Association Among Cardiorespiratory Fitness, Body Fat, and Bone Marker Measurements in Healthy Young Females

Debra A. Mowry, DO
Megan M. Costello, MAEd
Kate A. Heelan, PhD

Context: Serum vitamin D (25-hydroxyvitamin D [25(OH)D]) is important for maintaining bone health, as well as for influencing a multitude of nonosseous effects. Associations between 25(OH)D and other bone marker measurements have not been well studied in relation to obesity and cardiorespiratory fitness in healthy young females.

Objective: To determine if there is an association of cardiopulmonary fitness and body fat with bone marker measurements (ie, 25(OH)D and calcium) in healthy young females at peak bone mass.

Methods: Females younger than 25 years were recruited as volunteer participants. From July 2007 to October 2007, participants were assessed for cardiopulmonary fitness by measuring maximal oxygen consumption (VO₂ max). In addition, their levels of serum 25(OH)D and serum total calcium were analyzed. Finally, percent body fat was measured using dual-energy x-ray absorptiometry of the whole body. Pearson correlation coefficients were used to evaluate the association of fitness and body fat with bone marker measurements.

Results: A total of 59 adolescent girls and young women were recruited. A statistically significant, positive association was found between VO₂ max and serum 25(OH)D levels (Pearson r=0.36; P<.05), but not between VO₂ max and serum total calcium levels (Pearson r=-0.10; P>.05). Percent body fat was inversely associated with both VO₂ max (Pearson r=-0.70, P<.05) and 25(OH)D levels (Pearson r=-0.44, P<.05).

Conclusion: In healthy young females, better cardiopulmonary fitness, as measured by VO₂ max level, and lower percent body fat are associated with higher serum 25(OH)D level. This response is independent of serum total calcium level.


Osteopenia and osteoporosis are common conditions that affect more than 30 million women and more than 10 million men aged 50 years or older in the United States. Approximately 40% of postmenopausal women are estimated to have osteoporosis. Maximizing peak bone mass, 90% of which is obtained by age 18 years, may lessen osteoporosis risk.

Factors that contribute to maximizing peak bone mass include percent body fat and levels of serum total calcium, serum 25-hydroxyvitamin D (25(OH)D), and parathyroid hormone. A decrease in serum total calcium level results in an increase in the release of parathyroid hormone—which, in turn, enhances the renal conversion of 25(OH)D to 1,25-dihydroxyvitamin D. This dihydroxy form of vitamin D is needed to enhance absorption of calcium and phosphorus from the intestine. Hence, 25(OH)D is an important contributor to bone mass.

Not only is 25(OH)D important for a person’s overall bone health, but our understanding of the importance of this micronutrient for the prevention of other types of illness and disease has increased in the past few years. Forman et al reported that individuals with serum 25(OH)D levels of less than 15 ng/mL had a greater risk for incident hypertension than those with serum 25(OH)D levels of 30 ng/mL or greater (relative risk, 6.13; 95% confidence interval, 1.00-37.8). Similarly, Zhou et al concluded that higher serum 25(OH)D levels were possibly associated with improved survival in persons with early nonsmall-cell lung cancer.

Increased levels of serum 25(OH)D have also been shown to be indicative of improved health of individuals with colorectal disease, multiple sclerosis, periodontal disease, and other conditions. Some investigators have suggested that serum 25(OH)D levels less than 15 ng/mL do not support adequate bone health or overall health, and that serum 25(OH)D levels greater than 30 ng/mL are beneficial not only to bone health, but also to general disease prevention.

In fact, 25(OH)D appears to have a beneficial impact overall on bone health and disease prevention. An emerging area of research involves evaluating the benefits of 25(OH)D on musculoskeletal function. Bischoff-Ferrari et al showed that serum 25(OH)D levels greater than 40 nmol/L resulted in better lower-extremity function in individuals older than 60 years, as measured by a sit-to-stand test and an 8-foot-walk test, compared with individuals in the same age range with...
lower serum 25(OH)D levels ($P<.001$). However, only limited information is available on the association of exercise, cardiorespiratory fitness status, or both with serum 25(OH)D levels.

Several investigators$^{10-12}$ have measured exercise intensity patterns and fitness levels of study participants using interviews or questionnaires, by evaluating participation in structured exercise classes, or by measuring maximal oxygen consumption ($\text{VO}_2\text{max}$). The results of exercise intensity and fitness level investigations were then compared with participants' bone mineral density (BMD) levels. In studies where interviews or questionnaires were used to determine subject fitness level,$^{10-12}$ men were found to demonstrate a better correlation of cardiorespiratory activity to BMD than did women—though this correlation may have been the result of small sample size for female participants or certain limitations of the questionnaire.

Physical fitness classes have been shown to produce a beneficial impact on BMD in men.$^{13}$ However, a more rigorous measure of cardiorespiratory fitness can be obtained by measuring VO$_2$ max levels of study participants. Vico et al$^{14}$ reported that elderly postmenopausal women with higher VO$_2$ max levels had increased femoral, radial, and tibial BMDs. In a study of perimenopausal and early postmenopausal women aged between 45 and 54 years, Lynch et al$^{15}$ found that total BMD was “related indirectly” to VO$_2$ max level. Friedlander et al$^{16}$ also found a positive relationship between BMD and VO$_2$ max level in women aged between 20 and 35 years.

Although much research has been conducted on the relationship between exercise intensity and BMD, little is understood about the association of cardiorespiratory fitness, as determined by VO$_2$ max, with bone marker measurements in young women at peak bone mass. In addition, considering the emerging knowledge of the importance of 25(OH)D in relation to nonosseous health conditions, such as diabetes mellitus and hypertension,$^{4,17}$ it is important to examine the relationships of fitness, body fat, and 25(OH)D with calcium levels in young women.

The purpose of the present study, therefore, is to determine if there is an association of cardiorespiratory fitness, as measured by VO$_2$ max level, and percent body fat with levels of bone markers (ie, 25(OH)D and calcium) in healthy young females.

**Methods**

Females aged 16 to 24 years were recruited as volunteer participants. Volunteers were recruited through telephone calls to students who had participated—as middle school students—in a previous study at the University of Nebraska at Kearney, as well as through posters displayed on the university campus and through word-of-mouth recruitment. Medical histories of the volunteers were obtained to determine that they were free of serious health conditions—that is, they were not under the care of physicians for acute conditions that would put them at risk in the cardiorespiratory fitness assessment portion of the study. Any such individuals were excluded from participation.

The study, which was approved by the University of Nebraska at Kearney Institutional Review Board, was conducted from July 2007 to October 2007 to reduce seasonal variation of participants' serum values.

**Assessments**

Body weight of each subject was determined using a portable platform scale (model PS-6600; Befour Inc, Saukville, Wisconsin) that was accurate to within 0.1 kg. Subject height was assessed measuring to the nearest 0.25 inch with a standard stadiometer. Both body weight and height were measured before exercise testing with each subject wearing undergarments, a T-shirt, and shorts, but not wearing shoes. Body mass index (BMI) was calculated as weight (kg) divided by height squared (m$^2$).

Dual-energy x-ray absorptiometry of the whole body—which is considered the “gold standard” for body fat assessment—was performed using a densitometer (Lunar Prodigy Advance Plus; General Electric Co, Madison, Wisconsin). Percent body fat and fat-free mass were determined from this assessment.

Cardiorespiratory fitness, represented by VO$_2$ max, was determined by each subject’s completion of a maximal-graded treadmill test. Maximal oxygen consumption was considered the highest observed value as measured by a metabolic cart (TrueOne 2400; ParvoMedics, Sandy, Utah).

Nonfasting blood samples—approximately 10 mL from each subject—were collected using sterile techniques. The samples were analyzed at ARUP Laboratories (Salt Lake City, Utah) for serum 25(OH)D level by chemiluminescent immunoassay and for serum total calcium level by electrochemiluminescent immunoassay.

**Statistical Analysis**

In a bivariate analysis, Pearson correlation coefficients were used to determine the association of VO$_2$ max and percent body fat with bone markers (ie, 25(OH)D and calcium). A $P$ value of less than .05 was considered statistically significant. All analyses were performed using SAS statistical software (version 9.0 for Windows; SAS Institute, Cary, North Carolina).

**Results**

A total of 59 adolescent girls and young women—55 white and 4 Asian—were accepted to participate in the present study. Their ages ranged from 16 to 24 years, with a mean (SD) age of 19.86 (2.13) years.

Three of the included subjects were currently using inhalers as occasional treatment for asthma, 1 subject smoked occasionally, 15 subjects used birth-control pills, and 8 subjects...
supplemented their diets with multivitamins. The types of multivitamins varied among subjects and included Centrum Complete (Wyeth Pharmaceuticals, Collegeville, Pennsylvania) and One-a-Day Women’s (Bayer Healthcare LLC, Morris-town, New Jersey). Some of the subjects did not know the types of multivitamins they took. No subjects took separate calcium supplements. Diabetes mellitus, cardiovascular conditions, epilepsy, and other serious medical conditions were not present in the study population. One potential subject was excluded from participation in the current investigation due to pregnancy. A few subjects opted out of study participation after reading the informed consent statement regarding the required blood draws.

Mean (SD) baseline measurements of participants’ cardiorespiratory fitness, body fat, and bone marker measurements are shown in Table 1. Nineteen subjects (32%) were categorized as having poor cardiorespiratory fitness, with a VO2 max level of less than or equal to 35 mL/kg/min. Fourteen subjects (24%) had an excellent fitness classification, with a VO2 max level between 44 and 49 mL/kg/min. Thirty-one subjects (53%) had serum 25(OH)D at recommended levels of greater than 15 ng/mL. None of the subjects had serum 25(OH)D levels of less than 15 ng/mL.

Table 2 presents the Pearson correlation coefficients between participants’ cardiorespiratory fitness, body fat, and bone marker measurements. Cardiorespiratory fitness, as measured using VO2 max data, was significantly associated with percent body fat and 25(OH)D levels (P<.05), but it was not significantly associated with serum total calcium levels. Interestingly, percent body fat was inversely associated with 25(OH)D level (Pearson r = -0.44, P<.05).

A simple linear regression model of the relationship between VO2 max and percent body fat is shown in Figure 1, while the relationship between serum 25(OH)D and percent body fat is depicted in Figure 2. A simple linear regression model of the relationship between serum 25(OH)D and VO2 max is shown in Figure 3.

Comment
The purpose of the present investigation was to determine whether cardiorespiratory fitness and body fat were associated with bone marker measurements in healthy young females at peak bone mass. We found that serum 25(OH)D levels were significantly and positively associated with cardiovascular fitness and inversely associated with percent body fat, while serum total calcium levels were not significantly associated with either of these variables. There has been little research previously published on the association between fitness and serum 25(OH)D levels, though previous research did find a positive association between muscle strength and serum 25(OH)D levels in elderly individuals.

Vitamin D receptors have been found on skeletal muscle cells. Bischoff-Ferrari et al9 reported that, in women, the aging process led to decreased receptor expression in skeletal muscle cells. In a study by Sorensen et al,10 11 elderly patients were treated with a vitamin D analog (1-alpha-hydroxycholecalciferol) and calcium supplements, resulting in an increase in the number of fast-twitch type IIa muscle fibers and a decrease in the number of type IIB muscle fibers.

In our study, we assessed aerobic fitness, in which type IIa muscle fibers are important. Therefore, serum 25(OH)D level may have been related to better cardiovascular fitness, in part, because of the fiber type involved. In cardiovascular disease, the relation of 25(OH)D to fitness and fiber type may be important for understanding and developing preventive care models.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>19.86 (2.13)</td>
</tr>
<tr>
<td>Body Fat, %</td>
<td>36.16 (8.19)</td>
</tr>
<tr>
<td>Cardiorespiratory Fitness</td>
<td></td>
</tr>
<tr>
<td>VO2 max, mL/kg/min</td>
<td>39.10 (7.18)</td>
</tr>
<tr>
<td>Bone Marker Measurements</td>
<td></td>
</tr>
<tr>
<td>Serum 25(OH)D, ng/mL</td>
<td>46.19 (20.14)</td>
</tr>
<tr>
<td>Serum total calcium, ng/dL</td>
<td>9.57 (0.38)</td>
</tr>
</tbody>
</table>

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; VO2 max, maximal oxygen consumption.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Body Fat</th>
<th>25(OH)D</th>
<th>Calcium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pearson r</td>
<td>P Value</td>
<td>Pearson r</td>
</tr>
<tr>
<td>VO2 max</td>
<td>-0.70</td>
<td>&lt;.05</td>
<td>0.36</td>
</tr>
<tr>
<td>Bone Marker Measurements</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum 25(OH)D</td>
<td>-0.44</td>
<td>&lt;.05</td>
<td>NA</td>
</tr>
<tr>
<td>Serum total calcium</td>
<td>0.04</td>
<td>&gt;.05</td>
<td>NA</td>
</tr>
</tbody>
</table>

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; NA, not applicable; VO2 max, maximal oxygen consumption.
level of 17 ng/mL and a maximum level of 103 ng/mL. Multiple regression analysis suggested that body fat accounted for 53% of the variance in participants’ VO2 max levels (P<.05) and 20% of the variance in serum 25(OH)D levels (P<.05).

Our results did not show a relationship between subjects’ cardiorespiratory fitness and serum total calcium levels. As previously mentioned, 25(OH)D is involved in influencing nonosseous activity.4,17 Thus, the present study may provide another example of 25(OH)D functioning independently of the serum total calcium bone marker.

We found that serum 25(OH)D and body fat appeared to account for approximately 29% of the variance in BMD (P<.05), with cardiorespiratory fitness accounting for a nonsignificant 4.9% of the variance. Our data suggest that 25(OH)D, body fat, and cardiovascular fitness are all contributors to the development of peak bone mass in young women—independent of other bone marker measurements. The observed relationship between higher percent body fat and lower cardiorespiratory fitness level was expected.

The data in the present study also revealed that young females with higher percent body fat had lower serum 25(OH)D levels. Other investigators have found a similar relationship.24 Several reasons for this association have been proposed. In older persons, there may be decreased synthesis or ingestion of—or decreased ability to modify—25(OH)D.24,25 In younger persons as well as elderly individuals, bioavailability of the fat-soluble vitamin D may also be an important issue. Wortsman et al26 conducted a study in which obese and nonobese subjects were exposed to the same amount of simulated sunlight. The study revealed that though obese individuals had larger body surface area for sun exposure and, therefore, greater potential for skin synthesis of vitamin D, their serum levels of 25(OH)D were lower than those of individuals of normal weight.26 The authors26 proposed that storage of 25(OH)D in body fat accounted for this finding. Wortsman et al26 also evaluated oral challenges of 25(OH)D, noticing that serum peak 25(OH)D levels were the same in obese persons and persons of normal weight. Yet,

Vitamin D receptors have also been found on cardiac myocytes,21 and low serum 25(OH)D levels have been associated with congestive heart failure and high blood pressure.22,23 Perhaps improved cardiac function, as noted by increased levels of VO2 max, is, in part, related to increased 25(OH)D activity on cardiac myocytes.

Our sample of healthy young females demonstrated a large variation in serum 25(OH)D levels, with a minimum
individuals with higher BMI had lower levels of serum 25(OH)D.26

In the present study, we did not specifically recruit females with higher BMI for the purpose of studying the effect of obesity on serum 25(OH)D levels. A further study examining a larger population of young females at peak bone mass could be of interest. As obesity levels in young people continue to increase in the United States,27 improved understanding of the impact of obesity on 25(OH)D levels might help to prevent a multitude of health problems that are common in this demographic group.

Recently, the American Academy of Pediatrics28 increased its recommendation for minimum daily intake of vitamin D in children from 200 IU to 400 IU. Some researchers29 have also recommended increased daily intake of vitamin D for adults. However, the correlation between recommended ingested amounts of vitamin D and 25(OH)D levels is currently not well understood.

The US Department of Health and Human Services30 has increased its physical activity recommendations for all age groups. Increased physical activity could lead to a reduction in numerous chronic medical conditions. Understanding the relationship between 25(OH)D and cardiorespiratory fitness in all age groups may not only help decrease the prevalence of these conditions in the overall population, it may well influence patient care models.

Limitations to the present study include its small sample size, involving only 59 participants. The study was also limited by evaluating only two of the common bone markers—serum 25(OH)D and serum total calcium levels. Other bone markers, such as serum parathyroid hormone and phosphorus levels or BMD, were not analyzed in the present investigation.

Another limitation of the present study was the use of multivitamins by 8 subjects. We did not determine the dosage amounts of vitamin D or calcium in the supplements ingested by these subjects, nor did we examine the frequency of supplement usage (ie, regular vs intermittent). Also unexamined were amounts of vitamin D and calcium in the diets of subjects. Therefore, the amounts of ingested vitamin D and calcium by supplemental or dietary means was not reported in this study.

Conclusions
In the present study, a trend toward higher serum 25(OH)D levels was noted in females with lower levels of body fat. However, the sample size of the present investigation was small, making it impossible to determine the amount of body fat associated with normal serum 25(OH)D levels. Our population was healthy. Given the recent emergence of the importance of 25(OH)D in reducing the risk of nonosseous medical conditions, future research might evaluate the influence of 25(OH)D and cardiorespiratory fitness levels in young women at peak bone mass who have chronic health problems, such as asthma or diabetes mellitus.

Based on the results of the present study, it appears that in healthy young females, lower levels of body fat and greater cardiorespiratory fitness, as measured by VO2 max, are associated with higher levels of serum 25(OH)D. This response is independent of serum total calcium levels.

Additional research is necessary for improved understanding of the impact of serum 25(OH)D level, percent body fat, and cardiorespiratory fitness on women’s health. Such research might focus on determining if an increase in ingested vitamin D impacts 25(OH)D levels or influences cardiorespiratory fitness in young women at peak bone mass. Determining if ingested vitamin D influences health outcomes in obese young women would also be a valuable avenue for future research efforts.

Acknowledgments
We would like to thank the Research Services Council at the University of Nebraska at Kearney for its research assistance, and Stacey L. Bonner, BS, for her editorial assistance.

References