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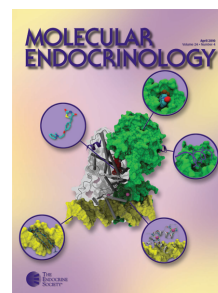
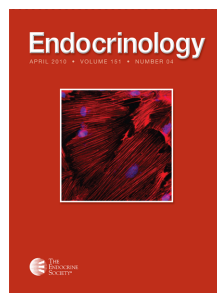
THE JOURNAL  
OF CLINICAL  
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J. Clin. Endocrinol. Metab. 2010 95:1595-1601 originally published online Feb 17, 2010; , doi: 10.1210/jc.2009-2309

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## Vitamin D Status and Its Relation to Muscle Mass and Muscle Fat in Young Women

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**Context:** Vitamin D insufficiency has now reached epidemic proportions and has been linked to increased body fat and decreased muscle strength. Whether vitamin D insufficiency is also related to adipose tissue infiltration in muscle is not known.

**Objective:** The objective of the study was to examine the relationship between serum 25-hydroxyvitamin D (25OHD) and the degree of fat infiltration in muscle.

**Design:** This was a cross-sectional study.

**Outcome Measures and Subjects:** Measures were anthropometric measures, serum 25OHD radioimmunoassay values, and computed tomography (CT) values of fat, muscle mass, and percent muscle fat in 90 postpubertal females, aged 16–22 yr, residing in California.

**Results:** Approximately 59% of subjects were 25OHD insufficient ( $\leq 29$  ng/ml), of which 24% were deficient ( $\leq 20$  ng/ml), whereas 41% were sufficient ( $\geq 30$  ng/ml). A strong negative relationship was present between serum 25OHD and CT measures of percent muscle fat ( $r = -0.37$ ;  $P < 0.001$ ). In contrast, no relationship was observed between circulating 25OHD concentrations and CT measures of thigh muscle area ( $r = 0.16$ ;  $P = 0.14$ ). Multiple regression analysis indicated that the relation between 25OHD and muscle adiposity was independent of body mass or CT measures of sc and visceral fat. Percent muscle fat was significantly lower in women with normal serum 25OHD concentrations than in women with insufficient levels and deficient levels ( $3.15 \pm 1.4$  vs.  $3.90 \pm 1.9$ ;  $P = 0.038$ ).

**Conclusions:** We found that vitamin D insufficiency is associated with increased fat infiltration in muscle in healthy young women. (*J Clin Endocrinol Metab* 95: 1595–1601, 2010)

Vitamin D, a key regulator of bone metabolism, is also known to be significantly associated with muscle strength (1). A lack of vitamin D can cause myopathy (2, 3), which tends to be more marked in the proximal muscles (4). In the elderly, vitamin D deficiency is linked to muscle weakness, increased body sway, and increased susceptibility to falls and fractures, which are improved by the administration of vitamin D with calcium (5–13). Vitamin D levels have also been shown to be significantly associated with muscle strength in healthy postmenarchal girls, suggesting that muscle contractility may be affected by

vitamin D status (1). The mechanisms underlying the effect of vitamin D on muscle strength are not fully understood but could be related to an independent effect on muscle mass, or alternatively, to enhancement of muscle function mediated through the effect of vitamin D. Nonetheless, vitamin D action requires activation of the vitamin D receptor, which is widely distributed in various tissues including skeletal muscle (14, 15).

Available data indicate that a higher muscle lipid content, measured as muscle attenuation with computed tomography (CT), is associated with lower levels of muscle

strength and physical performance, independent of muscle mass (16, 17). Additionally, several studies in patients with neuromuscular disorders have demonstrated skeletal muscle attenuation determined by CT to be strongly reciprocally associated with muscle lipid content (18–20).

In the current study, we propose that 25-hydroxyvitamin D (25OHD) concentrations are reciprocally related to adipose tissue infiltration in muscle independently of muscle mass. To test this hypothesis, we examined the relation between vitamin D and skeletal muscle lipid content and muscle mass using CT in a cohort of healthy young women living in California who were recently studied and found to have a reciprocal relation between vitamin D status and measures of adiposity (21).

## Subjects and Methods

### Study subjects

The sample of this study was the same 90 postpubertal healthy females who have been more thoroughly described in a previous investigation on the relation of vitamin D to body fat and bone (21). This study was approved by the institutional review board at our institution, and informed consent was obtained from all parents and/or subjects.

Candidates were excluded if they had a diagnosis of any underlying disease or chronic illness, had been ill for longer than wk during the 6 months before examination, had been admitted to the hospital at any time during the prior 3 yr, or were taking any medications including oral contraceptives. Candidates who were pregnant, had ever been pregnant, or had absence of menses for more than 4 consecutive months were also excluded from the study. To decrease the seasonal variability in biochemical determinations, all appointments were scheduled between May and October. In addition, all subjects had normal kidney function and normal liver function tests, and there was no evidence of liver abnormalities detected by CT. All participants had reached sexual maturity, defined as Tanner V of breast development (22), and skeletal maturity, defined as epiphyseal closure in the phalanges and metacarpals using the radiographic atlas of Greulich and Pyle (23).

Levels of physical activity in 83 participants were examined using a 7-d physical activity recall questionnaire. Participants were asked to indicate the number of times in the past week that they had engaged in strenuous, moderate, and mild forms of physical activity for more than 15 min and the daily time spent watching television and/or on the computer. This measure represents frequency, intensity, and duration elements of physical activity and inactivity with a test-retest reliability coefficient of 0.81 (24, 25).

### Muscle and fat measurements

CT measurements of muscle and sc fat were determined using a Hilite Advantage scanner (General Electric Healthcare, Milwaukee, WI) with a standardized reference phantom for simultaneous calibration. At the level of the umbilicus, measurements of the abdominal visceral fat (VF; square centimeters) and sc fat (SF; square centimeters) were obtained. For the purpose of this

study, SF was defined as the amount of adipose tissue located between the skin and rectus muscles of the abdomen, the external oblique muscles, the broadest muscles of the back, and the erector muscles of the spine at the level of the umbilicus. VF was defined as the intraabdominal adipose tissue surrounded by the rectus muscles of the abdomen, the external oblique muscles, the lumbar quadratus muscle, the psoas muscles, and the lumbar spine at the same level. The coefficient of variation (CV) for repeated measures of VF and SF has been reported to range from 1.5 to 3.5% (26). Additionally, measures of sc thigh fat and thigh muscle area were obtained at the midshafts of the femurs, and values from both the right and left leg measurements were averaged for analysis; the CVs for repeated CT measurements of sc fat and muscle in the thigh were previously reported to fall between 1 and 2% (27). Measures of thigh muscle area included the vastus muscles, adductors, rectus femoris, sartorius, gracilis, and semitendinosus and semimembranosus muscles.

The tissue densities of muscle and fat were determined by converting CT values expressed as Hounsfield units into measures of tissue density using an external phantom (28). All muscle and fat measures were obtained from a 2-cm<sup>2</sup> region of the rectus femoris and a 2-cm<sup>2</sup> region of adjacent sc fat. Based on the tissue densities of fat and muscle, the percentage of adipose tissue in the muscle was calculated according to the formula:  $(M-x)/(M-y) \times 100$ , where M represents the density of muscle without fat, x represents the density of the muscle, and y represents the density of fat. For the purpose of this study, the highest muscle density was used as the reference value representing muscle tissue with no fat infiltration. The CV for muscle attenuation from single-slice CT scans of the midthigh have previously been reported to be 0.51% for test-retest variability and 3.3% for within-subject variance (18). The inter- and intraclass correlation coefficients for measurements of adiposity in soft tissues using CT and a reference phantom are outstanding at greater than 0.98 (29). The time taken to complete the CT scans was approximately 10 min and the effective radiation dose was approximately 0.1 mSv (30).

### Biochemical determinations

Serum levels of 25OHD were assayed using a RIA as described by Hollis *et al.* (31). The lower limit of detection was 5 ng/ml (12.5 nmol/liter). Goat anti-25OHD was a gift from Dr. Bruce Hollis, the Director of Pediatric Nutritional Sciences at the Medical University of South Carolina. <sup>125</sup>I-25-(OH)D<sub>3</sub> and donkey antigoat secondary antibody were purchased from Diasorin (Stillwater, MN). This assay recognizes 25OHD<sub>2</sub> and 25OHD<sub>3</sub> equally and shows no bias when compared with HPLC (32). Calculated assay precision for within-assay variation averages of 6% and interassay of 16%. For the purpose of this study and according to the current consensus, subjects were divided into a 25OHD sufficient, or normal, group ( $\geq 30$  ng/ml) and an insufficient group ( $\leq 29$  ng/ml). Intact PTH (1–84) was measured with an electrochemiluminescent assay (33). The sensitivity of the assay is 1.2 pg/ml (0.127 pmol/liter) and intra- and interassay variations are 1.9–4 and 2.6–6.5%, respectively. To minimize interassay variability, all samples were analyzed simultaneously.

### Statistical analysis

Statistical analysis was carried out using Statview (version 5.0.1; SAS Institute Inc., Cary, NC). Data were analyzed using simple linear regression analysis, multiple regression analysis, and unpaired *t* tests. All values are expressed as mean  $\pm$  SD.

## Results

Age, anthropometric characteristics, 25OHD, and CT measures of muscle and muscle fat, separated based on 25OHD concentrations, are described in Table 1. Thirty-seven women (41%) had 25OHD concentrations 30 ng/ml or greater, whereas 57 women (59%) had insufficient 25OHD concentrations (<29 ng/ml), of which 22 women (24%) were vitamin D deficient ( $\leq 20$  ng/ml). Compared with women with normal 25OHD values, vitamin D-insufficient subjects were significantly shorter and heavier and had greater body mass index (BMI) and abdominal SF and VF.

CT measures of muscle attenuation were significantly lower in the 25OHD-insufficient group when compared with women with normal 25OHD values. The percentage of fat infiltration of the muscle derived from the attenuation values was also greater in women with lower 25OHD concentrations. In contrast, there were no differences in CT values for muscle area in the thighs between women in the two vitamin D groups. This was true whether the right or left thighs were analyzed independently (data not shown) or both extremities analyzed together.

Significant inverse correlations were observed between 25OHD and BMI, SF, VF, sc thigh fat, the degree of inactivity, and percent muscle fat; the strongest with percent muscle fat (Table 2 and Fig. 1). In contrast, neither age nor CT measures of muscle area were associated with 25OHD levels. Measures of muscle fat were unrelated to BMI, SF, VF, sc thigh fat, and measures of inactivity but inversely related to muscle area (Table 2).

Multiple regression analysis indicated that the relation between 25OHD levels and percent muscle fat was independent of body mass and measures of inactivity (Table 3). Similar results were found when measures of sc and visceral adiposity replaced BMI as an independent variable in this model (data not shown).

A significant inverse correlation was found between 25OHD and PTH ( $r = -0.27$ ;  $P = 0.01$ ), and PTH values were higher in the insufficient than in the sufficient group ( $2.28 \pm 0.88$  and  $1.92 \pm 0.90$ , respectively;  $P = 0.025$ ).

## Discussion

In this study, we used CT to investigate whether muscle mass and muscle adiposity in healthy young adult females are associated with vitamin D. Our data demonstrated that serum 25OHD was inversely related to the percent fat of skeletal muscle, a relation that was independent of body mass or CT measures of sc and visceral fat. Compared with women with normal 25OHD values, vitamin D-insuffi-

**TABLE 1.** 25OHD values, age, anthropometric characteristics, and CT measures of muscle for 90 women separated by 25OHD concentration groups

	All (n = 90)	Sufficient (n = 37)	Insufficient (n = 53)	P values
25OHD (ng/ml)	30.1 ± 13.0 (6.7–69.6)	42.4 ± 10.1 (30.0–69.6)	21.5 ± 5.9 (6.7–29.6)	<0.001
Age (yr)	19.4 ± 1.5 (16.3–22.8)	19.2 ± 1.6 (16.3–22.8)	19.5 ± 1.4 (17.0–22.87)	0.408
Weight (kg)	68.3 ± 17.5 (45.5–126.0)	63.9 ± 11.9 (45.6–113.0)	71.3 ± 20.0 (45.5–126.0)	0.046
Height (cm)	162.9 ± 4.7 (153.9–171.8)	164.1 ± 3.9 (156.8–170.3)	162.1 ± 5.1 (153.9–171.8)	0.048
BMI	25.7 ± 6.3 (16.7–44.5)	23.7 ± 4.6 (16.7–43.9)	27.1 ± 7.1 (17.6–44.5)	0.014
SF (cm <sup>2</sup> )	252.8 ± 152.7 (59.1–799.7)	203.3 ± 98.9 (59.1–431.9)	288.1 ± 174.0 (63.9–799.7)	0.029
VF (cm <sup>2</sup> )	36.46 ± 42.89 (2.8–240.8)	24.74 ± 33.88 (5.6–218.2)	44.81 ± 46.83 (2.8–240.8)	0.009
Thigh musculature (cm <sup>2</sup> )	87.49 ± 33.92 (33.5–177.4)	92.98 ± 33.50 (36.5–151.9)	83.66 ± 34.01 (33.5–177.4)	0.202
Subcutaneous thigh fat (cm <sup>2</sup> )	118.9 ± 61.0 (14.4–324.0)	104.8 ± 45.5 (26.6–219.5)	128.7 ± 69.4 (14.4–324.0)	0.067
Muscle fat (%)	3.59 ± 1.70 (0.0–9.5)	3.15 ± 1.37 (0.4–5.8)	3.90 ± 1.85 (0.0–9.5)	0.038
Inactivity (h/wk) <sup>a</sup>	7.8 ± 5.1 (1.5–31.8)	6.8 ± 10.4 (3.0–16.0)	8.4 ± 36.5 (1.5–31.8)	0.161

<sup>a</sup> Measures of inactivity were obtained in 83 subjects: 34 in the sufficient and 49 in the insufficient group.

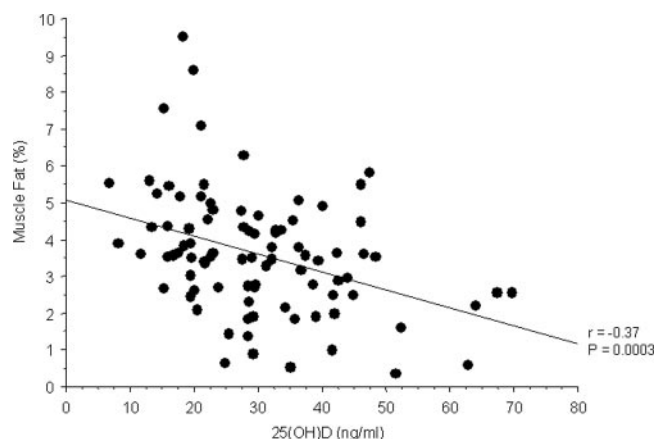
**TABLE 2.** Relations between 25OHD, anthropometric characteristics, and CT measures of muscle and fat in 90 women

	r	P
25OHD (ng/ml)		
Age (yr)	−0.17	0.101
BMI (kg/m <sup>2</sup> )	−0.35	<0.001
SF (cm <sup>2</sup> )	−0.36	<0.001
VF (cm <sup>2</sup> )	−0.28	0.007
Thigh muscle area (cm <sup>2</sup> )	0.16	0.138
Subcutaneous thigh fat (cm <sup>2</sup> )	−0.32	0.002
Inactivity (h/wk) <sup>a</sup>	−0.27	0.013
Muscle fat (%)		
Age (yr)	0.28	0.007
BMI (kg/m <sup>2</sup> )	−0.09	0.411
SF (cm <sup>2</sup> )	−0.08	0.459
VF (cm <sup>2</sup> )	−0.04	0.690
Thigh muscle area (cm <sup>2</sup> )	−0.24	0.024
Subcutaneous thigh fat (cm <sup>2</sup> )	−0.05	0.634
Inactivity (h/wk) <sup>a</sup>	0.17	0.122

<sup>a</sup> Measures of inactivity were obtained in 83 subjects.

cient women had approximately 24% greater muscle fat infiltration than women with normal serum 25OHD concentration. In contrast, we found no relation between thigh muscle area and serum levels of vitamin D and no significant differences in the cross-sectional area of thigh muscles between women with and without vitamin D insufficiency.

It should be noted that none of the young women had symptoms of overt vitamin D insufficiency or complained of muscle weakness, yet we were able to show the association of 25OHD with fat accumulation in skeletal muscle. However, muscle adiposity is known to strongly influence muscle strength, and available data indicate that im fat accumulation increases with reduced physical activity and decreases with exercise (16, 34, 35). It has also been shown that exercise training in both lean and obese subjects significantly improved the ability to oxidize lipids in skeletal muscle (34, 36). Although we did not measure



**FIG. 1.** Relation between vitamin D concentrations and CT measures of muscle fat infiltration.

**TABLE 3.** Multiple regression analysis with serum levels of vitamin D as the dependent variable in 83 women

	β	SE	P value	R <sup>2</sup>
25OHD (ng/ml)				
Muscle fat (%)	−2.94	0.72	<0.001	0.29
BMI (kg/m <sup>2</sup> )	−0.65	0.20	0.001	
Inactivity (h/wk)	−0.29	0.25	0.254	

muscle strength, a recent study by Ward *et al.* in a group of healthy postmenarchal adolescents (1) demonstrated serum 25OHD to be positively related to muscle power, force, velocity, and jump height. Because these associations, like those found in the current study, were independent of BMI, they cannot be ascribed to overall body adiposity.

In the current study, we found no relation between vitamin D levels and the cross-sectional area of the muscles in the thigh. However, vitamin D supplementation has been reported to enhance muscle strength without any apparent changes in muscle mass (11, 37, 38). Indeed, whereas muscle mass and strength are often associated, this association is relatively weak (39), and muscle strength can significantly improve without significant changes in muscle mass (40–42). Whereas measures of inactivity were positively related to weight and reciprocally related to vitamin D, neither CT measures of muscle area nor muscle adiposity was associated with time spent watching television and on the computer. Hence, it is possible that the muscle fat content in our subjects was a direct consequence of vitamin D insufficiency rather than the result of reduced vitamin D associated with increased adiposity due to inactivity.

Although the mechanism(s) underlying the vitamin D-muscle fat link is unknown, it is possible that im adipose tissue accumulation could regulate muscle metabolism. Vitamin D has been shown to mediate protein synthesis and cellular ATP accumulation (43), increase troponin C (44), and increase actin and sarcoplasmic protein expression (45) in striated muscles; whether these and/or other cellular events are influenced by im fat requires further study. Regardless of the mechanism by which vitamin D influences skeletal muscle composition, im lipids play an important role in the function of myocytes. Skeletal muscle fat infiltration impairs skeletal muscle mitochondrial function (46) and decreases the rate of mitochondrial oxidative phosphorylation (47). Furthermore, muscle triglyceride content reduces insulin-stimulated glucose uptake within muscle (48, 49). Other studies indicate that im adipose tissue deposition is associated with insulin resistance and diabetes (50–52) and accompanied by decreased glucose use, decreased signaling through the phosphatidylinositol 3-kinase/Akt pathway,

and decreased insulin-stimulated glycogen synthesis (52, 53). Additionally, low levels of circulating 25OHD have also been associated with glucose intolerance (54, 55) and insulin resistance (56) in adults, suggesting a possible link between vitamin D status, muscle fat content, and glucose homeostasis.

The high prevalence of vitamin D insufficiency in this young population, living in a sun-rich area, even during spring and summer months when vitamin D synthesis is greatest, is surprising and likely multifactorial. Close to 60% of women studied had insufficient 25OHD concentrations (<29 ng/ml), and close to 25% were vitamin D deficient ( $\leq 20$  ng/ml). These findings are, however, consistent with international data suggesting that even in the sunniest areas of the world, vitamin D deficiency is common. Studies in Turkey, Lebanon, Saudi Arabia, United Arab Emirates, India, and Australia show 30–50% of children and young adults to have 25OHD levels less than 20 ng/ml (57). Moreover, a recent study found that a substantial proportion (60%) of healthy adolescents living in a sunny Brazilian climate had vitamin D insufficiency (58).

There are limitations to our study, including its cross-sectional design and the inability to establish a causal relation between skeletal muscle adipose tissue infiltration and vitamin D. We used a single CT measure obtained at the mid thigh as a surrogate of muscle volume to minimize radiation exposure and were able to measure only a relatively small depot of skeletal muscle adipose tissue. However, previous studies noted a relatively strong correlation between the skeletal muscle fat content at different sites (59). Moreover, our study subjects were not recruited from the community at large and were limited to young females. Future studies are needed to determine whether vitamin D influences fat infiltration in the muscle in males and the elderly. Nevertheless, serum 25OHD is related to overall physical fitness in postmenopausal women (60), and ample data indicate that inadequate vitamin D status is related to muscle weakness and the risk of falls in older persons, regardless of gender (35). Lastly, studies are needed to determine whether the increased muscular adiposity (17, 31, 49) and decreased muscle strength and mobility (57–60) with aging are associated with vitamin D status. This is particularly relevant because vitamin D insufficiency is highly prevalent in the elderly population (61, 62).

In conclusion, our data show that vitamin D insufficiency is significantly and inversely associated with the degree of fat infiltration in skeletal muscle, a relation that is independent of body mass. This reciprocal association between vitamin D status and muscle fat was not previously reported and is unexplained and intriguing. Further

studies are needed to characterize the possible mechanistic relationship between muscle fat and vitamin D.

## Acknowledgments

The authors express their gratitude to Dr. Timothy Reinhardt for providing the measures of serum 25OHD.

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This work was supported by National Institutes of Health Grant 1R01 AR052744-01, Department of the Army Grant DAMD17-01-1-0817, Canadian Institutes of Health Research Grant MT-10839, Natural Science and Engineering Research Council/Dairy Farmers of Canada, and Natural Sciences and Engineering Research Council and Dimensional Fund Advisors Canada.

Disclosure Summary: V.G., A.K., A.O.M., T.A.L.W., and R.K. have nothing to declare.

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