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Vitamin D status is related to intramyocellular lipid in older adults

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Abstract

Vitamin D and intramyocellular lipid (IMCL) both affect muscle function, but the relationship between vitamin D status and IMCL has not been established. To assess the relationship between vitamin D [measured as 25-hydroxy-vitamin-D (25(OH)D)] and IMCL, 20 community-dwelling adults between the ages of 65 and 85 were recruited. Serum 25(OH)D, and gastrocnemius IMCL and extramyocellular lipid (EMCL) were measured with magnetic resonance spectroscopy and fat ratio segmentation. A lifestyle questionnaire assessed physical activity. Muscle strength (1-repetition maximum) and physical function tests (timed up and go, timed sit to stand, four square step test, and gait speed) were also performed. Mean 25(OH)D was 37.9 ± 13.1 ng/mL with a range of 19–68 ng/mL. Soleus and gastrocnemius IMCL to water ratio was 1.04 ± 0.43 and 0.53 ± 0.22 , respectively, but only gastrocnemius IMCL was correlated with 25(OH)D ($R^2 = 0.39$; $p = 0.02$). This relationship was independent of body mass index ($p > 0.14$), physical activity level ($p > 0.08$), and sex ($p > 0.13$). 25(OH)D did not correlate with EMCL ($R^2 = 0.007$; $p = 0.78$). The four square step test was the only performance or strength test correlated with 25(OH)D ($R^2 = 0.26$; $p = 0.023$). Muscle strength and physical function measures were not correlated with IMCL or EMCL. These data suggest that vitamin D status may influence gastrocnemius IMCL content independent of body mass and physical activity. Future studies should consider exploring whether vitamin D has an independent role in affecting muscle lipid metabolism and function.

Keywords

Vitamin D; Intramyocellular lipid; Extramyocellular lipid; Muscle strength; Physical function

Conflict of interest The authors declare that there are no conflicts of interest.

Introduction

Vitamin D deficiency is estimated to occur in approximately 20–100 % of community-dwelling elderly, and reports of vitamin D insufficiency are becoming more prevalent [1]. Traditionally, vitamin D deficiency has been documented as a cause of poor bone health [2]. Recently, researchers have established that compromised vitamin D status [measured as 25-hydroxy-vitamin-D (25(OH)D)] also contributes to poor skeletal muscle health [1, 3–5]. Vitamin D deficiency [defined as 25(OH)D<20 ng/mL] in the elderly has been associated with reduced muscle strength and physical function [3–8]; however, these negative outcomes appear to be partially mitigated with vitamin D repletion [4, 9, 10]. Vitamin D-induced changes in muscle tissue are thought to be explained by both genomic and non-genomic interaction between vitamin D and its receptor (VDR). Through these mechanisms, vitamin D has been shown to influence muscle lipid metabolism and improve muscle contraction by mediating intramyocellular calcium [11–13]. Vitamin D repletion has also been shown to act through skeletal muscle VDR to alter the size and number of Type II muscle fibers [11, 14], and by doing so contribute to the maintenance of optimal power and speed of muscle contraction [15]. Although emerging evidence supports a role for vitamin D in promoting preservation of muscle strength and function [4, 9, 10], few human studies have explored the relationship between vitamin D status and age-associated changes in muscle lipid depots. Given vitamin D involvement in promoting improvement in muscle function, it seems conceivable that some of these changes may be related to healthful mediation of muscle lipid metabolism that may have a significant impact on preserving muscle metabolic function in aging.

Age-related derangements in muscle lipid metabolism are a contributing factor to muscle dysfunction and similar to vitamin D deficiency are also linked with impaired muscle strength and physical function [16, 17]. Both extramyocellular (EMCL) and intramyocellular lipid (IMCL) depots have been linked to functional outcomes in muscle, but the connection between these muscle lipid depots and vitamin D has not been fully elucidated. EMCL is located outside the muscle cell and is defined as portions of adipose tissue in the interstitial layers of muscle [18]. EMCL has been inversely associated with 25(OH)D and physical function in both the young [19] and the elderly [17]. In contrast, IMCL is the fat within the muscle cell stored near the mitochondria [16, 18]. IMCL is a topic of heavy research interest and has been targeted as a key player in muscle metabolism due to its paradoxical association with both insulin sensitivity and resistance [20, 21]. Although usually considered negative, particularly in obesity, IMCL accretion in aged subjects as a result of exercise has been shown to increase the concentration of energy-producing substrates and improve metabolic profiles [20, 22, 23]. Although studies show that lipid metabolism can be altered with exercise across the lifespan, human studies have not observed a relationship between vitamin D and lipid metabolism independent of exercise. Currently, animal studies support a role for vitamin D in multiple lipid metabolic pathways involving diacylglycerol acyltransferase (DGAT), peroxisome proliferator-activated receptor α (PPAR α), and its target gene carnitine palmitoyltransferase-1 (CPT-1) [24, 25]. The aforementioned findings may suggest a supportive role of vitamin D repletion in altering EMCL and IMCL depots that may be further augmented by an exercise stimulus.

In particular, if vitamin D can independently influence IMCL accretion, then when exercise therapy is combined with vitamin D repletion, this could provide future insight to combating age-related decrements in muscle function.

A better understanding of the contributing factors to muscle metabolic dysfunction in the elderly will limit loss of muscle strength and function [4, 26–28]. The primary aim of this cross-sectional study was to determine the relationship between 25(OH)D and different muscle lipid depots (IMCL and EMCL) in healthy aged individuals. A secondary aim was to investigate the relationship between 25(OH)D, muscle strength, and physical function.

Methods

Subjects

Twenty community-dwelling 65–85 year olds residing in the Southeastern United States (latitude 38°N) were recruited to participate. Exclusion criteria included any medical condition that could compromise the safety of participation or confound study results, such as history of kidney disease, myopathy, and neurologic disorders. Additional exclusion criteria included injury or surgery to the lower extremities or resistance training 3 months prior to participation. The university institutional review board approved this study and written consent was obtained from each participant. All institutional and governmental regulations concerning the use of human subjects were followed during this study. Study data were collected and managed using REDCap electronic data capture tools hosted at the University of Kentucky [29].

Study design

An initial phone screening for eligibility was conducted, which included a medical history and physical activity questionnaire. Using the ACSM's Guidelines for Exercise Testing and Prescription, our unvalidated questionnaire inquired about prescription medication, dietary supplements, and physical activity habits, including frequency, duration, and type of physical activity. Prior to the first visit, a physician clearance form was obtained to further screen for eligibility. During the first visit, the consenting process, anthropometric measures, and a blood draw were completed. During the second visit, magnetic resonance imaging was scheduled; and during the third and final visit, physical function and muscle strength assessments were completed. All study measurements were completed between January and May.

Clinical assessments

Height and weight measurements were obtained using a wall-mount stadiometer (DETECTO 3P, Webb City, MO) and Tanita 800S scale (Tokyo, Japan). At the Center for Clinical and Translational Science (CCTS), trained phlebotomists followed standard guidelines and collected ~6.5 mL of whole blood. The University Biospecimens Core of CCTS completed all blood analysis. Plasma 25(OH)D was analyzed by liquid chromatography/tandem mass spectrometry with an assay coefficient of variation (CV) at 10 %. Parathyroid hormone (PTH) immunoassay had a CV of 4 % (intact PTH; Siemens, Tarrytown, NY). Although there is no consensus on a definite cutoff for vitamin D status,

researchers suggest that less than 20 ng/mL, 20–30 ng/mL, and levels greater than 40 ng/mL are defined as 25(OH)D deficiency, insufficiency, and optimal [1].

Muscle lipid assessment

Magnetic resonance spectroscopy (MRS) was used to assess IMCL in the soleus and gastrocnemius following an overnight fast. These muscles were chosen as both muscles play an important role for aging adults [30, 31]. The participants were positioned within a 3.0T TIM TRIO scanner (Siemens) in supine orientation with their lower left leg in a fixed dorsiflexed position placed in the radiofrequency coil. IMCL was quantified using single voxel placed in the gastrocnemius or soleus muscle (TR/TE = 2,000/30 ms, 96 averages, voxel size = 8 mm³, and water suppression) to obtain MRS data and distinguish IMCL from EMCL. To achieve optimal IMCL/EMCL separation, gastrocnemius IMCL was obtained either from the lateral or medial gastrocnemius. Quantification of IMCL was performed using spectral fitting by jMRUI (Graveron-Demilly group, Claude Bernard University Lyon 1, France, version 5). Sets of T1-weighted images (TR/TE = 650/22 ms and resolution = 0.4 mm×0.4 mm×5 mm) for high-resolution anatomy were collected to measure the area of contractile muscle tissue, subcutaneous and intramuscular adipose tissue. To compare lipid compartments in subjects with gastrocnemius IMCL measures, gastrocnemius EMCL was quantified. EMCL was quantified using a fat segmentation program (FMRIB Software Library, version 5.0) following manually drawn region of interest around the gastrocnemius.

Physical function assessment

Functional measures for each participant were conducted by the same physical therapist. Measurers included timed up and go (TUG), timed sit to stand (TSS), four square step test (FSST), and an 18-meter gait speed test. Each physical function test is reliable, valid, and is frequently utilized to assess physical function for community-dwelling older adults [32–34].

Muscle strength assessment

Dynamic upper and lower body strength was measured with 1-repetition maximum (1-RM) testing. One repetition maximum was defined as the maximal load that a subject can lift, at a single repetition. All strength tests were performed on Keiser Pneumatic weight lifting equipment (Keiser Corp., Fresno, CA) and included a biaxial upper back row, chest press, lateral pull down, and a bilateral leg press, curl, and extension. Participants were guided through warm-up sets at 40–60 and 70–80 % estimated 1-RM. Successive 1-RM attempts were performed until failure as described previously [35].

Statistical analysis

Data were analyzed using the Statistical Package for Social Sciences software (version 20.0) and included descriptive statistics, t test, and linear regression. Differences in subject characteristics between males and females and reported physical activity levels were compared by an independent t test. A sample size of 20 was estimated to allow a Fisher z test to detect Pearson Correlations between 25(OH)D, IMCL, and other outcome variables at 80 % power. Vitamin D was analyzed as a continuous variable. Linear regression was employed to investigate the relationship between our variables of interest. Statistical

significance was defined as $p < 0.05$ for all tests. All values are expressed as mean $\pm$ standard deviation.

Results

Subject characteristics

A total of 73 individuals were screened between January and April (Fig. 1). Twenty participants were asked to complete all study procedures, and soleus IMCL data were obtained from each participant; while gastrocnemius IMCL was obtained from 13 of the 20 participants. Subject characteristics, age, BMI, and 25(OH)D were not different between males and females (Table 1). Height ($p < 0.001$) and weight ($p < 0.001$) were statistically different between males and females. BMI ranged from normal weight to obese (19.5–36 kg/m²), with 16 of 20 participants having a BMI less than 30 kg/m². PTH levels ranged from 26 to 67 pg/mL with an average of 39.8 ± 12 pg/mL. From the physical activity questionnaire, 16 of 20 (80 %) participants reported being “active” and 4 participants reported being “inactive.”

Vitamin D status

25(OH)D was assessed in all 20 eligible participants (Table 1). Based on their 25(OH)D assessment, 1 participant was vitamin D deficient [25(OH)D < 20 ng/mL]. Six of 20 and 7 of 20 were considered vitamin D insufficient [25(OH)D between 20 and 30 ng/mL] and vitamin D sufficient [25(OH)D > 40 ng/mL], respectively. There were no statistically significant correlations between 25(OH)D and PTH, BMI, or age ($p > 0.09$).

Muscle lipid

Muscle lipid (IMCL and EMCL) was assessed in both the soleus and gastrocnemius. Soleus IMCL did not correlate with 25(OH)D (Table 2). A significant positive linear relationship was observed between 25(OH)D and gastrocnemius IMCL ($p = 0.02$) (Fig. 2). A linear regression was completed between our independent variable (gastrocnemius IMCL) and our dependent variables (vitamin D, BMI, physical activity, and sex). We found a significant relationship between IMCL and 25(OH)D ($p = 0.01$) and no significant relationship was observed between physical activity (0.08), BMI (0.14), sex (0.13), and IMCL. Based on reported physical activity levels, 25(OH)D, IMCL, and EMCL did not significantly differ ($p > 0.2$). 25(OH)D did not correlate with gastrocnemius EMCL ($p > 0.5$) (Table 3).

Performance measures

Muscle strength (1RMs) measures were not correlated with 25(OH)D, IMCL, or EMCL ($p > 0.1$). The FSST was the only physical function measure that significantly correlated with 25(OH)D ($R^2 = 0.26$; $p = 0.023$). The average time to complete the FSST was 8.6 ± 1.8 s with a range of 6.3–13.2 s.

Discussion

A positive linear relationship between 25(OH)D and gastrocnemius IMCL was observed in our healthy elderly cohort. To our knowledge, these are the first data to describe a

significant relationship between vitamin D status and IMCL. Although we did not observe a relationship between 25(OH)D and EMCL, previous research has documented an inverse relationship in post-pubertal females [19] and in older, previously hospitalized patients [17].

Recently, IMCL has been described to have a paradoxical association with both insulin sensitivity and resistance, sparking significant research interest as a key player in both normal and abnormal muscle metabolism [20, 21]. Several factors are thought to increase IMCL and potentially cause abnormal muscle metabolism such as a high BMI, advanced age, and a sedentary lifestyle. Conversely, increased IMCL contributing to normal muscle metabolism has been seen in active individuals. In this study, we show that physical activity levels and BMI did not influence the positive linear relationship we describe between gastrocnemius IMCL and vitamin D. Therefore, we interpret these data as a potentially positive insight that vitamin D may be independently involved in augmenting healthful IMCL accumulation. If vitamin D can independently alter the IMCL depot this could have significant implications on metabolic function in aged subjects trying to preserve muscle metabolism. Furthermore, this may be an interesting hypothesis to test, particularly in future work combining vitamin D repletion with a progressive aerobic training program. Since exercise is thought to impart a wide range of influences on muscle lipid content and metabolism [36], concomitant vitamin D repletion in aged subjects may uncover an exercise–vitamin D synergy that promotes preservation of muscle metabolic function via changes in muscle lipid metabolism and mitochondrial function.

The literature also suggests that relatively small amounts of moderate-intensity aerobic exercise have been shown to not only increase IMCL, but also to improve oxidative capacity, overall fitness, and insulin sensitivity [20–22]. These changes have been shown to occur quickly, as a 7-day aerobic training program promoted significant changes in [IMCL]/[EMCL] ratio in obese sedentary elderly adults [22]. The authors concluded that the IMCL pool increased as a result of EMCL to IMCL repartitioning. Independent of these findings, it has been proposed that a high turnover rate of pooled lipid metabolites, induced by exercise, assists in the maintenance of insulin sensitivity and that the regulation of lipid turnover and oxidation of lipids are important factors in maintaining insulin sensitivity [16, 37]. In summary, exercise is clearly an important stimulus to induce changes in muscle lipid that in turn influences metabolic function; but in this study, vitamin D status was the only predictor of IMCL in a sample of participants who were not exercising and had similar physical activity patterns.

The mechanistic connection between vitamin D and IMCL to explain our findings has yet to be fully elucidated. Vitamin D has been shown to play a key role in pathways that regulate lipid metabolism. Through its receptor, vitamin D can act on pathways involving DGAT, PPAR α , and its target gene CPT-1 [24, 25], but requires further investigation in human studies. Taken together with these molecular findings from other investigators [24, 25], we interpret our correlative data to support the hypothesis of vitamin D-induced IMCL accumulation to maximally serve as an energy substrate depot that supports normal muscle metabolic function and mitochondrial oxidation of lipid. We also propose that if vitamin D repletion has an independent effect on IMCL levels, vitamin D supplementation combined with aerobic training may have profound additive effects on improving metabolic outcomes.

Additionally, a recent study observed that vitamin D repletion improved mitochondrial oxidative phosphorylation. The study by Sinha et al. [38] documented an improvement in phosphocreatine recovery time, a marker of mitochondrial oxidation function, following 12 weeks of vitamin D supplementation in severely vitamin D-deficient adults. After vitamin D repletion, mitochondrial number, enzyme content, and oxygen supply increased compared to baseline [38]. The authors concluded that vitamin D deficiency may have a detrimental impact on mitochondrial function and that vitamin D repletion mitigated the loss of metabolic function.

In contrast to IMCL, the metabolic role of EMCL remains largely unknown [22, 39]. However, some studies suggest that EMCL is associated with impaired physical function and insulin resistance [22, 40] with an inverse relationship between EMCL and vitamin D status [17, 19]. We did not observe a significant relationship between vitamin D and EMCL. The discrepancy between our findings and other work may be attributed to different subject characteristics, extreme vitamin D deficiency, and different techniques used to assess EMCL. Tagliafico et al. [17] observed an increase in fatty degeneration of the thigh muscles in elderly adults, but, in contrast to our subjects, were vitamin D deficient and completing a follow-up visit after previous hospitalization. Our study sample was composed of a diverse range of 25(OH)D concentrations. Furthermore we manually completed fat segmentation, followed by EMCL quantification, whereas previous studies utilized computed tomography [19] or subjectively graded assessments of MR images [17]. Due to these varied assessments of EMCL it is difficult to compare results across studies.

It is unclear why we observed a relationship between 25(OH)D and gastrocnemius IMCL, but not soleus IMCL. The soleus muscle is a more oxidative mitochondrial-rich muscle [41], whereas the gastrocnemius is a mixture of Type I and II muscle fibers [41]. We speculate that it may be more difficult to see a relationship with soleus IMCL, as previous studies with varied interventions did not identify a relationship [39, 42, 43]. However, further investigations between IMCL and muscle types are warranted.

Several studies have suggested that vitamin D status effects muscle strength and physical function in the elderly [1, 3–9]. In this study we observed a positive relationship between 25(OH)D and the FSST. The FSST is a reliable and valid tool in assessing balance deficits and risks of falls [44] and has been previously associated with 25(OH)D [45]. This is an important finding as vitamin D deficiency is prevalent among the elderly [1] and lower vitamin D status may contribute to fall risk. We may not have observed significant relationships between 25(OH)D, and 1-RM test or other physical function measures due to our sample size and the higher vitamin D status of our cohort. Previous literature describes significant variability between vitamin D status and muscle strength and function [3–6]. Variability in study findings may be attributed to confounding factors such as differences in baseline strength, fitness level, and 25(OH)D levels ranging from deficiency to higher vitamin D status. Larger randomized control trials with a homogenous sample and significant vitamin D repletion are crucial to advance the understanding of the effects of vitamin D repletion on physical function and muscle strength in older individuals.

To our knowledge, this is the first study that has investigated the relationship between vitamin D and IMCL in a generally healthy older sample. Other strengths of this study include our homogenous group of healthy community-dwelling elderly participants, which minimized the variability in the functional ability associated with large age differences. Also, our analysis of EMCL by fat segmentation was robust and may suggest that vitamin D plays a minimal role in promoting EMCL reduction without concomitant exercise. Furthermore, with the use of MRS we have removed the invasive attainment of muscle tissue via biopsy. This is important for future studies to consider in a potentially frail, older population.

We acknowledge that multiple limitations exist for this cross-sectional study. The sample size was relatively small and underpowered for some analyses. In addition, subjects in this study were either vitamin D sufficient or insufficient, which did not allow for comparisons between outcomes across a wide range of vitamin D status, including vitamin D deficiency. With regards to body composition, a more sensitive measure such as the use of dual-energy X-ray absorptiometry would provide a better estimate of lean and fat mass compared to our use of BMI. Finally, a more thorough evaluation of physical activity levels with concomitant use of accelerometry and a validated questionnaire would provide a more robust analysis of physical activity.

Future work in this area should include study designs with larger sample sizes that stratify for sex. The incorporation of robust body composition measurements to examine how regional fat and muscle distribution predict changes in muscle lipid would also add to future investigation. Finally, to further evaluate the relationship between 25(OH)D, IMCL, and the possible metabolic effects of EMCL, future studies should expand on our findings and recent literature [17, 19, 22] by exploring the effectiveness of vitamin D repletion on muscle lipid repartitioning and the possible synergy between vitamin D repletion and exercise on metabolic function [38].

In conclusion, we observed a positive linear relationship between vitamin D and IMCL in healthy elderly individuals. This relationship was independent of reported physical activity, BMI, or age. Although the mechanisms explaining vitamin D contributions to muscle lipid metabolism are still under investigation, vitamin D could potentiate healthful IMCL accrual—a phenomenon that could play an important role in preserving muscle metabolic health in aging.

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Fig. 1.
Consort diagram

Fig. 2.
Relationship between 25(OH)D and gastrocnemius IMCL

Table 1

Subject characteristics, M(SD)

	Female (n = 15)	Male (n = 5)	Total (n = 20)
Age (years)	71.3 (4.0)	72.4 (4.8)	71.6 (4.1)
Height (cm)	161 (5.6) *	176.3 (5.4) *	164.8 (8.7)
Weight (kg)	68 (12.2) *	88.8 (7) *	73.2 (14.3)
Body mass index (kg/m ²)	26.3 (4.7)	28.6 (3.1)	26.9 (4.4)
25(OH)D (ng/mL)	36.7 (12.5)	40.6 (15.8)	37.7 (13.1)
	Range: 19–57	Range: 29–68	Range: 19–68
Race (n)			
White	14	5	19
Asian	1	0	1

* Significant differences between females and males in height and weight ($p < 0.002$)

Table 2

Soleus and gastrocnemius muscle lipid

Muscle lipid	Soleus	Gastrocnemius		
		Medial gastrocnemius	Lateral gastrocnemius	Total gastrocnemius
IMCL: water ratio $\times 100$ (au)	1.04 ± 0.43 (n = 20)	0.49 ± 0.17 (n = 6)	0.56 ± 0.26 (n = 7)	0.53 ± 0.22^a (n = 13)
EMCL % fat (n = 13)	–	10.3 ± 2.3 %	8.6 ± 2.4 %	19 ± 4.1 % ^b
		Range: 6.3–14.9 %	Range: 2.7–12.3 %	Range: 9–26 %

Au: arbitrary units

^aTotal gastrocnemius IMCL = average of medial and lateral gastrocnemius^bTotal gastrocnemius EMCL = (medial EMCL + lateral EMCL)/(n = 13)

Table 3

Pearson correlations for 25(OH)D, muscle strength, and physical performance

	Upper body strength				Lower body strength				Physical function			
	Back row	Chest press	Lat pull down	Leg press	Leg curl	Leg extension	TUG	TSS	FSST	Gait speed		
Female (N = 15)	-0.252 (p = 0.364)	0.015 (p = 0.957)	-0.441 (p = 0.100)	0.017 (p = 0.951)	-0.227 (p = 0.416)	-0.370 (p = 0.213)	-0.295 (p = 0.286)	-0.094 (p = 0.739)	-0.469 (p = 0.078)	0.195 (p = 0.485)		
Male (N = 5)	-0.004 (p = 0.995)	0.711 (p = 0.178)	-0.275 (p = 0.654)	0.447 (p = 0.451)	0.381 (p = 0.527)	-0.051 (p = 0.935)	-0.277 (p = 0.652)	-0.235 (p = 0.704)	-0.782 (p = 0.118)	0.136 (p = 0.827)		
Total (N = 20)	0.024 (p = 0.921)	0.265 (p = 0.259)	-0.046 (p = 0.849)	0.213 (p = 0.367)	0.095 (p = 0.691)	-0.052 (p = 0.837)	-0.300 (p = 0.199)	-0.133 (p = 0.577)	-0.507* (p = 0.023)	0.150 (p = 0.529)		

TUG: timed up and go; TSS: timed sit to stand; FSST: four-squared step test

* Significant correlation $p < 0.5$