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Tanning Predicts Bone Mass but Not Structure in Adolescent Females Living in Hawaii

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Objectives: To evaluate the relationship between facultative skin pigmentation, which predicts circulating levels of plasma 25-hydroxymitamin D, and several measures of bone mass and structure in a cross sectional sample of adolescent females living in Hawaii.

Methods: Our sample was composed of adolescent females (n = 94) living in Hawaii where seasonal sun exposure is minimal, and who self-identified as either white (n = 16) or Asian (n = 78). Bone mineral content (BMC) of the total body, the lumbar spine and the hip, and cross sectional area (CSA) and section modulus (Z) at the proximal femur were quantified using DXA. Facultative skin pigmentation was measured at the forehead and non-facultative skin pigmentation was measured at the inner arm using a Chroma Meter CR-200b colorimeter.

Results: There were no significant differences between ethnic groups in terms of skin pigmentation. The difference between a^* taken at the forehead and inner upper arm significantly predicted BMC at the lumbar spine, total hip, and total body. Other measures of skin pigmentation were not significant predictors of any other measure of skeletal integrity.

Conclusions: The difference between facultative and non-facultative skin pigmentation for a^* is a significant predictor BMC, but not bone structure. Our findings are limited by an inability to control for long term UVA and UVB exposure and lack of a measure of serum 25(OH)D status. Further research is needed to examine these questions, particularly in populations who live at high latitudes where a winter season limits vitamin D_3 synthesis.

Adult skeletal integrity is influenced by lifestyle throughout the growth period. Determinants of bone mass, however, remain poorly understood. It is clear that measurable differences in bone quantity and structure exists between ethnic groups in adulthood and that these differences manifest early in life (Vidulich et al., 2007; Weaver et al., 2007). Bone strength tends to be highest in individuals who identify as African American and fracture rates are lowest (Finkelstein et al., 2002). In general, Asians tend to have the lowest bone mass, but do not experience as many fractures as do whites. Individuals who identify as white and non-white Hispanic are intermediate in terms of bone strength, with whites suffering from the highest fracture rate (Bachrach et al., 1999; Finkelstein et al., 2002; Weaver et al., 2007; Wu et al., 2003). Body size, and thus bone size, explains much of the discrepancy in bone strength between these groups (Lee et al., 2010). However, there is some evidence that Asians are less susceptible to fracture than are whites because the former maintain thicker trabecular bone (Wang et al., 2009). Although most researchers assume an underlying genetic basis to these differences, relatively few have studied actual genes in this pursuit. Lifestyle factors associated with contemporary Western society, such as dietary intake and physical activity level, may influence susceptibility to skeletal fragility. Many studies have found that Asians tend to be less physically active and consume less calcium than whites or Hispanics (Weaver et al., 2007). Interestingly, lack of calcium intake among adolescents appears to be due in part to perceived lactose intolerance. That is, many adolescents believe they are lactose intolerant when they are not (Matlik et al., 2007). This suggests persistence of a cultural belief that likely limits the amount of calcium consumed. Physical activity has a positive effect on bone mass so it is reasonable to assume that a more sedentary lifestyle would increase the likelihood of skeletal fragility. The cumulative effects of nutrition and activity across generations, likely influence bone mass and structure as well.

Another potential contributor to inadequate bone mass is lack of vitamin D_3. When skin is penetrated by ultraviolet-B (UVB) radiation, 7-dehydrocholesterol is converted to previtamin D_3 via temperature-dependent isomerization (Holick, 2007; Holick et al., 1980). Subsequently, previtamin D_3 is converted to vitamin D_3, which is ultimately metabolized in the liver to 25-hydroxycholecalciferol (25(OH)D_3),and in the kidney to its biologically active form 1,25-dihydroxycholecalciferol (1,25(OH)2D_3), or calcitriol. Although vitamin D_3 can also be acquired through diet, dietary intake provides substantially less vitamin D_3 than UVB exposure (Hollis, 2005). Vitamin D_3 serves many regulatory purposes, including absorption of calcium and phosphorus in the intestine and re absorption of calcium in the kidney. Vitamin D_3 also promotes

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bone formation and mineralization (Harkness and Cromer, 2004). Insufficient vitamin D_3 results in increased parathyroid hormone (PTH), in order to initiate bone resorption and to maintain calcium homeostasis. Individuals with more recent African ancestry (i.e., African Americans) achieve calcium homeostatis more efficiently than do whites (Weaver et al., 2008). Insufficient vitamin D_3 may constrain bone mass (Cashman et al., 2008; Nieves et al., 2008; Tangpricha et al., 2004), but this relationship may be dependent on calcium intake (Weaver, 2007). Although vitamin D_3 is fat soluble, and thus can be stored, stores can be exhausted with insufficient vitamin D synthesis over time.

The capacity for vitamin D synthesis varies with season, latitude, pigmentation, age, and sex. Vitamin D_3 synthesis did not occur between November and February in cities at 42.2 degrees north (e.g., Boston, MA), a seasonal effect related to the tilting of the earth's axis during winter (Webb et al., 1988). In addition, winter weather complicates analyses, as the amount of surface area exposed to UV radiation typically decreases. Furthermore, UV intensity varies at a given location from year to year, so the assumption that UV intensity on a specific day will be similar one year to the next is not tenable (Jablonski and Chaplin, 2010).

Skin pigmentation effectively filters UV radiation, so vitamin D_3 synthesis is limited in individuals with greater amounts of pigmentation (Chen et al., 2007; Jablonski and Chaplin, 2000, 2010; Rockell et al., 2008). Skin is pigmented by hemoglobin and carotene, but the predominant chemical responsible for pigmentation is melanin. Melanin is produced by cells in the stratum basale of the epidermis, termed melanocytes, when these cells are exposed to UVB radiation (Jablonski and Chaplin, 2000, 2010). Skin pigmentation is sexually dimorphic, with females on average having less pigmentation than males, likely due to fluctuating hormone levels (Zouboulis et al., 2007). Further, skin loses its capacity for vitamin D_3 synthesis with aging (Holick, 2007).

Populations with long evolutionary histories at a particular region demonstrate a strong negative association between latitude and pigmentation. The evolution of depigmentation has been a matter of debate largely focused on the adaptive significance of UV induced vitamin D_3 in homeostasis, particularly as it relates to skeletal integrity. For instance, some have suggested a negligible role for vitamin D in this process, suggesting that depigmentation has occurred through sexual selection (Aoki, 2002; Frost, 1988, 2007). Robins (1991, 2009) has also argued that the vitamin D hypothesis of depigmentation is not supported, suggesting that rickets is a disease of industrialization and age related bone loss occurs too late in life to be a strong selective pressure. However, Chaplin and Jablonski (2009) offer a strong rebuttal of Robins' (1991, 2009) critique of the vitamin D hypothesis providing not only support for its role in osteogenesis, but also pointing out the pivotal role Vitamin D_3 plays in regulation of immune function, cellular differentiation, cancer prevention, normal functioning of several organs, and susceptibility to infectious disease. Convincing evidence in support of the vitamin D hypothesis continues to grow and illuminate the importance of the vitamin's role in immune function (Baeke et al., 2008; Bikle, 2008; Cantora and Mahon, 2005; Fernandes de Abreu et al., 2009; Jablonski and Chaplin, 2000, 2010). In so far as bone is concerned, the mechanism is sound: with insufficient synthesis of vitamin D_3, poor skeletal health will result.

The amount of acceptable exposure to UV radiation varies across cultures and is often mediated by perceptions of beauty and, more recently, by concern over risk for skin cancer. Exposure to UV radiation may be limited by the type and timing of outdoor activities, the amount of concealing clothing worn, direct avoidance or through the use of sunscreens. Therefore, barriers to vitamin D synthesis are complex and may include aspects of culture and evolutionary history. Understanding how skin pigmentation, UVB, and vitamin D interact may provide insight into skeletal variation in contemporary populations, as well as in our evolutionary past.

In this article we examine the relationship between skin reflectance, as a proxy for melanin content of the skin, and BMC (the amount of mineral per square centimeter in a section of bone controlling for the width of the bone) and bone structural geometry, in a sample of Asian and white adolescent females living in Hawaii. We aim (1) to evaluate skin reflectance within and between ethnic groups and (2) to determine whether measures of skin reflectance predict, BMC at the lumbar spine, total hip, and total body, as well as cross sectional area of the proximal femur and section modulus at the proximal femur. We test the hypothesis that facultative (i.e., tanned) skin pigmentation is associated with greater BMC and structural geometry in these non-African populations, controlling for differences in body size, pubertal development, and lifestyle. Alternatively, darker non facultative skin pigmentation may be a barrier to greater BMC.

MATERIALS AND METHODS

Subjects were healthy females in the sixth grade, with age at measurement ranging from 10 to 13 years. Subjects identified as Asian or white, though inclusion criteria were that a subject be 75% of a given group based on her parents' reported ethnicity. Subjects were recruited from area middle schools within a 1 h driving distance from Manoa, HI (21°N). Letters requesting consent were sent to parents of the interested students and they were enrolled into the program subsequent to completion of required informed consent documentation. The project was approved by the Committee on Human Studies at the University of Hawaii and by the Institutional Review Board of Hawaii Pacific Health. Subjects took a urine pregnancy screening test prior to scanning and were excluded from measurement if the test was positive.

To quantify bone mass, the following outcome measures were evaluated: bone mineral content (BMC) at the lumbar spine, total hip, and total body; and cross sectional area and section modulus at the femoral neck. Dual energy X-ray absorptiometry (DXA; Lunar Prodigy, software version 6.5 and 6.7 GE Medical Instruments, Madison, WI) was used to derive BMC, while the Advanced Hip Analysis (AHA) program provided measures of structural geometry at the femoral neck.

The AHA software calculated several measures of structural geometry using data derived from the DXA X-ray absorption curves (Faulkner et al., 2006; Martin and Burr, 1984; Yoshikawa et al., 1994). From this information, the following measures of structural geometry were used in this analysis:

1. Cross sectional moment of inertia (CSMI, mm^4) was measured at the section of minimum CSMI within the re-

gion of interest (ROI) on the femoral neck. This measure reflects the distribution of material around the neck axis and is necessary in order to calculate a bone's resistance to bending stress:

$$\text{CSMI} = \frac{k(dx)}{\rho}\left[\sum \text{PBM}x^2 - \left(\frac{\sum \text{PBM}x}{\text{PBM}}\right)^2\right] \text{mm}^4$$

where PBM is a pseudo-bone mineral value based on the X-ray absorption data, k is a PBM to BMC conversion factor, ρ is the average physical density of bone (1.85 g cm^{-3}), and dx is the distance between scan lines.

2. Section modulus (Z, mm^4) was derived using CSMI. Section modulus is a ratio of CSMI to the distance of the outer most point on the periosteum. Section modulus evaluates the size and shape of the cross section in a given ROI while controlling for distance from the neutral axis (y) to the periosteal diameter:

$$Z = \frac{\text{CSMI}}{y}$$

where y is the distance from the neutral axis to the periosteal margin.

Cross sectional area (CSA, mm^2) was used to measure the amount of cortical bone at the section of minimum CSMI within the ROI on the femoral neck.

Weight was measured wearing minimal clothing and without shoes on an electronic scale and recorded to the nearest 0.1 kg. Measurements not within 0.2 kg were repeated until two measures were obtained within a 0.2 kg agreement and the median was used for analysis. Height was measured with the subject standing barefoot against a wall with her head approximating the Frankfurt horizontal plane. A stadiometer was used to measure height and measurements were recorded to the nearest 0.1 cm. Measurements not within 0.2 cm were repeated until two measures were obtained within a 0.2-cm agreement and the median was used for analysis. Protocols for measuring height and weight follow the instructions provided in the Anthropometric Standardization Reference Manual (Lohman et al., 1988).

Pubertal maturation was assessed via self reported Tanner stage (Tanner, 1961) for development of pubic hair (adapted from Grumbach and Styne, 1992). Scores were provided for both the breast and pubic region on a scale from 1 to 5 with 1 being pre-pubertal and 5 being fully mature. Subjects were asked about menarcheal status and were designated as premenarche (n = 74) or postmenarche (n = 19).

Dietary calcium intake from the previous month was estimated using an electronic semi-quantitative food frequency questionnaire (eFFQ), based on a FFQ developed for and evaluated with Asian, Hispanic, and non-Hispanic white youth (Wong et al., 2008). The eFFQ was administered through an interactive multi-media computer program that provided a picture of a food or food grouping with a text message of the serving size(s). Respondents were instructed to indicate how often a food was consumed (e.g., never or less than once per month, one to three servings per month, one serving per week, two to four servings per week, five or more servings per week). Approximately 81 food items containing calcium, as well as foods that may interfere with calcium consumption, such as soft drinks and non-dairy sources of calcium-rich foods that are consumed among Asians in Hawaii, were included in the eFFQ. In this analysis, total calcium from food estimated from the eFFQ was used as a predictor variable.

Physical activity was estimated for the previous year by a validated self-administered modifiable activity questionnaire for adolescents (Aaron et al., 1995, 1993). Energy expenditure was calculated from the metabolic equivalent (MET) values from the Compendium of Physical Activities (Ainsworth et al., 2000). The MET values are expressed as hours of energy expenditure/week for all activities. The sum of all MET values was used as a proxy for physical activity in the past year. Physical activity questionnaires were entered and checked for accuracy. Inconsistent or incomplete questionnaires were returned for further clarification from the subject. Physical activity data were log transformed to account for the skewness in this measure.

Skin pigmentation was quantified using a Chroma Meter CR-200b colorimeter. The skin reflectance outcome variables used as proxies for skin pigmentation were L^* and a^*. Subjects with less skin pigmentation will have higher L^* values while subjects with more red in their skin pigmentation will have greater a^* values. To assess tanning, facultative (tanned) pigmentation was measured at the forehead. To assess nonfacultative (non-tanned) pigmentation, the inner upper arm was measured. Measurement of skin pigmentation took place three times at each location and the results were averaged. To examine the magnitude of tanning we used the difference in reflectance between the upper inner arm and the forehead for both L^* and a^*. Subjects with greater difference between facultative and nonfacultative skin pigmentation would have negative values for the difference in L^* (an indicator of whiteness) and positive values for the difference in a^* (an indicator of redness), (Hall et al., 2010).

Baseline data were summarized using means, standard deviations and minimum and maximum values. Outliers ≥2.5 standard deviations from the mean were removed from the sample (n = 94). Simple between group differences were evaluated with independent sample t tests (α = 0.05). Linear regression was used to evaluate the effects of a group of predictor variables on different bone mass outcomes. Each regression model consisted of a group of predictor variables (age, physical activity, calcium intake, Tanner score, body weight) known to influence BMC. The effects of these predictor variables on outcome measures of skeletal integrity were evaluated prior to inclusion of skin reflectance variables. Variations to this model were subsequently evaluated upon including skin reflectance, a measure of skin pigmentation. Statistics were performed using SPSS Version 17 (SPSS, Chicago, Ill.).

RESULTS

Descriptive statistics are provided in Table 1 and Pearson's correlations are listed in Table 2. Asians and whites did not differ significantly from one another in any measure of skin reflectance, skeletal integrity, anthropometry, physical activity, or calcium intake.

In regression models predicting BMC by anatomical site,

Table 1. Characteristics among early adolescent girls residing in Hawaii

	Asian (n = 78)	White (n = 15)	Total (n = 94)
Age (years)	11.04 ± 0.5	10.87 ± 0.4	11.01 ± 0.5
Tanner stage	2.12 ± 0.8	2.27 ± 0.8	2.14 ± 0.8
Height (cm)	145.9 ± 7.0	149.3 ± 8.4	146.4 ± 7.3
Weight (kg)	40.7 ± 10.1	41.4 ± 8.4	40.8 ± 9.8
Total-body BMC (g)	1455 ± 305	1494.4 ± 296.7	1461.5 ± 302.2
L1-L4 BMC (g)	25.8 ± 7.0	25.4 ± 7.4	25.7 ± 7.0
Total Hip BMC	31.4 ± 6.3	33.4 ± 5.8	31.7 ± 6.2
CSA	100.0 ± 17.5	108.1 ± 17.5	101.3 ± 17.7
Z	338.4 ± 83.1	376.1 ± 83.7	344.5 ± 83.9
Calcium (mg)	937.4 ± 518.0	1031.2 ± 410.4	952.5 ± 501.4
Physical Activity (MET hrs/wk)	39.5 ± 32.4	47.9 ± 41.2	40.9 ± 33.9
Forehead *L**	56.4 ± 3.4	62.3 ± 2.2	57.4 ± 3.9
Forehead *a**	11.3 ± 1.4	11.5 ± 1.6	11.3 ± 1.4
Upperarm *L**	60.7 ± 4.3	65.7 ± 3.6	61.5 ± 4.5
Upperarm *a**	8.0 ± 1.4	6.9 ± 1.8	7.9 ± 1.6

Table 2. Pearson correlation coefficients between parameters among Asian and non-Hispanic white early adolescent girls residing in Hawaii (n = 94)

	Height	Weight	TBBMC	L2L4BMC	Hip BMC	CSA	*Z*	Calcium	Physical activity	Forehead *L*	Forehead *a*	Upperarm *L*	Upperarm *a*
Height	1												
Weight	0.66[a]	1											
TBBMC	0.77[a]	0.81[a]	1										
L2L4BMC	0.74[a]	0.66[a]	0.93[a]	1									
Total hip BMC	0.71[a]	0.72[a]	0.92[a]	0.88[a]	1								
CSA	0.72[a]	0.73[a]	0.90[a]	0.86[a]	0.97[a]	1							
Z	0.74[a]	0.75[a]	0.89[a]	0.84[a]	0.93[a]	0.96[a]	1						
Calcium	0.04	-0.07	0.03	0.07	0.14	0.14	0.1	1					
Physical activity	0.03	-0.11	-0.01	0.05	0.04	0.06	0.03	0.21	1				
Forehead *L*	0.09	0.03	0.11	0.12	0.14	0.20	0.15	0.11	-0.01	1			
Forehead *a*	0.08	0.24	0.29	0.25	0.21	0.19	0.17	-0.01	0.05	-0.27[a]	1		
Upperarm *L*	0.1	0.06	0.11	0.13	0.09	0.1	0.09	-0.08	-0.18	0.56[a]	0.03	1	
Upperarm *a*	-0.17	-0.03	-0.15	-0.16	-0.17	-0.19	-0.16	0.03	0.25	-0.45[a]	0.22[a]	-0.81[a]	1

[a]Significant at 0.01 level.

height and weight are correlated; weight was selected as the measure of body size, as it is important to mechanical loading of bone. Tanner stage and menarcheal status are also correlated; menarcheal status was selected as the indicator of pubertal maturation. Likewise, indicators of skin reflectance were correlated and thus were only included individually in regression models.

In base models evaluating BMC at the lumbar spine and total body (TBBMC), menarcheal status and weight were significant predictors (Table 3). For total hip BMC, weight, calcium intake, ethnicity, and menarcheal status were significant predictors in the baseline regression model. Comparisons of actual BMC versus predictive baseline models are provided in Figure 1A-C. Inclusion of *L** at the forehead to the model did not substantially change the predictive power or modify the relationship between predictors and outcome measures in any of the models, although it approaches significance in the model for lumbar BMC. With *a** at the forehead in regression models, the adjusted R^2 value was unaffected and the relationship between predictor variables and BMC did not change. The difference between *L** measured at the forehead and inner upper arm was not a significant predictor of any measure of BMC. However, the difference between *a** measured at the forehead and inner upper arm was a significant predictor of lumbar spine, total hip, and total body BMC. Given that the difference between facultative *a** and nonfacultative *a** were significant predictors, comparisons of actual BMC versus predictive BMC for models incorporating this difference are provided in Figure 1D-F.

Weight, ethnicity, calcium intake and menarcheal status were significant predictors of CSA and *Z* in their respective base models (Table 3). In terms of ethnicity, whites had, on average, larger CSA values than Asians in this sample. No indicator of skin reflectance was a significant predictor of CSA or *Z* and the predictive power of their models did not change appreciably upon the inclusion of *L**or *a**. Comparisons of actual CSA and *Z* with their predicted values are provided in Figure 2A, B.

DISCUSSION

In this sample, skin pigmentation was not significantly different between Asian and white adolescent girls. In observing the average values of *L** and *a** in these groups, the average value for *L** is slightly higher for white adolescent girls and the average value of *a** is nearly identical. The slightly higher average for *L** equates with lighter skin. These results have been observed elsewhere, with *L** in Asians and whites being similar and measures of *a** being similar across many ethnic groups (Alaluf et al., 2002). Further, Norton et al., (2007) found genetic evidence of convergent evolution for light skin pigmentation in Europeans and East Asians, further supporting a basis for similarity in skin pigmentation. While there is still some disagreement over what selective pressure(s) led to depigmentation, the vitamin D hypothesis appears to be the most tenable model (Chaplin and Jablonski, 2009; Jablonski and Chaplin, 2000, 2010). However, there is still some question as to what degree skin pigmentation and latitude have affected variation in bone mass and structure.

Non-facultative skin pigmentation, as measured by skin reflectance at the inner upper arm, approximates the amount of melanin present in skin that has received little to no exposure to UVB. Thus, one might anticipate that greater non-

Table 3. Results of multivariate linear regression among Asian and non-Hispanic white early adolescent girls residing in Hawaii (n = 94)

Model	Lumbar BMC		Hip BMC		Total body BMC		Cross sectional area		Section modulus	
	Significant predictors	Adjusted R^2	Significant predictors	Adjusted R^2	Significant predictors	Adjusted R^2	Significant predictors	Adjusted R^2	Significant predictors	Adjusted R^2
Baseline	Weight, menarche	0.56	Weight, calcium, menarche	0.61	Weight, menarche	0.71	Weight, ethnicity, calcium, menarche	0.63	Weight, ethnicity, calcium, menarche	0.65
Facultative L^*	Weight, menarche	0.57	Weight, calcium, menarche	0.61	Weight, menarche	0.72	Weight, calcium, menarche calcium,	0.63	Weight, ethnicity, menarche	0.64
Facultative a^*	Weight, menarche	0.55	Weight, calcium, menarche	0.61	Weight, menarche	0.72	Weight, ethnicity, calcium, menarche	0.63	Weight, ethnicity, calcium, menarche	0.65
Non-facultative L^*	Weight, menarche	0.57	Weight, calcium, menarche	0.61	Weight, menarche	0.72	Weight, ethnicity, calcium, menarche	0.63	Weight, ethnicity, calcium, menarche	0.65
Non-facultative a^*	Weight, menarche	0.57	Weight, calcium, menarche	0.61	Weight, menarche	0.72	Weight, ethnicity, calcium, menarche	0.64	Weight, ethnicity, calcium, menarche	0.65
L^* forehead − inner upper arm	Weight, menarche	0.55	Weight, calcium, menarche	0.60	Weight, menarche	0.71	Weight, ethnicity, calcium, menarche	0.63	Weight, ethnicity, calcium, menarche	0.64
a^* forehead − inner upper arm	Weight, menarche	0.58	Weight, calcium, menarche, Diff. a^*	0.63	Weight, calcium, menarche, Diff. a^*	0.74	Weight, ethnicity, calcium, menarche	0.63	Weight, ethnicity, calcium, menarche	0.65

facultative skin pigmentation would be a barrier to vitamin D synthesis. If this is the case, it does not appear to have a significant effect on bone mass or structure in this sample, though it is possible that there was not sufficient variability to show this in the sample. Thus, non-facultative skin pigmentation may not be an indicator of vitamin D production from sun exposure.

Facultative skin pigmentation is a measure of vitamin D synthesis that has occurred or is occurring (Rockell et al., 2008); however, it does not necessarily reflect long term vitamin D synthesis and does not provide a direct measure of serum 25-hydroxyvitamin D (25OHD). If facultative skin pigmentation is an indicator of long term exposure to UVB, and thus of vitamin D synthesis, then it would be reasonable to expect a positive relationship with bone mass, where darker facultative skin pigmentation being associated with greater measures of BMC, controlling for covariates. Although facultative skin pigmentation shares a positive relationship (25OHD) (Rockell et al., 2008), vitamin D levels were shown to be low in active young adults living in Hawaii who received abundant sun exposure, suggesting that even with regular UV exposure it is still possible to be vitamin D deficient (Binkley et al., 2007). With these barriers in mind, facultative skin pigmentation measured at the forehead, a proxy for melanin present in skin exposed to UVB, was not a significant predictor of any measure of bone mass or structure in this analysis. L^* approaches significance in the lumbar BMC regression model (P = 0.052). As a measure of darkness, L^* likely reflects melanin content in the skin while a^*, a measure of redness, reflects pheomelanin and hemoglobin (Jablonski and Chaplin, 2000, 2010). The combined use of these indicators may provide a better overall measure of skin pigmentation, but because they are correlated, their use in a single regression model would be difficult to interpret. Valuable further work might involve development of a scale that combines the two indicators. The difference between L^* measured at the forehead and inner upper arm was not a significant predictor of any measure of BMC. However, the difference between a^* at the forehead and upper inner arm was a significant predictor of all measures of BMC. This difference reflects the amount of tanning by evaluating facultative skin pigmentation relative to non-facultative skin pigmentation. If greater tanning corresponds with increased vitamin D synthesis then it would seem that this variable might be useful in illuminating the relationship between skin pigmentation, vitamin D and bone. However, because we do not have a measure of serum 25(OH)D this point cannot be definitively established and thus remains hypothetical, although worthy of further investigation.

As with BMC, neither facultative nor non-facultative skin pigmentation predicted structural geometry at the femoral neck in this study. The difference between L^* and a^* at the forehead and inner upper arm did not significantly predict structural geometry at the femoral neck. Bending rigidity, a measure of strength, is largely determined by the distribution of bone around a cross section. Some of the variation in bone structural geometry can be explained by body size alone, but activity, genes and diet can also affect where bone is deposited (Ruff, 2003).

Although skin pigmentation was not a significant predictor of bone mass or structure, weight significantly predicted

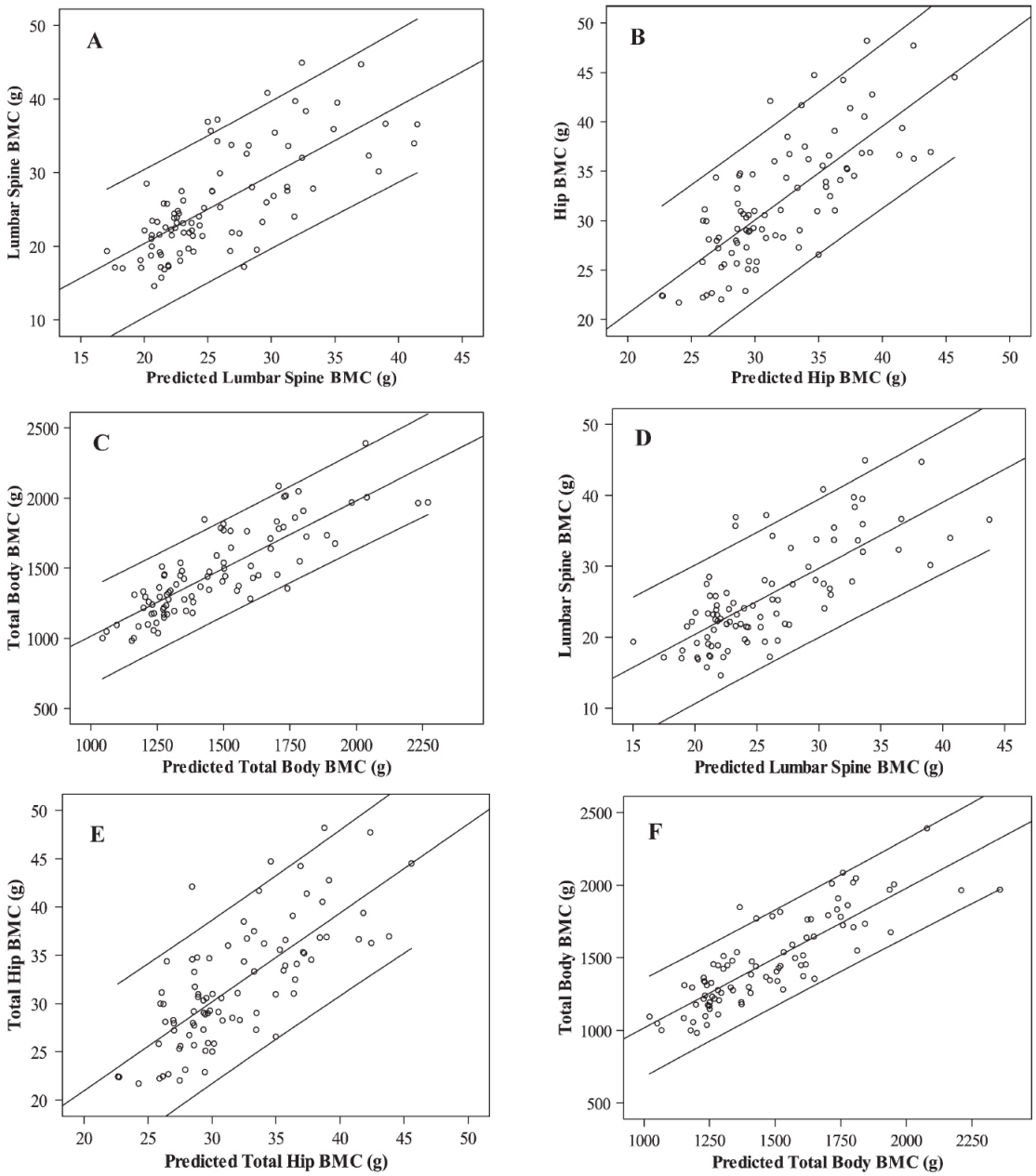

Figure 1. Comparison of actual versus predicted BMC for multiple regression models at baseline for (A) lumbar spine; (B) Total Hip; and (C) total body; and for multiple regression models including the difference between facultative and nonfacultative *a** for (D) lumbar spine; (E) total hip; and (F) total body.

BMC at all sites, CSA, and Z, supporting previous research indicative of a body size effect on measures of skeletal integrity (Ruff, 2003; Weaver et al., 2007). Given that body size is a proxy for bone outcome variables used here, lifelong variation in nutrition and physical activity, including intrauterine exposure, likely play a role in this variation as well (Vidulich et al., 2007). Further, body size can increase on a generational basis without significant gene flow from previously isolated populations (e.g., Bogin et al., 2002), so some of this variation likely reflects environmental and dietary change across generations, thus making bone mass and structure a developmentally plastic trait.

Ethnicity was a significant predictor of total hip BMC, CSA, and Z at the proximal femur, but not BMC at any site. The relationship between ethnicity and CSA is similar to what has been found in Chinese and white adult women (Yan et al., 2004). Further, Finkelstein et al. (2002) also found significantly smaller bone areas in Chinese and Japanese women compared to whites. The results produced here suggest that this difference has arisen by adolescence. However, the sample in this study uses the category "Asian" and is almost certainly more diverse than the samples used by Finkelstein et al., (2002) and Yan et al., (2004). Finkelstein et al. (2002) found significant differences between Chinese and Japanese women in some measures of bone mass at the lumbar spine and femur, suggesting that the pooling of these individuals into

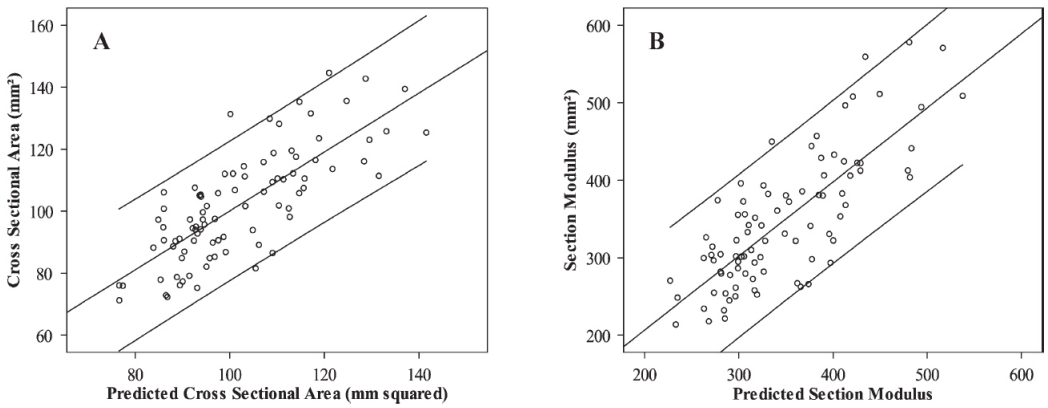

Figure. 2. Comparison of actual versus predicted (A) cross sectional area and (B) section modulus at the femoral neck for multiple regression models at baseline.

a single group may miss important variation. Furthermore, Wang et al., (2009) recently discovered, on average, thicker trabecular bone in Asians than in whites. In their analysis, Wang et al. (2009) quantified bone tissue using high resolution peripheral quantitative computed tomography (HR-pQCT); it is possible that this technology is better equipped to examine trabecular bone than DXA. Results derived using DXA are at odds with previously published findings on the effects of ethnicity on BMC in these anatomical regions, perhaps due to insufficient power (Weaver et al., 2007; Yan et al., 2004). Genes might account for some of the difference between these groups. However, lifelong and intergenerational nutrition and physical activity also play a role in these differences.

Interestingly, physical activity was not a significant predictor of any measure of bone mass or structure. Although results have varied, physical activity generally has a positive effect on BMC and structural geometry (Faulkner et al., 2006; MacDonald et al., 2008; MacKelvie et al., 2001; Matkovic et al., 2004). The proximal femur is loaded during many bouts of activity, making it a prime candidate for activity related effects. The manner in which physical activity is quantified here may partly explain this, as activity is quantified as a metabolic score and is not differentiated by activity type, duration, or intensity. The effects of physical activity on bone mass, structure and maintenance are dependent on activity type, duration, directionality, and the intensity of exertion (Robling et al., 2002; Shaw and Stock, 2009).

The effects of calcium on bone mass and structure are also peculiar in this study. Calcium normally has a positive effect on BMC (Weaver et al., 2007), but was only observed for total hip BMC, not lumbar spine BMC or TBBMC, likely due to insufficient statistical power. Although the effect of calcium intake on structural geometry can vary (Kardinaal et al., 2000), these variables often share a positive relationship (Zhu et al., 2005). While it makes intuitive sense to assume that calcium is directly affecting bone mass and structure, it is possible that other factors, such as protein content associated with dairy foods, may also play a role in shaping this variation.

The results could be interpreted as support the vitamin D hypothesis as it pertains to skeletal growth, assuming a relationship between skin pigmentation, vitamin D and bone. But given the limitations of this study (e.g., no control for long term UVB exposure, no control for UVA exposure, no measure of serum 25(OH)D status), further research must take place to evaluate this hypothesis. The sample used herein should not be considered representative of a larger population, but presents an intriguing set of variables, with ethnic diversity and substantial relatively constant UV exposure compared to most published populations, which is worthy of further evaluation. Future research should increase sample size and ethnic diversity. Because our subjects lived in the sub-tropical environment of Hawaii, these results may not applicable to populations living at high latitudes, particularly in large cities where lifestyle and environment may limit exposure to UVB. Likewise, subjects used in this analysis are localized US citizens and thus may not reflect cultural variation of these ethnic populations living in their ancestral homes.

Our study suggests several directions for future research on skin pigmentation, vitamin D, and bone. Given the culture of sun avoidance, particularly in high UV environments, future study of the relationship between facultative skin pigmentation and bone would benefit by considering sun exposure history and practices. This is particularly true for Hawaii where awareness of the damaging effects of high UV exposure lead some to guard against exposure to the extent of low vitamin D status. When combined with skin reflectance, history and practice of UVB avoidance could be used to estimate the amount of UVB exposure and perhaps estimate vitamin D production potential. In addition, methodological development for quantifying skin pigmentation may yield different results.

Control for the ratio of UVA/UVB would help to evaluate vitamin D synthesis and destruction potentials. This could be achieved with UV dosimeters that quantify the amount of UVA and UVB a subject is exposed to throughout the course of the day. Measures of serum 25(OH)D status permit definitive statements to be made regarding vitamin D status and improve our understanding of the relationship between skin pigmentation, UVR, and bone.

In addition to examining the amount of vitamin D available to the subject, consideration of vitamin D receptor polymorphisms (VDRp) would be necessary to truly understand the influence vitamin D has on bone. There is evidence that VDRp are associated with fracture rates both in association

with and independently of bone mass, suggesting that VDRp play an alternate role in determining bone strength (Bezerra et al., 2008; Garnero et al., 2005; Yu et al., 2011). In a multiethnic sample Esterle et al., (2009) found an interaction between calcium intake and the VDRp-1012. Interestingly, the authors report a higher frequency (70%) of the VDRp genotype associated with greater calcium requirements in white females than in Asians or Africans (Esterle et al., 2009). These results may explain why Asian and African adolescents can consume less calcium than whites without an associated decrease in osteogenesis. Methylation in VDRp have been documented in placental tissue but not offspring, suggesting the potential disruption of vitamin D homeostasis *in utero* (Novakovic et al., 2009). These added controls would improve our ability to make definitive statements regarding skin pigmentation, vitamin D synthesis, and bone.

CONCLUSION

Facultative and non-facultative skin pigmentation were not significant predictors of bone mass or structure in Asian and white early adolescent girls in Hawaii. The difference between *a** measured at the forehead and inner upper arm was a significant predictor of all measures of BMC, but not structural geometry. Our sample was limited in geographic scope and ethnic diversity. Further research is needed on diverse populations, particularly at high latitude, to better understand the relationship between skin pigmentation, bone mass and structure. This research is important given the large scale culture changes in UVB avoidance and increased population size in urban environments that are limited in UVB exposure.

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