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Vitamin D metabolite ratio as a marker of nutritional bone health in adolescents

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ABSTRACT

Vitamin D deficiency is a widespread public health issue among Saudi adolescents, posing significant risks to bone health and long-term well-being. Traditional markers such as serum 25-hydroxyvitamin D [25(OH)D] may not fully capture functional vitamin D status, particularly during critical growth periods such as adolescence. This study aimed to evaluate vitamin D metabolite levels and their association with calcium intake and bone health markers in Saudi adolescents, with a focus on the vitamin D metabolite ratio (VMR) as a potential indicator of functional vitamin D sufficiency. A cross-sectional analysis was conducted involving 949 (54 % females) Saudi adolescents. Serum levels of vitamin D metabolites, including 25(OH)D; 24,25-dihydroxyvitamin D [24,25(OH)₂D], and 25(OH)D₂, were measured using liquid chromatography–tandem mass spectrometry (LC-MS/MS). VMR was calculated as the ratio of 24,25(OH)₂D to 25(OH)D. Dietary calcium intake was assessed via validated questionnaires. Parathyroid hormone (PTH) and serum calcium levels were analyzed in a subset of participants. Vitamin D deficiency was highly prevalent, with 86.5 % of participants showing insufficient 25(OH)D levels. Over 93 % had low 24,25(OH)₂D, 99.7 % had undetectable 25(OH)D₂, and 74.9 % exhibited low VMR. VMR was significantly associated with serum and dietary calcium intake among girls, but not boys, suggesting potential sex-specific metabolic differences. Additionally, only VMR showed a significant association with serum calcium. An inverse association between PTH and both total vitamin D ($r = -0.28$, $p < 0.05$) and serum calcium ($r = -0.34$, $p < 0.01$) was observed in the subset analysis. In conclusion, VMR may serve as a useful sex-specific biomarker of functional vitamin D status in adolescents. The findings highlight the importance of addressing both intake and metabolic processing of vitamin D to optimize bone health during adolescence.

1. Introduction

The vitamin D metabolite ratio (VMR), defined as the ratio of 24,25-dihydroxyvitamin D₃ [24,25(OH)₂D] to 25-hydroxyvitamin D [25(OH)D], has emerged as a promising biomarker for assessing functional vitamin D status and skeletal health [1,2]. Unlike total serum 25-hydroxyvitamin D [25(OH)D], the conventional indicator of vitamin D sufficiency, VMR provides a more dynamic measure of vitamin D metabolism [3]. Specifically, it reflects the efficiency of 25(OH)D catabolism via the CYP24A1 enzyme pathway, which generates 24,25(OH)₂D as the predominant metabolite. Because this pathway is regulated by the active form of vitamin D [1,25(OH)₂D], the balance between 25(OH)D and 24,25(OH)₂D provides insight into how effectively vitamin D is metabolised, rather than simply indicating its storage pool [1,4,5]. This biological nuance has positioned VMR as a functional biomarker that may better capture clinically meaningful vitamin D activity than 25(OH)D

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alone.

In adult populations, a growing body of evidence demonstrates that VMR is more strongly associated with bone health outcomes than total 25(OH)D concentrations [5]. For instance, longitudinal studies have shown that lower VMR values predict declines in bone mineral density (BMD) at clinically important skeletal sites, including the hip, thoracic spine, and lumbar spine [6]. Moreover, reduced VMR has been linked to an increased incidence of osteoporotic fractures, whereas total serum 25 (OH)D concentrations have shown inconsistent associations with these outcomes [7–9]. Although 25(OH)D is the standard biomarker used to define vitamin D status, it reflects circulating levels rather than metabolic utilization [2,10]. VMR may serve as a more functional indicator of vitamin D metabolism because it reflects CYP24A1-mediated catabolism, the rate-limiting step in vitamin D breakdown [11]. In chronic kidney disease, a reduced VMR reflects impaired CYP24A1 activity and altered vitamin D catabolism [12–14]. Conversely, in cases of vitamin-D–mediated hypercalcemia due to CYP24A1 loss-of-function mutations, VMR is markedly suppressed and has diagnostic value [15]. In supplementation trials, VMR has demonstrated greater responsiveness to vitamin D dose changes compared with total 25(OH)D, reflecting dynamic metabolic turnover [16,17].

VMR therefore represents not only the availability of vitamin D substrate but also its metabolic processing, potentially offering better physiological insight than either metabolite measured independently. These findings signify that VMR integrates metabolic information beyond static measures of vitamin D storage, providing a physiologically relevant indicator of skeletal health. Prior work in adults also suggests that VMR may relate more closely to biological outcomes such as calcium homeostasis and parathyroid hormone (PTH) regulation [1,18]; however, these associations have not been investigated in adolescents, a developmental period characterized by rapid bone accrual and high vitamin D demand.

Despite these advances in adult cohorts, the utility and biological relevance of VMR in younger populations—particularly adolescents—remain poorly characterized. This represents an important gap in knowledge, as adolescence is a critical window for skeletal development [19]. During this period, rapid bone accrual occurs, with up to 90 % of peak bone mass being attained by the end of adolescence [20]. Peak bone mass serves as a key determinant of long-term skeletal resilience, influencing fracture risk and susceptibility to osteoporosis in later life. Inadequate vitamin D status during adolescence may therefore compromise this crucial process, with lasting implications for lifelong skeletal health [21]. Global health authorities, including the World Health Organization (WHO), have highlighted adolescence as a priority period for intervention [22].

Vitamin D deficiency is a recognized global public health concern, particularly in regions with high rates of limited sun exposure, conservative clothing practices, and dietary insufficiencies [23]. In Saudi Arabia and the broader Middle East, studies have consistently reported alarmingly high prevalence rates of vitamin D deficiency among adolescents, often exceeding 80–90 % [24,25]. The consequences of this deficiency extend beyond skeletal outcomes, as vitamin D insufficiency during adolescence has been associated with increased risks of metabolic dysfunction, impaired immunity, and cardiometabolic disorders later in life [26]. Traditionally, serum 25(OH)D concentration has been the gold standard for assessing vitamin D status. However, several limitations, one of which is that 25(OH)D provides only a static measure of circulating vitamin D stores and does not account for individual variability in vitamin D metabolism, and that it may not fully capture the functional adequacy of vitamin D in contexts where metabolism or responsiveness is altered, such as during growth, puberty, or chronic disease states. These limitations signify the need for more functional markers, such as VMR, which can reflect the efficiency of vitamin D metabolic pathways and potentially improve the identification of individuals at risk.

Although direct investigations of VMR in adolescents are currently lacking, the compelling evidence from adult populations signifies the need to explore its relevance in younger cohorts. Furthermore, identifying early biomarkers of functional vitamin D sufficiency may help stratify risk and guide preventive strategies at a population level. This is particularly critical in countries like Saudi Arabia, where the prevalence of deficiency is high and the burden of bone-related disorders is projected to increase. Therefore, the present study aims to address this critical gap by evaluating vitamin D metabolite levels, including VMR, in a large cohort of Saudi adolescents.

2. Methods

2.1. Subjects, vitamin D supplementation, and clinical assessment

Clinical data from the present cross-sectional study were taken from the biochemical Osteomalacia database of the Chair for Biomarkers of Chronic Diseases (CBCD) in King Saud University (KSU), Riyadh, Kingdom of Saudi Arabia (KSA) [27,28]. In brief, the biochemical Osteomalacia project was done in collaboration with the Saudi Ministry of Education, where healthy Saudi adolescents aged 12–17 years from over 60 preparatory and secondary schools in Riyadh were recruited to assess the prevalence of biochemical Osteomalacia in KSA and its association with dietary vitamin and mineral intake. Eligible participants were free of acute medical conditions, physically and mentally fit for school activities, and provided informed consent/assent, fasting blood samples, and dietary recall data. Exclusion criteria included non-Saudis, lack of consent, medical unfitness, or non-Saudi nationality.

2.2. Data collection

Demographic and anthropometric information were taken from the database. Biochemical data (fasting glucose, HbA1c, lipids, alkaline phosphatase, and serum calcium) as well as dietary calcium were assessed previously [27,28]. Circulating parathyroid hormone (PTH) and total vitamin D were assessed at CBCD (a DEQAS (vitamin D External Quality Assessment Scheme) participating laboratory), KSU, Riyadh, KSA. Serum PTH measurement was analyzed using a Liaison 1–84 PTH immunoassay on Liaison XL analyzer (DiaSorin, Italy). Serum total VitD levels were measured using a chemiluminescent immunoassay (DiaSorin, Saluggia, Italy), with inter-assay and intra-assay coefficients of variation of 10.6 % and 5.4 %, respectively, and a lower detection limit of 10 nmol/L.

2.3. Vitamin D metabolites

Assessment of circulating vitamin D metabolites was done at the Department of Clinical Chemistry, CIRM, University of Liège, Belgium. Serum 25-hydroxyvitamin D [25(OH)D], vitamin D_2, and 24,25-dihydroxyvitamin D [24,25$(OH)_2$D] were quantified using a validated and Center for Disease Control and Prevention (CDC) certified liquid chromatography–tandem mass spectrometry (LC–MS/MS) method [9]. The VMR was calculated using the formula: VMR = [24,25$(OH)_2$D/25(OH)D] × 100. For the present study, VD deficiency cut-offs were defined at 25(OH)D < 50nmol/L, 24,25(OH)VD < 3.0nmo/L, and VMR < 4 % based on proposed cut-offs [29–31].

2.4. Data analysis

Data were analyzed using SPSS (version 22, Chicago, IL, USA). Continuous data were presented as mean ± standard deviation (SD) for Gaussian variables, and non-Gaussian variables were presented in median (25th and 75th percentiles). Categorical data were presented as frequencies (n) and percentages (%), and association was analyzed by the Chi-square and Fisher's Exact test. All continuous variables were checked for normality using the Kolmogorov-Smirnov test. Non-Gaussian variables were log-transformed prior to parametric analysis.

Independent Student T-Test and Mann-Whitney *U* test were performed to compare mean and median differences in Gaussian and Non-Gaussian variables. Spearman's and Pearson's correlation analyses were performed. A p-value < 0.05 was considered statistically significant.

3. Results

3.1. Participant characteristics

The clinical characteristics of the 949 adolescents (513 girls, 436 boys) were compared according to vitamin D status in Table 1. The overall prevalence of Vitamin D deficiency (<50 nmol/L) was 86.5 % (n = 821). There were no significant differences in BMI and other anthropometrics, with the exception of height, which was significantly lower in the VD-deficient group (p = 0.002). VD-deficient participants also had a significantly higher diastolic blood pressure ($p = 0.04$) than their VD-sufficient counterparts, but did not differ significantly in most biochemical parameters. As expected, all measured vitamin D metabolites were markedly lower in the VD-deficient group, including 25 (OH) D, 24,25$(OH)_2$D, vitamin D_2, and VMR, all with p-values < 0.01. Dietary Ca was also significantly higher in the sufficient group than the VD-deficient participants (p = 0.007).

3.2. Prevalence of vitamin D metabolites

Table 2 shows the prevalence of vitamin D metabolites based on established cut-offs stratified by vitamin D status [29–31]. The prevalence of low 24,25(OH)2D (<3 nmol/L) was significantly higher in the deficient group (97.9 %, n = 804) as compared to the sufficient group (62.5 %, n = 80) ($p < 0.001$), and nearly all participants (~99 %) had vitamin D_2 levels below LOQ < 1.8 nmol/L. Low VMR (≤ 4 %) was present in 81.6 % (n = 670) of VD-deficient participants, and this was significantly higher than in sufficient participants, where low VMR was present in only 32 % (n = 41) ($p < 0.001$). The proportion of participants with normal 24,25$(OH)_2$D or VMR was significantly greater among those with sufficient 25(OH)D levels ($p < 0.001$ for both; Table 2).

Table 1
Clinical characteristics of subjects.

Parameters	Deficient < 50 (nmol/l)	Sufficient ≥ 50 (nmol/l)	*p*-Value
N (F/M)	821 (468/353)	128 (45/83)	
Age (years)	14.9 ± 1.7	14.8 ± 1.6	0.81
Height (cm)	156.6 ± 10.3	159.7 ± 9.4	**0.002**
Weight (kg)	57.8 ± 17.4	57.4 ± 13.7	0.79
BMI (kg/m^2)	23.37 ± 6.1	22.32 ± 4.3	0.062
Waist (cm)	70.6 ± 18.6	69.4 ± 17.4	0.52
Hips (cm)	85.3 ± 21.7	82.1 ± 20.6	0.12
WHR	0.84 ± 0.3	0.85 ± 0.1	0.69
Systolic BP (mmHg)	117.9 ± 14.4	115.4 ± 14.1	0.08
Diastolic BP (mmHg)	71.7 ± 11.9	69.4 ± 9.9	**0.04**
Glucose (mmol/l)	5.28 ± 1.2	5.17 ± 0.9	0.34
HbA1C (%)	5.09 ± 0.8	5.11 ± 0.9	0.81
Biochemical Parameters			
Total Cholesterol (mmol/l)	4.47 ± 0.9	4.64 ± 1.2	0.06
HDL-Cholesterol (mmol/l)	1.06 ± 0.3	1.05 ± 0.4	0.75
Triglycerides (mmol/l)	1.12 ± 0.6	1.17 ± 0.5	0.36
Calcium (mmol/l)	2.48 ± 0.4	2.51 ± 0.4	0.36
Dietary Calcium (mg/day)	303 (129.8–764)	465.9 (254.7–909)	**0.007**
Phosphorous (mmol/l)	1.53 ± 0.4	1.57 ± 0.4	0.26
ALP (U/l)	62.31 ± 36.7	63.34 ± 33.4	0.77
Vitamin D metabolites			
25 (OH)D (nmol/l)[a]	28 (21−37)	59 (53−67)	**< 0.001**
24, 25 $(OH)_2$D (nmol/l)[a]	0.67 (0.33–1.22)	2.59 (1.92–3.44)	**< 0.001**
VTD D2 (nmol/l)[a]	2.26 ± 0.2	2.44 ± 1.6	**0.007**
VMR	2.36 (1.5–3.6)	4.72 (3.5–5.9)	**< 0.001**
Total Vit D (nmol/l)[b]	29.4 (21.7–38.6)	56 (47.9–66.1)	**< 0.001**

Note: Data presented as Frequency N and N (%) where applicable. The superscripts 'a' and 'b' represent that the vitamin D metabolites were measured using LC–MS/MS and chemiluminescence assays respectively.

3.3. Associations of VMR with clinical, biochemical, and dietary parameters

Overall, VMR showed weak but statistically significant positive correlations with serum calcium ($r = 0.063$, $p < 0.05$), serum phosphorus ($r = 0.114$, $p < 0.01$), and dietary calcium intake ($r = 0.124$, $p < 0.01$) (Table 3). VMR was inversely associated with BMI ($r = –0.105$, $p < 0.01$) and fasting glucose ($r = –0.088$, $p < 0.01$). Sex-stratified analyses revealed distinct patterns. In boys, VMR correlated inversely with BMI ($r = –0.204$, $p < 0.01$), fasting glucose ($r = –0.169$, $p < 0.01$), HbA1c ($r = –0.108$, $p < 0.05$), and alkaline phosphatase ($r = –0.225$, $p < 0.01$), while showing a positive association with serum phosphorus ($r = 0.186$, $p < 0.01$). In girls, VMR was positively correlated with HDL cholesterol ($r = 0.123$, $p < 0.01$) and dietary calcium intake ($r = 0.223$, $p < 0.01$), but inversely associated with waist-to-hip ratio ($r = –0.101$, $p < 0.05$). Notably, serum calcium correlated significantly with VMR in the overall sample but not within sex-specific subgroups. We have performed a statistical comparison of correlation analysis and added two comparative correlation tables as supplementary tables (S1 and S2) showing correlation coefficients for VMR and total 25(OH)D (both measured by LC-MS/MS) vs. serum calcium and dietary calcium intake in the total samples and also in the subset group with PTH measurement. This allows direct comparison of the associations.

3.4. Subset analysis with PTH measurements

In the subset of participants (n = 64) with PTH data (Table 4), PTH was inversely correlated with serum calcium overall ($r = –0.34$, $p < 0.01$) and in both deficient ($r = –0.54$, $p < 0.05$) and sufficient ($r = –0.44$, $p < 0.05$) vitamin D status groups. PTH also showed inverse associations with Total Vit D in the overall sample ($r = –0.28$, $p < 0.05$) and in deficient participants ($r = –0.39$, $p < 0.05$). Overall, dietary calcium intake correlated positively with VMR ($r = 0.12$, $p < 0.01$), 24,25$(OH)_2$D ($r = 0.17$, $p < 0.01$), and 25(OH)D ($r = 0.17$, $p < 0.01$). Similar trends were observed within the deficient group, where dietary calcium was associated with 25(OH)D ($r = 0.15$, $p < 0.01$) and total vit D ($r = 0.11$, $p < 0.05$). In girls, dietary calcium was positively correlated with VMR ($r = 0.22$, $p < 0.05$), 24,25$(OH)_2$D ($r = 0.25$, $p < 0.01$), and 25(OH)D ($r = 0.22$, $p < 0.05$), but no such relationships were detected in boys. Serum calcium showed weak but significant positive correlations with VMR ($r = 0.06$, $p < 0.05$) and 24,25$(OH)_2$D ($r = 0.07$, $p < 0.05$) in the total sample, and with total vit D in girls only ($r = 0.17$, $p < 0.05$). Interestingly, VMR didn't show a significant correlation with PTH either in overall or in the subgroups (deficient, sufficient, boys, or girls), reflecting metabolic processing rather than PTH-driven regulation. These findings further support a modest but sex-specific link between dietary calcium intake and vitamin D metabolite status, particularly in adolescent girls.

4. Discussion

In this large cross-sectional study of Saudi adolescents, we found an alarmingly high prevalence of vitamin D deficiency, with more than 85 % of participants exhibiting insufficient serum 25(OH)D levels. The findings of this study highlight the urgent need to address vitamin D deficiency among Saudi adolescents, a significant public health concern with long-term consequences for bone health and overall well-being. Importantly, our data show that low levels of the VMR were also widespread, affecting nearly three-quarters of the cohort, and that VMR demonstrated stronger associations with serum calcium and dietary calcium intake than 25(OH)D concentrations, particularly among girls. These findings extend the evidence base for VMR, previously studied primarily in adult populations, by demonstrating its potential utility as a functional biomarker of vitamin D sufficiency in adolescents. To our

Table 2
Associations of 25 (OH)D deficiency with LOQ and Function VMR.

	Types	25 (OH) Vitamin D		P-Value
		Deficient < 50 (nmol/l) 821	Sufficient ≥ 50 (nmol/l) 128	
24, 25 $(OH)_2D$ (nmol/l)	Normal ≥ 3 nmol/l	17 (2.1)	48 (37.5)	< 0.001
	Low < 3 nmol/l	804 (97.9)	80 (62.5)	
	LOQ < 0.26 (Yes)	406 (49.5)	9 (7.0)	< 0.001
VTD D2 (nmol/l)	LOQ< 1.8 (Yes)	820 (99.9)	126 (98.4)	0.04
25 (OH)D (nmol/l)	Deficient ≤ 50 nmol/l	764 (93.1)	43 (33.6)	< 0.001
	Sufficient >50 nmol/l	57 (6.9)	85 (66.4)	
VMR	Normal VMR > 4	151 (18.4)	87 (68.0)	< 0.001
	Low VMR ≤ 4	670 (81.6)	41 (32.0)	
Total Vit D (nmol/l)	Deficient ≤ 50 nmol/l	764 (93.1)	45 (35.2)	< 0.001
	sufficient> 50.1–74.5 nmol/l	57 (6.9)	83 (64.8)	
	LOQ<0.98 (Yes)	0	0	

Note: Data presented as N (%). *P*-value is significant at 0.05 and 0.01 levels.

Table 3
Correlation analysis of VMR with other parameters.

Parameters	All	Boys	Girls
N	949	436	513
Age (Years)	0.03	-0.01	0.08
BMI (kg/m^2)	**-0.105****	**-0.204****	-0.06
WHR	0.05	-0.06	**-0.101***
Systolic BP (mmHg)	-0.04	-0.08	-0.05
Diastolic BP (mmHg)	-0.04	-0.05	0.01
Glucose (mmol/l)	**-0.088****	**-0.169****	-0.06
HbA1C (%)	-0.01	**-0.108***	-0.04
Biochemical Parameters			
Total Cholesterol (mmol/l)	0.05	0.03	0.08
HDL-Cholesterol (mmol/l)	0.06	0.04	**0.123****
Triglycerides (mmol/l)	0.00	-0.05	-0.03
Calcium (mmol/l)	0.063*	0.00	**0.09***
Phosphorous (mmol/l)	**0.114****	**0.186****	-0.03
ALP (U/l)	-0.04	**-0.225****	0.00
Dietary Ca (mg/day)	**0.124****	-0.05	**0.223****

Note: Data presented the R coefficient of determination. * and ** represented p-value significant at 0.05 & 0.01 level.

knowledge, this is one of the first studies to examine vitamin D metabolites and VMR in a large adolescent cohort from the Middle East, highlighting both the magnitude of deficiency and the value of exploring metabolic markers beyond traditional 25(OH)D measurements.

The findings of this study are particularly alarming considering that adolescence is a critical period for bone mass accrual, which plays a foundational role in determining bone health in later life. The World Health Organization's Decade of Healthy Ageing (2021–2030) emphasizes the importance of early-life interventions to support musculoskeletal health across the lifespan [27]. Failure to achieve optimal peak bone mass during this developmental window is a well-established risk factor for osteoporosis and fractures in later adulthood [28,29]. Booth et al. (2024) similarly found that low vitamin D levels in young orthopaedic trauma patients were associated with reduced bone mineral density, further reinforcing the critical importance of maintaining sufficient vitamin D during youth [32].

Support for the relevance of vitamin D to bone health in adolescents comes from several population-based studies. For example, the Korea National Health and Nutrition Examination Survey (KNHANES), which included 1063 adolescents aged 12–18 years, demonstrated significant positive associations between serum 25(OH)D levels and BMD Z-scores at multiple skeletal sites, including the lumbar spine, total femur, and femoral neck [7]. Importantly, these associations persisted even after adjusting for potential confounders such as BMI, dietary calcium intake, physical activity, and body composition. These findings suggest that vitamin D sufficiency plays a meaningful role in supporting bone health during adolescence, reinforcing the importance of monitoring and addressing deficiency during this developmental stage. Further insights are provided by studies in young adults, which highlight the complex relationships between vitamin D metabolites, bone health, and body composition [33,34]. One investigation involving males with a mean age of 22.6 years found that serum 25(OH)D levels were positively associated with BMD across all measured skeletal sites and with lean body mass [8]. In contrast, some studies show that $VitD_2$ levels are negatively associated with total body and spine BMD, raising the possibility that different vitamin D metabolites exert differential effects on bone outcomes [35]. Other findings emphasize the sex- and site-specific determinants of bone health [36]. These findings highlight the multifactorial influences on bone health during early life and the potential for

Table 4
Correlations of PTH and dietary calcium with VD metabolites in a subset with PTH measurements.

Parameters	All			Deficient			Sufficient			Boys			Girls		
	PTH	Diet Ca	Ca	PTH	Diet Ca	Ca	PTH	Diet Ca	Ca	PTH	Diet Ca	Ca	PTH	Diet Ca	Ca
PTH#	1			1			1			1			1		
Dietary Ca (mg/day)	0.06	1		0.03	1		-0.19	1		0.04	1		0.07	1	
Calcium (mmol/l)	**-0.34****	-0.03	1	-0.22	-0.03	1	**-0.54***	0.02	1	**-0.44***	-0.03	1	-0.30	-0.10	1
VMR#	0.03	**0.12****	**0.06***	0.17	0.08	0.06	-0.06	0.13	-0.04	0.13	-0.05	0.002	-0.07	**0.22***	0.06
24, 25 $(OH)_2D$ (nmol/l)[a]#	0.03	**0.17****	**0.07***	0.14	**0.12***	**0.071***	-0.14	0.08	-0.10	0.12	-0.04	-0.01	0.05	**0.25****	0.03
25 (OH) D (nmol/l)[a]#	**-0.28***	**0.17****	0.06	**-0.388***	**0.15****	0.05	-0.11	-0.21	0.02	-0.22	-0.01	0.03	-0.40	**0.22****	-0.01
Total Vit D[b] (nmol/l) #	0.02	**0.18****	0.05	0.06	**0.11***	0.06	-0.12	0.04	**-0.19***	0.06	0.02	-0.02	0.23	**0.17****	-0.02

Note: Data presented the Coefficient (r). * and ** represent P-value significant at 0.01 and 0.05 levels. # denotes the variables were log-transformed before analysis. The superscripts 'a' and 'b' represent that the vitamin D metabolites were measured using LC–MS/MS and chemiluminescence assays respectively.

differential effects of vitamin D metabolites between sexes and skeletal sites.

In our study, VMR exhibited a modest but statistically significant association with both serum and dietary calcium intake in girls, suggesting a potential sex-specific role of VMR as a nutritional marker for bone metabolism during adolescence. This aligns with earlier findings that demonstrated gender differences in vitamin D metabolism and calcium utilization, potentially due to hormonal influences and variations in body composition [37]. Notably, all vitamin D metabolites, excluding $VitD_2$, were significantly associated with dietary calcium intake in girls but not in boys, a pattern echoed by us earlier, where we reported significant sex-specific associations between calcium intake and bone mineralization markers in Saudi adolescents [38]. Together, these observations point to complex interactions between sex hormones, calcium metabolism, and vitamin D pathways during adolescence, suggesting that girls may be particularly sensitive to nutritional deficiencies or imbalances.

VMR has been proposed as a more informative indicator of vitamin D status than total 25(OH)D because it incorporates both substrate availability [25(OH)D] and its metabolic conversion via the CYP24A1 pathway to $24,25(OH)_2D$ [39,40]. Unlike total 25(OH)D, which reflects the storage pool, VMR reflects the rate at which vitamin D is metabolized and therefore provides insight into functional vitamin D sufficiency [40]. The present study also demonstrates that VMR exhibits stronger physiological associations with markers of bone mineral homeostasis than 25(OH)D alone. VMR correlated significantly with serum calcium and dietary calcium intake (in girls), whereas total 25(OH)D did not. These findings support the premise that VMR better reflects functional vitamin D activity, consistent with the fact that VMR integrates both the substrate availability and metabolic conversion via CYP24A1. This is in line with reports from adult data of the SarcoPhAge cohort, where VMR was proposed as a more reliable biomarker of functional vitamin D status than 25(OH)D alone [32,41,42]. Our findings suggest that this may also apply to adolescents, although further research is needed to confirm its utility in younger populations. The implication is that VMR may serve as a more sensitive indicator of the functional adequacy of vitamin D metabolism and its role in maintaining calcium homeostasis during growth, compared with static measures of 25(OH)D alone.

In the subset of participants with PTH measurements, VMR remained associated with serum calcium, whereas 25(OH)D did not, further reinforcing its biological relevance. We initially hypothesized that VMR would be inversely associated with PTH, given that PTH increases under conditions of inadequate vitamin D activity to maintain calcium homeostasis. However, while expected inverse correlations were observed between PTH and both serum calcium and 25(OH)D, no correlation was observed between PTH and VMR. The absence of a significant correlation with PTH suggests that VMR reflects vitamin D metabolic processing rather than PTH-driven feedback, aligning with previous observations in adults [43,44]. Although we initially considered insufficient power (because of low sample size for this subset analysis) as a possible explanation for the lack of association between VMR and PTH, further review suggests that this is unlikely. The same subset (~64 participants) demonstrated significant inverse correlations between PTH and both 25(OH)D and serum calcium, indicating adequate power to detect physiologically meaningful associations. Instead, the lack of correlation appears to be due to restricted variability in vitamin D-related metabolites within this subgroup; participants displayed uniformly low levels of 25(OH)D and $24,25(OH)_2D$, resulting in a narrow VMR range. Also, VMR was derived from LC–MS/MS quantification of $24,25(OH)_2D$ and 25(OH)D, whereas PTH and serum calcium were measured using automated immunoassay platforms. Because LC–MS/MS detects low circulating metabolites with high analytical precision, the limited dispersion in values may mask associations with PTH. This further suggests that VMR may reflect metabolic vitamin D utilization independent of PTH-mediated feedback. While the strength of correlations observed was modest, this is expected in adolescents due to rapid skeletal turnover, hormonal influences, and variability in calcium intake during growth. A longitudinal follow-up may be needed to confirm whether VMR predicts changes in bone mineral density over time.

Beyond biological factors, environmental and lifestyle determinants also likely contribute to the high prevalence of deficiency. Limited sun exposure due to climate, cultural clothing practices, low physical activity, and limited consumption of fortified foods may exacerbate the problem. Socioeconomic disparities and indoor-oriented lifestyles further compound the challenge [45]. These contextual factors highlight that vitamin D deficiency among Saudi adolescents is not only a biomedical issue but also a sociocultural and environmental phenomenon, requiring comprehensive and context-specific interventions.

The findings in this study signify the need for targeted public health strategies. Population-level measures such as vitamin D supplementation programs, school-based nutrition initiatives, and widespread food fortification policies could help mitigate vitamin D deficiency. For clinical practice, evaluating VMR alongside 25(OH)D could offer a more nuanced understanding of vitamin D status and its functional implications, allowing earlier identification of at-risk adolescents. In particular, the potential of VMR as a sensitive biomarker for bone-related outcomes, especially among females, warrants exploration in larger, prospective, and ideally longitudinal studies.

This study benefits from a large adolescent sample and the comprehensive assessment of multiple vitamin D metabolites beyond the conventional 25(OH)D measure. The inclusion of sex-stratified analyses and integration of anthropometric, biochemical, and dietary data further strengthens its contribution, providing in-depth insights into vitamin D metabolism during adolescence. Nevertheless, several limitations should be acknowledged. The cross-sectional design precludes inference about causality, and the availability of PTH data in only a small subset restricts the scope of secondary analyses. Additionally, key determinants such as sun exposure, physical activity, socioeconomic status, and seasonality were not assessed, which may confound associations. Most observed correlations were modest despite statistical significance, suggesting the need for cautious interpretation. Furthermore, reliance on self-reported dietary intake introduces potential recall bias, and the recruitment of participants from a single region limits the generalizability of the findings to all Saudi adolescents.

5. Conclusion

In conclusion, our findings provide new evidence that VMR, a functional marker of vitamin D metabolism, may have clinical utility in adolescents, particularly in relation to calcium status and sex-specific metabolic patterns. With vitamin D deficiency remaining highly prevalent in Saudi youth, there is an urgent need for coordinated strategies to improve vitamin D intake and optimize bone health during this critical life stage. By integrating traditional markers such as 25(OH)D with novel metabolic indices like VMR, clinicians and policymakers may be better equipped to address the challenge of vitamin D deficiency and to safeguard skeletal health across the lifespan.

CRediT authorship contribution statement

Yousef Al-Saleh: Writing – review & editing, Methodology, Investigation. **Kaiser Wani:** Writing – original draft, Methodology, Investigation. **Shaun Sabico:** Writing – review & editing, Investigation, Conceptualization. **Amal M. Alenad:** Writing – original draft, Supervision. **Leena T. Fakhurji:** Writing – review & editing, Methodology, Investigation. **Abdullah M. Alnaami:** Writing – review & editing, Methodology, Investigation. **Malak N.K. Khattak:** Writing – review & editing, Formal analysis. **Al-Daghri Nasser:** Writing – review & editing, Funding acquisition, Conceptualization. **Etienne Cavalier:** Writing – review & editing, Project administration, Conceptualization. **Jean-Yves Reginster:** Writing – review & editing, Supervision, Investigation.

Majed S. Alokail: Writing – review & editing, Validation, Supervision.

Statement of human rights

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008. Written informed consent was obtained from each participant in this study. Ethical approval was obtained from the Institutional Review Board (IRB) of the College of Medicine, KSU (E-21–6095; approved 18 January 2019, amended 7 April 2022).

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jsbmb.2025.106918.

References

[1] J. Aloia, M. Fazzari, A. Shieh, R. Dhaliwal, M. Mikhail, A.N. Hoofnagle, L. Ragolia, The vitamin D metabolite ratio (VMR) as a predictor of functional biomarkers of bone health, Clin. Endocrinol. 86 (2017) 674–679.
[2] M. Herrmann, C.-J.L. Farrell, I. Pusceddu, N. Fabregat-Cabello, E. Cavalier, Assessment of vitamin D status–a changing landscape, Clin. Chem. Lab. Med. (CCLM) 55 (2017) 3–26.
[3] J.C. Tang, S. Jackson, N.P. Walsh, J. Greeves, W.D. Fraser, The dynamic relationships between the active and catabolic vitamin D metabolites, their ratios, and associations with PTH, Sci. Rep. 9 (2019) 6974.
[4] C. Ginsberg, A.N. Hoofnagle, R. Katz, J. Hughes-Austin, L.M. Miller, J.O. Becker, S. B. Kritchevsky, M.G. Shlipak, M.J. Sarnak, J.H. Ix, The vitamin D metabolite ratio is associated with changes in bone density and fracture risk in older adults, J. Bone Miner. Res. 36 (2020) 2343–2350.
[5] C. Ginsberg, T. Blackwell, J.H. Cheng, O.A. Potok, J.A. Cauley, K.E. Ensrud, S. Hsu, D.M. Kado, E. Orwoll, P.M. Cawthon, The vitamin D metabolite ratio is associated with volumetric bone density in older men, J. Clin. Endocrinol. Metab. 109 (2024) e513–e521.
[6] A. Darelid, M. Nilsson, J.M. Kindblom, D. Mellström, C. Ohlsson, M. Lorentzon, Bone turnover markers predict bone mass development in young adult men: a five-year longitudinal study, J. Clin. Endocrinol. Metab. 100 (2015) 1460–1468.
[7] K. Song, A. Kwon, H.W. Chae, J. Suh, H.S. Choi, Y. Choi, J.S. Oh, H.-S. Kim, Vitamin D status is associated with bone mineral density in adolescents: findings from the Korea National Health and Nutrition Examination Survey, Nutr. Res. 87 (2021) 13–21.
[8] M. Högström, A. Nordström, P. Nordstr, öm, Relationship between vitamin D metabolites and bone mineral density in young males: a cross-sectional and longitudinal study, Calcif. Tissue Int. 79 (2006) 95–101.
[9] A. Kopiczko, M. Łopuszańska-Dawid, K. Gryko, Bone mineral density in young adults: the influence of vitamin D status, biochemical indicators, physical activity and body composition, Arch. Osteoporos. 15 (2020) 45.
[10] N. Alonso, S. Zelzer, G. Eibinger, M. Herrmann, Vitamin D metabolites: analytical challenges and clinical relevance, Calcif. Tissue Int. 112 (2023) 158–177.
[11] G. Jones, M. Kaufmann, Diagnostic aspects of vitamin D: clinical utility of vitamin D metabolite profiling, J. Bone Miner. Res. 5 (2021) e10581.
[12] C. Ginsberg, L.R. Zelnick, G.A. Block, G.M. Chertow, M. Chonchol, A. Hoofnagle, B. Kestenbaum, I.H. de Boer, Differential effects of phosphate binders on vitamin D metabolism in chronic kidney disease, Nephrol. Dial. Transpl. 35 (2020) 616–623.
[13] S. Lee, H.J. Chung, S. Jung, H.N. Jang, S.H. Chang, H.J. Kim, M.C. Cho, 24,25-dihydroxy vitamin D and vitamin D metabolite ratio as biomarkers of vitamin d in chronic kidney disease, Nutrients 15 (2023).
[14] M.E. Turner, T.S. Rowsell, M. Kaufmann, P.A. Norman, K. Neville, S. Sarabia, C. A. White, M. Petkovich, G. Jones, M.A. Adams, R.M. Holden, The 1,24,25(OH)(3)D (3) metabolite in clinical and experimental CKD: Impact of calcitriol treatment, J. Steroid Biochem Mol. Biol. 226 (2023) 106207.
[15] M. Kaufmann, J.C. Gallagher, M. Peacock, K.-P. Schlingmann, M. Konrad, H. F. DeLuca, R. Sigueiro, B. Lopez, A. Mourino, M. Maestro, Clinical utility of simultaneous quantitation of 25-hydroxyvitamin D and 24, 25-dihydroxyvitamin D by LC-MS/MS involving derivatization with DMEQ-TAD, J. Clin. Endocrinol. Metab. 99 (2014) 2567–2574.
[16] S. Zelzer, A. Meinitzer, D. Enko, M.H. Keppel, M. Herrmann, V. Theiler-Schwetz, C. Trummer, L. Schmitt, A. Tomaschitz, P. Sadoghi, J. Dierkes, P. Pludowski, A. Zittermann, W. März, S. Pilz, Classification of vitamin D status based on vitamin D metabolism: a randomized controlled trial in hypertensive patients, Nutrients 16 (2024).
[17] V. Francic, S.R. Ursem, N.F. Dirks, M.H. Keppel, V. Theiler-Schwetz, C. Trummer, M. Pandis, V. Borzan, M.R. Grübler, N.D. Verheyen, W. März, A. Tomaschitz, S. Pilz, A.C. Heijboer, B. Obermayer-Pietsch, The Effect of Vitamin D supplementation on its metabolism and the vitamin D metabolite ratio, Nutrients 11 (2019).
[18] G. Lombardi, E. Ziemann, G. Banfi, S. Corbetta, Physical activity-dependent regulation of parathyroid hormone and calcium-phosphorous metabolism, Int. J. Mol. Sci. 21 (2020) 5388.
[19] M.E. Lewis, Exploring adolescence as a key life history stage in bioarchaeology, Am. J. Biol. Anthropol. 179 (2022) 519–534.
[20] T. Chevalley, R. Rizzoli, Acquisition of peak bone mass, Best. Pract. Res. Clin. Endocrinol. Metab. 36 (2022) 101616.
[21] P. Proia, A. Amato, P. Drid, D. Korovljev, S. Vasto, S. Baldassano, The impact of diet and physical activity on bone health in children and adolescents, Front. Endocrinol. 12 (2021) 704647.
[22] D. Hargreaves, E. Mates, P. Menon, H. Alderman, D. Devakumar, W. Fawzi, G. Greenfield, W. Hammoudeh, S. He, A. Lahiri, Strategies and interventions for healthy adolescent growth, nutrition, and development, Lancet 399 (2022) 198–210.
[23] N.M. Al-Daghri, S.D. Hussain, M.G. Ansari, M.N. Khattak, N. Aljohani, Y. Al-Saleh, M.Y. Al-Harbi, S. Sabico, M.S. Alokail, Decreasing prevalence of vitamin D deficiency in the central region of Saudi Arabia (2008-2017), J. Steroid Biochem. Mol. Biol. 212 (2021) 105920.
[24] M.S. Alhamed, F. Alharbi, A. Al Joher, S. Dhahry, A.A. Fallatah, O.H. Alanazi, J. M. Almutiri, S.S. Albaradie, B.A.A. Al Enezi, M.S. Albishi, Vitamin D deficiency in children and adolescents in Saudi Arabia: a systematic review, Cureus 16 (2024).
[25] N.M. Al-Daghri, Vitamin D in Saudi Arabia: prevalence, distribution and disease associations, J. Steroid Biochem. Mol. Biol. 175 (2018) 102–107.
[26] J.B. Daniel, P.R. de Farias Costa, M. Pereira, A.M. Oliveira, Vitamin D deficiency and cardiometabolic risk factors in adolescents: systematic review and meta-analysis, Rev. Endocr. Metab. Disord. 23 (2022) 995–1010.
[27] N.M. Al-Daghri, S. Yakout, S. Sabico, K. Wani, S.D. Hussain, N. Aljohani, S. Uday, W. Högler, Establishing the prevalence of osteomalacia in Arab adolescents using biochemical markers of bone health, Nutrients 14 (2022) 5354.
[28] N.M. Al-Daghri, S. Sabico, K. Wani, S.D. Hussain, S. Yakout, N. Aljohani, S. Uday, W. Högler, Association of bone mineralization markers with dietary nutrient intake in adolescents with and without biochemical osteomalacia, Front. Nutr. 10 (2023) 1206711.
[29] A. Ladang, A.-S. Gendebien, S. Kovacs, C. Demonceau, C. Beaudart, S. Peeters, M. S. Alokail, N.M. Al-Daghri, C. Le Goff, J.-Y. Reginster, Investigation of the Vitamin D Metabolite Ratio (VMR) as a Marker of Functional Vitamin D Deficiency: findings from the SarcoPhAge Cohort, Nutrients 16 (2024) 3224.
[30] A. LADANG, O. ROUSSELLE, L. Huyghebaert, A.-C. BEKAERT, S. KOVACS, C.L. E. GOFF, E. CAVALIER, 24, 25 (OH) 2D as a marker of vitamin d deficiency in children: a retrospective, Euromedlab 2019 (2019).
[31] J.C. Tang, H. Nicholls, I. Piec, C.J. Washbourne, J.J. Dutton, S. Jackson, J. Greeves, W.D. Fraser, Reference intervals for serum 24, 25-dihydroxyvitamin D and the ratio with 25-hydroxyvitamin D established using a newly developed LC–MS/MS method, J. Nutr. Biochem. 46 (2017) 21–29.
[32] M. Booth, K. Sabacinski, C. Watkins, E. Butcho, E. Kramer, L. Meadows, M. A. Bramer, Vitamin D levels and bone mineral density: a prospective cross-sectional analysis of young orthopedic trauma patients at a rural United States trauma center, J. Trauma Inj. 37 (2024) 276.
[33] I. Sioen, E. Lust, S. De Henauw, L. Moreno, D. Jiménez-Pavón, Associations between body composition and bone health in children and adolescents: a systematic review, Calcif. Tissue Int. 99 (2016) 557–577.
[34] K.V. Giudici, R.M. Fisberg, D.M.L. Marchioni, B.S.E. Peters, L.A. Martini, Crosstalk between bone and fat tissue: associations between vitamin D, osteocalcin, adipokines, and markers of glucose metabolism among adolescents, J. Am. Coll. Nutr. 36 (2017) 273–280.
[35] C. Li, P. Chen, X. Duan, J. Wang, B. Shu, X. Li, Q. Ba, J. Li, Y. Wang, H. Wang, Bioavailable 25 (OH) D but not total 25 (OH) D is an independent determinant for bone mineral density in Chinese postmenopausal women, EBioMedicine 15 (2017) 184–192.
[36] S. Ouyang, D. Cao, Y. Yin, F. Ma, G. Ren, Sex differences in physical activity dose-response effects on site-specific bone mineral density during childhood and adolescence, Sci. Rep. 15 (2025) 17003.
[37] K. Zhu, W.H. Oddy, P. Holt, W.C.S. Ping-Delfos, J. Mountain, S. Lye, C. Pennell, P. H. Hart, J.P. Walsh, Tracking of vitamin D status from childhood to early

adulthood and its association with peak bone mass, Am. J. Clin. Nutr. 106 (2017) 276–283.
[38] K.J. Segheto, M. Pereira, D.C.Gd Silva, C.Jd Carvalho, F.R. Massardi, A. M. Kakehasi, L.L. Juvanhol, G.Z. Longo, Vitamin D and bone health in adults: a systematic review and meta-analysis, Ciência SaúDe. Coletiva 26 (2021) 3221–3244.
[39] L.H. Ahmed, A.E. Butler, S.R. Dargham, A. Latif, O.M. Chidiac, S.L. Atkin, C. Abi Khalil, Vitamin D3 metabolite ratio as an indicator of vitamin D status and its association with diabetes complications, BMC Endocr. Disord. 20 (2020) 161.
[40] S. Sabico, N. Al-Daghri, A. Alenad, Y. Al-Saleh, M.N. Khattak, K. Wani, A. Alnaami, J.-Y. Reginster, M.S. Alokail, E. Cavalier, Sexual Dimorphism in LC-MS/MS-Based Vitamin D Metabolite Profiling and Nutrition-Acquired Biochemical Osteomalacia among Adolescents, Front. Nutr. 12 (2025) 1696230.
[41] A. Dugar, A.N. Hoofnagle, A.P. Sanchez, D.M. Ward, J. Corey-Bloom, J.H. Cheng, J. H. Ix, C. Ginsberg, The vitamin D metabolite ratio (VMR) is a biomarker of vitamin D status that is not affected by acute changes in vitamin D binding protein, Clin. Chem. 69 (2023) 718–723.
[42] S.M. Kim, P.L. Lutsey, E.D. Michos, Vitamin D and cardiovascular disease: can novel measures of vitamin D status improve risk prediction and address the vitamin D racial paradox? Curr. Cardiovasc. risk Rep. 11 (2017) 3.
[43] C. Cipriani, J. Pepe, L. Colangelo, S. Minisola, Vitamin D and secondary hyperparathyroid states, Front Horm. Res 50 (2018) 138–148.
[44] S. Lemoine, L. Figueres, J. Bacchetta, S. Frey, L. Dubourg, Calcium homeostasis and hyperparathyroidism: nephrologic and endocrinologic points of view. Annales d'Endocrinologie, Elsevier, 2022, pp. 237–243.
[45] L. Alfredsson, B.K. Armstrong, D.A. Butterfield, R. Chowdhury, F.R. de Gruijl, M. Feelisch, C.F. Garland, P.H. Hart, D.G. Hoel, R. Jacobsen, Insufficient sun exposure has become a real public health problem, Int. J. Environ. Res. Public Health 17 (2020) 5014.