Relationship Between Vitamin D Status From Childhood to Early Adulthood With Body Composition in Young Australian Adults

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Context: Vitamin D plays a role in the differentiation and metabolism of skeletal muscle and, possibly, adipose tissue; however, the relationship between vitamin D status during growth and body composition in early adulthood is unclear.

Objective: We examined associations between vitamin D status in childhood, adolescence, and early adulthood with body composition at age 20 years.

Design, Setting, Participants: We studied 821 offspring (385 females) of the Western Australian Pregnancy Cohort Study who had ≥3 serum 25-hydroxyvitamin D [25(OH)D] at age 6, 14, 17, and 20 years and body composition assessed at age 20 using dual-energy x-ray absorptiometry. The participants were grouped into four vitamin D status trajectories: consistently lower, decreasing, increasing, and consistently higher.

Results: The mean serum 25(OH)D at the study visits was 72.7 to 86.8 nmol/L. In males, serum 25(OH)D at 17 and 20 years was positively associated with lean body mass (LBM), and 25(OH)D at age 20 correlated negatively with fat body mass (FBM). Males with a consistently higher 25(OH)D trajectory had a 2.3- to 3.7-kg greater LBM and 4.1- to 6.0-kg lower FBM at 20 years compared with those with consistently lower or decreasing trajectories (P < 0.05 for all). In females, 25(OH)D at 14, 17, and 20 years was negatively associated with FBM. Females with increasing or consistently higher 25(OH)D trajectories had a 5.2- to 6.8-kg lower FBM at age 20 compared with those with a consistently lower trajectory (P < 0.05 for all).

Conclusions: In the present predominantly white, relatively vitamin D-replete cohort, a higher vitamin D status trajectory from childhood to early adulthood was associated with a greater LBM in males and lower FBM in both sexes at age 20.

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; ALM, appendicular lean mass; BMI, body mass index; DXA, dual-energy x-ray absorptiometry; EIA, enzyme immunoassay; FBM, fat body mass; LBM, lean body mass; LC-MS/MS, liquid chromatography/tandem mass spectrometry; Raine study, Western Australia Pregnancy Cohort study; TV, television.
Vitamin D has well-established physiological roles in calcium homeostasis and bone metabolism, and considerable evidence has supported its importance in the differentiation and metabolism of skeletal muscle cells [1]. Despite this, studies examining the relationship between vitamin D status and lean body mass (LBM) in adults have yielded inconsistent results, with some showing positive associations [2, 3], and others showing null associations with muscle mass [4] or area [5].

It has been hypothesized that during growth, vitamin D might have a positive influence on the accumulation of lean mass and that this might partly mediate the positive effect of vitamin D on bone mineral accretion [1]. However, no prospective studies have examined the relationship between vitamin D status during the developmental years and LBM in young adults.

Individuals who are obese tend to have lower vitamin D levels than those who are lean, and an inverse relationship between the body mass index (BMI) and serum 25-hydroxyvitamin D [25(OH)D] is well-established [6–8]. The proposed mechanisms for this have included reduced subcutaneous synthesis of vitamin D owing to less time spent outdoors, sequestration of 25(OH)D in adipose tissue [9], and volumetric dilution [10]. It is also possible that a low vitamin D status increases the risk of developing obesity, although the evidence has been conflicting. In the HUNT study (Nord-Trøndelag Health Study), participants with serum 25(OH)D <50 nmol/L at baseline had a substantially increased odds ratio for new-onset obesity during 10 years of follow-up [11]. In contrast, in an intervention study, cholecalciferol supplementation in overweight and obese adults for 1 year did not substantially affect body weight or body fat percentage compared with placebo; however, the mean serum 25(OH)D concentration was >50 nmol/L at baseline [12]. A Mendelian randomization analysis of vitamin D and obesity concluded that a higher BMI could lead to a lower 25(OH)D but that any effects of a lower 25(OH)D in increasing the BMI were likely to be small [13]. However, BMI has well-recognized limitations as a measure of body fatness, and it remains possible that vitamin D status affects body composition (i.e., the proportion of LBM and FBM) without altering the body weight or BMI. In support of this, in a 12-week randomized controlled trial of 77 healthy, premenopausal overweight and obese women with a serum 25(OH)D concentration <50 nmol/L, supplementation with vitamin D3 at 1000 IU daily substantially reduced the FBM, without substantial effects on body mass or BMI [14]. In young women, vitamin D insufficiency has been associated with a greater total FBM measured using DXA, with visceral and subcutaneous fat measured using computed tomography [15]. Data are lacking on whether the vitamin D status during childhood, adolescence, and early adulthood is associated with the body composition in young adults.

We previously reported that in the offspring (generation 2) of the Western Australia Pregnancy Cohort (Raine) study, a higher vitamin D status during childhood and adolescence was associated with substantially greater bone mass at age 20 years in men but not in women [16]. Because of the negative health consequences associated with a low muscle mass and high body fat in later life, in the present study, we examined the relationships between vitamin D status during growth and development with LBM, ALM (a surrogate of muscle mass), FBM, and trunk/limb fat mass ratio (a surrogate of visceral fat) [17] in young adults.

1. Subjects and Methods

A. Participants

The present longitudinal, prospective study included data from 821 offspring (436 males and 385 females) from the Raine study. The original study had recruited 2900 pregnant women
from the antenatal clinic at King Edward Memorial Hospital and nearby private clinics in Perth, Western Australia from May 1989 to November 1991, as previously reported [18]. All offspring were invited to attend periodic follow-up examinations. Compared with the general Western Australian population, the characteristics of the Raine participants at birth were similar to all Western Australian contemporaneous births, except that the Raine participants had had slightly more pregnancies with complications and cesarean deliveries and had had more first-time mothers and unmarried mothers. However, comparisons of the participants remaining in the study at the 14-, 17-, and 20-year follow-up evaluations suggested that attrition resulted in a cohort comparable with the general population [19]. A total of 1306 offspring participated in the clinical component of the 20-year follow-up evaluation, and 1183 had a valid whole body DXA scan available. The present study was restricted to those participants who had undergone a whole body DXA scan at 20 years and also had three or more measurements of serum 25(OH)D available from the study time points of age 6, 14, 17, and 20 years. Each follow-up protocol had been approved by the human research ethics committee of Princess Margaret Hospital (years 6, 14, and 17) or the University of Western Australia (year 20). At each study visit, the parents and/or offspring, as appropriate, had provided written informed consent.

B. Vitamin D Status at 6, 14, 17, and 20 Years

Fasting venous blood samples were collected at age 6, 14, 17, and 20 years, and the serum was then securely stored at −80°C. The assay method for serum 25(OH)D has been previously reported in detail [16]. In brief, at 6 and 14 years of age, serum 25(OH)D was measured using the enzyme immunoassay (EIA; Immunodiagnostics Systems, Ltd., Scottsdale, AZ), which uses an antibody (RRID:AB_2756867 [20]). At 17 and 20 years of age, 25(OH)D was measured using isotope-dilution liquid chromatography/tandem mass spectrometry (LC-MS/MS; RMIT Drug Discovery Technologies, Melbourne, Victoria, Australia) according to the report method [21]. The sample analysis at age 14 years showed excellent agreement between the two methods ($r^2 = 0.933$; intraclass correlation coefficient, 0.940), with no systematic bias [22]. In contrast, samples from age 6 years showed a positive bias in EIA results compared with LC-MS/MS. Accordingly, a weighted Deming regression [23] was used to calculate standardized 25(OH)D values at year 6: standardized 25(OH)D = 22.3 + 0.58 × EIA. The interassay coefficient of variation for the EIA ranged from 4.6% to 8.7% and for the LC-MS/MS from 5.0% to 8.8% at different 25(OH)D concentrations. For both methods, the internal quality control tests showed that the quality control samples passed the acceptance criteria. To convert 25(OH)D from nmol/L to ng/mL, the value is multiplied by 0.4.

Latent class growth analysis was used to identify trajectories of vitamin D status, and, as previously reported [16], four vitamin D status trajectories were identified: (i) consistently lower [most serum 25(OH)D values in the two bottom quartiles; n = 259]; (ii) decreasing (moving from the two top quartiles to the two bottom quartiles over time; n = 125); (iii) increasing (moving from the two bottom quartiles to the two top quartiles over time; n = 138); and (iv) consistently higher (most values in the two top quartiles; n = 299).

C. Whole Body DXA at 20 Years

Whole body scanning was performed at the 20-year follow-up visit using DXA with a Norland XR-36 densitometer (Norland Medical Systems, Inc., Fort Atkinson, WI), in accordance with the manufacturer-recommended procedures. Analysis of the scans was performed using built-in machine software (version 4.3.0), and the regions of interest for the head, trunk, arms, and legs were manually placed by trained study staff using a standard analysis protocol. The analysis provided estimates of LBM, FBM, and the lean mass of the arms and legs, which were summed to provide the appendicular lean mass (ALM; reflecting the skeletal muscle mass). The trunk/limb fat mass ratio was calculated as the trunk fat mass divided by the fat mass of the arms and legs and was used as the surrogate for visceral fat [17]. Calibration was performed daily before each scanning session, and the interscan coefficient of variation was <2%. 

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D. Other Assessments

At age 6, 14, 17, and 20 years, the participants’ body weight was measured to the nearest 0.1 kg with the subjects dressed in light clothes. Their height was measured using a stadiometer to the nearest 0.1 cm. The weight/height ratio was calculated as the weight in kilograms divided by the height in meters. The BMI calculated as the weight in kilograms divided by the height in square meters. In addition, data on organized sports participation (except for at 20 years) and television (TV) watching were collected using a questionnaire. At 14 years, the month and year of menarche for the girls were recorded. At age 20 years, a validated semiquantitative food frequency questionnaire from the Cancer Council Victoria [24] was used to assess dietary intake. The physical activity level was assessed using the International Physical Activity Questionnaire (categorized as low, medium, and high according to the International Physical Activity Questionnaire scoring protocol [25]), and oral contraceptive use in females was recorded by questionnaire.

E. Statistical Analysis

The variables are presented as the mean ± SD or estimated mean ± SEM for the adjusted analysis, unless otherwise stated. The normality of the continuous variables was checked through the construction of histograms. The characteristics of the participants included in the present study were compared with those of the entire Raine study cohort to determine whether participants were representative of the broader cohort using the Student t test and \( \chi^2 \) test. Because we found significant interactions between sex and the vitamin D status trajectories for the associations with LBM and BMI, the data from the males and females were analyzed separately.

Comparisons among the four vitamin D status trajectories on the anthropometric and lifestyle characteristics at ages 6, 14, 17, and 20 years and body composition outcomes at age 20 years were performed using ANOVA with the Tukey post hoc test or Mantel-Haenszel linear-by-linear association, as appropriate. Further comparisons of the body weight, weight/height ratio, BMI, and body composition measures at 20 years were performed using analysis of covariance with the Bonferroni post hoc test adjusted for age, height (for the models of body weight, LBM, and FBM), TV watching, physical activity level, energy intake, and protein intake at 20 years. In addition, linear regression analyses were used to evaluate the associations between the serum 25(OH)D at different ages (6, 14, 17, and 20 years) and body weight, weight/height ratio, BMI, and body composition measures at age 20 years. These were adjusted for covariates, including the season of blood sampling, TV watching, and organized sports participation at the 25(OH)D assessment and age, height (for models for body weight, lean and fat mass), TV watching, physical activity level, energy intake, and protein intake at 20 years. The models for LBM were also adjusted for FBM and vice versa. The models for females were also adjusted for age at menarche and oral contraceptive use at 20 years. The statistical significance level was set at \( P < 0.05 \) (two-tailed). All analyses were performed using SPSS, version 22 (IBM Corp., Armonk, NY), and R, version 3.4.2 (R Foundation for Statistical Computing, Vienna, Austria).

2. Results

A. Clinical Characteristics and Vitamin D Trajectories

Of the 821 participants (436 males and 385 females) included in the present study, 499, 778, 775, and 793 had had serum 25(OH)D measured at ages 6, 4, 17, and 20 years, and the mean 25(OH)D levels were 82.3 ± 18.5, 86.8 ± 30.0, 74.9 ± 27.5, and 72.7 ± 25.3 nmol/L, respectively. At the 20-year follow-up visit, the mean age of the participants was 20.4 ± 0.4 years, and the mean BMI was 23.9 ± 4.3 kg/m\(^2\). No significant differences were found in age, BMI, or sex- and height-adjusted total LBM and FBM between the individuals included in the
The present study and the 362 participants who had undergone a whole body DXA assessment at age 20 years but were not included.

The comparison of characteristics among the four vitamin D status trajectory groups at each time point for the males and females is presented in Table 1. No significant differences were found among the four groups for all characteristics studied at age 6 years in either sex, except that for the females, the increasing vitamin D status trajectory group had a significantly lower BMI compared with the consistently lower group. From the consistently lower and decreasing trajectories to the increasing and consistently higher trajectories, a trend was found for increased organized sports participation or physical activity level at ages 14, 17, and 20 years in both sexes, and a trend toward less TV watching at age 17 and 20 years in females. Compared with the consistently lower trajectory group, the males in the consistently higher trajectory group had a significantly lower BMI at age 14 years and significantly greater energy intake at age 20 years. The females in the consistently higher and increasing trajectory groups had a significantly lower BMI at age 14 and 20 years and significantly lower body weight at age 20 y, and females in the increasing trajectory group also had significantly lower BMI at age 17 y and were significantly taller at ages 17 and 20 y.

B. Serum 25(OH)D and Body Composition

In males, after adjustment for covariates, serum 25(OH)D at ages 17 and 20 years (but not 6 or 14 years) was positively associated with LBM and ALM at age 20 years (Table 2). For serum 25(OH)D measured at age 17 years, each additional 25 nmol/L of 25(OH)D was associated with a 0.91-kg additional LBM and a 0.43-kg additional ALM at age 20 years. In contrast, for 25(OH)D measured at age 20 years, the corresponding data were 2.12 kg and 1.01 kg. Furthermore, 25(OH)D measured at age 20 years was negatively associated with fat mass, such that each additional 25 nmol/L of 25(OH)D at 20 years was associated with 3.15 kg less FBM and a trunk/limb fat mass ratio that was 0.07 unit lower.

In females, the serum 25(OH)D concentrations at ages 6, 14, 17, and 20 years were not a significant predictor of LBM at age 20 years. However, 25(OH)D at ages 14, 17, and 20 years was negatively associated with BMI and FBM at age 20 years, such that each additional 25 nmol/L of 25(OH)D was associated with a 0.54-, 0.81-, and 0.77-kg/m² lower BMI and 1.33-, 1.92-, and 2.31-kg lower FBM, respectively. In addition, 25(OH)D at ages 17 and 20 years was negatively associated with body weight and weight/height ratio at age 20 years, and 25(OH)D at age 17 years (but not at the other study visits) was significantly associated with lower trunk/limb fat mass ratio [0.04 unit lower per 25 nmol of 25(OH)D]; Table 2.

C. Vitamin D Trajectories and Body Composition

In the unadjusted analysis, males in the consistently higher vitamin D status trajectory had a significantly greater mean LBM (by 3.5 kg) and ALM (by 1.4 kg) at age 20 years compared with those in the consistently lower trajectory and significantly lower FBM (by 4.9 to 5.8 kg) and trunk/limb fat mass ratio (by 0.11 to 0.14 unit) compared with the consistently lower and decreasing trajectory groups. The increasing vitamin D status trajectory resulted in a significantly lower FBM (by 3.9 to 4.8 kg) compared with the consistently lower and decreasing trajectories (Table 3). After adjustment for covariates, the associations were attenuated; however, males in the consistently higher vitamin D status trajectory group maintained a significantly greater LBM (by 2.3 to 3.7 kg) and significantly lower FBM (by 4.1 to 6.0 kg) compared with the consistently lower and decreasing trajectories and significantly lower trunk/limb fat mass ratio (by 0.11 unit) compared with the consistently lower trajectory (Table 3).

In females, we found no significant differences in LBM or ALM at age 20 years among the four vitamin D trajectories. In the unadjusted analysis, the consistently higher and the increasing vitamin D status trajectories had significantly lower FBM (by 5.4 and 6.7 kg, respectively), body weight, weight/height ratio, BMI, and lower trunk/limb fat mass ratio at 20 years compared with the consistently lower group (Table 3). After covariate adjustment,
Table 1. Comparison of Characteristics Among the Four Vitamin D Status Trajectory Groups at Each Time Point

| Variable                        | Males                          | Females                         |
|---------------------------------|--------------------------------|---------------------------------|
|                                 | Consistently Lower (n = 136)   | Consistently Lower (n = 123)    |
|                                 | Decreasing (n = 62)            | Decreasing (n = 63)             |
|                                 | Increasing (n = 64)            | Increasing (n = 74)             |
|                                 | Consistently Higher (n = 174)  | Consistently Higher (n = 115)   |
|                                 |                                |                                |
| Age 6 y, n                       | 86                             | 70                              |
| Serum 25(OH)D, nmol/L            | 67.5 ± 7.4                     | 68.6 ± 8.8                      |
| Age, y                           | 6.0 ± 0.2                      | 5.9 ± 0.2                       |
| Height, cm                       | 116.3 ± 5.1                    | 115.5 ± 4.6                     |
| Weight, kg                       | 21.5 ± 3.4                     | 21.3 ± 3.2                      |
| BMI, kg/m²                       | 15.8 ± 1.6                     | 15.9 ± 1.9                      |
| Watch TV ≥2 h/d, %               | 33.3                           | 20.9                            |
| Organized sports, %              | 32.1                           | 29.9                            |
| Age 14 y, n                      | 127                            | 115                             |
| Serum 25(OH)D, nmol/L            | 65.2 ± 14.1                    | 64.0 ± 12.4                     |
| Age, y                           | 14.1 ± 0.2                     | 14.0 ± 0.2                      |
| Height, cm                       | 165.2 ± 8.5                    | 161.6 ± 6.7                     |
| Weight, kg                       | 59.2 ± 14.2                    | 57.8 ± 12.4                     |
| BMI, kg/m²                       | 21.5 ± 3.9                     | 22.1 ± 4.1                      |
| Watch TV ≥2 h/d, %               | 47.2                           | 50.0                            |
| Organized sports, %              | 90.6                           | 83.3                            |
| Age at menarche, y               | NA                             | 12.8                            |
| Age 17 y, n                      | 130                            | 117                             |
| Serum 25(OH)D, nmol/L            | 51.5 ± 14.1                    | 54.1                            |
| Age, y                           | 17.0 ± 0.2                     | 17.1 ± 0.3                      |
| Height, cm                       | 176.8 ± 7.0                    | 165.4 ± 7.1                     |
| Weight, kg                       | 71.7 ± 14.8                    | 64.2 ± 13.5                     |
| BMI, kg/m²                       | 22.8 ± 3.9                     | 23.5 ± 4.8                      |
| Watch TV ≥2 h/d, %               | 29.6                           | 39.0                            |
| Organized sports, %              | 78.6                           | 65.3                            |
| Age 20 y, n                      | 136                            | 117                             |
| Serum 25(OH)D, nmol/L            | 51.2 ± 15.5                    | 54.0 ± 14.7                     |
| Age, y                           | 20.0 ± 0.4                     | 20.1 ± 0.4                      |
| Height, cm                       | 177.2 ± 6.9                    | 164.9 ± 6.9                     |
| Weight, kg                       | 77.3 ± 16.1                    | 68.4 ± 16.3                     |
| BMI, kg/m²                       | 24.5 ± 4.4                     | 25.2 ± 5.9                      |

(Continued)
Table 1. Comparison of Characteristics Among the Four Vitamin D Status Trajectory Groups at Each Time Point (Continued)

| Variable                           | Males                                                                 | Females                                                                 |
|------------------------------------|----------------------------------------------------------------------|------------------------------------------------------------------------|
|                                    | Consistently Lower (n = 136) | Decreasing (n = 62) | Increasing (n = 64) | Consistently Higher (n = 174) | Consistently Lower (n = 123) | Decreasing (n = 63) | Increasing (n = 74) | Consistently Higher (n = 125) |
| Watch TV ≥2 h/d, %                 | 27.0                        | 29.8                   | 33.3                   | 23.0                   | 32.5                        | 28.6                   | 20.9                   | 21.7c                      |
| Physical activity, %               | Low                        | 12.6                    | 6.4                    | 2.4                    | 6.5                        | 17.5                    | 19.6                    | 7.5                        | 8.7                        |
|                                   | Moderate                   | 34.2                    | 46.8                    | 38.1                   | 27.3                        | 56.1                    | 53.6                    | 47.8                        | 51.3                        |
|                                   | High                       | 53.2                    | 46.8                    | 59.5                   | 66.2b                      | 26.3                    | 26.8                    | 44.8                       | 40.0b                       |
| Energy intake, kJ/d                | 8946 ± 3080                | 8958 ± 2840            | 9165 ± 2748            | 10087 ± 3382a          | 7011 ± 1802                | 6715 ± 2111            | 7130 ± 2548             | 7127 ± 2567                |
| Protein intake, g/d                | 106 ± 37                    | 103 ± 37                | 111 ± 37                | 118 ± 46               | 81 ± 26                     | 78 ± 26                 | 84 ± 31                 | 82 ± 31                     |
| Oral contraceptive use, %          | NA                         | NA                      | NA                      | NA                     | 38.6                        | 53.6                    | 73.1                    | 66.1b                       |

To convert 25(OH)D from nmol/L to ng/mL, the value is multiplied by 0.4.
Abbreviation: NA, not applicable.

aSignificantly different from that of the consistently lower group, \( P < 0.05 \) (ANOVA with Tukey post hoc test).
b\( P \leq 0.01 \), statistically significant using Mantel-Haenszel linear-by-linear association within sex.

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b\( P \leq 0.01 \), statistically significant using Mantel-Haenszel linear-by-linear association within sex.

\( cP < 0.05 \), statistically significant using Mantel-Haenszel linear-by-linear association within sex.
the differences in FBM, weight/height ratio, and BMI among the vitamin D trajectories status were similar; however, the differences in the trunk/limb fat mass ratio were no longer statistically significant, and the consistently higher trajectory only showed a nonstatistically significant trend toward lower body weight compared with the consistently lower trajectory ($P = 0.07$).

### 3. Discussion

To the best of our knowledge, the present longitudinal study is the first to examine the relationship between serum 25(OH)D in childhood and adolescence and the body composition of young adults. We found that in males, serum 25(OH)D at ages 17 and 20 years was substantially and positively associated with LBM and ALM at 20 years. In contrast, at 20 years, 25(OH)D was negatively associated with the FBM and trunk/limb fat mass ratio. Males in the consistently higher vitamin D status trajectory had substantially greater LBM and substantially lower FBM than those with consistently lower or decreasing vitamin D status and substantially lower trunk/limb fat mass ratio compared with the consistently lower group. In females, serum 25(OH)D and vitamin D trajectories were not significantly associated with LBM. However, serum 25(OH)D at 14, 17, and 20 years was significantly and negatively associated with FBM at age 20 years. Also, females in the consistently higher or increasing vitamin D status trajectory had substantially lower FBM at 20 years than those with consistently lower vitamin D status. In females, but not in males, the consistently higher and increasing vitamin D status trajectory groups had lower weight/height ratio and BMI at 20 years, suggesting that vitamin D was mainly associated with the relative proportions of FBM and LBM in males but with fat mass accumulation in females.
Table 3. Association of Vitamin D Status Trajectory Class With Body Weight, BMI, and Body Composition Measures at 20 Years

| Variable             | Males                                   | Females                                  |
|----------------------|-----------------------------------------|------------------------------------------|
|                      | Consistently Lower (n = 136)            | Consistently Lower (n = 123)             |
|                      | Consistently Decreasing (n = 62)        | Consistently Decreasing (n = 63)         |
|                      | Consistently Increasing (n = 64)        | Consistently Increasing (n = 74)         |
|                      | Consistently Higher (n = 174)           | Consistently Higher (n = 125)            |
|                      | Unadjusted                              | Covariate-adjusted                       |
| Body weight, kg      | 77.3 ± 16.1                             | 76.3 ± 1.4                               |
|                      | 79.1 ± 15.0                             | 79.2 ± 2.3                               |
|                      | 75.9 ± 12.4                             | 76.8 ± 2.4                               |
|                      | 76.0 ± 12.6                             | 75.9 ± 1.3                               |
| Weight/height ratio, kg/m² | 43.5 ± 8.3                             | 42.9 ± 0.7                               |
| BMI, kg/m²            | 24.5 ± 4.4                              | 24.2 ± 0.4                               |
|                      | 24.5 ± 4.4                              | 24.5 ± 0.6                               |
|                      | 23.9 ± 3.1                              | 24.0 ± 0.7                               |
|                      | 23.7 ± 3.6                              | 23.8 ± 0.3                               |
| LBM, kg               | 55.0 ± 8.5                              | 55.7 ± 0.6                               |
|                      | 55.7 ± 7.1                              | 54.3 ± 1.0                               |
|                      | 57.3 ± 7.5                              | 57.1 ± 1.0                               |
|                      | 58.5 ± 7.8                             | 58.0 ± 0.5                               |
| ALM, kg               | 26.0 ± 4.4                              | 26.2 ± 0.3                               |
|                      | 26.5 ± 4.0                              | 25.7 ± 0.5                               |
|                      | 26.8 ± 3.8                              | 26.7 ± 0.5                               |
|                      | 27.4 ± 4.3                             | 27.3 ± 0.3                               |
| FBM, kg               | 19.8 ± 10.7                             | 19.2 ± 0.9                               |
|                      | 20.7 ± 12.5                             | 21.1 ± 1.5                               |
|                      | 15.9 ± 8.6                             | 16.8 ± 1.6                               |
|                      | 14.9 ± 8.0                             | 15.1 ± 0.8                               |
| Trunk/limb fat mass ratio | 0.95 ± 0.30                           | 0.93 ± 0.03                             |
|                      | 0.92 ± 0.25                             | 0.90 ± 0.05                              |
|                      | 0.88 ± 0.29                             | 0.82 ± 0.02                             |

Data presented as mean ± SD for unadjusted analysis (ANOVA with Tukey post hoc test) and estimated mean ± SEM for adjusted analysis (analysis of covariance with Bonferroni post hoc test), adjusted for age, TV watching, physical activity level, energy intake, protein intake, height (for models for LBM, ALM, and FBM), FBM (for models for LBM and ALM), and LBM (for models for FBM) at 20 years, with models for females additionally adjusted for age at menarche and oral contraceptive use at 20 years.

Significantly different statistically from that of the consistently lower group, P < 0.05.

Significantly different statistically from that of the decreasing group, P < 0.05.
In the present study, a higher vitamin D status was associated with greater LBM in males but not in females. The lack of an association between the circulating 25(OH)D concentration and LBM in females is consistent with the results from a previous cross-sectional study in young women aged 16 to 22 years, which reported no significant correlation between serum 25(OH)D and computed tomography measures of the thigh muscle area [5]. In contrast, we observed a positive association between 25(OH)D and LBM/muscle mass in young men. Similarly, in a previous analysis of the present cohort, we found that higher vitamin D status during childhood and adolescence was associated with a substantially greater bone mass at age 20 years in males but not in females [16]. It has been hypothesized that during growth, vitamin D might have a positive influence on the accumulation of LBM, which might partly mediate the positive effect of vitamin D on bone mineral accretion [1]. Therefore, our data support that a role of vitamin D on muscle accumulation is plausible. A physiological role for vitamin D in muscle development is supported by several lines of evidence, including abnormal skeletal muscle development in vitamin D receptor knockout mice [26], reversal of myopathy with the correction of vitamin D deficiency in humans [27], and in vitro evidence that 1,25(OH)2D enhances the protein-stimulating effects of insulin and leucine in myotubes by actions in the Akt/mTOR pathway [28]. Whether such effects of vitamin D on muscle are direct or indirect remains uncertain, and the presence and physiological relevance of vitamin D receptors in skeletal muscle is controversial [29–31]. Although this question is subject to continuing inquiry, increasing evidence has suggested that the vitamin D receptor is expressed within muscle precursor cells and in developing muscle fibers [1, 32]. The positive association we observed between 25(OH)D and LBM in males but not females could reflect an interaction of vitamin D with testosterone, which has anabolic effects on muscle [33]. Supporting this, in males, the association with body composition was observed for 25(OH)D measured at ages 17 and 20 years but not at younger ages, which could be related to the increment in LBM associated with puberty in males. A cross-sectional study of 2299 men showed a positive association between serum 25(OH)D and testosterone [34]. However, vitamin D supplementation studies of serum testosterone concentrations in men have yielded inconsistent results, with positive [35] and null [36] effects reported. Alternatively, the lack of association between vitamin D status and LBM in females could result from the effects of estrogen on vitamin D metabolism and signaling. Estrogen stimulates renal 1-α-hydroxylase activity, increasing conversion of 25(OH)D to the more biologically active hormone 1,25(OH)2D [37] and might increase vitamin D receptor expression via activation of the ERK1/2 signaling pathway [38]. In a randomized controlled trial of girls aged 10 to 17 years, vitamin D supplementation increased LBM in premenarcheal girls but not in postmenarcheal girls, suggesting that circulating estrogen might (to some extent at least) counteract the musculoskeletal effects of vitamin D deficiency [39]. Further research is needed to explore the mechanisms underlying the sex differences observed in the present study.

Our longitudinal data showed that higher vitamin D status from 14 years onward in girls and from 17 years onward in boys was associated with a lower FBM at 20 years of age. This is consistent with the results from previous cross-sectional studies in which lower circulating concentrations of 25(OH)D were independently associated with higher total FBM in young women [15], higher BMI and waist circumference in children and adolescents [40], and greater BMI and FBM in adults [41]. In addition, the association between 25(OH)D and FBM, which was evident at younger ages in females than in males, could reflect the earlier onset of puberty in girls. A role of vitamin D status on fat mass accumulation is plausible. Adipose tissue expresses vitamin D receptors and has the ability to synthesize 1,25-dihydroxyvitamin D. Also, in vitro studies have suggested that 1,25-dihydroxyvitamin D could inhibit adipogenesis and lipid accumulation [42, 43]. It has also been hypothesized that a decrease in circulating 25(OH)D level could increase the set point for body weight and increase the accumulation of the fat mass [44]. Low vitamin D status could also cause an increase in PTH levels, which might interfere with catecholamine-induced lipolysis and, thus, favor lipid storage metabolism [45]. In young healthy women, positive correlations between fasting serum PTH and FBM and changes in PTH and body fat during a 12-month period were
observed [46]. In older men and women, the body fat percentage was positively associated with PTH levels [8].

We evaluated the association of vitamin D status with the trunk/limb fat mass ratio, a surrogate of visceral fat [17], in the present study. We found that males in the consistently higher vitamin D status trajectory group had a substantially lower trunk/limb fat mass ratio compared with those in the consistently lower trajectory group. In contrast, in females, only 25(OH)D measured at 17 years associated with a lower trunk/limb fat mass ratio at 20 years in the covariate-adjusted analysis. This is somewhat in contrast to the findings from middle-age, South Asian Americans, in whom vitamin D deficiency was associated with a greater visceral fat area in women but not in men [47] and might arise from the differences in ethnicity and age between the studies. Furthermore, it has been reported that for a given BMI, men will have more visceral adipose tissue [48].

The findings of our study suggest that a consistently higher vitamin D status from childhood to early adulthood will be associated with better muscle development in males and less fat accumulation in both sexes. However, because body composition was only assessed at 20 years, our results could be explained by reverse causation (i.e., that a lower fat mass during growth and development led to higher vitamin D status, rather than vice versa). This might be because individuals with a lower fat mass have more sun exposure, resulting in more vitamin D synthesis, or that such individuals are more physically active, because evidence has shown that intense physical activity is itself positively associated with circulating 25(OH)D [49]. In our data set, we found evidence of greater participation in organized sports and less TV watching in the higher vitamin D trajectory classes. However, the associations between vitamin D status and body composition remained significant after accounting for these factors.

Both sarcopenia (loss of skeletal muscle mass and strength) with older age [50] and obesity are major public health problems. In healthy adults, the body fat percentage is associated with risk factors for cardiovascular disease and metabolic syndrome in both men and women [51]. In middle-age women, higher body fat for BMI was associated with lower bone mineral density [52]. Therefore, our finding that higher vitamin D status from adolescence to early adulthood was associated with greater LBM and reduced FBM in early adulthood could be of public health relevance for the prevention of sarcopenia and high body fat-related adverse health outcomes in later life.

The strengths of our study included the large sample size, measurement of serum 25(OH)D at multiple time points from childhood to early adulthood, analysis by the trajectory of 25(OH)D across these time points, inclusion of both male and female participants, and the detailed data collection, which allowed for adjustment for multiple potential confounders at each developmental stage. Our study also had limitations. First, because of its observational nature, we could not assume that the relationships between vitamin D status and body composition are causal, and, as discussed, the relationship could reflect reverse causality, be bidirectional, or reflect residual confounding. Second, most participants were white, with median serum 25(OH)D concentrations of 70 to 80 nmol/L at different time points. Thus, the study findings might not be applicable to other ethnic groups or communities with a substantially different vitamin D status. Finally, two different methods were used to measure serum 25(OH)D, which could have affected the measured 25(OH)D values but should have had minimal to no influence on the ranking and trajectory.

In conclusion, in the present cohort study, we have shown that consistently higher vitamin D status from childhood to early adulthood was associated with greater LBM in males and less FBM in both sexes at 20 years of age.

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