Correlates of 25-hydroxyvitamin D and breast cancer stage in the Women's Healthy Eating and Living Study

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Abstract

Inverse associations between circulating 25-hydroxyvitamin D [25(OH)D] and breast cancer stage have been reported, thus it is critical to understand the variables that contribute to 25(OH)D levels among women with breast cancer. Among 904 women in the Women's Healthy Eating and Living Study, plasma 25(OH)D concentrations were measured and data on demographic characteristics, diet, physical activity, and tumor characteristics *were collected at study entry*. Statistically significant associations with 25(OH)D concentrations were observed for body mass index (BMI), body surface area (BSA), height, smoking, total vitamin D intake, physical activity, and race or ethnicity. Of the correlates of 25(OH)D, BMI, BSA, height, physical activity, and study site were associated with stage of breast cancer; however, concentrations of 25(OH)D were not significantly related to stage. In fully adjusted logistic regression models, the ORs (95% CIs) for the association between vitamin D deficiency and Stage II and III cancers were 0.85 (0.59-1.22) and 1.23 (0.71-2.15), respectively (p-trend=0.59), compared to Stage I. This study confirms previous work regarding the correlates of 25(OH)D concentrations but does not provide support for an association between vitamin D status and breast cancer stage.

Introduction

Circulating concentrations of the vitamin D metabolite 25-hydroxyvitamin D [25(OH)D] have been observed to be inversely associated with breast cancer stage or grade(1-4). The mechanism by which vitamin D status may be associated with risk for disease progression is yet to be elucidated, and an alternative explanation that these associations are the result of confounding has yet to be ruled out. Further, characteristics other than vitamin D intake or UV exposure appear to be among the determinants of circulating concentrations of 25(OH)D(5-7). Giovannucci and colleagues first created a model to predict levels of 25(OH)D of participants in the Health Professionals Follow-Up Study(8), and several other studies have also identified of correlates of circulating concentrations of 25(OH)D in North

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American cohorts(6, 7, 9-17), revealing several patient characteristics to be significantly associated with 25(OH)D concentrations. Specific positive correlates identified through these studies include physical activity, dietary or supplemental vitamin D intake, white race/ethnicity, and greater sun exposure; while body mass index (BMI), African-American or Hispanic race or ethnicity, and smoking are generally inversely related(6-17). Given the reported associations between 25(OH)D concentrations and breast cancer stage or grade(1-4), it is critical to understand the correlates of this biomarker in women who have been diagnosed with breast cancer, as well as to further explore the potential effects of these variables on the relationship between 25(OH)D concentration and breast cancer stage.

The objective of the current work was to further elucidate the correlates of circulating 25(OH)D concentrations among women who were diagnosed with breast cancer within the Women's Healthy Eating and Living Study (WHEL) and treated for their disease on average 2 years prior to study enrollment. In addition, we sought to investigate the relationship between 25(OH)D concentrations and breast cancer stage at diagnosis, and to identify and assess the impact of potentially confounding variables on this association.

Methods

Study population

Study participants were from the Women's Healthy Eating and Living (WHEL) Study, a multi-center, randomized, controlled dietary intervention trial conducted in Arizona, California, Oregon and Texas. The WHEL Study, described in detail previously(18), enrolled 3088 breast cancer survivors who had completed primary treatment for early stage breast cancer within the previous four years. Eligibility criteria included evidence from the medical record of a diagnosis within the past four years of primary operable invasive breast carcinoma categorized using the American Joint Committee on Cancer criteria as Stage I tumor (1 cm), Stage II, Stage IIIA or Stage IIIC; aged 18 to 70 years at diagnosis; treated with axillary dissection and total mastectomy or lumpectomy followed by primary breast radiation; not scheduled for or currently undergoing chemotherapy; no evidence of recurrent disease or new breast cancer since completion of initial local treatment; and no other invasive cancer in the past 10 years(23).

Beginning in 1995, eligible women were randomized to either an intervention group, which received intensive counseling for adoption of a diet very high in vegetables, fruit, and fiber and low in fat; or a comparison group counseled to follow the National Cancer Institute's 5-A-Day dietary guidelines(18). Complete endpoint data were obtained for 96% of the study participants. In a report of the primary findings of the project, no effect of the very high vegetable, low fat dietary intervention was detected(19), and circulating concentrations of 25(OH)D were not associated with risk for breast cancer recurrence or survival(3). The Human Subjects Committees/Institutional Review Boards for each of the seven recruitment sites approved the study protocol prior to study initiation.

Dietary and Demographic Data

The Arizona Food Frequency Questionnaire (AFFQ), as described elsewhere(20, 21), was employed for the collection of dietary data at baseline. In addition, at baseline, each participant completed a set of four 24-hour dietary recalls, in which they reported supplement name, number of pills, brand or manufacturer for all dietary supplement formulations they had taken during the previous day. A comprehensive supplement database maintained by the WHEL coordinating center, with nutrient content in standardized units per tablet or capsule, and verified by bottle labels and manufacturer websites for more than 9000 dietary supplement formulations, allowed us to quantify the total amount of supplemental vitamin D from multivitamins, mineral (e.g. calcium) supplements or single ingredient vitamin D formulations for each day. Vitamin D supplement intake was then averaged over the 4 recall days. Details on the WHEL dietary supplement database have been published previously(22, 23). Additional questionnaires for ascertainment of demographic and lifestyle data were administered at baseline, including the Health Status Questionnaire, Personal Habits Questionnaire and the Lifestyle Questionnaire(18). The Health Status Questionnaire was prepared specifically for the WHEL Study and was used to collect data regarding hospitalizations, breast cancer events, medical conditions, and use of prescription medications(18). The Personal Habits Questionnaire was adapted from the Women's Health Initiative trial questionnaires(18). Specifically, the Personal Habits Questionnaire queried study participants regarding behaviors associated with cancer risk, including tobacco use, alcohol use, and physical activity. Body mass index (BMI) was calculated as kg/m^2 from measured data for height and weight collected at clinic visits, while body surface area (BSA) was calculated using the formula of Mosteller(24).

Plasma 25(OH)D Measurement

Blood samples were drawn upon randomization into the parent trial and were stored at $-80°C$ until employed for analysis of 25(OH)D. The mean length of time between breast cancer diagnosis and entry into the WHEL Study was 2 years. Thus, for the current work, 25(OH)D was measured an average of 2 years post- diagnosis, similar to the study of Neuhouser et al.(4). The chemiluminescent DiaSorin assay, a direct, competitive chemiluminescence immunoassay was employed for 25(OH)D analysis in the current work and has been described in detail elsewhere(3, 25, 26). Briefly, the assay range was 7.0 -150 ng/mL and the lowest reportable value was 7.0 ng/mL. National Institute of Standards and Technology (NIST) level 1 and level 2 Vitamin D standard reference material were used to compute the between-run and within-run coefficients of variation (CVs) for the assay, which were 8.0% (low) and 2.7% (high); and 6.1% (low) and 5.4% (high), respectively.

Statistical Methods

Logistic regression models were employed to evaluate the association between characteristics of WHEL participants and odds for vitamin D deficiency, as defined by a concentration of 25(OH)D < 20.0 ng/ ml. Several variables were considered as possible correlates of vitamin D deficiency, including study site, race or ethnicity, total vitamin D intake (dietary plus supplements), latitude, education, stage of breast cancer, physical activity (metabolic equivalent tasks [METS]), use of anti-estrogen medication, height, age,

and menopausal status. If a variable was statistically significantly related to vitamin D deficiency in unadjusted analyses, it was included in the final multivariate model. Variables that were significantly related to vitamin D deficiency were then examined for associations with breast cancer stage. Means and standard deviations for continuous variables were calculated for each stage, and regression analyses were employed for calculation of statistically significant trends for continuous variables. Chi-square analyses were used to assess the relationship between categorical variables and breast cancer stage. Multinomial logistic regression was employed to examine the association between vitamin D deficiency and breast cancer stage, with Stages I, II, and III as the outcome variable. Potential confounders identified from the analyses described above as significantly associated with both vitamin D deficiency and breast cancer stage were included in the adjusted models.

Results

Table 1 presents the unadjusted and adjusted odds ratios and 95% confidence intervals for vitamin D deficiency, defined as 25(OH)D concentrations of less than 20.0 ng/ml. Variables that were significantly associated with increased odds for vitamin D deficiency in unadjusted models were BMI (*p*-trend <0.001) and BSA (*p*-trend <0.001), African-American race (OR=5.10; 95% CI=2.44-10.63), or Hispanic ethnicity (OR=2.54; 95% CI=1.41-4.60); while height (*p*-trend <0.05), total vitamin D intake from diet and supplements (*p*-trend <0.001) and physical activity (*p*-trend <0.001) were related to a lower odds for deficiency. Adjusted models were constructed separately for BMI and BSA due to the relatively high correlation between these two variables (r^2=0.85). BMI remained statistically significantly associated with vitamin D deficiency in the adjusted model, as did total vitamin D intake, physical activity, African-American race, and Hispanic ethnicity. Winter season was also significantly associated with an increased risk for deficiency compared to Fall. The results for BSA were similar to that of BMI for all variables, with the exception of height, which remained statistically significantly related to vitamin D deficiency in the adjusted model for BSA but not BMI.

As shown in Table 2, several variables associated with vitamin D deficiency in the WHEL Study were also related to breast cancer stage at diagnosis. Body mass index (p-trend <0.05), body surface area (p-trend <0.05), and height (p <0.05) exhibited statistically significant increasing trends with increasing stage of breast cancer. Conversely, physical activity after diagnosis was significantly reduced with increasing stage (p-trend <0.05). Variation in stage of diagnosis was also apparent by study site. For example, there was a greater proportion of Stage III cancers at Arizona (15.7%) compared to Kaiser Oakland (4.6%). There were no statistically significant differences in breast cancer stage by smoking, total vitamin D intake, season, or race/ethnicity. Results by tumor grade were similar to those for stage (data not shown).

Table 3 presents the unadjusted and adjusted associations between vitamin D deficiency and stage of breast cancer at diagnosis. Among women with Stage III cancers, there was a greater proportion with vitamin D deficiency (40.5%) compared to Stage II (31.2%) or Stage I (31.6%) cancer. However, in the unadjusted model of vitamin D deficiency and breast cancer stage, there was no statistically significant association between deficiency and breast

cancer stage. Compared to women with Stage I cancers, the unadjusted ORs (95% CIs) for vitamin D deficiency were 0.98 (0.70-1.37) and 1.47 (0.88-2.45) among Stage II and Stage III cancers, respectively (p-trend=0.28). After adjustment for BMI or BSA only, or with inclusion of all confounding variables, these results were not materially altered.

Discussion

The results of the present study indicate that correlates of plasma 25(OH)D concentrations among women diagnosed with breast cancer are similar to those reported in North American populations without cancer(6, 7, 9-17), as well as those reported among breast cancer survivors(2, 4, 27-29). Variables observed to be statistically significantly related to odds for vitamin D deficiency in women from the WHEL Study included BMI , BSA, height, African-American race or Hispanic ethnicity, season of blood collection, total vitamin D intake, and physical activity. Although there was a greater proportion of women with Stage III cancers who were vitamin D deficient as compared to Stage II and Stage I cancers, this result was not statistically significant, and similar associations were observed for tumor grade (data not shown).

Previous studies of circulating 25(OH)D concentrations among women with breast cancer(2-4, 27, 28) have revealed that body size or adiposity, usually assessed by BMI, are consistently inversely related to 25(OH)D levels(2-4, 27, 30), and the results of the present study support this observation. However, the mechanism of action for this relationship remains unknown. It has been hypothesized that increased body size results in greater sequestration of 25(OH)D in the adipose tissue. Wortsman et al.(31) compared the response of obese ($n = 19$, BMI 30 kg/m^2) and matched lean control ($n = 19$, BMI 25 kg/m^2) subjects to UVB irradiation and an oral dose (50,000 IU) of vitamin D_2. The obese group showed an attenuated response in vitamin D_3 concentration following UVB irradiation, and peak serum vitamin D_2 after oral intake was significantly correlated with BMI ($r = –0.56$, $P = 0.007$). In a recent study, the response to a daily oral dose of 50,000 IU vitamin D_2 or D_3 for 12 weeks was examined in 33 healthy adults with a mean BMI of 25.5 kg/m^2, and both circulating concentrations and adipose tissue calciferol content was measured[30]. Extrapolation using estimated mean total body fat content suggested that only 17% of the administered dose of vitamin D_3 appeared to have been sequestered by fat tissue during that time frame, and the authors concluded that little vitamin D appears to be sequestered in adipose tissue(32). Other possibilities for the inverse association between body size and 25(OH)D concentrations include lower UV exposure among those with higher BMI(33) or a potential hemodilution effect(34, 35), the latter of which was explored using BSA in the current work. BSA is strongly positively correlated with blood volume(36); for this reason, BSA has been employed for calculation of chemotherapeutic doses(36), though this approach is not without controversy(37). Recently, Song et al.(34) reported that serum prostate-specific antigen levels were more strongly correlated with BSA than with BMI. The authors speculated that BSA is a surrogate measure for blood volume, and thus the lower PSA concentrations they observed in obese men could be the result of hemodilution(34). Although in the present study BMI and BSA had similar associations with 25(OH)D concentrations, they were also highly correlated with one another (r^2=0.85), and

hemodilution remains a possibility for the consistently observed inverse association for body size and 25(OH)D.

Physical activity was significantly inversely associated with odds for vitamin D deficiency in the present study, as has been shown in other work(3, 6, 8), though it is not yet clear whether this relationship is due to sun exposure, smaller body size, or another mechanism. African-American race and Hispanic ethnicity were both statistically significantly associated higher odds for vitamin D deficiency, as has been reported elsewhere(9) (11, 14, 38). Differences in 25(OH)D by race or ethnicity are believed to result from greater skin pigmentation(39), though other characteristics such as diet and body size may also contribute.

Several of the risk factors for vitamin D deficiency were also observed to be statistically significantly related to stage of breast cancer at diagnosis, including BMI, BSA, height, and physical activity. BMI has previously been demonstrated to be associated with severity of disease at diagnosis(40). Related to body size, physical activity was lowest in women with stage III cancer, followed by stage II; lower physical activity among women with more advanced cancers has previously been described(41). Each of these potentially confounding variables is a critical consideration in light of prior findings that concentrations of 25(OH)D are significantly related to breast cancer stage(4, 28). A better understanding of the interactions between body size, 25(OH)D, and breast cancer risk is critical, particularly in light of the potential for vitamin D metabolites to suppress leptin, a hormone associated with both body size and cancer risk(42-44).

Among women who were diagnosed with a Stage III breast cancer in the WHEL Study, approximately 40% were vitamin D deficient, as compared to approximately 32% in those with stage I or II cancers, but this association was not statistically significant in either unadjusted or adjusted regression models. Because there were comparatively few women in the WHEL Study with stage III cancers, there may not have been enough statistical power to detect a significant difference between the groups. Some published studies of 25(OH)D and breast cancer stage or grade have shown a statistically significant association wherein more advanced cancers are associated with lower 25(OH)D concentrations(1, 2, 4, 28), while others have not(45, 46). Some of the statistically significant results for studies suggesting an association between 25(OH)D levels and breast cancer stage were reported as baseline descriptions of a study population, and for this or other reasons the association was not evaluated in the context of a multivariate adjusted model(1, 2, 28). Conversely, in another study(4) the association between 25(OH)D and cancer stage was investigated with a multivariate adjusted regression model. Among women in the Health, Eating, Activity and Lifestyle (HEAL) study, Neuhouser et al.(4) reported significantly lower 25(OH)D concentrations among women with regional as opposed to *in situ* breast cancers; these estimates were not materially altered by adjustment for age, BMI, race/ethnicity, geographic site, physical activity, season of blood draw, treatment, or tamoxifen use. The reasons for the discordant results between these the current work and that of the HEAL study likely lies in the selection of study participants. In our trial, women were required to have a prior diagnosis of Stage I,II or IIIA invasive breast cancer, while the HEAL study included women with carcinoma-in-situ through highly malignant Stage IV disease.

The strengths of the current work include the availability of detailed questionnaire data as well as blood samples from the WHEL intervention trial, which allowed for thorough investigation the correlates of concentrations of plasma 25(OH)D concentration. One limitation of the current work is that the women in the WHEL study all had relatively early stage cancers. It is possible that significant differences in 25(OH)D concentrations would have been observed in comparisons with women with later-stage cancers, though a causal relationship could not have been established with such a study design.

In summary, challenges in identifying the mechanism of action for the effect of various correlates on circulating 25(OH)D concentrations remain; however, a better understanding of these relationships is critical for understanding associations between circulating 25(OH)D and cancer. Caution remains warranted for assigning a causal relationship between vitamin D and breast cancer or breast cancer stage until the effects of 25(OH)D correlates are more fully understood(5, 41, 47); this is particularly important in light of the demonstrated variation in results for vitamin D and breast cancer by study design, and the potential for reverse causation in this association(3, 48, 49).

Acknowledgments

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Table 1

Association of participant characteristics and vitamin D deficiency (plasma 25(OH)D concentrations < *20 ng/ml)* in WHEL Study women (n=904) in unadjusted and adjusted regression models.

Variable	<20 ng/ml n, %	Unadjusted OR (95% CI)	Adjusted[1] OR (95% CI)	Adjusted[2] OR (95% CI)
Body Mass Index (kg/m^2)				
<25	78 (21.4)	1.00	1.00	na
25 BMI < 30	82 (30.6)	1.61(1.13 - 2.32)	1.52 (1.01-2.29)	na
>30	131 (50.0)	3.67 (2.59 -5.19)	3.14 (2.09-4.70)	na
p-trend[3]		<0.001	<0.001	na
Body Surface Area (m^2)				
1.36-1.72	74 (24.0)	1.00	na	1.00
1.73-1.90	99 (33.0)	1.56 (1.10 - 2.23)	na	1.67 (1.10-2.55)
>1.90-2.64	122 (41.4)	2.24 (1.58 - 3.23)	na	2.52 (1.64-3.87)
p-trend		<0.001	na	0.001
Height (cm)				
<161.6	114 (37.5)	1.00	1.00	1.00
161.6-167.3	92 (30.5)	0.73 (0.52 – 1.02)	0.77 (0.52 -1.16)	0.65 (0.43 -0.98)
>=167.4	89 (29.9)	0.71 (0.51 – 1.00)	0.92 (0.61–1.38)	0.63 (0.42–0.96)
p-trend[3]		<0.05	0.65	<0.05
Smoking (pack years)				
0	150 (30.1)	1.00	1.00	1.00
0.1-19.9	26 (28.0)	0.90 (0.55- 1.48)	0.78 (0.44-1.39)	0.79 (0.45-1.40)
20	112 (38.1)	1.43 (1.08-1.94)	1.42 (1.00-2.01)	1.38 (0.98-1.95)
p-trend[3]		<0.05	0.07	0.09
Total vitamin D intake (IU/d) mean ± SD				
<240	121 (40.2)	1.00	1.00	1.00
240-534.9	85 (30.6)	0.66 (0.46-0.92)	0.60 (0.41 – 0.88)	0.61 (0.41 – 0.89)
>=535	69 (25.7)	0.51 (0.36-0.73)	0.53 (0.35 – 0.79)	0.55 (0.37 – 0.82)
p-trend[3]		<0.001	<0.001	<0.01
Physical activity (MET min/wk): mean ± sd				
0-285	140 (39.8)	1.00	1.00	1.00
290 – 930	96 (33.9)	0.78 (0.56 – 1.08)	1.02 (0.69 – 1.49)	0.94 (0.64 – 1.37)
>= 945	59 (21.9)	0.43 (0.30 – 0.61)	0.63 (0.41 - 0.96)	0.53 (0.35 - 0.79)
p-trend[3]		<0.001	<0.05	<0.01
Season				
Fall	74 (31.2)	1.00	1.00	1.00
Winter	76 (38.0)	1.35 (0.91 – 2.01)	1.79 (1.12 – 2.85)	1.85 (1.17 – 2.95)
Spring	82 (36.1)	1.25 (0.85 - 1.83)	1.48 (0.94 – 2.35)	1.54 (0.98 – 2.44)
Summer	63 (26.3)	0.78 (0.53 – 1.17)	0.99 (0.62 – 1.57)	1.03 (0.65 – 1.62)

Variable	<20 ng/ml n, %	Unadjusted OR (95% CI)	Adjusted[1] OR (95% CI)	Adjusted[2] OR (95% CI)
Race/Ethnicity				
White	224 (29.1)	1.00	1.00	1.00
Black	23 (67.7)	5.10 (2.44-10.63)	4.67 (2.11 – 10.30)	4.59 (2.11 – 10.00)
Hispanic	24 (51.1)	2.54 (1.41 – 4.60)	2.40 (1.19 – 4.83)	2.21 (1.10 – 4.44)

[1] Adjusted model includes BMI, height, smoking, total vitamin D intake, physical activity, season of blood collection, study site, race/ethnicity, and stage of breast cancer at diagnosis.

[2] Adjusted model includes BSA, height, smoking, total vitamin D intake, physical activity, season of blood collection, study site, race/ethnicity, and stage of breast cancer at diagnosis.

[3] Tests for trend were conducted for appropriate characteristics using the regression model and a categorical variable for each dependent variable

Table 2

Participant characteristics related to vitamin D deficiency and breast cancer stage at diagnosis in the WHEL Study[1].

	Breast Cancer Stage			
Variable	Stage I (n=202)	Stage II (n=617)	Stage III (n=85)	p-value
Body Mass Index (kg/m²)[2]	27.0 ± 5.9	27. ± 6.0	28. ± 7.0	<0.05
Body Surface Area (m²)	1.80 ± 0.2	1.84 ± 0.2	1.90 ± 0.2	<0.05
Height (cm)	163.9 ± 6.4	164.6 ± 6.8	165.1 ± 6.2	<0.05
Smoking (pack-years)	5.4 ± 11.1	6.0 ± 13.1	8.0 ± 14.2	0.17
Total vitamin D intake (IU/d): mean ± sd	389.4 ± 346.8	385.7 ± 312.5	441.6 ± 397.1	0.41
Physical Activity (mets/d)	741.5 ± 755.5	784.0± 845.1	645.1 ± 698.5	<0.01
Season				
Fall	54 (22.8)	157 (66.2)	26 (11.0)	
Winter	48 (21.2)	158 (69.6)	21 (9.3)	
Spring	57 (23.8)	162 (67.5)	21 (8.8)	
Summer	43 (21.5)	140 (70.0)	17 (8.5)	0.95
Study site				
San Diego	47 (23.9)	131 (66.5)	19 (9.6)	
Kaiser Oakland	45 (34.1)	81 (61.4)	6 (4.6)	
UC Davis	37 (22.0)	119 (70.8)	12 (7.1)	
Arizona	32 (21.8)	92 (62.6)	23 (15.7)	
Stanford	28 (20.1)	100 (71.9)	11 (7.9)	
MD Anderson	6 (7.7)	61 (78.2)	11 (14.1)	
Kaiser Portland	7 (16.3)	33 (76.7)	3 (7.0)	<0.01
Race/ Ethnicity				
White, not Hispanic	170 (22.1)	525 (68.2)	75 (9.7)	
African-American	9 (26.5)	22 (64.7)	3 (8.8)	
Hispanic	8 (17.0)	34 (72.3)	5 (10.6)	0.90

[1] n=904; analysis includes only those women for whom 25(OH)D measurements were available.

[2] Values shown are mean ± SD for continuous variables and n(%) for categorical variables.

Table 3

Crude and adjusted odds ratios (95% CIs) for vitamin D deficiency and stage of breast cancer at diagnosis.

	Stage I	Stage II	Stage III	p-value
Vitamin D Deficiency n(%)	**68 (31.6)**	**203 (31.2)**	**36 (40.5)**	
Unadjusted model	1.00	0.98 (0.70-1.37)	1.47 (0.88-2.45)	0.28
BMI only[1]	1.00	0.93 (0.66-1.32)	1.26 (0.74-2.15)	0.65
BSA only[2]	1.00	0.92 (0.65-1.29)	1.26 (0.75-2.14)	0.63
Full model with BMI[3]	1.00	0.86 (0.60-1.24)	1.23 (0.70-2.17)	0.58
Full model with BSA[4]	1.00	0.85 (0.59-1.22)	1.23 (0.71-2.15)	0.59

[1] Model for association between vitamin D deficiency and breast cancer stage adjusted for BMI only.

[2] Model for association between vitamin D deficiency and breast cancer stage adjusted for BSA only.

[3] Fully adjusted model with BMI includes smoking, total vitamin D intake, physical activity, race/ethnicity, and study site.

[4] Fully adjusted model with BSA includes smoking, total vitamin D intake, physical activity, race/ethnicity, and study site.