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Serum 25-hydroxyvitamin D and intact parathyroid hormone influences muscle outcomes in children and adolescents

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Abstract

Increases in 25-hydroxyvitamin D concentrations are shown to improve muscle strength in adults; however, data in pediatric populations are scant and equivocal. In this ancillary study of a larger-scale, multi-sited, double-blind, randomized, placebo-controlled vitamin D intervention in US children and adolescents, we examined the associations between changes in vitamin D metabolites and changes in muscle mass, strength, and composition following 12-weeks of vitamin D3 supplementation. Healthy male and female, black and white children and adolescents between the ages of 9-13 years old from two US states (Georgia 34°N and Indiana 40°N) were enrolled in the study and randomly assigned to receive an oral vitamin D3 dose of 0, 400, 1,000, 2,000 or 4,000 IU/d for 12 weeks between the winter months of 2009 to 2011 (N=324). Analyses of covariance, partial correlations, and regression analyses of baseline and 12-week changes (post-baseline) in vitamin D metabolites (serum 25(OH)D, $1,25(OH)_2D$, intact parathyroid hormone (iPTH)) and outcomes of muscle mass, strength, and composition (total body fat-free soft tissue (FFST), handgrip strength, forearm and calf muscle cross-sectional area (MCSA), muscle density, and intermuscular adipose tissue (IMAT)) were assessed. Serum 25(OH)D and $1,25(OH)_2D$, but not iPTH, increased over time, as did fat mass, FFST, forearm and calf MCSA, forearm IMAT, and handgrip strength ($p<0.05$). Vitamin D metabolites were not associated with muscle strength at baseline nor following the 12-week intervention. Changes in serum 25(OH)D correlated with decreases in forearm IMAT, while changes in serum iPTH predicted increases in forearm and calf

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MCSA and IMAT ($p<0.05$). Overall, increases in 25(OH)D did not influence muscle mass or strength in vitamin D sufficient children and adolescents; however, the role of iPTH on muscle composition in this population is unknown and warrants further investigation.

Keywords

Vitamin D; muscle health; pediatrics; parathyroid hormone; muscle composition

Introduction

Vitamin D insufficiency (< 50 nmol/L) is highly prevalent amongst older adults[1] and is becoming more commonly documented in children[2]. Classically, vitamin D plays an essential role in calcium regulation and bone metabolism, and vitamin D insufficiency is associated with low bone mass and an increased risk of skeletal fracture[3]. However, over the past few decades there has been increased interest in the non-skeletal effects of vitamin D. Muscle has been of particular interest as vitamin D deficiency (< 20 nmol/L) is associated with proximal limb myopathy[4] and vitamin D insufficiency with impaired muscle function[5].

Older adults with low circulating 25-hydroxyvitamin D (25(OH)D), indicative of vitamin D insufficiency, have decreased muscle strength and poor muscle function illustrated by decreased thigh muscle strength[6] and power[7], weak handgrip strength[8], reduced gait speed[6], and a prolonged sit-to-stand time[9]. In adolescents, lower circulating 25(OH)D has likewise been associated with decreased muscle strength and poor muscle function[10-12]. Though vitamin D supplementation is shown to improve muscle-related outcomes in adults[13], only two randomized clinically controlled trials have examined this relationships in adolescents[14,15]. In the first study, vitamin D insufficient (35 nmol/L) Lebanese adolescent females (N=168, aged 10-17 years) were supplemented with weekly oral vitamin D_3 doses of 1,400 IU, 14,000 IU or placebo for one year. Following the intervention, significant increases in total body lean muscle mass were observed in pre-menarcheal, but not post-menarcheal females[14]. In the second study, a group of predominately vitamin D deficient (18 nmol/L) South Asian adolescent females (N=69, aged 12-14 years) received an oral vitamin D_2 dose of 150,000 IU or placebo every three months for one year. Following the intervention, an improvement in movement efficiency, indicating an improvement in flexibility and neuromuscular coordination, was observed[15].

The two previously described trials were limited to adolescent females with insufficient or deficient baseline 25(OH)D concentrations and were predominately in those of Middle Eastern or South Asian ancestry. Whether increasing serum 25(OH)D concentrations improves muscle-related outcomes in vitamin D sufficient (>50 nmol/l) children and adolescents, of both sexes, is unknown. Additionally, no studies to date have examined the relationship between 25(OH)D and muscle mass, strength, or composition in black children or adolescents, which is particularly relevant given their high prevalence of low serum circulating 25(OH)D concentrations[16]. Therefore, the purpose of this ancillary analysis of a 12-week randomized, placebo-controlled vitamin D intervention was to determine if changes

in vitamin D metabolites were associated with changes in muscle-related outcomes including size, strength, and composition in black and white, male and female children and adolescents. We hypothesized that increases in circulating 25(OH)D concentrations would improve muscle-related outcomes following the 12-week intervention.

Materials and Methods

Study Participants and Design

Data from The University of Georgia, Purdue University, and Indiana University (GAPI) 12-week vitamin D intervention in US children ages 9 to 13 years (N=324) from two different latitudes (34°N or 40°N) were used for this ancillary study[17]. Briefly, participants were healthy white (n=159) and black (n=165), males (n=162) and females (n=162) from southern (n=161) or northern (n=163) locations who following enrollment during the winter months (October through December) of 2009 to 2010 and 2010 to 2011, when serum 25(OH)D is at its nadir, were randomly assigned to receive an oral vitamin D_3 dose of 0, 400, 1,000, 2,000 or 4,000 IU/d for 12 weeks. Pubertal status was kept consistent between participants by recruiting those with sexual maturity ratings of 2 and 3, estimated using a validated self-assessment questionnaire for genitalia or breast development in a mixed ethnic population[18-20] Participants taking nutritional supplements were enrolled after a 4-week washout period, and all participants agreed to not alter their dietary or physical activity patterns throughout the 12-week intervention. Additional details regarding the study participants, recruitment, blinding, randomization scheme and allocation, and participant flow were previously reported in Lewis et al.[17]

Exclusion criteria included achievement of menarche (for females), pre-existing muscle or bone disorders, growth disorders, or medications/supplements known to influence vitamin D metabolism. Serum 25(OH)D, 1,25-dihydroxyvitamin D [$1,25(OH)_2D$], intact parathyroid hormone (iPTH), indices of total body composition (fat-free soft tissue mass (FFST), fat mass, body fat percent), forearm and calf muscle cross-sectional area (MCSA), muscle density (MD), intermuscular adipose tissue (IMAT), and handgrip strength were assessed at baseline and following the 12-week intervention. Dietary intake and physical activity data were also collected for use as possible covariates in the analyses. Each university's Institutional Review Board on Human Subjects Research approved the current study.

Statistical power calculations were not conducted for this ancillary analysis. Original power calculations are published elsewhere and were based upon fractional calcium absorption, 25(OH)D, $1,25(OH)_2D$, and iPTH, with 25(OH)D acting as the primary sample size determinant [17]. However, the allotted sample size provided the parent study more than 90% power ($\alpha = 0.05$) to detect differences in 25(OH)D concentrations among the five-supplementation groups with forty-eight participants per group. Retrospectively, a 7.4% difference in total lean mass change following a +27.5 nmol/L (placebo) versus +69.9 nmol/L (2,000 IU/d) increase in 25(OH)D concentrations in 15 participants could be detected with 90% power (2-sided significance), according to a previous vitamin D supplementation trial in school children[14].

Biochemical Analyses

Blood samples were collected following an overnight fast and left to sit for 30-60 minutes to coagulate before centrifuging at 4°C and 1,800 RPMs for 15 minutes. All samples were then prepared for storage and frozen at < −70°C until further analyses. Reference controls (kits) and internal controls (in-house pooled samples) were included with each assay run for quality control. All assays were conducted in duplicate using a block design, such that baseline and 12-week samples from the same subject were assayed at the same time using the same kit by the same investigator who was blinded to participant race, sex, and treatment. Repeat analyses were conducted if duplicate samples differed by >10%. Serum 25(OH)D was assessed using a 2-step radioimmunoassay (DIASORIN, 25 hydroxyvitamin D hydroxyvitamin D ^{125}I RIA kit, no.68100, Stillwater, MN, 100 tube kit). The inter- and intra-assay coefficients of variation (CV) were 5.2 – 8.7% and 5.5 – 8.2%, respectively, and analytical reliability was further monitored through laboratory participation in DEQAS (Vitamin D External Quality Assessment Scheme). Serum 1,25(OH)$_2$D was assessed using a 2-step radioimmunoassay (DIASORIN, 1,25-dihydroxyvitamin D ^{125}I RIA kit, no. 65100E, Stillwater, MN, 100 tube kit). The inter- and intra-assay CV were 12.4% to 17.6% and 11.6%, respectively. Serum iPTH was assessed using an immunoradiometric assay (DIASORIN, N-tact iPTH SP kit, no. 26100, Stillwater, MN 100 beads/kit). The inter- and intra-assay CV were 0.5 to 3.0% and 3.1 to 9.4%, respectively.

Whole Body Composition

Dual energy X-ray absorptiometry (DXA)—Total body FFST (kg), fat mass (kg) and percent body fat (%) were measured using DXA (Delphi A Hologic Inc. [University of Georgia site]; GE Healthcare Lunar iDXA, GE Medical Instruments [Purdue University site]; Hologic Discovery-W [Indiana University site]) at baseline and 12 weeks. For consistency, the same technician at each study site performed all analyses. DXA quality control for soft tissue was assured by standard protocols. Intra-class correlation coefficients (ICCs) were calculated in young females 5 to 8 years of age (n=10) scanned twice at the University of Georgia site during a 7-day period for measures of body composition (ICC≥ 0.98). Short- and long-term DXA precision at the Purdue and Indiana University's sites demonstrates errors of less than 2%. Instruments from all three sites were cross-calibrated and study-specific conversion equations were developed to standardize the baseline and 12-week change data for whole body composition outcomes for all statistical analyses[21].

Muscle Composition

Peripheral quantitative computed tomography (pQCT)—Non-dominant calf and forearm MCSA (mm^2), MD (g/cm^2) and intermuscular adipose tissue (IMAT, mm^2) were measured at 66% of the bone length from the distal radius and tibia by pQCT (XCT-2000; Stratec Medizintechnic, Pforzheim, Germany) at baseline and 12 weeks. MCSA, MD, and IMAT were each assessed through multiple analyses of the same tomographic slice. MCSA of the forearm and calf are shown to be surrogate markers of muscle strength[22]. To measure MCSA, both subcutaneous fat and bone area were individually quantified and subtracted from the total volume of interest. Subcutaneous fat was quantified by contour mode 1 and peel mode 1 using a threshold of 34 mg/cm^3, while bone area was quantified

using a threshold of 710 mg/cm^3. To assess MD, muscle bone area (MBA) and density (MBD) were first quantified and separated from adipose tissue using a threshold of 40 mg/cm^3. Additional indices of bone area (BA) and bone density (BD) were further quantified using a threshold of 150 mg/cm^3 and MD was calculated from the following equation: Muscle Density = ((MBD + (MBA / BA)) − BD)/((MBA − BA) / BA). To assess IMAT, defined in this manuscript as adipose tissue located beneath the fascia and in-between or inside muscle fibers, a contour mode 3 and peel mode 2 non-filtered analysis using a threshold of −101 mg/cm^3 and an inner threshold of 40 mg/cm^3 was used to separate total fat from muscle and bone. Subcutaneous fat (threshold −101 mg/cm^3, inner threshold 40 mg/cm^3) and bone marrow (threshold 710 mg/cm^3, inner threshold −101 mg/cm^3) were then subtracted from total fat to produce an IMAT value.

Scans with excessive motion (radius, n = 34; tibia, n = 16) were not included in any muscle analyses. Comparability of muscle data from each study site/scanner was achieved through a minimum of 20 phantom scans per scanner. Quality assurance was verified with daily phantom scans. Test-retest measurements were performed on five females, aged 18 to 24 years, to determine reliability of the pQCT at the University of Georgia site. ICCs for all pQCT measurements were calculated as ICC ≥ 0.97[23]. Similar reliability measures were shown at Indiana sites, with a root mean square CV of <1% for bone density, mass, and structure, and <1.5% for MCSA in 30 healthy individuals scanned six times with interim repositioning[24].

Handgrip strength—Maximum isometric strength of the non-dominant hand was assessed using a handgrip dynamometer (Jamar Plus+ Digital Hand Dynamometer; Patterson Medical, Bolingbrook, IL) by the same technician at each site at baseline and 12 weeks. Handgrip strength is a valid measurement of total body muscle strength[25] and physical fitness (speed, agility, flexibility, jumping)[26] in pediatric populations. At baseline, the technician adjusted the handle of the dynamometer for the most comfortable gripping position for each subject (1st and 2nd position for children), noted the subject specific setting, and repeated the same setting at 12 weeks. Three measurements were taken 30 seconds apart and the average of the three tests was recorded. Test- retest measurements in 11 participants (4 white males, 3 white females, 3 black males, and 1 black female) aged 9 to 13 years were conducted in a single day (3 hours apart) with a 30-second rest period between triplicate measurements and showed an average ICC for handgrip strength of ICC=0.98. The correlation coefficient of the handgrip dynamometer in assessing grip strength of the dominant and non-dominant hand in children has been reported as 0.97 (95% confidence interval: 0.95 to 0.98) and 0.95 (95% confidence interval: 0.92 to 0.96), respectively[27].

Dietary Intake & Physical Activity

Three-day food records (2 weekdays and 1 weekend day) were completed by participants and their parents at both baseline and 12 weeks to assess intakes of energy (kcal), vitamin D (IU) and calcium (mg). Three-day food records have been shown in adolescents as reliable tools for estimating energy and nutrient intakes[28]. Diet records were analyzed using Food Processor SQL version 9.7.3 (ESHA Research). The three-day physical activity recalls (3DPAR, 1 weekend day and 2 weekdays)[29] were administered by a trained interviewer

and used to quantify each participant's type, duration and intensity of physical activity at baseline and 12 weeks. A metabolic equivalent (MET) value was assigned to each 30-minute time block based upon activity type, duration and level of intensity.

Statistics

The objective of this ancillary study was to determine the relationship between vitamin D metabolites (serum 25(OH)D, 1,25(OH)2D, iPTH) and muscle-related outcomes (muscle mass, strength, and composition) both at baseline and their changes over the 12-week intervention. Therefore, the effect of vitamin D_3 dose on both independent and dependent variables were not examined. This effect of vitamin D_3 dose on vitamin D metabolites and the overall compliance to supplementation have previously been published[17]. Baseline group differences between race and sex were examined using two-way ANOVA. Changes over the 12-week intervention were determined by paired *t*-test, and a two-way ANOVA was used to determined differences in change values due to race and sex. Serum and muscle-related outcomes were expressed as continuous variables and were tested for normality by the Shapiro-Wilk and the Dixon tests to reveal a non-normal distribution for all serum and muscle-related outcomes. Therefore, a two-step power transformation was applied to satisfy the assumption of normally distributed errors[30]. The presence of outliers were also noted for multiple muscle-related outcomes and removed according to the extreme studentized deviate test[31]. Physical activity and dietary intake did not change over the 12-week intervention and were not associated to vitamin D metabolites or muscle-related outcomes at baseline or 12-weeks. Therefore, physical activity and diet were not included as covariates in future analyses. Partial correlation analyses, adjusted for race, sex, age, and latitude, were performed to assess relationships between baseline and 12-week changes (i.e., post-pre) in vitamin D metabolites (25(OH)D, $1,25(OH)_2D$, iPTH), and baseline and 12-week changes (i.e., post-pre) in muscle-related outcomes (muscle mass, strength, and composition). Multiple linear regression analyses, adjusted for race, sex, age, and latitude, were further performed to assess the effect of changes in vitamin D metabolites on changes in muscle-related outcomes. Statistical analyses were performed using the SPSS statistical package (IBM SPSS Statistics for Windows, Version 22.0 [IBM Corp: Armonk, NY]), and statistical significance was denoted upon a p-value ≤ 0.05.

Results

Baseline Characteristics (Table 1)

Black vs. white subjects were generally heavier, younger, and less physically active, with greater forearm and calf IMAT concentrations and lower muscle density, stronger grip strength, lower calcium intake, lower serum 25(OH)D, but higher serum iPTH and $1,25(OH)_2D$ concentrations. Males vs. females were older, taller, lighter for their age, displayed greater lean mass, less fat mass, larger forearms with higher IMAT concentrations, higher daily energy intakes, and lower serum $1,25(OH)_2D$ concentrations. Mean 25(OH)D for the overall sample at baseline was ~70 nmol/L. Subjects with serum 25(OH)D concentrations below 50 nmol/L and 30 nmol/L were 13% and 2%, respectively.

Baseline Partial Correlations

Partial correlations between baseline vitamin D metabolites and muscle-related outcomes, adjusted for covariates (age, race, sex, and latitude), are presented in Table 2. Baseline serum iPTH was positively correlated with FFST (r=0.145,/p=0.030) and forearm MCSA (r=0.152, p=0.022).

12-week Changes Following Intervention (Table 3)

304 participants completed the 12-week intervention, yielding a 94% retention rate and is described in detail by Lewis et al.[17]. Weight, BMI-For-Age, fat mass, FFST, handgrip strength, forearm MCSA ($p \leq 0.001$) and IMAT ($p = 0.001$), as well as calf MCSA ($p = 0.025$) increased over the 12-week intervention. Mean serum 25(OH)D and 1,25$(OH)_2$D, but not iPTH, increased from baseline to 12 weeks (p<0.0001). Changes in serum and muscle-related outcomes did not differ between males and females nor whites and blacks over the 12-week intervention.

12-week Change Partial Correlations

Partial correlations for 12-week absolute changes in vitamin D metabolites and muscle-related outcomes (i.e., post-pre) are shown in Table 4. Age and race were positively correlated with changes in FFST (Age: r=0.166,p=0.005; Race: r=−0.156,p=0.008) and arm MCSA (Age: r=0.14,p=0.019; Race: r=−0.128,p=0.031). Changes in serum 25(OH)D were negatively correlated with changes in forearm IMAT (r= −0.173, p=0.029). Changes in serum iPTH, unadjusted or adjusted, were positively correlated with changes in forearm and calf MCSA, and forearm and calf IMAT. Neither baseline nor changes in dietary intakes of calcium and vitamin D or physical activity were associated with changes in serum vitamin D metabolites or muscle-related outcomes.

Multiple Linear Regression: Effects of vitamin D metabolites on muscle-related outcomes

Results from multiple linear regression analyses between 12-week changes in vitamin D metabolites (independent variable) and 12-week changes in muscle-related outcomes (dependent variable), adjusted for covariates (age, race, sex, and latitude), are presented in Table 5. Increases in serum iPTH predicted increases in calf MSCA (β=0.180,p=0.003), as well as forearm (β=0.135,p>=0.035) and calf IMAT (β=0.135,p=0.038). A trend was also present for serum iPTH and FFST (β=0.112,pτ=0.054).

Discussion

Changes in muscle mass and strength were not related to changes in 25(OH)D, despite large increases in serum 25(OH)D concentrations up to 155.7 nmol/L following the 12-week vitamin D3 intervention. These results contradict previous vitamin D interventions in adults, where increases in serum 25(OH)D were associated with increases in muscle fiber size[32] and improvements in muscle strength[33] and power[34]. In-vitro studies further support this notion showing alterations in calcium signaling pathways and myocellular gene expression following 25(OH)D or 1,25$(OH)_2$D exposure[35-37]. However, in agreement with the current study, improvements in serum 25(OH)D have also shown a neutral effect on muscle-related outcomes including a lack of an effect on muscle strength[38,39], muscle function, or the risk

of falling[40,41]. Though alleviating vitamin D deficiency (<20 nmol/L) through supplementation improves muscle-related outcomes[32-34,38,42,43], whether such effects occur in the absence of deficiency in both adolescents and adults (>20 nmol/L) is still unclear.

The current study is one of only three randomized clinically-controlled trials that examined the influence of changes in vitamin D metabolites on muscle-related outcomes in adolescents[14,15]. The study by El-Hajj Fuleihan et al.[14] showed that increases in 25(OH)D increased total body FFST in Lebanese females, while the study by Ward et al.[15] showed improvements in movement efficiency in a predominately South Asian female population. In contrast, the current study showed no such improvements in muscle mass or strength which may be explained by the length of the vitamin D intervention (i.e., 12-weeks vs. 12-months) and baseline vitamin D status of each study population (i.e., sufficient vs. insufficient).

Though shorter in duration in comparison to the two previous 12-month interventions[14,15], the current study demonstrated increases in 25(OH)D, $1,25(OH)_2D$, FFST, handgrip strength, and forearm and calf MCSA following the 12-week vitamin D intervention. These results are reasonable given the rapid rate of growth associated with the early stages of puberty[44]. However, baseline 25(OH)D concentrations in the two previous interventions[14,15] were considerably lower in comparison to the current study (i.e., average 35 nmol/L and 18 nmol/L vs. ~70 nmol/L, respectively). The lack of association between increasing serum 25(OH)D concentrations and changes in muscle mass and strength in the current study suggest that changes in muscle-related outcomes are only apparent when baseline 25(OH)D concentrations are insufficient[14] or deficient[15].

Akin to cross-sectional studies in adults[45-47], increases in serum 25(OH)D were correlated with decreases in forearm IMAT over the 12-week vitamin D intervention following adjustment for race, sex, age, and latitude. The etiology of IMAT accumulation is still unknown, but is believed to be the result of impairments in mitochondrial function and muscle lipid metabolism[48]. Vitamin D supplementation has shown to improve mitochondrial function[49] and regulate genes involved in lipogenesis and fatty acid metabolism[50], which could explain the current study results. Yet, associations between serum 25(OH)D and forearm or calf IMAT concentrations were not present following regression analyses. Therefore, though improvements in muscle composition following increases in 25(OH)D are present and plausible, further investigation is needed to confirm the current study result.

This study is among the first to report a positive relationship between iPTH and muscle-related outcomes in a pediatric population. Twelve-week changes in iPTH, after adjusting for covariates, were positively correlated with and even predicted changes in forearm and calf MCSA and IMAT; suggesting an overall bigger and fatter muscle with higher serum iPTH. In conjunction with a trend between iPTH and FFST, results from the current study mirror those in adults showing an independent association between iPTH concentrations and skeletal muscle mass[51]. However unlike adults[52], iPTH concentrations in the current study were not associated with handgrip strength. Furthermore, based upon adult data alone, the positive relationship between iPTH concentrations and IMAT would suggest poor muscle

strength[53] as well as an increased risk of diabetes in this early adolescent population[54]. To our knowledge, there are no studies that have addressed the effect of IMAT on muscle strength in a non-diabetic adolescent population, and whether it elicits a negative effect or is simply a part of the normal growth process. Similarly, it is important to note that a transient diabetic-like state of insulin resistance occurs during early adolescence[55]. Whether or not this diabetic-like state and potentially a iPTH-induced increase in IMAT are related is unclear. Therefore, more research is needed to clarify the role of iPTH and IMAT in early adolescent muscle and their potential interaction during the transient diabetic-like state.

Limitations of the present study include: 1) the exclusion of select muscle data due to motion artifacts in pQCT-derived images (forearm, n = 34; calf, n = 16), 2) the length of the study intervention, 3) participant's sufficient baseline vitamin D status, and the 4) lack of data on prior dietary supplement usage. Though there were significant changes in vitamin D metabolites and muscle-related outcomes following the 12-week intervention, based upon previous data in adolescents[14,15] a longer study intervention may have generated larger changes in outcome variables. Additionally, though a unique aspect of the current study, conducting the 12-week intervention in a vitamin D deficient or insufficient population may have yielded a greater association between 25(OH)D and muscle-related outcomes, similar to previous data in adolescents[14,15] and adults[32-34,38,42,43]. Finally, though all participants consuming dietary supplements prior to randomization completed a 4-week washout period and baseline dietary intakes were not associated with baseline or 12-week changes in any study outcome, future researchers should collect data on prior dietary supplement usage as a potential covariate in final analyses. The primary strength of this study is that it was a multi-site vitamin D intervention trial that assessed changes in muscle-related outcomes in both black and white and male and female vitamin D sufficient adolescents in the early stages of puberty. To our knowledge, this study was also the first to measure IMAT using pQCT in a pediatric population. Though a novel approach in pediatric populations, the assessment of IMAT using pQCT has previously been documented in adult populations [54,56] and in the present study changes in IMAT concentrations were inversely correlated with changes in MD and positively correlated with changes in MCSA.

In summary, increases in 25(OH)D were correlated with decreases in forearm IMAT, whereas iPTH was positively associated and predicted changes in forearm and calf MCSA and IMAT in this diverse early adolescent population. Additional research is needed to clarify the relationship between 25(OH)D and IMAT accumulation, the influence of iPTH on muscle in early adolescence, and whether these are meaningful relationships.

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Abbreviations:

25(OH)D	25-hydroxyvitamin D
1,25(OH)$_2$D	1,25-dihydroxyvitamin D
RIA	Radioimmunoassay
iPTH	Intact Parathyroid Hormone
IRMA	Immunoradiometric Assay
DXA	Dual-energy X-ray Absorptiometry
pQCT	Peripheral Quantitative Computed Tomography
FFST	Fat-Free Soft Tissue
MCSA	Muscle Cross-Sectional Area
MD	Muscle Density
IMAT	Intermuscular Adipose Tissue

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Table 1.

Subject Characteristics

			Males		Females		Two-Way ANOVA		
Variable[1]	**N**	**Overall**	**White**	**Black**	**White**	**Black**	**S x R**	**Sex (S)**	**Race (R)**
Age (years)	323	11.3 ± 1.2	12.3 ± 0.9	11.6 ± 1.1	10.9 ± 1.0	10.7 ± 1.0	NS[4]	**<0.001**	**<0.001**
Whole Body Composition									
Weight (kg)	323	47.4 ± 12.2	45.4 ± 11.0	45.5 ± 12.0	43.3 ± 8.4	46.8 ± 10.4	NS	NS	**0.014**
Height (cm)	323	151 ± 9	153 ± 8	150 ± 9	149 ± 9	148 ± 8	NS	**0.022**	NS
BMI-For-Age (%)	323	68.0 ± 29.2	51.6 ± 30.7	64.2 ± 28.8	66.4 ± 26.3	75.0 ± 28.3	NS	**0.002**	**0.009**
Fat Mass (kg)[2]	317	14.5 ± 6.9	12.2 ± 7.0	12.3 ± 7.8	14.6 ± 5.2	16.7 ± 6.2	NS	**<0.001**	NS
Percent Body Fat[2]	319	29.8 ± 9.4	27.6 ± 9.2	24.9 ± 9.6	32.7 ± 7.4	32.4 ± 7.8	NS	**<0.001**	NS
Fat-Free Soft Tissue (kg)[2]	315	30.0 ± 6.2	31.1 ± 5.6	29.9 ± 6.0	27.8 ± 5.2	28.3 ± 5.6	NS	**0.002**	NS
Muscle Composition									
Forearm MCSA (cm^2)[3]	319	21.2 ± 4.7	20.8 ± 3.8	22.4 ± 3.9	17.9 ± 3.5	21.2 ± 3.4	NS	**<0.001**	**<0.001**
Forearm MD (mg/cm^3)[3]	321	74.6 ± 13.7	81.8 ± 11.5	71.0 ± 12.7	76.8 ± 8.0	67.5 ± 14.1	NS	**0.012**	**<0.001**
Forearm IMAT (cm^2)[3]	289	2.6 ± 1.3	2.0 ± 0.9	3.3 ± 1.2	1.7 ± 0.8	2.9 ± 1.1	NS	**0.043**	**<0.001**
Calf MCSA (cm^2)[3]	315	47.9 ± 9.8	48.3 ± 9.1	46.8 ± 9.0	46.5 ± 8.6	46.1 ± 9.4	NS	NS	NS
Calf MD (mg/cm^3)[3]	318	73.3 ± 3.2	74.8 ± 2.2	72.4 ± 3.2	74.4 ± 1.8	71.5 ± 2.7	NS	NS	**<0.001**
Calf IMAT (cm^2)[3]	296	10.3 ±3.6	9.5 ±3.2	11.0 ± 3.1	9.2 ±3.1	11.0 ± 3.9	NS	NS	**0.001**
Handgrip Strength (kg)	288	18.0 ± 9.5	16.3 ± 8.5	22.8 ± 7.2	15.1 ± 7.9	20.6 ± 8.7	NS	NS	**<0.001**
Dietary Intake									
Energy (kcal)	307	2001 ± 556	2092 ± 519	2035 ± 646	2010 ± 530	1885 ± 477	NS	NS	NS
Calcium (mg)	307	901 ± 395	938 ± 420	801 ± 403	993 ± 444	860 ± 290	NS	NS	**0.013**
Vitamin D (IU)	300	173 ± 123	191 ± 77	148 ± 81	152 ± 99	167 ± 100	NS	NS	NS
Physical Activity iMETs/d)	304	62.2 ± 10.0	63.0 ± 9.1	60.1 ± 8.6	64.7 ± 11.0	58.2 ± 8.0	NS	NS	**<0.001**
Biochemical Variables									
25(OH)D (nmol/L)	318	69.9 ± 18.5	79.8 ± 13.0	62.0 ± 15.2	80.5 ± 16.1	58.9 ± 18.2	NS	NS	**<0.001**
iPTH (pg/mL)	314	26.9 ± 9.9	23.8 ± 8.8	28.2 ± 9.6	25.6 ± 8.4	30.0 ± 8.1	NS	NS	**<0.001**

			Males		Females		Two-Way ANOVA		
Variable[1]	**N**	**Overall**	**White**	**Black**	**White**	**Black**	**S x R**	**Sex (S)**	**Race (R)**
1,25$(OH)_2$D (pg/mL)	318	55.4 ± 16.5	48.4 ± 15.3	54.2 ± 16.6	55.3 ± 14.6	59.7 ± 14.8	NS	**0.004**	**0.019**

[1] Values are Mean ± SD. White (n=159) and black (n=165), males (n=162) and females (n=162)

[2] Measured by dual-energy X-ray absorptiometry

[3] Measured by peripheral quantitative computed tomography; MCSA, muscle cross-sectional area; MD, muscle density; IMAT, intermuscular adipose tissue

[4] NS, Non-significant (p-value > 0.05); Statistically significant if p-value ≤ 0.05 and in bold font

Table 2.

Partial correlations of baseline serum and muscle-related measurements

		25(OH)D (N=318)[1]				$1,25(OH)_2D$ (N=318)[1]				iPTH (N=318)[1]			
		Unadjusted		Adjusted		Unadjusted		Adjusted		Unadjusted		Adjusted	
Variable[2]	**N**	**r**	***P***	**r**	***P***	**r**	***P***	**r**	***P***	**r**	***P***	**r**	***P***
Fat-Free Soft Tissue (kg)	320	−0.12	**0.038**	−0.02	0.826	0.12	**0.037**	0.10	0.130	0.12	**0.037**	0.15	**0.030**
Handgrip Strength (kg)	303	−0.21	**<0.001**	0.04	0.602	0.14	**0.019**	0.12	0.083	0.21	**0.001**	0.07	0.25
Forearm MCSA (mm^2)	321	−0.32	**<0.01**	−0.02	0.826	0.13	**0.028**	0.12	0.270	0.23	**<0.001**	0.15	**0.022**
Forearm MD (mg/cm^3)	321	0.34	**<0.001**	0.09	0.166	−0.04	0.509	−0.03	0.620	−0.12	**0.034**	<0.01	0.99
Forearm IMAT (mm^2)	297	−0.33	**<0.001**	−0.04	0.517	0.10	0.101	0.04	0.529	0.13	**0.034**	0.07	0.314
Calf MCSA (mm^2)	317	−0.13	**0.026**	−0.06	0.356	0.12	**0.040**	0.08	0.233	0.18	**0.001**	0.12	0.066
Calf MD (mg/cm^3)	318	0.39	**<0.01**	0.11	0.103	0.01	0.934	<0.01	0.948	−0.16	**0.006**	−<0.01	0.962
Calf IMAT (mm^2)	308	−0.21	**<0.01**	−0.08	0.230	0.12	**0.045**	0.06	0.349	0.13	**0.030**	0.07	0.268

1. Partial correlations of unadjusted and adjusted (for race, sex, age, and latitude, N=223) between vitamin D metabolites and muscle-related outcomes. Statistically significant if $P \leq 0.05$ and in bold font. Partial Correlation Coefficient (r) displays strength of correlation. MCSA, muscle cross-sectional area; MD, muscle density; IMAT, intermuscular adipose tissue

Table 3.

12-week changes in body weight, composition, muscle and biochemical outcomes

				Paired T-Test
Variable[1]	**N**	**Δ**	**% Change**	**Time**
Whole Body Composition				
Weight (kg)	304	1.6 ± 2.9	3.6 ± 4.7	**<0.001**
BMI-For-Age (%)	304	2.2 ± 6.4	9.7 ± 27.6	**<0.001**
Fat Mass (kg)[2]	296	0.57 ± 0.93	3.4 ± 52.2	**<0.001**
Fat-Free Soft Tissue (kg)[2]	293	1.11 ± 0.91	1.3 ± 3.5	**<0.001**
Muscle Composition				
Forearm MCSA (cm^2)[3]	296	0.83 ± 1.24	4.4 ± 11.8	**<0.001**
Forearm MD (mg/cm^3)[3]	297	−0.85 ± 13.75	1.5 ± 16.4	NS
Forearm IMAT (cm^2)[3]	255	11.24 ± 37.56	14.6 ± 122.5	**0.001**
Calf MCSA (cm^2)[3]	293	0.07 ± 0.25	1.4 ± 8.2	**0.025**
Calf MD (mg/cm^3)[3]	295	0.05 ± 0.77	−0.3 ± 6.0	NS
Calf IMAT (cm^2)[3]	265	10.85 ± 102.70	3.1 ± 25.4	NS
Handgrip Strength (kg)	266	0.74 ± 2.06	1.8 ± 123.2	**<0.001**
Biochemical Variables				
25(OH)D (nmol/L)	294	22.37 ± 32.99	34.9 ± 55.9	**<0.001**
iPTH (pg/mL)	294	0.08 ± 8.46	4.9 ± 32.2	NS
$1,25(OH)_2D$ (pg/mL)	299	9.92 ± 17.15	27.0 ± 53.3	**<0.001**

1. Mean ± SD; Δ = Post-Baseline values; % Change = ((Post-Baseline)/Baseline)* 100; Two-Way ANOVA revealed no differences in change values due to race and sex (p-value > 0.05).

2. Measured by dual-energy X-ray absorptiometry

3. Measured by peripheral quantitative computed tomography; MCSA, muscle cross-sectional area; MD, muscle density; IMAT, intermuscular adipose tissue

4. NS, Non-significant (p-value > 0.05); Statistically significant if p-value ≤ 0.05 and in bold font.

Table 4.

Partial correlation of 12-week change in serum and muscle-related outcomes

		25(OH)D (N=302)[1]				$1,25(OH)_2D$ (N=302)[1]				iPTH (N=302)[1]			
		Unadjusted		Adjusted		Unadjusted		Adjusted		Unadjusted		Adjusted	
ΔMuscle Outcomes[2]	**N**	**r**	***P***	**r**	***P***	**r**	***P***	**r**	***P***	**r**	***P***	**r**	***P***
Fat-Free Soft Tissue (kg)	300	−0.04	0.564	−0.03	0.688	<0.01	0.975	0.03	0.718	<0.01	0.975	0.10	0.231
Handgrip Strength (kg)	285	0.04	0.585	0.02	0.841	0.07	0.270	0.10	0.214	0.05	0.439	<0.01	0.956
Forearm MCSA (mm^2)	299	0.03	0.666	−0.05	0.560	0.11	0.077	0.08	0.321	0.10	0.103	0.19	**0.019**
Forearm MD (mg/cm^3)	299	0.04	0.478	0.06	0.462	−0.03	0.652	0.01	0.862	0.01	0.939	0.01	0.922
Forearm IMAT (mm^2)	265	−0.05	0.487	−0.17	**0.029**	0.09	0.139	0.01	0.913	0.13	**0.039**	0.17	**0.029**
Calf MCSA (mm^2)	296	0.01	0.858	−0.04	0.581	0.09	0.154	−0.07	0.376	0.18	**0.002**	0.19	**0.016**
Calf MD (mg/cm^3)	296	−0.05	0.424	−0.10	0.192	0.02	0.693	0.07	0.362	−0.10	0.104	−0.07	0.414
Calf IMAT (mm^2)	280	0.06	0.386	0.04	0.612	0.03	0.678	−0.08	0.305	0.14	**0.029**	0.16	**0.037**

1. Partial correlations of unadjusted and adjusted (for race, sex, age, and latitude, N =239) between N Vitamin D metabolites and muscle-related outcomes. Statistically significant if $P \leq 0.05$ and in bold font. Partial Correlation Coefficient (r) displays strength of correlation.

2. MCSA, muscle cross-sectional area; MD, muscle density; IMAT, intermuscular adipose tissue.

Table 5.

Multiple linear regression: Effect of vitamin D metabolites on muscle-related outcomes

		Δ25(OH)D (N=302)		Δ1,25(OH)$_2$D (N=302)		ΔiPTH (N=302)	
ΔMuscle Outcomes	**N**	**Beta**	***p*-value**	**Beta**	***p*-value**	**Beta**	***p*-value**
Fat-Free Soft Tissue (kg)	300	−0.034	0.562	−0.003	0.954	0.112	0.054
Handgrip Strength (kg)	285	0.028	0.653	0.074	0.232	0.046	0.464
Forearm MCSA (mm^2)	299	0.025	0.678	0.102	0.086	0.097	0.101
Forearm MD (mg/cm^3)	299	0.049	0.415	−0.029	0.629	0.002	0.971
Forearm IMAT (mm^2)	265	−0.053	0.413	0.093	0.147	0.135	**0.035**
Calf MCSA (mm^2)	296	0.015	0.811	0.085	0.158	0.180	**0.003**
Calf MD (mg/cm^3)	296	−0.038	0.526	0.025	0.677	−0.101	0.090
Calf IMAT (mm^2)	280	0.065	0.323	0.024	0.707	0.135	**0.038**

1. Δ = Post-Baseline values

2. Multiple linear regression controlling for race, sex, age, and latitude; Change in serum and muscle-related outcomes (post - baseline). Statistically significant if $P \leq 0.05$ and in bold font. Beta coefficient displays strength or influence of the independent variable over the dependent variable.

3. MCSA, muscle cross-sectional area; MD, muscle density; IMAT, intermuscular adipose tissue