An Association between Bioavailable 25-Hydroxyvitamin D and Bone Mineral Density in a Diverse Cohort of Collegiate Athletes

MICHELLE S. ROCKWELL[1,2], SAMANTHA B. KOSTELNIK[3], RYAN P. MCMILLAN[1,4], MELANIE LANCASTER[3], D. ENETTE LARSON-MEYER[3], and MATTHEW W. HULVER[1,3]

[1]Fralin Life Sciences Institute, Virginia Tech, Blacksburg, VA; [2]Department of Family and Community Medicine, Virginia Tech Carilion School of Medicine, Roanoke, VA; [3]Department of Human Nutrition, Foods, and Exercise, Blacksburg, VA; and [4]The Metabolism Core at Virginia Tech, Blacksburg, VA

ABSTRACT

ROCKWELL, M. S., S. B. KOSTELNIK, R. P. MCMILLAN, M. LANCASTER, D. E. LARSON-MEYER, and M. W. HULVER. An Association between Bioavailable 25-Hydroxyvitamin D and Bone Mineral Density in a Diverse Cohort of Collegiate Athletes. *Med. Sci. Sports Exerc.*, Vol. 54, No. 3, pp. 371–376, 2022. **Introduction:** Although vitamin D is intimately involved in bone metabolism, the relationship between vitamin D status, as measured by serum total 25-hydroxyvitamin D [25(OH)D] concentration, and bone mineral density (BMD) is weak, particularly in non-White populations. Measurement of bioavailable 25(OH)D has been suggested as a better indicator of vitamin D status than total 25(OH)D concentration. To date, the bioavailable 25(OH)D biomarker has been explored minimally in athletic populations. The purpose of this study was to investigate the relationship between total and bioavailable 25(OH)D concentrations and BMD in collegiate athletes. **Methods:** NCAA Division I basketball and swimming athletes served as study participants ($n = 53$; 28 females, 25 males; 28 basketball players, 25 swimmers). All participants completed dual-energy x-ray absorptiometry scans for analysis of BMD, blood draws for vitamin D measures, and diet/lifestyle questionnaires. **Results:** Overall, total 25(OH)D was 80.0 + 13.9 $\text{nmol}\cdot\text{L}^{-1}$ and bioavailable 25(OH)D was 6.0 ± 1.9 $\text{nmol}\cdot\text{L}^{-1}$. There was strong disagreement between total 25(OH)D and bioavailable 25(OH)D concentrations ($\kappa = -0.299$, $r = -0.129$) ($P = 0.100$); 53% of total participants and 77% of Black participants were classified differently (low vs normal vitamin D status) based on total and bioavailable 25(OH)D criteria. Black participants had significantly lower total 25(OH)D and higher bioavailable 25(OH)D concentrations than White participants (59.5 vs 102.5 $\text{nmol}\cdot\text{L}^{-1}$ and 7.9 vs 5.4 $\text{nmol}\cdot\text{L}^{-1}$, respectively) ($P < 0.001$). Total 25(OH)D and total BMD were not correlated, but bioavailable 25(OH)D and total BMD demonstrated a positive correlation ($r = 0.618$, $P < 0.01$). **Conclusions:** These results suggest that bioavailable 25(OH)D concentration may be a better clinical measure of vitamin D status than total 25(OH)D as related to BMD in collegiate athletes, particularly in Black athletes. Further research on the utility of the bioavailable 25(OH)D biomarker in athletes is needed. **Key Words:** VITAMIN D, VITAMIN D–BINDING PROTEIN, FREE 25(OH)D, BIOMARKER

Vitamin D has long been known for its role in bone health and metabolism (1) and is increasingly recognized for influencing the performance, health, and well-being of athletes (2–6). Consumed through the diet or synthesized in the skin upon exposure to ultraviolet-B (UVB) light, vitamin D is converted to 25-hydroxyvitamin D [25(OH)D] in the liver, and then to its biologically active form, 1,25-dihydroxyvitamin D [$1,25(OH)_2D$], in the kidneys (7). Vitamin D status is commonly assessed via serum total 25(OH)D concentration because it is less tightly regulated and has a longer half-life than $1,25(OH)_2D$ (weeks vs hours) (8).

Testing for low total 25(OH)D concentration has become commonplace in sports medicine (9). Based on the total 25(OH)D concentration, vitamin D deficiency and insufficiency are frequently observed among athletes (10,11). However, there is some evidence that measurement of bioavailable or free 25(OH)D concentration may be a better indicator of vitamin D status than total 25(OH)D concentration (12,13). In circulation, approximately 85% of 25(OH)D is bound to vitamin D–binding protein (DBP). Of the remaining 25(OH)D in circulation, 10% to 15% is loosely bound to albumin and less than 1% remains "free" or unbound (14). Bioavailable 25(OH)D

Address for correspondence: Michelle S. Rockwell, Ph.D., R.D., Virginia Tech, 315-C Steger Hall, Blacksburg, VA 24061; E-mail: msrock@vt.edu.
Submitted for publication February 2021.
Accepted for publication September 2021.

0195-9131/21/5403-0371/0
MEDICINE & SCIENCE IN SPORTS & EXERCISE®

DOI: 10.1249/MSS.0000000000002807

reflects the 25(OH)D loosely bound to albumin plus free 25(OH)D because these forms are available for conversion to $1,25(OH)_2D$ and interaction with vitamin D receptors (VDR). The assessment of bioavailable 25(OH)D has been suggested to best reflect true vitamin D status and better correlate with health outcomes, including bone mineral density (BMD), particularly in Black and African American individuals (2,12,13,15).

Bone health is of high importance to athletes as stress fracture and acute fracture rank among the most common sports injuries (16,17). Further, athletes participating in non–weight bearing sports, such as swimming, have been shown to exhibit decreased BMD (18). Given the role of vitamin D in calcium and phosphorus metabolism, bone remodeling, and association with stress fracture risk in some studies (19–21), the maintenance of optimal vitamin D status is recommended to support athletes' bone health (22). Interestingly, some research does not support a relationship between total 25(OH)D and BMD in athletes (23–26). Allison et al. (15), for example, reported no relationship between total 25(OH)D and BMD in a racially diverse cohort of more than 600 male athletes. However, a significant positive association between bioavailable 25(OH)D and BMD was observed. Fields et al. (25), by contrast, were unable to confirm these findings in a small group of female collegiate athletes.

The purpose of the present study was to further explore the relationship between total 25(OH)D and bioavailable 25(OH)D concentrations, and BMD, in a racially diverse group of male and female collegiate athletes. Basketball and swimming athletes were selected for the investigation because of their differences in habitual training (weight bearing vs not) and expected differences in BMD.

METHODS

Participants. Male and female student-athletes from an NCAA Division I program in Virginia (35° N latitude) volunteered to serve as participants for this study. Participants were ≥18 yr of age and current members of the university's basketball or swimming teams. Participants self-reported their race and ethnicity. Those who had taken vitamin D or calcium supplements or medications that may influence vitamin D status or BMD in the previous 90 d were excluded from participating. This investigation was approved by the Institutional Review Board of Virginia Polytechnic Institute and State University (IRB no. 17-009), and all participants provided written, informed consent before the start of the study.

Procedures. Participants completed two sequential days of testing in April, at the conclusion of the competitive season for both teams. Day 1 of testing involved a health screening, diet and lifestyle questionnaires, and a blood draw for analysis of multiple vitamin D–related measures. On day 2 of testing, dual-energy x-ray absorptiometry (DXA) scans for analysis of BMD and body composition were completed. Participants fasted overnight and abstained from exercise for at least 12 h before both days of testing.

Diet and lifestyle questionnaires. Daily dietary vitamin D and calcium consumption were assessed using a food frequency questionnaire previously validated to assess vitamin D and calcium intakes in college-age women with anorexia nervosa (27). UVB exposure was assessed using a survey instrument based on a portion of the tool developed by Halliday et al. (28) for use with college athletes.

Blood sampling and analysis. Approximately 20 mL of blood was obtained from the antecubital vein. One tube was set aside for analysis of serum total 25(OH)D concentration, which took place within 2 d after blood draws at a Clinical Laboratory Improvement Amendments–certified commercial laboratory (LabCorp, Burlington, NC) using an immunochemiluminometric assay (DiaSorin Liaison, Saluggia, Italy). Remaining tubes were allowed to clot at room temperature in a vertical position for 20–30 min, centrifuged at 4°C for 15 min at 3200 rpm (Eppendorf 5810R, Hauppage, NY), and stored at −80°C for later analysis. Serum intact parathyroid hormone (PTH) was measured using an Immulite immunoassay 1000 analyzer (Siemens, Erlangen, Germany) and commercially available kits (Siemens, Erlangen, Germany). Commercial enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN) was used for measurement of serum DBP concentration, and serum albumin concentration was measured via enzymatic–colorimetric assay (Thermo Fisher Scientific, Waltham, MA). The coefficient of variation was <8% for all analyses.

Calculation of bioavailable 25(OH)D [albumin-bound 25(OH)D + free 25(OH)D] was based on methodology developed by Vermeulen et al. (29) and adapted and validated by Powe et al. (14), based on the free hormone hypothesis.

Calculation equations were as follows:

$$[\text{Free } 25(\text{OH})\text{D}] = \frac{[\text{Total } 25(\text{OH})\text{D}]}{1 + (K_{\text{Alb}} \times [\text{Alb}]) + (K_{\text{DBP}} \times [\text{DBP}])}$$

$$[\text{Bioavailable } 25(\text{OH})\text{D}] = [\text{Free } 25(\text{OH})\text{D}] + [\text{D}_{\text{Alb}}]$$
$$= (K_{\text{alb}} \times [\text{Alb}] + 1) \times [\text{Free } 25(\text{OH})\text{D}]$$

where K_{VDBP} is the affinity constant between 25(OH)D and VDBP (i.e., $7 \times 108\ \text{M}^{-1}$); K_{Alb} is the affinity constant between 25(OH)D and albumin (i.e., $6 \times 105\ \text{M}^{-1}$); and [Alb] is the concentration of albumin.

Total and bioavailable 25(OH)D were classified as low or normal (Table 1) (30–32).

Bone mineral density, body composition, and anthropometric measures. BMD measurement (total body, anteroposterior [AP] spine, and dual hip) and body composition (percent body fat and fat-free mass) were conducted in the morning after an overnight fast via a series of DXA scans (Lunar Prodigy Advance software version 8.10e; General Electric, Madison, WI). All DXA scans were conducted by a technician certified by the International Society for Clinical Densitometry and licensed by the Virginia Department of Health. The DXA scanner was calibrated daily before beginning scans. Body weight was measured to the nearest 0.1 lb on a digital scale (Model 5002; Scale-Tronix, White Plains,

TABLE 1. Classification of low vs normal total 25(OH)D and bioavailable 25(OH)D.

	Low	Normal
Total 25(OH)D	Deficient: 0 to 49.9 nmol·L^{-1} Insufficient: 50 to 74.9 nmol·L^{-1}	≥75 nmol·L^{-1}
Bioavailable 25(OH)D	0 to 4.9 nmol·L^{-1}	≥5 nmol·L^{-1}

NY), and height was measured to the nearest 0.1 inch using a wall-mounted stadiometer (Scale-Tronix, Wheaton, IL).

Data analysis. Statistical Package for the Social Sciences (version 26; SPSS; Chicago, IL) was used for statistical analysis, with significance set at $P < 0.05$. Descriptive statistics were expressed as mean ± SD. Group differences (sex, race, and sport) were assessed via independent Student *t*-tests with Bonferroni correction applied. Asian participants were excluded from race-specific analyses because of low sample size ($n = 2$). Pearson's correlation analysis was used to evaluate relationships among variables. Cohen's kappa statistic was used to evaluate agreement between total and bioavailable 25(OH)D classifications (low vs normal). Cohen's kappa results were interpreted as no agreement to slight agreement ($\kappa = 0$ to 0.20), fair to moderate agreement ($\kappa = 0.21$ to 0.60), and substantial to perfect agreement ($\kappa = 0.61$ to 1.00) (33). Negative kappa values were interpreted as disagreement ($\kappa = -0.10$ to 0) and strong disagreement (<−0.10) (33).

RESULTS

Fifty-three participants (28 females, 25 males; 28 basketball, 25 swimming) completed the study. Descriptive data about the participants are shown in Table 2.

Serum vitamin D and PTH concentration. Overall total 25(OH)D concentration was 80.0 ± 34.8 nmol·L^{-1} (Table 3). Seventy-four percent of participants ($n = 39$) were classified as having low vitamin D status (0 to 74.9 nmol·L^{-1}; 11 deficient and 28 insufficient) and 26% ($n = 14$) as normal (≥75 nmol·L^{-1}) based on total 25(OH)D concentration. Females had higher total 25(OH)D concentration than males (95.3 vs 62.8 nmol·L^{-1}) ($P < 0.001$); swimming participants had higher total 25(OH)D than basketball participants (97.5 vs 65.0 nmol·L^{-1}) ($P < 0.001$); and White participants had higher total 25(OH)D concentration than Black participants (102.5 vs 59.5 nmol·L^{-1}) ($P < 0.001$). Overall PTH was 4.14 pmol·L^{-1}, with no differences between groups and no association with total 25(OH)D. No participants exhibited elevated PTH (>7.0 pmol·L^{-1}).

TABLE 3. Vitamin D metabolite concentrations.

Vitamin D Metabolite	Mean ± SD	Range
Total 25(OH)D (nmol·L^{-1})	80.0 ± 34.8	18.9–187.5
DBP (μg·mL^{-1})	181.3 ± 47.2	31.7–279.0
Bioavailable 25(OH)D (nmol·L^{-1})	6.0 ± 1.9	1.2–9.2
Free 25(OH)D (nmol·L^{-1})	0.0378 ± 0.009	0.0091–0.0812

Low bioavailable serum 25(OH)D concentration was observed in 19% ($n = 10$) of participants. Bioavailable 25(OH)D did not differ by sex (6.2 nmol·L^{-1} for males vs 5.8 nmol·L^{-1} for females) or sport (6.6 nmol·L^{-1} for swimming vs 5.7 nmol·L^{-1} for basketball). However, Black participants had significantly higher bioavailable 25(OH)D than White participants (7.9 vs 5.4 nmol·L^{-1}) ($P < 0.05$). There were no significant group differences in DBP (211.2 μg·mL^{-1} for White participants vs 172.9 μg·mL^{-1} for Black participants, $P = 0.44$; 225.16 μg·mL^{-1} for swimming vs 171.8 μg·mL^{-1} for basketball, $P = 0.32$; 173.77 μg·mL^{-1} for males vs 227.98 μg·mL^{-1} for females, $P = 0.39$).

Based on total 25(OH)D vs bioavailable 25(OH)D classifications (Table 1) in the overall group, 53% ($n = 28$) were classified differently, with 32% ($n = 8$) of White participants and 76.9% ($n = 20$) of Black participants varying in classification.

Total serum 25(OH)D concentration was moderately correlated with dietary calcium and dietary vitamin D ($r = 0.475$ and $r = 0.424$, respectively) ($P < 0.05$), but not with leisure time sun exposure or tanning bed use. Bioavailable 25(OH)D, DBP, and PTH were not associated with dietary calcium, dietary vitamin D, leisure time sun exposure, or tanning bed use.

Association between vitamin D metabolites and bone mineral density. Table 4 summarizes the BMD results by sport, race, and sex. All participants demonstrated normal total BMD based on *T*-score except for two female swimmers who showed evidence of osteopenia (*T*-scores of −1.4 and −1.9). There was no significant relationship between total 25(OH)D concentration and total BMD ($r = -0.256$, $P = 0.221$), but total 25(OH)D concentration was negatively correlated with AP spine and dual hip BMD ($r = -0.444$ and $r = -0.409$, respectively, $P < 0.05$). Bioavailable 25(OH)D concentration was positively

TABLE 2. Anthropometric, dietary intake, and ultraviolet-B exposure in NCAA Division I basketball and swimming study participants.

	Overall ($N = 53$)	Basketball ($n = 28$)		Swim ($n = 25$)	
Sex	Male ($n = 25$)	Male ($n = 14$)	Female ($n = 14$)	Male ($n = 11$)	Female ($n = 14$)
Age (yr)	19.9 ± 1.3	19.9 ± 0.4	19.7 ± 0.3	20 ± 0.2	19.8 ± 0.4
Race	25 White/26 Black/2 Asian	0 White/14 Black/0 Asian	3 White/11 Black/0 Asian	8 White/1 Black/2 Asian	14 White/0 Black/0 Asian
Height (cm)	179.3 ± 12.9	191.8 ± 3.4[a]	173.2 ± 3.4	181.5 ± 4.3	171.3 ± 1.6
Weight (kg)	77.8 ± 15.8	92.4 ± 4.1[b]	69.7 ± 4.0	82.9 ± 2.2	67.3 ± 2.0
Body fat (%)	20.0 ± 6.0	15.5 ± 0.8	23.7 ± 1.2	14.1 ± 0.5[c]	25.5 ± 1.2
Fat-free mass (kg)	60.3 ± 21.2	79.7 ± 2.8	49.3 ± 4.6	71.1 ± 1.5	53.4 ± 5.0
Dietary calcium (mg)	1230.3 ± 154.1	260.4 ± 55.7[d]	532 ± 95.6[d]	2319.8 ± 315.2	2042.6 ± 234.0
Dietary vitamin D (IU)	430.8 ± 47.5	169.5 ± 31.2[d]	358.6 ± 49.6[d]	752.2 ± 122.5	511.9 ± 96.5
Tanning bed use in last 3 months (yes)	6	0	4	2	0
Leisure time outdoors in last 3 months (h·d^{-1})	0.59 ± 0.28	0.16 ± 0.48	0.44 ± 0.21	0.73 ± 0.19	0.91 ± 0.22

[a]Significant difference between female basketball and female swim participants ($P < 0.01$).
[b]Significant difference between female basketball participants ($P < 0.05$).
[c]Significant difference between female swim participants ($P < 0.05$).
[d]Significant difference between male and female swim participants ($P < 0.05$).

TABLE 4. Total bone mineral density, AP spine BMD, and dual hip BMD.

				Race		Sex	
Body Composition Measures	Overall (Mean ± SD)	Basketball (Mean ± SD)	Swim (Mean ± SD)	Black (Mean ± SD)	White (Mean ± SD)	Male (Mean ± SD)	Female (Mean ± SD)
Total BMD (g·cm^{-2})	1.388 ± 0.290	1.547 ± 0.246[a]	1.209 ± 0.227	1.549 ± 0.248[b]	1.235 ± 0.248	1.518 ± 0.245[c]	1.272 ± 0.281
AP spine BMD (g·cm^{-2})	1.429 ± 0.272	1.600 ± 0.215[a]	1.238 ± 0.189	1.603 ± 0.220[b]	1.263 ± 0.212	1.517 ± 0.240[c]	1.351 ± 0.279
Dual hip BMD (g·cm^{-2})	1.366 ± 0.316	1.539 ± 0.275[a]	1.172 ± 0.238	1.535 ± 0.265[b]	1.206 ± 0.285	1.457 ± 0.287[c]	1.284 ± 0.323

[a]Significant difference between basketball and swimming participants ($P < 0.05$).
[b]Significant difference between Black and White participants ($P < 0.05$).
[c]Significant difference between male and female participants ($P < 0.05$).

correlated with total BMD ($r = 0.618$, $P < 0.05$), AP spine, and dual hip BMD ($r = 0.596$ and $r = 0.641$, respectively, $P < 0.05$). PTH was not associated with BMD at any site.

DISCUSSION

In collegiate swimming and basketball athletes, we report no association between total serum 25(OH)D concentration, the common measure of vitamin D status, and total BMD. However, a positive association between bioavailable 25(OH)D in serum and total BMD was observed. Although 74% of participants exhibited low total 25(OH)D concentration, only 19% had low bioavailable 25(OH)D. There was less agreement between total and bioavailable 25(OH)D in Black athletes compared with White athletes. These results suggest that bioavailable 25(OH)D may be a better biomarker of vitamin D status than total 25(OH)D as related to bone health in athletes, and that current clinical practices may overestimate low vitamin D status in this population.

Assessment of vitamin D status. Bioavailable 25(OH)D reflects the amount of active vitamin D available for biological activity through interaction with VDR. By contrast, approximately 85% of total 25(OH)D is bound to DBP, which inhibits biological activity of 25(OH)D (34). Multiple (13–15), but not all, studies (25,35) have showed no association between total 25(OH)D and bioavailable 25(OH)D concentrations, likely because of the individual variation in DBP concentration. Single nucleotide polymorphisms in the DBP gene, known as the globulin complex (GC), are a key factor in this variation (34,36).

In the present study, there was no relationship between bioavailable and total serum 25(OH)D concentrations, nor between DBP and total 25(OH)D concentrations. Over half of participants classified with low total 25(OH)D were not classified similarly using bioavailable 25(OH)D. Although the cutoff we used for low versus normal bioavailable 25(OH)D is a preliminary recommendation based on limited evidence (32), there is reason to believe that in the clinical setting, athletes' vitamin D status may be misdiagnosed based on the biomarker being tested. Because the assessment of vitamin D status is so common among athletic populations and subsequent overtreatment with vitamin D supplements has the potential to cause harm (8,38), further research to identify the utility of bioavailable 25(OH)D for athletes is needed. Currently, assessment of DBP and bioavailable 25(OH)D is not readily available in commercial laboratory settings.

Relationship between vitamin D status and bone mineral density. Vitamin D plays an integral role in bone metabolism through enhancing intestinal and renal calcium absorption and promoting bone remodeling (37). However, several studies have identified no relationship between total 25(OH)D concentration and BMD (12,13,26). Our findings confirm those of Allison et al. (15) who observed an association between bioavailable 25(OH)D, but not total 25(OH)D, and BMD at multiple sites in professional male athletes in Qatar, and in studies involving nonathlete populations (13,14).

It is not clear why higher total 25(OH)D concentration was associated with lower BMD at two regional sites (AP spine and hip). High-dose vitamin D supplementation has been shown to negatively affect PTH and BMD in other studies (38,39), so it is possible that participants in this study previously took high dose vitamin D supplements, although they confirmed not using any vitamin D supplements for 90 d before the start of the study and none showed abnormal PTH. By contrast, bioavailable 25(OH)D concentration was found to be positively with total BMD at these same sites.

Weight-bearing exercise is known to enhance BMD (22). Nevertheless, stress fracture and other bone-related disorders are common among athletes (17), and well-trained athletes in non–weight bearing sports have higher rates of reduced BMD (40). Swimming participants in the present study had significantly lower BMD at all sites than basketball athletes, and two showed BMD indicative of osteopenia. Improving vitamin D status is widely promoted as a means of enhancing bone health in athletes (22). To address the independent contribution of vitamin D deficiency to decreased bone health and to design interventions to improve BMD, clinicians must be able to assess true vitamin D status.

Racial differences in vitamin D status. Compared with White participants, Black participants in the current investigation had significantly lower total 25(OH)D, a finding that is well documented in the literature (41,42). Black participants also had significantly higher BMD than White participants, which is also commonly observed (43). These findings illustrate the vitamin D paradox; that is, Black individuals experience better bone health and a lower rate of fracture compared with White individuals despite apparently inferior vitamin D status (41). Given this paradox, the clinical relevance of low total 25(OH)D for Black populations has been questioned (15,41,44).

Bioavailable 25(OH)D may be particularly valuable in assessing true vitamin D status of Black individuals. Despite having lower total 25(OH)D concentration, we observed

significantly higher bioavailable 25(OH)D concentration in Black compared with White athletes. Furthermore, Black participants were more likely to be classified differently based on total and bioavailable 25(OH)D than White participants. Racial differences in the globulin complex single nucleotide polymorphism rs7041 and rs4588 that influence the DBP concentration have been identified previously in the general population (44). Future investigations should explore vitamin D genetics in athletes and their relationship with vitamin D metabolites, BMD, and other aspects of health and performance.

Strengths and limitations. This study was one of the first to explore the bioavailable 25(OH)D biomarker in collegiate athletes. The inclusion of racially diverse participants from two sports with varying typical BMD is another strength, although one potential limitation is that racial breakdown was not balanced between sports. Consideration of diet and UVB and their relationship to vitamin D metabolite concentrations adds to the literature on vitamin D status and athletes. The main limitation of this preliminary research is a modest sample size, which somewhat limited statistical analysis and generalizability of results. We also assessed vitamin D status and BMD only at one time point, which was postseason for both sports and in early spring when UVB vitamin D synthesis is extremely minimal at our latitude. The food frequency questionnaire we used (27) was validated in women with a diagnosed eating disorder, a potential limitation because our participants were men and women who did not have the same diagnosis. However, the instrument developed by Taylor et al. (27) is very similar to that developed by Halliday et al. (28) in collegiate athletes. Given the similarity of these tools and the documented challenges associated with evaluating vitamin D content of foods and in validating a vitamin D–specific assessment nstrument (3,45), we feel confident that our assessment of dietary vitamin D intake is a good estimation of actual intake. Finally, although we report acceptable assay variance, challenges with measurement of vitamin D metabolites are well documented (46), and some have suggested that the assessment of DBP via polyclonal assay is superior to the monoclonal assay analysis that we and others have used (47).

CONCLUSIONS

In summary, there was strong disagreement between total serum 25(OH)D concentration, the common biomarker of vitamin D status, and bioavailable 25(OHD) concentration in a racially diverse cohort of collegiate athletes. Biomarker disagreement was greater in Black participants than White participants, potentially because of variation in vitamin D genetics. Bioavailable 25(OH)D concentration was associated with higher total, AP spine, and hip BMD, but total 25(OH)D concentration was not related to total BMD and was negatively associated with AP spine and hip BMD. Further research to explore the utility of the bioavailable 25(OH)D biomarker in racially diverse collegiate athletes is needed. In particular, exploration of vitamin D genetics and relationships between genetics, vitamin D biomarkers, and multiple aspects of health and performance may inform the assessment of vitamin D status in athletes and prevent overtreatment.

The authors thank the student-athletes for their participation in the study. This research was supported, in part, by the Center for Transformative Research on Health Behaviors at the Fralin Biomedical Research Institute at Virginia Tech Carilion and the Fralin Life Sciences Institute at Virginia Tech.

The authors have no conflicts of interest to report and declare that results are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

Results of the present study do not constitute endorsement by the American College of Sports and Medicine.

REFERENCES

1. Wicinski M, Adamkiewicz D, Adamkiewicz M, et al. Impact of vitamin D on physical efficiency and exercise performance—a review. *Nutrients*. 2019;11(11):2826.
2. Owens DJ, Allison R, Close GL. Vitamin D and the athlete: current perspectives and new challenges. *Sports Med*. 2018;48(1 Suppl):3–16.
3. Todd JJ, Pourshahidi LK, McSorley EM, Madigan SM, Magee PJ. Vitamin D: recent advances and implications for athletes. *Sports Med*. 2015;45(2):213–29.
4. He CS, Fraser WD, Tang J, et al. The effect of 14 weeks of vitamin D_3 supplementation on antimicrobial peptides and proteins in athletes. *J Sports Sci*. 2016;34(1):67–74.
5. Rockwell MS, Frisard MI, Rankin JW, et al. Effects of seasonal vitamin D3 supplementation on strength, power, and body composition in college swimmers. *Int J Sport Nutr Exerc Metab*. 2020;30(2):165–73.
6. Larson-Meyer E. Vitamin D supplementation in athletes. *Nestle Nutr Inst Workshop Ser*. 2013;75:109–21.
7. Jeon SM, Shin EA. Exploring vitamin D metabolism and function in cancer. *Exp Mol Med*. 2018;50(4):1–14.
8. Dirks NF, Ackermans MT, Lips P, et al. The when, what & how of measuring vitamin D metabolism in clinical medicine. *Nutrients*. 2018;10(4):482.
9. Rockwell M, Hulver M, Eugene E. Vitamin D practice patterns in National Collegiate Athletic Association Division I Collegiate Athletics Programs. *J Athl Train*. 2020;55(1):65–70.
10. Farrokhyar F, Tabasinejad R, Dao D, et al. Prevalence of vitamin D inadequacy in athletes: a systematic-review and meta-analysis. *Sports Med*. 2015;45(3):365–78.
11. de la Puente Yagüe M, Collado Yurrita L, Ciudad Cabañas MJ, Cuadrado Cenzual MA. Role of vitamin D in athletes and their performance: current concepts and new trends. *Nutrients*. 2020;12(2):579.
12. Bikle D, Bouillon R, Thadhani R, Schoenmakers I. Vitamin D metabolites in captivity? Should we measure free or total 25(OH)D to assess vitamin D status? *J Steroid Biochem Mol Biol*. 2017;173:105–16.
13. Johnsen MS, Grimnes G, Figenschau Y, Torjesen PA, Almås B, Jorde R. Serum free and bio-available 25-hydroxyvitamin D correlate better with bone density than serum total 25-hydroxyvitamin D. *Scand J Clin Lab Invest*. 2014;74(3):177–83.
14. Powe CE, Ricciardi C, Berg AH, et al. Vitamin D–binding protein modifies the vitamin D–bone mineral density relationship. *J Bone Miner Res*. 2011;26(7):1609–16.
15. Allison RJ, Farooq A, Cherif A, Hamilton B, Close GL, Wilson MG. 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. *Br J Sports Med*. 2018;52(8):522–6.
16. Fredericson M, Jennings F, Beaulieu C, Matheson GO. Stress fractures in athletes. *Top Magn Reson Imaging*. 2006;17(5):309–25.

17. Rizzone KH, Ackerman KE, Roos KG, Dompier TP, Kerr ZY. The epidemiology of stress fractures in collegiate student-athletes, 2004–2005 through 2013–2014 academic years. *J Athl Train*. 2017; 52(10):966–75.
18. Jakse B, Sekulic D, Jakse B, Cuk I, Sajber D. Bone health among indoor female athletes and associated factors; a cross-sectional study. *Res Sports Med*. 2020;28(3):314–23.
19. Miller JR, Dunn KW, Ciliberti LJ Jr, Patel RD, Swanson BA. Association of vitamin D with stress fractures: a retrospective cohort study. *J Foot Ankle Surg*. 2016;55(1):117–20.
20. Maroon JC, Mathyssek CM, Bost JW, et al. Vitamin D profile in National Football League players. *Am J Sports Med*. 2015;43(5):1241–5.
21. Lappe J, Cullen D, Haynatzki G, Recker R, Ahlf R, Thompson K. Calcium and vitamin D supplementation decreases incidence of stress fractures in female navy recruits. *J Bone Miner Res*. 2008;23(5):741–9.
22. Sale C, Elliott-Sale KJ. Nutrition and athlete bone health. *Sports Med*. 2019;49(2 Suppl):139–51.
23. Allison RJ, Farooq A, Hamilton B, Close GL, Wilson MG. No association between vitamin D deficiency and markers of bone health in athletes. *Med Sci Sports Exerc*. 2015;47(4):782–8.
24. Dias Quiterio AL, Carnero EA, Baptista FM, Sardinha LB. Skeletal mass in adolescent male athletes and nonathletes: relationships with high-impact sports. *J Strength Cond Res*. 2011;25(12):3439–47.
25. Fields JB, Gallo S, Worswick JM, Busteed DR, Jones MT. 25-Hydroxyvitamin D, vitamin D binding protein, bioavailable 25-Hydroxyvitamin D, and body composition in a diverse sample of women collegiate indoor athletes. *J Funct Morphol Kinesiol*. 2020; 5(2):32.
26. Hamilton B, Grantham J, Racinais S, Chalabi H. Vitamin D deficiency is endemic in middle eastern sportsmen. *Public Health Nutr*. 2010;13(10):1528–34.
27. Taylor C, Lamparello B, Kruczek K, Anderson EJ, Hubbard J, Misra M. Validation of a food frequency questionnaire for determining calcium and vitamin D intake by adolescent girls with anorexia nervosa. *J Am Diet Assoc*. 2009;109(3):479–85, 485.e1–3.
28. Halliday TM, Peterson NJ, Thomas JJ, Kleppinger K, Hollis BW, Larson-Meyer DE. Vitamin D status relative to diet, lifestyle, injury, and illness in college athletes. *Med Sci Sports Exerc*. 2011;43(2):335–43.
29. Vermeulen A, Verdonck L, Kaufman JM. A critical evaluation of simple methods for the estimation of free testosterone in serum. *J Clin Endocrinol Metab*. 1999;84(10):3666–72.
30. Larson-Meyer DE, Woolf K, Burke L. Assessment of nutrient status in athletes and the need for supplementation. *Int J Sport Nutr Exerc Metab*. 2018;28(2):139–58.
31. Holick MF, Binkley NC, Bischoff-Ferrari HA, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab*. 2011; 96(7):1911–30.
32. Close GL, Allison R, Owens D. Vitamin D and the athlete—an oversimplified, complex biological problem. *Sports Sci Exch*. 2019;#191.
33. McHugh ML. Interrater reliability: the kappa statistic. *Biochem Med (Zagreb)*. 2012;22(3):276–82.
34. Malik S, Fu L, Juras DJ, et al. Common variants of the vitamin D binding protein gene and adverse health outcomes. *Crit Rev Clin Lab Sci*. 2013;50(1):1–22.
35. Thambiah SC, Wong TH, Gupta ED, et al. Calculation of free and bioavailable vitamin D and its association with bone mineral density in Malaysian women. *Malays J Pathol*. 2018;40(3):287–94.
36. Nimitphong H, Sritara C, Chailurkit LO, et al. Relationship of vitamin D status and bone mass according to vitamin D–binding protein genotypes. *Nutr J*. 2015;14:29.
37. Holick MF. Vitamin D status: measurement, interpretation, and clinical application. *Ann Epidemiol*. 2009;19(2):73–8.
38. Owens DJ, Tang JC, Bradley WJ, et al. Efficacy of high-dose vitamin D supplements for elite athletes. *Med Sci Sports Exerc*. 2017;49(2): 349–56.
39. Burt LA, Billington EO, Rose MS, Raymond DA, Hanley DA, Boyd SK. Effect of high-dose vitamin D supplementation on volumetric bone density and bone strength: a randomized clinical trial. *JAMA*. 2019;322(8):736–45.
40. Lee EC, Fragala MS, Kavouras SA, Queen RM, Pryor JL, Casa DJ. Biomarkers in sports and exercise: tracking health, performance, and recovery in athletes. *J Strength Cond Res*. 2017;31(10):2920–37.
41. Brown LL, Cohen B, Tabor D, Zappalà G, Maruvada P, Coates PM. The vitamin D paradox in Black Americans: a systems-based approach to investigating clinical practice, research, and public health—expert panel meeting report. *BMC Proc*. 2018;12(6 Suppl):6.
42. Forrest KY, Stuhldreher WL. Prevalence and correlates of vitamin D deficiency in US adults. *Nutr Res*. 2011;31(1):48–54.
43. Ettinger B, Sidney S, Cummings SR, et al. Racial differences in bone density between young adult Black and White subjects persist after adjustment for anthropometric, lifestyle, and biochemical differences. *J Clin Endocrinol Metab*. 1997;82(2):429–34.
44. Powe CE, Evans MK, Wenger J, et al. Vitamin D–binding protein and vitamin D status of Black Americans and White Americans. *N Engl J Med*. 2013;369(21):1991–2000.
45. Larson-Meyer DE, Douglas CS, Thomas JJ, et al. Validation of a vitamin D specific questionnaire to determine vitamin D status in athletes. *Nutrients*. 2019;11(11):2732.
46. Sempos CT, Carter GD, Binkley NC. 25-Hydroxyvitamin D assays: standardization guidelines, problems, and interpretation. In: Feldman D, editor. *Vitamin D (Fourth Edition)*. Academic Press; 2018. pp. 939–57.
47. Nielson CM, Jones KS, Chun RF, et al. Free 25-hydroxyvitamin D: impact of vitamin D binding protein assays on racial-genotypic associations. *J Clin Endocrinol Metab*. 2016;101(5):2226–34.