

Body Fat Content and 25-Hydroxyvitamin D Levels in Healthy Women

SONIA ARUNABH, SIMCHA POLLACK, JAMES YEH, AND JOHN F. ALOIA

Bone Mineral Research Center (S.A., S.P., J.Y., J.F.A.), Winthrop-University Hospital, Mineola, New York 11501; State University of New York at Stony Brook (J.F.A.), Stony Brook, New York 11790; and St. John's University (S.P.), Jamaica, New York 11439

Obesity is associated with alterations in the vitamin D endocrine system. Lower levels of serum 25-hydroxyvitamin D (25-OHD) in morbidly obese individuals may be secondary to an alteration in tissue distribution resulting from an increase in adipose mass. Therefore, morbidly obese individuals are expected to need higher doses of vitamin D supplementation than the general population. However, it is still unknown whether adiposity (or percentage body fat) should be taken into consideration while assessing vitamin D requirements in the general population. To study the relationship between 25-OHD levels and percentage body fat content in healthy women, we studied 410 healthy women between 20 and 80 yr of age with body mass index ranging from 17 to 30 kg/m². We analyzed the correlation between serum 25-OHD level and

percentage body fat measured by dual energy x-ray absorptiometry. We also analyzed the influence of season, dietary vitamin D intake, age, and race on this relationship. The levels of serum 25-OHD inversely correlated with percentage body fat. The correlation was -0.13 ($P = 0.013$) after adjusting for race, age, season, and dietary vitamin D intake. In a multiple stepwise regression, race and season were found to have a major influence on serum 25-OHD (cumulative $R^2 = 0.34$), and percentage body fat, although modest (additional $R^2 = 0.02$), also had an independent statistically significant influence on serum 25-OHD levels. We conclude, percentage body fat content is inversely related to the serum 25-OHD levels in healthy women. (*J Clin Endocrinol Metab* 88: 157–161, 2003)

OBESITY IS ASSOCIATED with alterations in the vitamin D endocrine system. The levels of serum intact PTH and 1,25-hydroxyvitamin D are elevated in morbidly obese patients, whereas levels of 25-hydroxyvitamin D (25-OHD) have been found to be low (1–4). There is also an increase in urinary cyclic adenosine cAMP levels and decrease in urinary calcium excretion in obese, compared with nonobese, age-matched men and women (5). The low levels of 25-OHD in obesity have been attributed to multiple factors like decreased exposure to sunlight because of limited mobility, negative feedback from elevated 1,25-hydroxyvitamin D and PTH levels on hepatic synthesis of 25-OHD (5), and excessive storage of vitamin D in the adipose tissue (4).

The first evidence of a relationship between vitamin D and body fat was described by Lumb *et al.* (6) in 1971. They hypothesized that vitamin D after absorption is sequestered and stored in tissues like fat and muscle and then released slowly into the circulation in which it is used biologically. Later the same group (7) demonstrated by injecting radioactively labeled vitamin D₃ in 60 individuals in whom the highest concentration of biological activity and radioactivity was seen in fat tissue. Similar evidence was found in animal models confirming that adipose tissue is the major storage site for vitamin D₃ and a source available for conversion to other metabolites during deprivation (8). A subsequent study on morbidly obese subjects also suggested that decline in 25-OHD levels in obesity may be secondary to alteration in tissue distribution resulting from increase in adipose

mass (4). More recently, Worstman *et al.* (9) confirmed that obesity-associated vitamin D insufficiency most likely is due to decreased bioavailability of vitamin D₃ from cutaneous and dietary sources because of its deposition in body fat compartments. Based on this evidence, it is expected that obese individuals need higher-than-usual doses of vitamin D for supplementation.

However, it still remains unclear whether adiposity (or percentage body fat) should be taken into consideration while assessing vitamin D requirements in the general population. We studied the relationship between 25-OHD levels and the percentage body fat content in healthy women from our community who ranged from lean to mildly obese. We also analyzed the influence of season, dietary vitamin D intake, age, race, and smoking on this correlation.

Subjects and Methods

We evaluated 410 healthy women between 20 and 80 yr of age among subjects enrolled in a study on body composition in healthy black and white women (10). There were 171 black and 239 healthy white women. Participants were recruited through advertising in the local media and a direct mail campaign. Exclusion criteria consisted of any chronic illness including hypertension, diabetes, coronary, hepatic, or renal disease; medications known to affect bone metabolism; and any use of oral contraceptives or hormonal replacement therapy. The study was approved by the Institutional Review Board of Winthrop-University Hospital. The subjects varied from lean to mildly obese with body mass index (BMI) ranging from 17–30 kg/m². None of the women were morbidly obese. A 3-d diet history was obtained and reviewed with the study dietitian using food models to estimate portion size. A 24-h food recall was completed with the assistance of a dietitian to record dietary vitamin D intake of the subjects. Nutritionist III software (First Data Bank, San Bruno, CA) was used to calculate the average daily intake of vitamin D. Smoking history was recorded during a detailed history at screening of participants.

Abbreviations: 25-OHD, 25-Hydroxyvitamin D; BMI, body mass index; DXA, dual-energy x-ray absorptiometry; %TBF, percentage body fat; TBF, total body fat mass.

Each participant had a total body scan using dual-energy x-ray absorptiometry (DXA) with a radiation densitometer (model DPX-L, software program 1.3Y, Lunar Corp., Madison, WI). Kilograms of total body fat and nonfat soft tissue weight were estimated by DXA on total body scan. Percentage body fat (%TBF) by DXA was calculated as [fat mass (g)/fat mass (g) + lean mass (g) + total bone mineral content (g)] × 100.

Blood samples were collected after an overnight fast for serum calcium, phosphorus, and 25-OHD. Serum 25-OHD was measured by RIA using a kit (DiaSorin, Inc. Corp., Stillwater, MN). The coefficient of variation for intraassay was 4.1% and interassay was 7.0%. Serum calcium was measured by atomic absorption spectrophotometry (model 560, Perkin Elmer Corp., Norwalk, CT). Serum inorganic phosphate was measured colorimetrically.

Statistical analysis

Some of the known influences on 25-OHD levels include season, race, age, and dietary vitamin D. In examining the relationship between 25-OHD levels and the percentage body fat, we statistically controlled for these variables in a multiple regression model, including them as covariates. In addition, we looked at univariate linear predictors of 25-OHD.

To study seasonal variation in New York (latitude 40 degrees, 42 min, 51 sec north; longitude 74 degrees, 0 min, 23 sec west), we subdivided the year into 4-month measurement periods: February-May, June-September, and October-January (11). These periods roughly approximate the seasonal changes in availability of sunlight (measurement of both direct and diffuse solar radiation assessed by pyranometers) throughout a year in the New York area. The amount of daily and seasonal solar radiation available at this latitude varies significantly among the three seasons defined above (12).

Statistical analyses were generated by SAS version 8.1 (SAS Institute, Inc., Cary, NC). Because nonparametric and parametric results were similar, only parametric results were reported. For purposes of reporting descriptive statistics, 25-OHD results were stratified by race and season. A nominal significance level of 0.05 (two-tailed) was used throughout. A stepwise multiple regression was run to determine whether other anthropometric variables were associated with 25-OHD. The significance level criterion for stepwise inclusion into the multiple regression model was 0.10. In addition to total body fat, other anthropometric measures like body weight, height, and BMI were included. These analyses were also controlled for race, season, age, and dietary vitamin D.

Results

The mean age of women in the study was 47.6 yr (range, 20–80 yr). The women ranged from lean to mildly obese with a mean BMI of 24 kg/m² (range, 17–30 kg/m²). The demographic profile of our study population is highlighted in Table 1. The mean total body fat mass (TBF) measured by DXA for the whole population was 23.6 ± 7.3 kg. Mean %TBF was 36.2% ± 7.4%. Dietary calcium intake on average was 667 ± 302 mg/d, and daily dietary vitamin D intake in the population was generally low, mean 2.7 ± 2.4 μg/d (108 ± 96 IU/d). Serum calcium and phosphorus levels were within normal limits. Ten percent of the women were current smokers with an average smoking history of 87.7 packs/yr. Fifty-

seven percent of the women were premenopausal and 43% were postmenopausal. There were fewer visits during the winter period of October-January (23% of total study subjects were studied during winter).

The levels of serum 25-OHD progressively decreased as the %TBF increased. Analyzing the group by quartiles of TBF, the mean 25-OHD levels in the group with less than 31% TBF was 56.6 nmol/liter, the group with 32–37% TBF was 52.6 nmol/liter, the group with 38–42% TBF was 50.8 nmol/liter, and the highest quartile of the %TBF (>42%) was 44.2 nmol/liter. The difference of 25-OHD between the highest and lowest quartile of %TBF was statistically significant (mean 44.2 nmol/liter vs. 56.6 nmol/liter, $P < 0.01$). There were also significant variations in 25-OHD levels among our patients observed across three seasonal intervals. The highest levels of serum calcidiol were seen in June-September and the lowest levels during the period February to May in both ethnic groups (Fig. 1). The changes in 25-OHD levels with season were more pronounced in white, compared with black, women. The mean increase in serum 25-OHD levels between nadir (21 nmol/liter) in winter and peak (40 nmol/liter) in summer was 19.4 nmol/liter in black women. However, white women not only had a higher serum 25-OHD levels during each season but also showed a higher increase from winter (53 nmol/liter) to summer (84 nmol/liter) season, with a mean increase of 31 nmol/liter. The difference from nadir to peak in blacks, compared with whites, reached a trend level of significance ($P < 0.10$).

When controlling for race by stratifying the sample into black and white patients, we found a statistically significant inverse relationship between fat quartile and the likelihood of reaching the 80 nmol/liter level, which is now considered the optimal serum levels for 25-OHD. In the summer season, 7.1% of blacks in the lowest quartile of fat reached 80 nmol/liter, but only 3.7% of those in the highest quartile achieved it. Among whites a similar pattern held. In the summer season, 73.3% of whites in the lowest quartile of fat reached 80 nmol/liter, but only 30.7% of those in the highest quartile achieved it. This trend for higher fat levels to produce lower 25-OHD values is significant across all three seasons (overall $P = 0.0006$, Cochran-Armitage exact permutation trend test).

Race, season, age, and dietary vitamin D were significant variables, in a bivariate analysis, predicting serum calcidiol levels in our population. Therefore, we statistically controlled for these variables to study the impact of %TBF on serum calcidiol levels. We found a significant linear negative correlation between 25-OHD levels and %TBF in the study population after adjusting for race, season, age, and dietary vitamin D. The partial correlation with %TBF was -0.13 ($P = 0.013$, Table 2). The levels of 25-OHD inversely correlated with BMI as well, although this was not statistically significant. We did not find a significant correlation between cigarette smoking and serum 25-OHD levels in the few smokers ($n = 40$) in our population ($r = 0.002$, $P = \text{ns}$).

To determine the most powerful predictor of 25-OHD among the various variables, a stepwise linear regression was run (Table 3). Race played the primary role in predicting 25-OHD. Second in importance was season with a cumulative R^2 of .34 between them (race and season). Among the various anthropomorphic variables (%TBF, body weight,

TABLE 1. Profile of healthy women in the study ($n = 410$)

Characteristic	Mean	±SD
Age (yr)	47.6	±14.8
BMI (kg/m ²)	23.9	±2.9
TBF (kg)	23.6	±7.3
%TBF	36.2	±7.4
Dietary vitamin D intake (μg/d)	2.7	±2.4
Serum 25-OHD (nmol/liter)	54.2	±34.7
Serum calcium (mg/dl)	9.3	±0.5
Serum phosphorus (mg/dl)	3.6	±0.4
Cigarette smoking (packs/yr)	87.7	±86.6

FIG. 1. Seasonal variation of unadjusted (mean + SEM) 25-OHD by race.

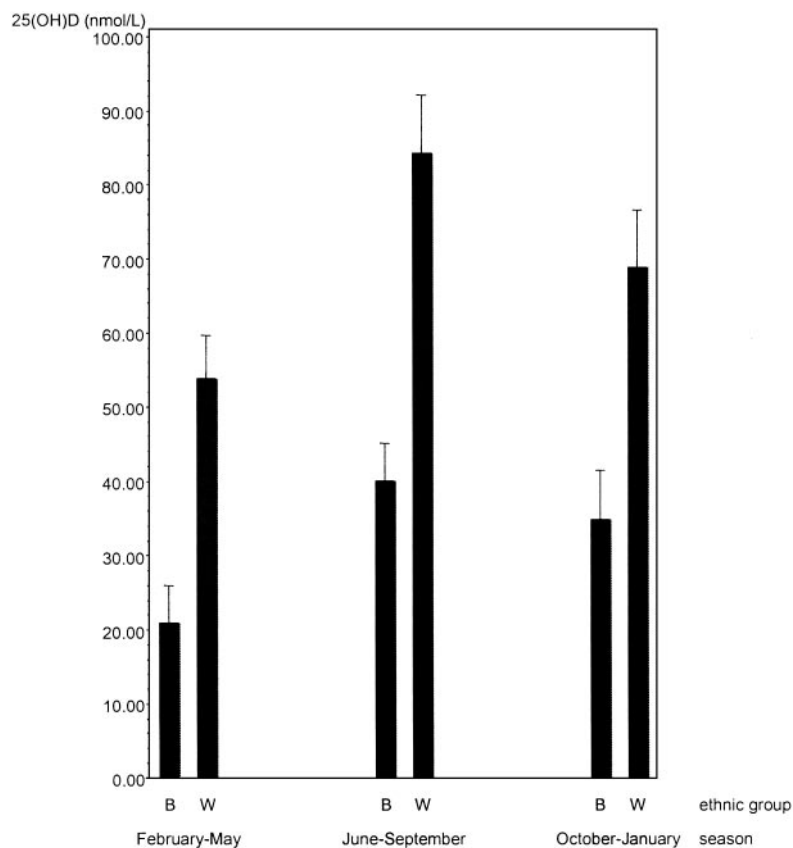


TABLE 2. Partial correlation of serum 25-OHD (adjusted for race, season, age, and dietary vitamin D) with other anthropometric parameters

Variable	Correlation (r)	P
Height (cm)	0.09	NS
Weight (kg)	-0.02	NS
BMI (kg/m ²)	-0.08	NS
%TBF	-0.13	0.013

NS, Not significant.

TABLE 3. Stepwise linear regression of factors associated with 25-OHD

Variable	Partial r ²	P
Race	0.24	0.0001
Season	0.10	0.001
Age	0.01	0.027
%TBF	0.02	0.011

The following independent variables were tested: race, season, age, dietary vitamin D, %TBF, height (cm), BMI (kg/m²), weight (kg), and smoking (packs/year).

height, and BMI), only %TBF was found to independently enter into the multiple regression equation predicting 25-OHD at the 0.05 level, increasing the cumulative partial R² by 0.02.

Discussion

This was a cross-sectional, population-based study that provided evidence that 25-OHD levels vary with adiposity (%TBF) in healthy women who are not morbidly obese. Al-

though a number of previously published studies (1–4, 13–16) reported that serum 25-OHD levels are inversely correlated with body fat, almost all of them compared serum 25-OHD levels in morbidly obese patients with nonobese subjects. Variables like season, dietary vitamin D intake, age, and race are already well known to influence the levels of serum 25-OHD in a healthy population of women. Our results imply that TBF is another variable that influences serum calcidiol levels with increasing adiposity associated with lower serum 25-OHD levels. The fact that serum 25-OHD levels are more strongly correlated with %TBF, compared with body weight or BMI, indicate that it is adiposity, not simply body mass, that influences the serum level of 25-OHD. This relationship seems to be related to differences in volume of distribution of 25-OHD in fat mass (6, 7).

The mechanism of variations in serum 25-OHD levels in both nonobese and obese individuals appears to be related to availability of adipose tissue leading to excessive storage of the precursor in the fat tissue. Some investigators (4) had suggested that serum 25-OHD increases appropriately in response to UV radiation in obese individuals, implying that low vitamin D levels do not result from impaired dermal production and delivery. A recent study by Worstman *et al.* (9) contradicts this statement. In dynamic testing on 13 obese (BMI > 30) white individuals and 13 controls to evaluate the blood levels of vitamin D and its response after exposure to UV-B radiation or an oral dose of vitamin D₂, they reported that the increase in vitamin D₃ levels was 57% less in the obese than in nonobese subjects post irradiation to UVB rays. This suggests that the sc fat, which is known to store vitamin

D₃, sequestered more of the synthesized vitamin D₃ in the obese than in the nonobese subjects because there was more fat available for this process. In contrast, supplementation with 50,000 IU oral vitamin D₂ resulted in an increase in serum vitamin D₂ levels in both obese and nonobese individuals with no significant difference between the peak serum vitamin D₂ levels in the two groups. The results of this study not only imply that obesity is associated with lower vitamin D levels secondary to the increase in adiposity but also suggest that oral vitamin D supplementation, in high doses, may be more bioavailable to obese individuals for conversion into 25-OHD, compared with vitamin D synthesized in the dermis.

Although Scragg *et al.* (17) had reported no correlation between serum vitamin D levels and BMI in a cross-sectional study of the healthy population in New Zealand, there is also evidence that indicates that increasing adiposity causes relative resistance to standard doses of vitamin D, *i.e.* the serum levels of 25-OHD do not rise as much in thinner individuals. In a study by Barger-Lux *et al.* (18), oral vitamin D₃ was given in graded doses to nonobese men aged 20–37 yr and the factors influencing the serum 25-OHD response to a given dose of vitamin D₃ were determined. Besides the dose of oral vitamin D₃ and basal level of serum 25-OHD, BMI, not weight, contributed significantly to the variance in 25-OHD response in an inverse relationship (<0.05). We analyzed our data to predict serum 25-OHD levels based on the daily dietary vitamin D intake per kilogram of body weight of the subjects. The results could be expressed as serum 25-OHD (nanomoles per liter) = $48.8 + 127.1$ [dietary vitamin D (in micrograms per day) per kilogram body weight]. This relationship is significant at P less than 0.01. We did not find as strong a correlation between serum 25-OHD levels and the vitamin D intake/kg/day as the previous study by Barger-Lux *et al.* (18). The rather small r^2 could be from several differences in our study compared with Barger-Lux *et al.* Our data analysis is cross-sectional and the range of dietary vitamin D intake in the study subjects was very small (with a mean of $2.7 \mu\text{g}/\text{d} \pm 2.4 \mu\text{g}/\text{d}$). Dietary vitamin D assessment was based on a recall method that gives only a rough estimate. Variations in the estimate of vitamin D intake derived from the 24-hr recall method may obscure significant relationships (19). Finally, our correlation is based on baseline dietary vitamin D consumption rather than oral vitamin D supplementation in a controlled fashion. Nevertheless, both these studies indicate that body weight, or more specifically body fat, have a significant influence on the serum 25-OHD levels not only in obese subjects but also in a healthy population.

The effect of seasonal changes on the serum 25-OHD levels was seen in the whole population but was less pronounced in blacks compared with white women. The peak levels were achieved during summer months when the sun exposure is greatest. As is well known cutaneous synthesis of vitamin D occurs through photoconversion of 7-dehydrocholesterol to precholecalciferol at wavelengths of light between 290–315 nm (20). Thermal isomerization of precholecalciferol to cholecalciferol occurs with about 50% of precholecalciferol (previtamin D₃) converting to cholecalciferol (vitamin D₃) over a three day period. During the winter months sunlight

is filtered at a more oblique angle through the stratospheric ozone layer, decreasing the UVB radiation (responsible for previtamin D₃ production) that reaches the earth's surface resulting in marked changes in the cutaneous vitamin D₃ synthesis varying with different latitudes (21). Some other factors that could effect cutaneous previtamin D₃ production include melanin (22) and aging (23). Katz *et al.* (24) prospectively measured seasonal changes in black adults. In this study, nine black men and women from South Carolina had increases in serum 25-OHD levels that were half those of the six white subjects. Similar racial differences have been reported by Scragg *et al.* (17) in New Zealand, and Harris and Dawson-Hughes (25) in Boston. In that study, 51 young black and 39 white women were studied longitudinally. The results were similar to our study. Both groups showed seasonal variation in 25-OHD concentrations, including the fact that the amplitude of change, *i.e.* summertime increase, was lower in black than white women. When UV exposure is sufficient, black adult populations can achieve mean concentrations of both vitamin D and 25-OHD that are similar to those of whites (26, 27). Thus, it appears that the more pigmented skin of black women allowed them to form substantially less previtamin D during summer months resulting in lower stores of previtamin D for conversion to vitamin D (22). There is also some suggestion that black women may also store less previtamin D, vitamin D, or vitamin D metabolites in body tissues for several months beyond the synthesis phase (5, 28), although definitive studies need to be done for this to be established.

The influence of age on 25-OHD has also been previously studied (23, 28, 29). The absolute concentration of 7-dehydrocholesterol (previtamin D₃) in the skin decreases with age. Aging *per se* does not appear to significantly alter the efficiency of conversion of provitamin D₃ to previtamin D₃ and vitamin D₃ (23). Also, older people tend to have lower dietary vitamin D intake and less exposure to sunlight. These observations are especially important for the elderly who rely on exposure to sunlight for their vitamin D requirements. If sufficient stores are not built up in the fat during summer months, it is likely that without vitamin D supplementation the elderly people will be at risk for developing vitamin D deficiency because exposure to sunlight during winter is ineffective for producing vitamin D₃ in skin. Absorption of vitamin D from the gut is not impaired by aging (28, 29), but dietary intake is often reduced.

In conclusion, this study suggests that percentage body fat is independently associated with serum levels of 25-OHD in healthy women besides other well-known factors such as dietary vitamin D intake, season, age, and race. Although the impact of body fat is relatively small, it is statistically significant and may influence the way we assess vitamin D nutrition and recommend supplementation in healthy women. Because optimal vitamin D nutrition is one of the goals to improve skeletal health, vitamin D supplementation may be a norm, especially during winter months for women living at higher latitudes. It is now an emerging consensus that 25-OHD level of at least 80 nmol/liter may be needed to ensure vitamin D sufficiency and prevent secondary hyperparathyroidism and its consequences on bone metabolism (30). It is clear from our study that greater the body fat, the

increased likelihood of failing to achieve an optimal 25-OHD of 80 nmol/liter, even during summer when it should be relatively easy to maintain blood levels, independent of race.

A remedy for this inadequacy may be oral vitamin D supplementation in women. Our study suggests that individuals with higher percentage body fat may require higher vitamin D intake to attain optimal 25-OHD levels, compared with lean individuals; therefore, body fat should be taken into consideration when assessing vitamin D requirements. But the exact impact of body fat on oral vitamin D supplementation to optimize 25-OHD could be obtained only from a longitudinal study with vitamin D supplementation. Needless to say, what seems to be a small correlation in our cross-sectional study may in fact have a greater impact when assessed in a controlled longitudinal study with vitamin D supplementation. Therefore, we think that a longitudinal study of oral vitamin D supplementation in the general population will be necessary before this information could be applied clinically.

Acknowledgments

Received June 25, 2002. Accepted October 3, 2002.

Address all correspondence and requests for reprints to: John F. Aloia, M.D., Winthrop-University Hospital, 259 First Street, Mineola, New York 11501.

This work was supported by NIH Grant RO1-AR37520-05.

References

1. Compston JE, Vedi S, Ledger JE, Webba, Gazet JC, Pilkington RE 1981 Vitamin D status and bone histomorphometry in gross obesity. *Am J Clin Nutr* 34:2359–2363
2. Buffington C, Walker B, Cowan Jr GS, Scruggs D 1993 Vitamin D deficiency in morbidly obese. *Obes Surg* 3:421–424
3. Bell NH, Epstein S, Greene A, Shary J, Oexmann MJ, Shaw S 1985 Evidence for alteration of the vitamin D endocrine system in obese subjects. *J Clin Invest* 76:370–373
4. Liel Y, Ulmer E, Shary J, Hollis BW, Bell NH 1988 Low circulating vitamin D in obesity. *Calcif Tissue Int* 43:199–201
5. Bell NH, Shaw S, Turner RT 1984 Evidence that 1, 25-dihydroxyvitamin D₃ inhibits the hepatic production of 25-hydroxyvitamin D in man. *J Clin Invest* 74:1540–1544
6. Lumb GA, Mawer EB, Stanbury SW 1971 The apparent vitamin D resistance of chronic renal failure: a study of the physiology of vitamin D in man. *Am J Med* 50:421–44
7. Mawer EB, Backhouse J, Holman CA, Lumb GA, Stanbury SW 1972 The distribution and storage of vitamin D and its metabolites in human tissues. *Clin Sci* 43:414–431
8. Rosenstreich SJ, Rich C, Volwiler W 1971 Deposition in and release of vitamin D₃ from body fat: evidence for a storage site in the rat. *J Clin Invest* 50:679–687
9. Worstman J, Matsuoka LY, Chen TC, Lu Z, Holick MF 2000 Decreased bioavailability of vitamin D in obesity. *Am J Clin Nutr* 72:690–693
10. Aloia JF, Vaswani A, Yeh JH, Flaster E 1996 Risk for osteoporosis in black women. *Calcif Tissue Int* 59:415–423
11. Dawson-Hughes B, Harris SS, Dallal GE 1997 Plasma calcidiol, season, and serum parathyroid hormone concentrations in healthy elderly men and women. *Am J Clin Nutr* 65:67–71
12. Duffie J, Beckman W 1980 Available solar radiation. In: Duffie J, Beckman W, eds. *Solar engineering of thermal processes*. New York: Wiley-Interscience Publication
13. Teitelbaum SL, Halverson JD, Bates M, Wise L, Haddad JG 1977 Abnormalities of circulating 25-OH vitamin D after jejunal bypass for obesity: evidence of an adaptive response. *Ann Intern Med* 86:289–293
14. Rickers H, Christiansen C, Balsev I, Rodbro P 1984 Impairment of vitamin D metabolism and bone mineral content after intestinal bypass surgery. *Scand J Gastroenterol* 19:184–189
15. Hey H, Stockholm KH, Lund BJ, Sorenson OH 1982 Vitamin D deficiency in obese patients and changes in circulating vitamin D metabolites following jejunoileal bypass. *Int J Obes* 6:473–479
16. Hyldstrup L, Andersen T, McNair P, Breum L, Transbol I 1993 Bone metabolism in obesity: changes related to severe overweight and dietary weight reduction. *Acta Endocrinol* 129:393–398
17. Scragg R, Holdaway I, Singh V, Metcalf P, Baker J, Dryson E 1995 Serum 25 hydroxyvitamin D₃ is related to physical activity and ethnicity but not to obesity in a multicultural workforce. *Aust N Z J Med* 25:218–223
18. Barger-Lux MJ, Heaney RP, Dowell S, Chen TC, Holick MF 1998 Vitamin D and its major metabolites: serum levels after graded oral dosing in healthy men. *Osteoporos Int* 8:222–230
19. Beaton GH, Milner J, McGuire V, Feather TE, Little JA 1983 Sources of variance in 24-hour dietary recall data: implications for nutrition study design and interpretation. *Carbohydrate sources, vitamins and minerals*. *Am J Clin Nutr* 37:986–995
20. Holick MF, MacLaughlin JA, Clark MB 1982 Photosynthesis of previtamin D₃ in human skin. *Science* 216:1001–1003
21. Webb AR, Kline L, Holick MF 1988 Influence of season and latitude on the cutaneous synthesis of vitamin D₃: exposure to winter sunlight in Boston and Edmonton will not promote vitamin D₃ synthesis in human skin. *J Clin Endocrinol Metab* 67:373–378
22. Holick MF, MacLaughlin JA, Doppelt SH 1981 Regulation of cutaneous previtamin D₃ photosynthesis in man: skin pigment is not an essential regulator. *Science* 211:590
23. MacLaughlin JA, Holick MF 1985 Aging decreases the capacity of skin to produce vitamin D₃. *J Clin Invest* 76:1536–1538
24. Katz BS, Jackson GJ, Hollis BW, Bell NH 1993 Diagnostic criterion of vitamin D deficiency. *Endocrinologist* 3:248–253
25. Harris SS, Dawson-Hughes B 1998 Seasonal changes in plasma 25 hydroxyvitamin D concentrations of young American black and white women. *Am J Clin Nutr* 67:1232–1236
26. Clemens TL, Adams JS, Henderson SL, Holick MF 1982 Increased skin pigmentation reduces the capacity of skin to synthesize vitamin D₃. *Lancet* 1:74–76
27. Brazero WF, McPhee AJ, Mimouni F, Specker BL, Tsang RC 1988 Serial ultraviolet B exposure and serum 25 hydroxyvitamin D response in young adult American blacks and white: no racial differences. *J Am Coll Nutr* 7: 111–118
28. Holick MF 1986 Vitamin D requirements for the elderly. *Clin Nutr* 5:121–129
29. Clemens TL, Zhou XY, Myles M, Endres D, Lindsay R 1986 Serum vitamin D₂ and vitamin D₃ metabolite concentrations and absorption of vitamin D₂ in elderly subjects. *J Clin Endocrinol Metab* 63:656–660
30. Heaney RP 1999 Lessons for nutritional science from vitamin D. *Am J Clin Nutr* 69:825–826