inter-assay CV 4.5%).

115 Albumin, creatinine, calcium and PTH were measured by autoanalyser (Cobas c701, Roche
116 Diagnostics, inter-assay precision <2.0% all tests).

117 DBP genotyping was done by Sheffield Children's Hospital, UK. The pyrosequencing assay
118 was developed using PSQ software version 1.0.6 (Qiagen) to detect rs4588 and rs7041
119 polymorphisms.

120 25OHD half-life was measured with a 24 mcg orally administered tracer stable isotope of
121 25OHD_3 (3-^{2}H-25-hydroxyvitamin D_3 (6, 19, 19-d3)). The tracer was given dissolved in olive
122 oil with a standard breakfast. Venous blood was taken at 6±1, 9±2, 27±2 and 30±2 days after

123 administration. $25OHD_3$ half-life was calculated from the terminal slope of the disappearance
124 of d3-$25OHD_3$, as t1⁄2=ln(2)/kB, where kB is the natural logarithm of the slope of the line of
125 best fit from day 5 to day 30 (28). Tracer preparation and LC-MS/MS measurements (29)
126 were performed at MRC Human Nutrition Research, Cambridge, UK.

127 Bone mineral density and fat mass were assessed by dual energy X-ray absorptiometry
128 (DXA) and high resolution peripheral quantitative tomography (HR-pQCT).

129 Whole body, lumbar spine and hip DXA were performed with a Discovery densitometer
130 (Hologic Inc, Waltham MA, USA). The short-term precision for the spine and hip are 1.0%
131 and 1.1%.

132 HR-pQCT images of the distal radius and tibia (4% site, non-dominant, non-fractured) were
133 obtained using XtremeCT (Scanco Medical AG, Switzerland). Images were analysed with
134 Scanco software (version 6). The short term precision of the BMD measurements is 0.2 to
135 5.5% (30).

136 *Statistics*

137 Normality was assessed using histograms. Skewed variables were log10 transformed for
138 analysis.

139 Variables that differed between the three BMI groups were identified with analysis of
140 variance (ANOVA). Effects of age group and gender were tested with analysis of covariance
141 (ANCOVA). Post-hoc testing for differences between pairs of BMI groups was adjusted for
142 multiple comparisons using the Tukey method.

143 Relationships between variables and BMI (as a continuous variable) were examined with
144 univariate linear models. Multiple linear regression models were used to adjust for age (as a
145 continuous variable) and gender.

146 Correlations between variables were calculated with Spearman's Rank test, and 95%
147 confidence intervals were calculated by bootstrapping.

148 Statistical analyses were performed with SPSS Version 21 and R Version 3.2.1.

149 The fall/spring study (n=223) had 90% power at 5% two-sided significance to detect a 0.22
150 correlation coefficient between BMI and 25OHD. For ANOVA, 65 participants per BMI
151 group had 90% power to detect a standardised effect size of 0.26 at 5% two-sided
152 significance.

153 The winter study (n=106) had 90% power at 5% two-sided significance to detect a 0.30
154 correlation coefficient between BMI and 25OHD. For ANOVA, 32 participants per BMI
155 group had 90% power to detect a standardised effect size of 0.37 at 5% two-sided
156 significance.

157 For missing data report see **Supplemental Table 2.**

158

159 **Results**

160 Characteristics of study participants are given in **Table 1**. Dietary calcium intake did not
161 differ between BMI groups (mg/day mean and 95% CI: normal weight 1072 (1002 to 1145),
162 overweight 1074 (998 to 1158), obese 1055 (1001 to 1112)). The subset also assessed in
163 winter were representative of the whole group (n=106: normal BMI = 34, overweight = 32,
164 obese = 40; younger = 46, older = 60; male = 50, female = 56).

165 Total $25OHD_3$ was lower in obese and overweight people than normal weight people in
166 fall/spring, but not in winter (**Figure 1**). In fall/spring, 56% of overweight and obese people
167 had $25OHD_3$ below 50nmol/l, compared with 37% of normal weight. In winter, 75% of

168 overweight and obese people had $25OHD_3$ below 50nmol/l, compared with 62% of normal
169 weight.

170 Total $25OHD_3$ in fall/spring was inversely correlated with BMI (adjusted for date of visit, age
171 and gender; model adjusted $R^2 = 0.339$, $p<0.001$). For every five unit increase in BMI, total
172 $25OHD_3$ decreased by 10.0% (95% CI: 5.7 to 14.0%, $p<0.001$). After the same adjustments,
173 total $25OHD_3$ was also negatively correlated with whole body fat mass (model adjusted R^2
174 $=0.334$, $p<0.001$). For every 10kg increase in fat mass, total $25OHD_3$ decreased by 11%
175 (95% CI: 6 to 15%, $p<0.001$).

176 Although total $25OHD_3$ did not differ by BMI group in winter, $25OHD_3$ was negatively
177 correlated with BMI (adjusted for age and gender; model adjusted R^2 0.172, $p<0.001$). For
178 every five unit increase in BMI, $25OHD_3$ decreased by 8.2% (95% CI: 0.5 to 15.3%,
179 $p=0.038$).

180 Dietary vitamin D and sunlight exposure did not differ by BMI group (**Table 2**). The average
181 hours of sunlight (irradiance measurement above 120 w/m2) in Sheffield during the period of
182 the study measurements were 4.6 in fall/spring and 1.9 in winter (Data kindly provided by
183 Weston Park Weather Station, Sheffield).

184 DBP and albumin did not differ by BMI group, and adjustment for age and gender did not
185 change this result (**Table 2**). DBP genotype distribution (Gc1-1 47%, Gc2-1 42%, Gc2-2
186 11%) was similar to other reported white European populations (16). Genotype distribution
187 did not differ by BMI group and BMI did not differ by genotype. Total $25OHD_3$
188 concentration did differ by genotype (mean nmol/l and 95% CI: Gc1-1 52.2 (47.2 to 57.6),
189 Gc2-1 45.3 (40.9 to 50.3), Gc2-2 39.4 (32.1 to 48.3) $p=0.024$).

190 $25OHD_3$ half-life did not differ by BMI group (**Table 2**).

191 Free 25OHD was lower in the obese and overweight groups than normal weight in
192 fall/spring. BMI was negatively correlated with free 25OHD (adjusted for date of visit, age
193 and gender; model adjusted $R^2 = 0.296$, $p<0.001$). For every five unit increase in BMI, free
194 25OHD decreased by 12.3% (95% CI: 7.7 to 16.6%, $p<0.001$). When total 25OHD was
195 added to the model the relationship between free 25OHD and BMI was no longer significant
196 ($R^2 = 0.619$, $p=0.16$).

197 Total 1,25$(OH)_2$D was also lower in the obese and overweight groups than normal weight in
198 fall/spring (**Table 3**).

199 PTH did not differ by BMI group (**Table 3**) and was not correlated with BMI. Adjusting for
200 age and gender did not change this result. CTX and osteocalcin were lower in the obese
201 group than normal weight and overweight. Bone ALP and PINP did not differ between BMI
202 groups (**Table 3**).

203 BMD by DXA at the whole body, lumbar spine and hip, and by HR-pQCT at the distal radius
204 and tibia was higher in the overweight and obese groups than normal weight (**Table 3**).

205 Grip strength did not differ by BMI group. Adjustment for age and gender did not change this
206 result. SPPB score was lower in the overweight and obese groups than normal weight.
207 However, SPPB score was not correlated with 25OHD (Spearman's rho -0.122, 95% CI: -
208 0.261 to 0.014, $p=0.073$).

209

210 **Discussion**

211 This is the first study to use the free 25OHD assay and stable isotope half-life method to
212 investigate the effect of body weight on vitamin D metabolism.

213 As expected, total serum 25OHD is was lower at higher body weight (lower in obese than
214 normal weight people in fall/spring, and negatively correlated with BMI in fall/spring and in
215 winter). We also identified that the biologically available free serum 25OHD and active
216 hormone 1,25$(OH)_2$D were lower in obesity. However, PTH was similar across BMI groups,
217 (other studies have described higher PTH in obesity (15, 20, 31)), bone turnover was not
218 higher (bone resorption was lower than normal weight and formation was similar), and BMD
219 by DXA and HR-pQCT was higher at all measured sites. We have previously shown that
220 bone microarchitecture is more favourable for bone strength in obese people, with greater
221 cortical thickness and trabecular number (31).

222 We investigated several possible mechanisms for the effects of body weight on vitamin D
223 status. Dietary vitamin D intake and sunlight exposure were similar across BMI groups. A
224 previous UK study also found that sunlight exposure did not vary with BMI (32).

225 Lower total 25OHD in obesity was not due to differences in protein binding; free 25OHD
226 was also lower and serum albumin, DBP and DBP genotype did not differ by BMI group.

227 $25OHD_3$ half-life did not differ by BMI group, so lower 25OHD in obesity is not due to more
228 rapid metabolic clearance.

229 After cutaneous synthesis and absorption, vitamin D is distributed into fat, muscle and other
230 tissues (33), and when volume of distribution is greater, less vitamin D may be available for
231 25-hydroxylation. 25OHD is also distributed into fat and muscle, and into serum (34) and all
232 of these compartments are increased in obesity. Consistent with this, other investigators have
233 reported that the summer rise in circulating 25OHD is blunted in obesity (32, 35). When
234 exposed to UV-B, normal weight and obese people have similar cutaneous synthesis of
235 vitamin D (49), but the serum 25OHD rise is smaller in obese people (18), consistent with our
236 observation that the 25OHD difference between normal weight and obese is greater in

237 fall/spring than in winter. This theory is supported by evidence that serum 25OHD response
238 to oral vitamin D dosing is BMI-dependent (31, 36).

239 Due to the greater volume of distribution, if whole body vitamin D and 25OHD were similar
240 in obese and normal weight people, measured serum concentrations would be lower in obese
241 people (and conversely, people with low BMI may have relatively high serum 25OHD but
242 lower whole body stores). Therefore, BMI may need to be considered when using serum
243 25OHD as a marker of vitamin D status.

244 It is possible that the lower serum 25OHD in obesity does reflect true vitamin D deficiency,
245 but that adverse skeletal effects are countered by positive skeletal effects of obesity, such as
246 increased loading, oestrogen synthesis from adipocyte aromatase, or adipocyte hormones
247 such as leptin.

248 Physical function score was poorer in obese people, but not correlated with 25OHD. Vitamin
249 D and calcium supplementation may improve physical functioning in older people, but there
250 is less evidence for benefit in young adults (34-36). Other factors such as less physical
251 activity and fat infiltration of muscle might contribute to poorer function. It is possible that
252 vitamin D maintains muscle integrity in older adults by preventing intramuscular fat
253 accumulation (37), which might be relevant to muscle function in obesity.

254 There are some limitations to this study. Dietary and sunlight exposure habits differ by
255 geography and culture, and it is very possible that lower dietary vitamin D and sunlight
256 exposure contribute to low 25OHD in obese people elsewhere. We did not measure volume
257 of distribution directly; this would require an intravenous isotope and there are none available
258 for human use. We did not measure intestinal calcium absorption. We used the R+D DBP
259 assay; other DBP assays may give different results because the influence of DBP genotype
260 varies by assay (38), but all participants were Caucasian which will have minimised genotype

261 variation (the genotype distribution varies by ethnic group) and DBP genotype distribution
262 did not differ between the BMI groups. We also excluded effects of protein binding by direct
263 measurement of free 25OHD.

264 We have not assessed effects of low 25OHD beyond the musculoskeletal system. Vitamin D
265 deficiency has been associated with diseases such as cancer and metabolic syndrome, where
266 obesity is also a risk factor. However, there is not yet evidence for a causative role of vitamin
267 D deficiency (39).

268 In conclusion, it is well recognised that total serum 25OHD is low in obesity, but we have
269 shown that biologically available free serum 25OHD and the active hormone 1,25$(OH)_2$D are
270 also lower at higher body weight. The likely cause of lower 25OHD in obesity is greater
271 volume of distribution. The lower 25OHD in obesity was not associated with higher PTH or
272 bone turnover, lower bone density or poorer physical function. BMI affects the relationship
273 between serum 25OHD and bone health and lower serum 25OHD at higher body weight may
274 not indicate at-risk skeletal health.

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References

1. Cranney A, Horsley T, O'Donnell S, Weiler H, Puil L, Ooi D, Atkinson S, Ward L, Moher D, Hanley D, et al. Effectiveness and safety of vitamin D in relation to bone health. Evidence report/technology assessment 2007(158):1-235.
2. Rosen CJ, Adams JS, Bikle DD, Black DM, Demay MB, Manson JE, Murad MH, Kovacs CS. The nonskeletal effects of vitamin D: an Endocrine Society scientific statement. Endocrine reviews 2012;33(3):456-92. doi: 10.1210/er.2012-1000.
3. Institute of Medicine. Dietary reference intakes for calcium and vitamin D. The National Academies Press; Washington DC. 2011.
4. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, Murad MH, Weaver CM, Endocrine S. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. The Journal of clinical endocrinology and metabolism 2011;96(7):1911-30. doi: 10.1210/jc.2011-0385.
5. Macdonald HM, Mavroeidi A, Barr RJ, Black AJ, Fraser WD, Reid DM. Vitamin D status in postmenopausal women living at higher latitudes in the UK in relation to bone health, overweight, sunlight exposure and dietary vitamin D. Bone 2008;42(5):996-1003. doi: S8756-3282(08)00066-5 [pii];10.1016/j.bone.2008.01.011 [doi].
6. Ardawi MS, Sibiany AM, Bakhsh TM, Qari MH, Maimani AA. High prevalence of vitamin D deficiency among healthy Saudi Arabian men: relationship to bone mineral density, parathyroid hormone, bone turnover markers, and lifestyle factors. OsteoporosInt 2011. doi: 10.1007/s00198-011-1606-1 [doi].
7. Shea MK, Houston DK, Tooze JA, Davis CC, Johnson MA, Hausman DB, Cauley JA, Bauer DC, Tylavsky F, Harris TB, et al. Correlates and prevalence of insufficient 25-hydroxyvitamin D status in black and white older adults: the health, aging and body composition study. JAmGeriatrSoc 2011;59(7):1165-74. doi: 10.1111/j.1532-5415.2011.03476.x [doi].
8. Rajakumar K, de las HJ, Chen TC, Lee S, Holick MF, Arslanian SA. Vitamin D status, adiposity, and lipids in black American and Caucasian children. JClinEndocrinolMetab 2011;96(5):1560-7. doi: jc.2010-2388 [pii];10.1210/jc.2010-2388 [doi].
9. Bolland MJ, Grey AB, Ames RW, Mason BH, Horne AM, Gamble GD, Reid IR. Determinants of vitamin D status in older men living in a subtropical climate. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA 2006;17(12):1742-8. doi: 10.1007/s00198-006-0190-2.
10. Lagunova Z, Porojnicu AC, Lindberg F, Hexeberg S, Moan J. The dependency of vitamin D status on body mass index, gender, age and season. Anticancer Res 2009;29(9):3713-20. doi: 29/9/3713 [pii].
11. McKinney K, Breitkopf CR, Berenson AB. Association of race, body fat and season with vitamin D status among young women: a cross-sectional study. ClinEndocrinol(Oxf) 2008;69(4):535-41. doi: CEN3233 [pii];10.1111/j.1365-2265.2008.03233.x [doi].
12. Palacios C, Gil K, Perez CM, Joshipura K. Determinants of vitamin D status among overweight and obese Puerto Rican adults. Annals of nutrition & metabolism 2012;60(1):35-43. doi: 10.1159/000335282.

13. Samuel L, Borrell LN. The effect of body mass index on optimal vitamin D status in U.S. adults: the National Health and Nutrition Examination Survey 2001-2006. Annals of epidemiology 2013;23(7):409-14. doi: 10.1016/j.annepidem.2013.05.011.
14. Kull M, Kallikorm R, Lember M. Body mass index determines sunbathing habits: implications on vitamin D levels. InternMedJ 2009;39(4):256-8. doi: IMJ1900 [pii];10.1111/j.1445-5994.2009.01900.x [doi].
15. Goldner WS, Stoner JA, Thompson J, Taylor K, Larson L, Erickson J, McBride C. Prevalence of vitamin D insufficiency and deficiency in morbidly obese patients: a comparison with non-obese controls. ObesSurg 2008;18(2):145-50. doi: 10.1007/s11695-007-9315-8 [doi].
16. Lauridsen AL, Vestergaard P, Hermann AP, Brot C, Heickendorff L, Mosekilde L, Nexo E. Plasma concentrations of 25-hydroxy-vitamin D and 1,25-dihydroxy-vitamin D are related to the phenotype of Gc (vitamin D-binding protein): a cross-sectional study on 595 early postmenopausal women. Calcified tissue international 2005;77(1):15-22. doi: 10.1007/s00223-004-0227-5.
17. Carpenter TO, Zhang JH, Parra E, Ellis BK, Simpson C, Lee WM, Balko J, Fu L, Wong BY, Cole DE. Vitamin D binding protein is a key determinant of 25-hydroxyvitamin D levels in infants and toddlers. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research 2013;28(1):213-21. doi: 10.1002/jbmr.1735.
18. Winters SJ, Chennubhatla R, Wang C, Miller JJ. Influence of obesity on vitamin D-binding protein and 25-hydroxy vitamin D levels in African American and white women. Metabolism 2009;58(4):438-42. doi: S0026-0495(08)00406-X [pii];10.1016/j.metabol.2008.10.017 [doi].
19. Taes YE, Goemaere S, Huang G, Van P, I, De BD, Verhasselt B, Van den Broeke C, Delanghe JR, Kaufman JM. Vitamin D binding protein, bone status and body composition in community-dwelling elderly men. Bone 2006;38(5):701-7. doi: S8756-3282(05)00426-6 [pii];10.1016/j.bone.2005.10.006 [doi].
20. Bolland MJ, Grey AB, Ames RW, Horne AM, Gamble GD, Reid IR. Fat mass is an important predictor of parathyroid hormone levels in postmenopausal women. Bone 2006;38(3):317-21. doi: S8756-3282(05)00371-6 [pii];10.1016/j.bone.2005.08.018 [doi].
21. Edelstein SL, Barrett-Connor E. Relation between body size and bone mineral density in elderly men and women. American journal of epidemiology 1993;138(3):160-9.
22. De LC, Kanis JA, Oden A, Johanson H, Johnell O, Delmas P, Eisman JA, Kroger H, Fujiwara S, Garnero P, et al. Body mass index as a predictor of fracture risk: a meta-analysis. OsteoporosInt 2005;16(11):1330-8. doi: 10.1007/s00198-005-1863-y [doi].
23. Cruz-Jentoft AJ, Baeyens JP, Bauer JM, Boirie Y, Cederholm T, Landi F, Martin FC, Michel JP, Rolland Y, Schneider SM, et al. Sarcopenia: European consensus on definition and diagnosis: Report of the European Working Group on Sarcopenia in Older People. Age and ageing 2010;39(4):412-23. doi: 10.1093/ageing/afq034.
24. Macdonald HM. Contributions of sunlight and diet to vitamin D status. Calcified tissue international 2013;92(2):163-76. doi: 10.1007/s00223-012-9634-1.
25. Finglas P.M. RMA, Pinchen H.M., Berry R., Church S.M., Dodhia S.K., FarronWilson, G MS. McCance and Widdowson’s The Composition of Foods. 7th Ed ed. Cambridge, UK The Royal Society of Chemistry, 2014.
26. Swinkels L MA, Martens M, Parsons G, Rosmalen F. An immunoassay for free 25-hydroxy vitamin D. 43rd Oak Ridge Conference Emerging Technologies for 21st Century Diagnostics: April 14–15 2011 American Association for Clinical Chemistry 2011.

27. Schwartz JB, Lai J, Lizaola B, Kane L, Markova S, Weyland P, Terrault NA, Stotland N, Bikle D. A Comparison of Measured and Calculated Free 25(OH) Vitamin D Levels in Clinical Populations. The Journal of clinical endocrinology and metabolism 2014;99(5):1631-7. doi: 10.1210/jc.2013-3874.
28. Jones KS, Schoenmakers I, Bluck LJ, Ding S, Prentice A. Plasma appearance and disappearance of an oral dose of 25-hydroxyvitamin D2 in healthy adults. The British journal of nutrition 2012;107(8):1128-37. doi: 10.1017/S0007114511004132.
29. Jones KS, Assar S, Harnpanich D, Bouillon R, Lambrechts D, Prentice A, Schoenmakers I. 25(OH)D2 half-life is shorter than 25(OH)D3 half-life and is influenced by DBP concentration and genotype. The Journal of clinical endocrinology and metabolism 2014;99(9):3373-81. doi: 10.1210/jc.2014-1714.
30. Paggiosi MA, Eastell R, Walsh JS. Precision of high-resolution peripheral quantitative computed tomography measurement variables: influence of gender, examination site, and age. Calcified tissue international 2014;94(2):191-201. doi: 10.1007/s00223-013-9798-3.
31. Gallagher JC, Yalamanchili V, Smith LM. The effect of vitamin D supplementation on serum 25(OH)D in thin and obese women. The Journal of steroid biochemistry and molecular biology 2013;136:195-200. doi: 10.1016/j.jsbmb.2012.12.003.
32. Macdonald HM, Mavroeidi A, Aucott LA, Diffey BL, Fraser WD, Ormerod AD, Reid DM. Skin color change in Caucasian postmenopausal women predicts summer-winter change in 25-hydroxyvitamin D: findings from the ANSAViD cohort study. JClinEndocrinolMetab 2011;96(6):1677-86. doi: jc.2010-2032 [pii];10.1210/jc.2010-2032 [doi].
33. Blum M, Dolnikowski G, Seyoum E, Harris SS, Booth SL, Peterson J, Saltzman E, Dawson-Hughes B. Vitamin D(3) in fat tissue. Endocrine 2008;33(1):90-4. doi: 10.1007/s12020-008-9051-4 [doi].
34. Heaney RP, Horst RL, Cullen DM, Armas LA. Vitamin D3 distribution and status in the body. Journal of the American College of Nutrition 2009;28(3):252-6.
35. Bolland MJ, Grey AB, Ames RW, Mason BH, Horne AM, Gamble GD, Reid IR. The effects of seasonal variation of 25-hydroxyvitamin D and fat mass on a diagnosis of vitamin D sufficiency. AmJClinNutr 2007;86(4):959-64. doi: 86/4/959 [pii].
36. Drincic A, Fuller E, Heaney RP, Armas LA. 25-hydroxyvitamin d response to graded vitamin d3 supplementation among obese adults. The Journal of clinical endocrinology and metabolism 2013;98(12):4845-51. doi: 10.1210/jc.2012-4103.
37. Scott D, Sanders KM, Ebeling PR. Vitamin D, muscle function, and falls in older adults: does reduced deposition of intramuscular adipose tissue influence the relationship? The Journal of clinical endocrinology and metabolism 2013;98(10):3968-70. doi: 10.1210/jc.2013-2560.
38. Nielson C WY, Swanson C, Lee C, Chun R, Hewison M, Adams J, Vanderschueren D, Bouillon R, Lapidus J, Cauley J, Orwoll E. Lack of concordance among vitamin D binding protein assays and effect on bioavailable 25OHD estimates. Journal of Bone and Mineral Research 2014;29, Suppl 1(Suppl 1):MO0334.
39. Autier PB, M.; Pizot, C.; Mullie, P. Vitamin D status and ill health: a systematic review. Lancet Diabetes Endocrinol 2014;2:76-89.

Table 1: Participant characteristics by BMI group.

BMI group	**Female/Male (number)**	**Age (years)**	**Height (m)**	**BMI (kg/m^2)**	**Fat mass (kg)**
Normal (18.5 to 24.9 kg/m^2)	43/34	55.9 (16.0)	1.68 (0.09)	22.8 (1.4)	19.2 (3.5)
Overweight (25.0 to 29.9 kg/m^2)	28/35	50.6 (15.2)	1.72 (0.09)	27.6 (1.3)	27.6 (5.7)
Obese (>30.0 kg/m^2)	42/41	56.6 (15.4)	1.69 (0.10)	35.4 (4.3)	40.7 (9.2)

Results given as mean (SD)

Table 2: Possible contributors to low vitamin D in obesity.

BMI group	**Normal** n = 77	**Overweight** n=63	**Obese** n=83
Dietary vitamin D intake (µg)	3.61 (3.01, 4.34)	3.05 (2.50, 3.72)	2.72 (2.24, 3.31)
Annual sunlight exposure score	90.48 (82.44, 98.53)	96.34 (86.69, 105.98)	92.33 (84.54, 100.13)
Summer sunlight exposure score	48.45 (43.96, 53.74)	51.15 (45.37, 56.94)	47.55 (42.65, 52.46)
Vitamin D binding protein (µg/ml)	136.0 (124.9,147.0)	124.9 (112.3, 137.6)	130.5 (120.7, 140.4)
Albumin (g/l)	46.0 (45.3, 46.8)	45.7 (45.0, 46.4)	45.1 (44.2, 45.9)
$25OHD_3$ half-life (days)	17.8 (16.6, 19.1)	17.0 (15.8, 18.2)	18.2 (17.0, 19.1)

Dietary vitamin D and sunlight scores given as geometric mean (95% CI). ANOVA all $p>0.05$.

Table 3: Possible consequences of low vitamin D in obesity

BMI group	Normal n=77	Overweight n=63	Obese n=83
Free 25OHD[2] (pmol/l)	10.6[a] (9.4, 12.0)	7.5[b] (6.5, 8.6)	7.8[b] (6.9, 8.8)
Total 1,25(OH)$_2$D[1] (pmol/l)	95.0[a] (87.1, 103.7)	79.4[b] (72.3, 87.1)	78.5[b] (72.3, 85.3)
PTH (ng/l)	41.4[a] (38.4, 44.7)	41.4[a] (37.6, 45.5)	43.5[a] (40.5, 46.7)
CTX[1] (ng/l)	0.45[a] (0.40, 0.50)	0.47[a] (0.43, 0.51)	0.38[b] (0.35, 0.42)
Osteocalcin[1] (ng/ml)	23.0[a] (21.3, 24.8)	22.0[a] (20.5, 23.6)	19.1[b] (18.0, 20.4)
PINP (ng/ml)	40.8[a] (36.9, 45.2)	41.4[a] (38.3, 44.8)	37.8[a] (34.8, 41.0)
Bone ALP (ng/ml)	12.8[a] (11.7, 13.9)	12.9[a] (11.8, 14.0)	12.7[a] (11.8, 13.7)
Whole body DXA BMD[2] (g/cm^2)	1.07[a] (1.05, 1.09)	1.14[b] (1.11, 1.16)	1.16[b] (1.13, 1.18)
Lumbar spine DXA BMD[2] (g/cm^2)	0.95[a] (0.91, 0.98)	1.04[b] (1.01, 1.08)	1.09[c] (1.06, 1.13)
Total hip DXA BMD[2] (g/cm^2)	0.88[a] (0.85, 0.91)	1.00[b] (0.97, 1.03)	1.06[c] (1.03, 1.09)
Distal radius HR-pQCT BMD[2] (mgHA/cm^3)	272.0[a] (258.6, 286.0)	303.0[b] (290.6, 315.9)	315.0[c] (303.9, 326.5)
Distal tibia HR-pQCT BMD[2] (mgHA/cm^3)	280.0[a] (269.5, 290.8)	312.2[b] (298.3, 326.7)	327.6[b] (316.8, 338.8)
Grip strength (kg)	22.1[a] (20.3, 23.9)	24.1[a] (21.6, 26.6)	23.1[a] (21.0, 25.1)
Short physical performance battery score[2]	9.5[a] (9.1, 9.9)	9.1[b] (8.7, 9.4)	8.3[c] (8.0, 8.7)

Measurements taken in fall/spring. Results given as geometric mean (95% CI). ANCOVA adjusted for age, gender (and date of visit for biochemistry) [1]$p<0.01$, [2]$p<0.001$.

Means not sharing a common superscript letter are significantly different at $p<0.05$ based on post-hoc testing adjusted for multiple comparisons using the Tukey method.

Figure legends

Figure 1: Total $25OHD_3$ (LC-MS/MS) by BMI group in fall/spring (n =223) (A) and in winter (n=106) (B).

Results shown as geometric mean and 95% confidence interval. ANCOVA adjusted for date of visit (April/May vs September/October), age group and gender.