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Why don't serum Vitamin D concentrations associate with BMD by DXA? A case of being 'bound' to the wrong assay? Implications for Vitamin D screening

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ABSTRACT

Background: The association between bone mineral density (BMD) and serum 25[OH]D concentration is weak, particularly in certain races (e.g. Black African vs. Caucasian) and in athletic populations. We aimed to examine if bioavailable vitamin D rather than serum 25[OH]D was related to markers of bone health within a racially diverse athletic population.

Methods: In 604 male athletes [Arab (n=327), Asian (n=48), Black (n=108), Caucasian (n=53) & Hispanic (n=68)], we measured total 25-hydroxyvitamin D (25[OH]D), vitamin D–binding protein, and bone mineral density (BMD) by (DXA). Bioavailable vitamin D was calculated using the free hormone hypothesis.

Results: From 604 athletes, 21.5% (n=130) demonstrated severe 25[OH]D deficiency, 37.1% (n=224) deficiency, 26% (n=157) insufficiency and 15.4% (n=93) sufficiency. Serum 25[OH]D concentrations were not associated with BMD at any site. After adjusting for age and race, bioavailable vitamin D was associated with BMD (spine, neck and hip). Mean serum vitamin D binding protein concentrations were not associated with 25[OH]D concentrations (p=0.392).

Conclusion: Regardless of age or race, bioavailable vitamin D and not serum 25[OH]D was associated with BMD in a racially diverse athletic population. If vitamin D screening is warranted, clinicians should use appropriate assays to calculate vitamin D binding protein and bioavailable vitamin D levels concentrations than serum 25[OH]D. In turn, prophylactic vitamin D supplementation to 'correct' insufficient athletes should not be based upon serum 25[OH]D measures.

INTRODUCTION

Vitamin D_3 (cholecalciferol) is a lipophilic pro-hormone produced in the skin from exposure to sunlight. Cholecalciferol is transported bound to vitamin D-binding protein (DBP), and is hydroxylated in the liver to form 25-hydroxyvitamin D (25[OH]D). 25[OH]D undergoes further hydroxylation in the kidney, to form the active hormone 1, 25-dihydroxyvitamin D [$1,25(OH)_2D$]. This bioactive metabolite, regulates intestinal calcium absorption, bone calcium resorption and renal calcium reabsorption to maintain calcium homeostasis, and promote skeletal mineralisation (1, 2). Accordingly, skeletal pathologies such as rickets and osteomalacia often present when 25[OH]D levels are consistently deficient (3).

Clinically to date, measuring serum 25[OH]D concentration provides the best estimate of vitamin D status as both circulating levels of cholecalciferol and $1,25(OH)_2D$ have short half-lives, approximately 24hrs and 4-6hrs, respectively (4, 5). Serum cholecalciferol levels can also be affected by recent sunlight exposure, and is difficult to measure due to the lipophilic nature of the molecule. Optimum concentrations of serum 25[OH]D for skeletal health are still debated (7). Many clinicians define vitamin D sufficiency as the lowest serum 25[OH]D concentration that maximally suppresses parathyroid hormone (PTH) secretion and/or optimises bone mineral density (BMD) (6-8). The generally accepted serum 25[OH]D concentration ranges are: severely deficient (<10 ng/mL), deficient (10–20 ng/mL), insufficient (20–30 ng/mL), or sufficient (>30 ng/mL); although of evidence supporting these categories are derived from elderly cohorts or groups with existing skeletal disorders (9-12).

Association between bone mineral density (BMD) and serum 25[OH]D concentration is weak (13-17). Our group previously demonstrated no association between serum 25[OH]D and markers of bone health in weight bearing athletes of different racial background, suggesting that markers of bone health are independent of serum 25[OH]D concentrations (18). It appears there is a 'paradoxical relationship' between race and vitamin D concentration that has largely been ignored, i.e. black individuals generally have the lowest serum 25[OH]D concentrations but the greatest BMD and reduced risk of fracture (14, 19). DBP may provide an insight as to why certain racial groups have distinct 25[OH]D and BMD relationships (20). DBP is the primary vitamin D carrier, binding 85%–90% of total circulating 25[OH]D, with the remaining unbound 25[OH]D considered to be bioavailable (21). Polymorphisms in the DBP coding genes (specifically rs4588 and rs7041) produce proteins that differ in affinity for 25[OH]D, and it is these polymorphisms that are known to differ between racial groups (22). Differentiating between total vitamin D (measured as 25[OH]D) and bioavailable vitamin D is crucial, given that the latest evidence suggests that DBP inhibits certain actions of vitamin D, since the bound fraction is unavailable to act on target cells. (20, 21, 23). Consequently, serum bioavailable vitamin D may have a better association with bone health than serum 25[OH]D concentration (20). To date, research on vitamin D status in athletes has focused solely upon serum 25[OH]D and as a consequence, the current advice and guidelines given to athletes and clinicians treating vitamin D deficiency may be inaccurate. Therefore, the present study set out to examine if bioavailable vitamin D is related to markers of bone health within a racially diverse athletic population. It was hypothesised that bioavailable vitamin D and not serum vitamin D would be associated with markers of bone health in a racially diverse athlete population.

METHODS

Participants

Six hundred and four male athletes registered with the Qatar Olympic Committee (QOC) [Arab (n=327), Asian (n=48), Black (n=108), Caucasian (n=53) and Hispanic (n=68)], exercising ≥6 h/week, presented for pre-competition medical assessment at Aspetar Sports Medicine Hospital, Qatar. All athletes undertook dual-energy X-ray absorptiometry for assessment of their bone mineral density, and a blood test to assess serum 25[OH]D concentration and vitamin D-binding protein. No athlete was taking vitamin D supplementation upon recruitment. Ethical approval was obtained from Qatar Anti-Doping Laboratory IRB (#F2014000062).

Laboratory Analyses

Serum 25-Hydroxyvitamin D (25[OH]D) and Parathyroid hormone (PTH)

Venous blood samples were collected from athletes following an overnight fast. The blood sample was separated into two aliquots (5mL SST tubes) of serum for analysis of 25[OH]D and one aliquot of plasma for PTH assessment. Samples were centrifuged for 10 minutes at 3000 RPM. Serum 25[OH]D was analysed utilising chemiluminescent immunoassay technology with sensitivity set at 7 ng/mL (Liaison® 25-OH Vitamin-D Total Assay; Diasorin Inc., Saluggia (Vercelli), Italy). The intra- and interassay CV was 7.6–9.4% and 9.8–13.4%, respectively. Based upon the serum 25[OH]D results, athletes were placed into four 25[OH]D categories; severely deficient (<10 ng/mL), deficient (10–20 ng/mL), insufficient (20–30 ng/mL), or sufficient (>30 ng/mL). Levels of intact PTH were measured with the use of the DiaSorin Liaison analyzer, chemiluminescence immunoassay (CLIA). The inter-assay CV was 2.5%.

Vitamin D-Binding Protein

Serum vitamin D-binding protein (DBP) concentrations (µg/mL) were determined using a commercially available kit (R&D Systems, UK). The limit of sensitivity was ≤0.65 $ng.mL^{-1}$ and an inter-assay coefficient of variation was 7.2%. An automatic enzyme-linked immunosorbent assay (ELISA) microplate reader (Infinite®200-PRO NanoQuant, Switzerland) and computer software Magellan Standard (v7.1) were used to analyse DBP.

Assessment of Bone Mineral Density

Dual-energy x-ray absorptiometry (Osteocore III, Perols, France, v5.22b) scanning was used to assess hip, femoral neck and lumbar spine bone mineral density (BMD). A certified technologist from the International Society of Clinical Densitometry performed all calibrations and measurements. Quality assurance was performed each morning before testing. The coefficient of variation for these records is <1.01% in Aspetar. BMD was calculated in g/cm^2 for spine (L2–L4), hip-neck and hip-total. In addition, the clinical age-matched and gender-specific Z-score index was used to classify the BMD. T-scores were calculated for those athletes older than 20 years as per WHO recommendations (24).

Calculation of Bioavailable 25-Hydroxyvitamin D

Bioavailable and DBP-bound 25[OH]D were calculated using equations from material provided by Powe et al., 2013 (20), and adapted from those described by Vermeulen and colleagues (25). These methods use the free hormone hypothesis to define bioavailable hormone, as the fraction

that is both free and albumin-bound, i.e., the fraction not bound to circulating binding proteins such as DBP. Free levels of 25[OH]D were calculated using the following equation:

$$[D_{free}] = \frac{\frac{[D_{DBP}]}{K_{DBP}}}{[Total\ DBP] - [D_{DBP}]}$$

After calculating free 25-hydroxyvitamin D, we used equation 2 to calculate the concentration of bioavailable (non-DBP bound vitamin):

$$[\text{Bio D}] = [D_{free}] + [D_{Alb}] = (K_{alb} * [Alb] + 1) * [D_{Free}])$$

Definitions

$[D_{Free}]$ = concentration of free (unbound) 25[OH] D

$[D_{DBP}]$ = concentration of vitamin D-binding protein-bound 25[OH] D

K_{DBP} = genotype-nonspecific affinity constant between 25[OH] D and DBP = $0.7 \times 10^9\ M^{-1}$

[Total DBP] = concentration of serum DBP in g/L

[Bio D] = concentration of bioavailable D

$[D_{Alb}]$ = concentration of albumin-bound vitamin D

Kalb = affinity constant between vitamin D and albumin = $6 \times 10^5\ M^{-1}$

Alb = albumin

Statistics

All data were coded and analysed using the SPSS (v21.0). Descriptive statistics were presented as mean and standard deviation (SD) for continuous variables. For categorical variables, frequency and percentage were reported. 25[OH]D data were skewed therefore a natural log-transformation

was applied prior to analysis. A one-way analysis of variance was performed to assess anthropometric differences between the four 25[OH]D groups (<10 ng/mL, 10-20 ng/mL, 20-30 ng/mL and >30 ng/mL). A post-hoc analysis with Bonferroni correction was used for further comparisons in the event of significance. To determine the relationship of bone health parameters with serum 25[OH]D, DBP and bioavailable 25[OH]D, Pearson's correlation coefficient was determined. Multiple linear regression analysis including covariates such as age and race was performed with bone health as a dependent variable. Parameter estimates along with 95% Confidence Intervals (CI) were reported. . Power and sample size calculations recommended that 543 subjects are sufficient for multiple regression to detect an effect size (Cohens f^2) as small as 0.02 with a significant alpha=0.05 and a statistical power 0.80 given only 3 predictors. A p-value<0.05 was used as a cut-off for statistical significance.

RESULTS

Participants

From 604 athletes, 21.5% (n=130) demonstrated severe 25[OH]D deficiency, 37.1% (n=224) deficiency, 26% (n=157) insufficiency and 15.4% (n=93) sufficiency. There was no difference in athlete age or body mass index across the four vitamin D status categories. However, 25[OH]D sufficient (>30ng/mL) athletes presented with significantly lower body fat % compared to insufficient (P=0.024), deficient (P=0.002) and severely deficient (P=0.001) athletes. Markers of bone health were normal for all athletes across all sites.

Vitamin D–Binding Protein, Bioavailable Vitamin D and Markers of Vitamin D Status

Whilst there was a positive linear association between 25[OH]D status and bioavailable vitamin D ($r=0.702$; $p<0.001$), DBP concentration was not associated with 25[OH]D concentrations ($r=-0.035$, $p=0.392$). PTH was significantly greater in severely 25[OH]D deficient athletes compared to insufficient ($P=0.010$), deficient ($P=0.005$) and sufficient ($P<0.001$) athletes. Albumin was significantly greater in sufficient athletes compared to 25[OH]D deficient athletes ($P=0.029$) (Table 1). Bioavailable vitamin D showed a strong association with DBP ($r=-0.733$; $P<0.001$) and PTH ($r=-0.310$; $P<0.001$) but not with $1,25[OH]_2D$ ($r=-0.023$, $P=0.738$).

Impact of Race upon Serum 25[OH]D, Bioavailable Vitamin D and BMD

Mean serum 25[OH]D concentrations significantly differed between race, with Caucasians and Hispanics presenting greater serum 25[OH]D levels than Arabs, Asians and Blacks (Table 2). Whilst $1,25(OH)_2D$ and PTH were not significantly different between racial groups, DBP was significantly lower in Black athletes compared to in Arabs ($P<0.001$), Caucasian ($P<0.001$) and Hispanic ($P<0.001$) athletes. Arab athletes presented with significantly lower bioavailable 25[OH]D concentrations compared to Black ($P<0.001$), Caucasian ($P<0.001$) and Hispanic ($P<0.001$) athletes. Arab athletes demonstrated significantly lower BMD scores across all sites (spine ($P<0.001$), neck ($P<0.001$) and total hip ($P<0.001$) compared to Caucasians, Blacks and Hispanic athletes. There was no difference in BMD across all sites between Black, Caucasian and Hispanic athletes. Finally, 25[OH]D sufficient Arab athletes presented with higher spine BMD than severely deficient Arabs athletes ($p=0.036$; 1.39 vs. 1.24 g/cm^3 respectively); this was not observed in any other race.

Relationship between Markers of Bone Health, Serum 25[OH]D, PTH and Bioavailable Vitamin D

Serum 25[OH]D, DBP and bioavailable vitamin D showed skewed distributions and were logarithmic transformed in order to meet the assumptions of the parametric statistical techniques. P-values and parameter estimates presented are based on log-transformed data. The age and ethnicity adjusted spine BMD increased [*B*=0.032 95% CI (0.012 to 0.051); P=0.001] with each one log unit change in bioavailable vitamin D.

Similarly, bioavailable vitamin D was also associated with spine [*B*=0.032 95% CI (0.012 to 0.051), P=0.001], neck [*B*=0.037 95% CI (0.016 to 0.057), P<0.001] and hip [*B*=0.035 95% CI (0.016 to 0.055), P<0.001] BMD (Table 3). DBP was negatively associated with spine [*B*=-0.035 95% CI (-0.059 to -0.012), P=0.004], neck [*B*=-0.040 95% CI (-0.065 to -0.016), P=0.001] and hip [*B*=-0.037 95% CI (-0.061 to -0.014), P=0.002] BMD. Serum 25[OH]D concentrations were not associated with BMD at any site. DBP was positively associated with neck (P=0.034) and hip T-score (P=0.040) but not spine (P=0.067). Serum 25[OH]D and bioavailable 25[OH]D were not associated with T-score at any site . PTH was not associated with BMD across all sites and T-scores of Spine (r=-0.029, P=0.641), Hip (r=-0.093, P=0.134) and Neck (R=-0.117, P=0.061).

DISCUSSION

The aim of the present study was to examine if bioavailable vitamin D was related to markers of bone health within a racially diverse athlete population. It was observed that after adjusting for age and race, bioavailable vitamin D was closely associated with BMD (spine, neck and hip), whilst there was no association between serum 25[OH]D concentration and BMD at any site . DBP was positively associated with neck and hip T-scores but not spine. Serum 25[OH]D and bioavailable 25[OH]D were not associated with T-scores at any site. Furthermore, mean serum DBP concentrations were not associated with 25[OH]D concentrations. For clinicians treating vitamin D insufficiency based on serum 25(OH)D measures, our data suggests that the current choice of assay is not fit for practice when examining the relationship between bone health and vitamin D concentrations.

The Role of Bioavailable Vitamin D upon Markers of Bone Health

Vitamin D is just one of many factors including energy availability and weight bearing exercise that can impact upon bone health. The role of vitamin D and calcium in bone development, growth and integrity have been well documented (1). Previous studies however, demonstrate inconsistent associations between serum 25[OH]D concentrations and BMD in general (16, 17) and athletic populations (26). It has be postulated that the osteogenic effect of weight-bearing exercise (i.e. loading the bones) may be sufficient to maintain markers of bone health, irrespective of 25[OH]D status in healthy adults (27, 28). Though the present data also suggests that the clinical utilisation of serum 25[OH]D could be an additional factor for this inconsistency, given that bioavailable

vitamin D and not serum 25[OH]D was associated with BMD at all sites in an racially diverse athletic population. Using the free hormone hypothesis, the unbound fraction (or bioavailable vitamin D) may exert a stronger biological effect on BMD than total 25[OH]D (21, 23). Since serum 25[OH]D is generally used in previous observational and interventional studies, it may explain such inconsistencies in the poor association between serum 25[OH]D and BMD (29, 30). Accordingly, our data supports the notion that current 25[OH]D reference ranges provide an inaccurate representation of true vitamin D status in athletes. In contrast to previous findings, DBP concentration was not associated with 25[OH]D concentrations (p=0.392) (29). Whilst there was a positive linear association between 25[OH]D status and bioavailable vitamin D, PTH levels were not associated with 25[OH]D levels, BMD or T-scores, suggesting that the association between bioavailable 25[OH]D levels and BMD is not mediated via PTH.

Impact of Race

Studies support that racial differences or a 'paradox' exists in the relationship between serum 25[OH]D concentrations and markers of bone health (14, 31, 32). Specifically, recent studies in the general population have demonstrated that polymorphisms in the DBP gene were associated with corresponding changes in total 25[OH]D levels and the risk of osteoporosis (22, 33). Aggarwal et al. demonstrated that bioavailable 25[OH]D was a better measure of vitamin D status with respect of BMD in patients with nephrotic syndrome (34). To our knowledge, this is the first study to examine DBP and bioavailable vitamin D alongside bone health as assessed by DXA in athletes. Our data supports these previous observations, with Hispanic athletes presenting with greater mean serum 25[OH]D levels (32.1 ng/mL) compared to Caucasian (26.2ng/mL) and Black

(19.9ng/mL) athletes, despite no difference in bioavailable vitamin D or BMD at any site. Bioavailable vitamin D is determined by vitamin D-binding protein concentration, which is encoded by the group-specific component (GC) gene (35). Polymorphisms in the GC gene, produce proteins that differ in affinity for 25[OH]D, and it is these polymorphisms that are known to differ between racial groups. Racial differences in the prevalence of common genetic polymorphisms provide a likely explanation for this observation. Consequently, our data have implications for the wider general population, with further research warranted an adolescent and female athletes.

Financial Considerations for Treating Clinicians

Screening athlete for vitamin D insufficiency is expensive. In our facility, 25[OH]D assessment costs $255USD per athlete. Since Aspetar screens approximately 1500 elite athletes per year, systematic 25[OH]D screening costs our facility $382,500USD per year. Since our data clinically questions the value of routine 25[OH]D screening, the financial burden of vitamin D testing only adds weight to the debate of whether clinicians should be testing for vitamin D insufficiency, since all athletes in the present study demonstrated normal bone health. Our data also questions the value of prophylactic vitamin D supplementation (typically 2000IU/d oral cholecalciferol) in insufficient athletes to 'correct' 25[OH]D status, but who show no symptoms of relative energy deficiency syndrome, poor bone health or musculoskeletal injury. Perhaps targeted vitamin D screening and supplementation should be reserved for those athletes presenting with stress fracture related injuries, or those competing in non-weight bearing sports such as cycling and swimming where the osteogenic effect of bone loading is sub-optimal. If testing is warranted however, clinicians

should use the appropriate assays to calculate DBP and bioavailable vitamin D status rather than serum 25[OH]D.

Limitations

The primary limitations of this study are the lack of data on DBP polymorphisms across racial groups and the cross-sectional design. The equations used to calculate free and bioavailable 25[OH]D in this study used the affinity constants of the Gc1F allele, as did the equations in Powe et al. (29). Whilst we acknowledge that training volume and intensity were not recorded, athletes were only included in the study if they were registered with the QOC, competed at national or international level and trained for more than 6 h per week. We also acknowledge that serum 25[OH]D measurement only offered a snapshot of current status. Thus, it is entirely possible for severely deficient (<10 ng/mL) and deficient (10-20 ng/mL) athletes in the present study to have spent many years sufficient (>30 ng/mL) prior to recruitment. An additional limitation is that only male athletes were studied, due to a lack of female athletes presenting for pre-competition medical assessment at the time of data analysis. Finally, the impact of training load and dietary intake data, to assess total energy and calcium intake, and the assessment of steroid hormones were not accounted for in this study. Although, our previous research reports no association between 25[OH]D and bone health (26), future longitudinal trials including genotype-specific binding affinity constants in the calculation for bioavailable vitamin D, training, dietary and hormone analysis in racially diverse populations are warranted.

CONCLUSION

In conclusion, regardless of age or race, bioavailable vitamin D and not serum 25[OH]D was associated with BMD in a racially diverse athletic population. Our data questions the value of routine 25[OH]D screening in athletes, since all athletes in the present study demonstrated clinically normal bone health, despite 85% of athletes presenting with either serum 25[OH]D insufficiency, deficiency or severe deficiency, respectively. This study suggests that bioavailable vitamin D, rather than the current standard of 25[OH]D is a better measure of vitamin D status with respect to BMD.

What are the findings?

- This study demonstrated that in a racially diverse athletic population, bioavailable vitamin D was closely associated with bone mineral density (BMD; spine, neck and hip).
- No association was observed between serum 25[OH]D concentration and BMD at any site.
- Mean serum DBP concentration were not correlated with 25[OH]D concentrations ($p=0.392$).

How might it impact on clinical practice in the near future?

- Determining bone health is multifactorial. Systematic screening to determine 25[OH]D concentrations in isolation is expensive, and demonstrates a poor relationship to bone health in an racially diverse athletic population.
- If testing is warranted, clinicians should use the appropriate assays to calculate DBP and bioavailable vitamin D concentration rather than total serum 25[OH]D.

- Screening athlete for vitamin D insufficiency is expensive. Targeted vitamin D screening and supplementation should be reserved for those athletes presenting with relative energy deficiency syndrome or those competing in non-weight bearing sports, such as cycling and swimming, where the osteogenic effect of bone loading is sub-optimal.

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Competing interests

None of the authors have any relevant conflicts of interest. No payments or services from a third party were received for any aspect of the submitted work.

Table 1: Measures of vitamin D, albumin and parathyroid hormone against 25[OH]D status categories

	Vitamin D category (ng/mL)			
	Severely Deficient <10 (n=130)	**Deficient 10-20 (n=224)**	**Insufficient 20-30 (n=157)**	**Sufficient >30 (n=93)**
Age Mean (SD)	23.7 (5.4)	24.6 (5.6)	25.4 (5.6)	25.0 (4.5)
Body mass index Mean (SD)	23.6 (3.9)	24.7 (3.4)	24.2 (3.5)	24.0(3.6)
Race n (%)				
Arab (n=327)	96 (29.4)	146 (44.6)	65 (19.9)	20 (6.1)
Asian (n=48)	10 (20.8)	17 (35.4)	14 (29.2)	7 (14.6)
Black (n=108)	17 (15.7)	43 (39.8)	32 (29.6)	16 (14.8)
Caucasian (n=53)	4 (7.5)	13 (24.5)	20 (37.7)	16 (30.2)
Hispanic (n=68)	3 (4.4)	5 (7.4)	26 (38.2)	34 (50.0)
Blood markers (mean±SD)				
$1,25(OH)_2D$	39.3 (14.3)	39.4 (14.9)	39.9(14.0)	34.9(9.9)
ALB (g/L)	42.1(2.4)	41.8 (2.4)	41.9(2.5)	42.6(2.8) [b]
PTH (pg/ml)	67.2 (50.8) [bcd]	52.9(16.2)	50.1(14.0)	41.3(8.3)
DBP (µg/ml)	478.5 (410.4)	385.1(304.0)	416.6(298.4)	370.5(327.1)
Bioavailable 25[OH]D (ng/mL)	0.7 (0.5)	1.5(0.9) [a]	2.6(1.7) [ab]	4.7(3.0) [abc]

ALB; albumin, PTH; parathyroid hormone, DBP; vitamin D binding protein.

a: significantly greater than <10ng/mL, b: significantly greater than 10-20ng/mL, c: significantly greater than 20-30ng/mL and d: significantly greater than >30ng/mL.

Table 2. Measures of vitamin D, BMD, albumin and parathyroid hormone by athlete race (Mean±SD)

	Athlete Race				
	Arab [A] (n=328)	**Asian [B] (n=48)**	**Black [C] (n=108)**	**Caucasian [D] (n=53)**	**Hispanic [E] (n=35)**
25[OH]D	15.9(8.8)	19.3 (11.8)	19.9 (10.4)[a]	26.2 (12.9)[abc]	32.1 (13.5)[abcd]
1,25(OH)$_2$D	39.6(14.7)	39.8 (14.4)	39.3 (14.6)	38.8 (10.4)	33.0 (11.7)
ALB (g/L)	41.8(2.32)	42.2 (2.1)	41.2 (2.5)	43.3 (2.6)[ac]	43.1 (2.7)[ac]
PTH (pg/ml)	60.7(39.5)	47.7 (11.1)	52.1 (15.4)	47.2 (14.9)	41.2 (8.2)
DBP (µg/ml)	449.8(314.6)[c]	376 (176.0)	288.2 (336.1)	422.2 (372.8)[c]	434.2 (416.4)[c]
Bioavailable 25[OH]D (ng/mL)	1.5(1.3)	1.7 (1.2)	3.3(2.7)[ab]	2.5 (1.7)[a]	3.3 (2.8)[ab]
Spine BMD	1.29 (0.21)	1.32 (0.16)	1.48(0.16)[ab]	1.50 (0.13)[ab]	1.45 (0.14)[ab]
Neck BMD	1.25 (0.21)	1.31 (0.19)	1.48 (0.17)[ab]	1.43 (0.14)[ab]	1.41 (0.17)[ab]
Hip BMD	1.26 (0.20)	1.31 (0.18)	1.47 (0.17)[ab]	1.40 (0.13)[ab]	1.40 (0.15)[ab]
Total BMD	1.26 (0.16)	1.28 (0.10)	1.43 (0.13)[ab]	1.42 (0.10)[ab]	1.38 (0.10)[ab]
Spine T-score	0.61 (1.20)	0.64 (1.19)	1.85 (1.43)[ab]	1.98 (1.33)[ab]	1.74 (1.16)[ab]
Neck T-score	1.22 (1.42)	1.53 (1.49)	2.72 (1.65)[ab]	2.25 (1.37)[a]	2.44 (1.42)[ab]
Hip T-score	1.02 (1.20)	1.20 (1.23)	2.18 (1.41)[ab]	1.68 (1.07)[a]	2.00 (1.12)[ab]
Total T-score	0.63 (1.21)	0.67 (0.96)	2.06 (1.39)[ab]	1.78 (1.24)[ab]	1.72 (1.07)[ab]

ALB; albumin, PTH; parathyroid hormone, DBP; vitamin D binding protein, BMD; bone mineral density. a: significantly greater than Arabs, b: significantly greater than Asian, c: significantly greater than Blacks and d: significantly greater than Caucasians.

Table 3. Parameter estimates (β 95% CI) of association of log transformed vitamin D parameters against independent bone health variables (spine, neck and hip BMD and T-scores) after adjusting for age and race

Dependent variables	**Vitamin D parameters**					
	Serum 25[OH]D *B (95% CI)*	P value	DBP *B (95% CI)*	P value	Bioavailable 25[OH]D *B (95% CI)*	P value
Spine BMD	0.022 (-0.007 to 0.052)	0.141	-0.035 (-0.059 to -0.012)	0.004	0.032 (0.012 to 0.051)	0.001
Neck BMD	0.028 (-0.002 to 0.059)	0.071	-0.040 (-0.065 to -0.016)	0.001	0.037 (0.016 to 0.057)	<0.001
Hip BMD	0.029 (0.000 to 0.059)	0.051	-0.037 (-0.061 to -0.014)	0.002	0.035 (0.016 to 0.055)	<0.001
Spine T-score	-0.030 (-0.229 to 0.170)	0.770	-0.151 (-0.313 to 0.011)	0.067	0.078 (-0.055 to 0.211)	0.250
Neck T–score	-0.006 (-0.244 to 0.231)	0.957	-0.209 (-0.401 to -0.016)	0.034	0.119 (-0.038 to 0.277)	0.138
Hip T-score	0.000 (-0.198 to 0.198)	1.000	-0.169 (-0.329 to -0.008)	0.040	0.098 (-0.034 to 0.230)	0.144

* log transformed covariates and adjusted for age and ethnicity

B: Unstandardised coefficient and (95 % confidence intervals.)

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