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13. SUPPLEMENTARY NOTES

14. ABSTRACT: Past studies suggest that early menarche, growth velocity, and specific hormonal patterns during breast development may be critical in determining risk of breast cancer later in life. Nutritional factors during childhood and puberty, and inherited genetic factors are suspected to interact in modulating these early-life exposures. However, the biological processes involved remain poorly understood. We propose to test the relationships between nutrition, genetic factors, hormonal levels and early life events contributing to breast cancer risk in a unique cohort of 323 adolescent girls of Caucasian or Asian ancestry originally recruited for the Female Adolescent Maturation (FAM) Study. These girls were studied twice, 2 years apart, for dietary intake, body size and composition, sexual maturation, growth and bone density. Data collection will be extended by conducting a third examination and obtaining blood samples for DNA genotyping and hormone analysis. A cross-sectional sample of 140 additional girls will also be recruited. Recruitment has been ongoing, as well as refining of recruitment methods. To date, 123 girls have come in for study visits and, as of May 30, twenty additional girls have been scheduled to come in at Kapiolani Clinical Research Center. Of the 123 girls, 57 are from the FAM cohort and 66 are new recruits from Kaiser. Portfolios with health education material have been sent out to those who have completed the study, and a database is being built for data entry. Questionnaires are being coded in preparation for data entry.

15. SUBJECT TERMS
Molecular Epidemiology, Nutrition, Genetics, Hormones

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INTRODUCTION

Past studies suggest that early menarche, growth velocity, and specific hormonal patterns during breast development may be critical in determining risk of breast cancer later in life (1-5). Nutritional factors (i.e., adiposity, physical activity and diet) during childhood and puberty, and inherited genetic factors are suspected to interact in modulating these early-life exposures (2, 6-15). However, the biological processes involved remain poorly understood. These gene-environment interactions may explain the remarkable increase in breast cancer incidence observed among US-born Asian women. We propose to test the relationships between nutrition, genetic factors, hormonal levels and early life events contributing to breast cancer risk in a unique cohort of 323 adolescent girls of Caucasian or Asian ancestry originally recruited for the Female Adolescent Maturation (FAM) Study (USDA NRI grant 99-00700). These girls were first studied in 1999-2000 at age 9-14 years and, again two years later in 2002-2003, for dietary intake, body size and composition, sexual maturation (Tanner staging), growth and bone density. Data collection will be extended by conducting a third examination and obtaining blood samples for DNA genotyping and hormone analysis at a time when most girls will have attained menarche. A cross-sectional sample of 100 additional girls will also be recruited.

BODY

Below are relevant updates on the research accomplishments as outlined in the approved Statement of Work through May 14, 2006.

Task 1. Plan Study and Procedures
   a. Purchase supplies and equipment
      a. Supplies for recruitment and visits, including gift certificates, copying of questionnaires, consent and assent forms, pregnancy test kits and mailing supplies have been ongoing.
      b. A modification in our recruitment procedure required the purchase of cell phone trinkets and reminder bands in an attempt to increase participation rates.

   b. Create tracking database
      a. Maintaining of central database for recruitment and study visits has been ongoing and maintained by the Lead Study Coordinator at the University of Hawaii. Study staff at Kapiolani Clinical Research Center and Cancer Research Center Hawaii tracks the portion of their study procedures, and continues to send them to the Lead Study Coordinator monthly.

   c. Wrote procedure manuals and finalize instruments
      a. Some minor changes have been made to the study protocol and questionnaires. All changes have been submitted an approved by all 3 IRBs.

   d. Maintain IRB Approvals for the Study: IRB renewals were obtained from the two local IRBs.

   e. Identify potential subjects for the new recruitment
      a. Kaiser is continuing to identify potential subjects from their database for potential recruitment. Members were identified based on ethnicity, age, gender, and living in the Honolulu area.

Task 2. Subject Recruitment and Data Collection
   a. Enroll FAM participants for the follow-up examination, 4 years after the baseline examination.
      a. Recruitment letters are being sent out monthly to FAM participants for follow-up visits. Letters were first sent to the oldest girls, before they potentially leave Hawaii for college, and recruitment letters are now sent in the chronological order of their FAM 1 visit. To date, 271 letters have been sent out.

   b. Enroll new participants

        a.   Kaiser is continuing to send recruitment letters to potential new recruits, and the Kaiser Study Coordinator has been calling the girls who indicated interest in order to screen them for eligibility.

  c. Schedule clinical exam and perform data collection
      a. A total of 123 girls have come in for the two study visits and, as of May 30, 2006, 20 more girls are scheduled to come in at Kapiolani Clinical Research Center.
      b. Of the 123 girls, 57 are from the original FAM cohort and 66 are new recruits.
      c. As of May 30, 14 more FAM cohort and 6 new recruits are scheduled to come in.
      d. Portfolios with health education material were sent out to those who have completed the study

  d. Collect blood and urine samples
      a. 60 girls have completed a blood draw. Of the 60, 48 have completed all 4 urine collections, 10 completed 3 urine collections and 2 completed 2 urine collections. 22 other girls who have completed the clinical study visits at Kapiolani have been scheduled for a blood and urine collection.

  e. Process biospecimen for storage
      a. All specimens have been processed for storage. Samples are stored at -80ºC.

  f. Enter data and maintain databases
      a. Questionnaires are being coded in preparation for data entry.
      b. A database is being built for data entry.
      c. We expect to be caught up with data entry by the end of the summer.

Task 3: Perform Genotyping assays:
      a. Extraction of DNA will start in June 2006.
      b. Plates will be prepared this summer.
      c. TaqMan assays will be ordered and run during the Fall.

Task 4: Perform Hormone Analyses: will be done in Spring 2007

Task 5. Interim Analyses
      a. Interim analyses will start this Fall, after the data entry is completed.
      b. Write annual reports: An annual report was completed May 14, 2005. This is the second annual report.
Task 6: Final Analyses and Report Writing: Will be done in ly summer 2007.

KEY RESEARCH ACCOMPLISHMENTS

1. Preliminary results from a feasibility study on 158 of the girls examined in 2002-2003 who donated a mouthwash sample show a direct association between the *CYP3A4 *1B* polymorphism with pubic hair Tanner stage ($p=0.01$), but no association of this variant with breast Tanner stage or age at menarche. This association is consistent with the role of CYP3A4 in the catabolism of testosterone and the involvement of this hormone in the early stages of puberty.

2. We analyze previously collected data on the FAM cohort and showed that Asian ethnicity and birth weight are both independently and positively associated with trunk fat measured by DEXA.

3. In the same data, protein intake was found to be positively associated with increased waist circumference.

REPORTABLE OUTCOMES

<u>Publication</u>
1. R Novotny, Y Daida, J Grove, L LeMarchand and V Vijayadeva. Asian Adolescents have a higher trunk:peripheral fat ratio than Whites. *J Nutr*136:642-647

<u>Abstracts</u>
1. R Novotny, Y Daida, J Grove, L LeMarchand and V Vijayadeva. Asian Ethnicity Is Positively Associated With Bone Mineral Content Of Female Adolescents**.** IUNS, South Africa, 2005.
2. Daida Y, Novotny R, Grove J, Le Marchand L, Vijayadeva V Birth weight is negatively associated with DXA trunk to periphery fat ratio of multiethnic adolescent girls. Experimental Biology, San Francisco, 2006.
3. Caryn Oshiro, Vinutha Vijayadeva, Yihe Daida, Rachel Novotny, John Grove, Loic LeMarchand. Protein intake is positively associated with waist circumference in female adolescents. Experimental Biology, San Francisco, 2006.

CONCLUSIONS

Further analyses should confirm ethnic differences in early risk factors for chronic diseases, including breast cancer, and provide support for the causal involvement of genetic variants and nutrition in early maturation, obesity patterns and bone density.

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APPENDICES

1. R Novotny, Y Daida, J Grove, L LeMarchand and V Vijayadeva. Asian Adolescents have a higher trunk:peripheral fat ratio than Whites. *J Nutr*136:642-647.

2. R Novotny, Y Daida, J Grove, L LeMarchand and V Vijayadeva. Asian Ethnicity Is Positively Associated With Bone Mineral Content Of Female Adolescents**. IUNS, South Africa, 2005.

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Asian Adolescents Have a Higher Trunk:Peripheral Fat Ratio than Whites[1]

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ABSTRACT   Body fat, especially in the upper body, has been associated with increased risk of chronic disease among adults. Factors associated with these traits among ethnically diverse populations are not well studied. We examined factors influencing body fat and weight among Asian and White adolescent girls from the female adolescent maturation longitudinal study (initial exam plus 2-y follow-up examination) in Hawaii. The objective of this study was to identify and compare influences on and differences in body size and fat distribution among Asian and White adolescent girls. Subjects were identified among age-eligible members of a large HMO. Of the 214 girls recruited for Exam 1, 107 girls returned for Exam 2. The girls' age, ethnicity, a 3-d diet record, and physical activity recall were obtained by questionnaire at both times, and Tanner pubic hair stage and anthropometry were clinically measured by trained staff at both exams. The ethnic proportion of the study sample was 57% Asian and 43% White. Each girl's ethnicity was based on the ethnic proportion of each biologic parent. The percentage of body fat was measured by Lunar Prodigy dual energy X-ray absorptiometry (DXA) at the follow-up exam only. Among various measures of skeletal size and adiposity, only leg length (inversely) and DXA trunk:peripheral fat ratio (directly) were associated with proportion of Asian ethnicity, after adjusting for age, Tanner pubic hair stage, physical activity, and energy intake. In a multivariate analysis focusing on the trunk:peripheral fat ratio, this measure of central obesity was positively associated with proportion of Asian ethnicity ($P = 0.001$) and bi-iliac breadth ($P = 0.002$), and negatively associated with birth weight ($P = 0.021$), after adjustment for Tanner pubic hair stage, physical activity, energy intake, biacromial breadth, and height. In conclusion, Asian adolescents have a higher trunk:peripheral fat ratio than Whites. Adolescent bi-iliac breadth (positively) and birthweight (negatively) are associated with more body fat on the trunk vs. periphery during adolescence.   J. Nutr. 136: 642–647, 2006.

KEY WORDS: • adolescent • ethnicity • DXA • Asian • trunk • fat

National data from the National Health and Nutrition Examination Survey (1999–2000) indicated a prevalence of overweight of 15.5% among children 12–19 y old, 15.3% among children 6–11 y old, and 10.4% among children 2–5 y old, compared with 10.5, 11.3, and 7.2%, respectively, in 1988–1994 (1). The prevalence of overweight among children in the United States has been increasing since the 1960s. It is of considerable importance to identify dietary and other factors that may influence body fat and weight to stop or reverse this trend and to identify subgroups of the population that should be targeted for prevention efforts.

There is increasing evidence for an emerging high prevalence of type 2 diabetes and increased cardiovascular risk factors in parts of Asia in which the mean BMI is lower than in the United States. For a given BMI, Asian adults were found to have a higher body fat percentage and more upper-body subcutaneous fat, than Whites (2). On the other hand, He et al. (3) reported that Asian girls had less skinfold-derived extremity and gynoid fat than Whites.

The purpose of this study was to examine influences on and differences in body size and fat distribution among Asian and White adolescent girls.

SUBJECTS AND MATERIALS

This study, the Female Adolescent Maturation study, was approved by the Institutional Review Board of Kaiser Permanente Hawaii [where the baseline examination (Exam 1) was conducted in 2000–2001], the University of Hawaii and Kapiolani Clinical Research Center [where the 2-y follow-up examination (Exam 2) was conducted]. Informed assent and consent was obtained from the child and both parents, respectively.

Only subjects who participated in Exam 1 were eligible for Exam 2. Adolescent girls 9–14 y old were selected from the Kaiser Permanente Oahu membership database for Exam 1. Subjects with any chronic diseases or use of steroids, asthma and antiepileptic medications were excluded. From an initial sample

of 1106 age- and gender-eligible Kaiser Permanente patients, 349 met the inclusion criteria and agreed to participate. Of the original cohort, 160 girls participated in Exam 2 (46%). The 2 examinations were timed 2 y (± 2 mo) apart. For the purpose of this analysis, we included girls who were 100% Asian, 100% White, or a mixture of Asian and White only (i.e., no other ethnic admixtures); there were 214 girls admitted on the basis of those criteria for Exam 1 and 107 of these returned for Exam 2.

Information on the girls' ethnicity, age, and menstrual status was obtained from their parents. Parents/guardians were asked to provide every ethnicity of the biologic parents of the subject, as a percentage. These proportions were classified as Asian, White, or a mixture of Asian and White and were derived from the summed combination of both mother's and father's ethnicity (4). For example, if a girl's father was 50% Asian and 50% White, and her mother was 25% Asian, 75% White, the girls' ethnicity was defined as 37.5% Asian and 62.5% White. Asian ethnicities included girls of Japanese, Korean, Chinese, Filipino, Indian, Thai, and Vietnamese origin. In multiple regression analysis, ethnicity was described as the proportion of Asian ethnicity.

A 3-d diet record at each exam (Exam 1 and 2) included 2 weekdays (Thursday and Friday) and 1 weekend day (Saturday), and was completed by the girls with their parents' assistance. A measuring cup, spoon, and ruled paper were provided to help the girls estimate the quantities of food items eaten. The nutrient analysis was done at the Cancer Research Center of Hawaii using the Nutrition Support Shared Resource's food composition database (5). Nutrient means of the 6 d of diet record were used in the analysis to estimate dietary energy intake over the 2-y interval.

Girls also completed a physical activity questionnaire at each examination (6). They were asked to fill in activities that they engaged in >10 times in the past year. For each activity they took part in, they were asked how many months a year, how many days a week, and how many minutes each day they spent doing that particular activity. The average number of hours per week doing a particular activity, during the past year, was calculated using the formula: mo × (4.3 wk/mo) × (d/wk) × (min/d)/(60 min/h)/(52 wk/y). The metabolic equivalent (MET) values for all activities were calculated for the specified duration (MET of each activity × duration of each activity). The sum of all MET values was used as a proxy for physical activity in the past year.

Anthropometric measures were taken during the visit to the Kapiolani Clinical Research Center. Weight was measured with a digital scale (Seca) in kilograms. Height and sitting height were measured using a digital stadiometer (Measurement Concepts). Biacromial and bi-iliac breadths were measured using a Lafayette Caliper. Each measurement was taken at least twice; a third measurement was taken if the 2 measures differed by ≥0.2 units, with the mean of the 2 closest values used in analysis.

The percentage of body fat of the total body, trunk, arms, and legs was measured by the Lunar Prodigy dual-energy X-ray absorptiometer (DXA) at Exam 2 only. The ratio of trunk:peripheral fat was calculated using DXA body fat output. Total body fat was calculated as the sum of trunk, left and right arms, plus left and right legs from the DXA output. Fat from arms and legs was first summed to estimate peripheral fat, and a trunk:peripheral fat ratio variable was created by dividing trunk regional fat (g) by peripheral regional fat (g).

Clinical breast and pubic hair staging were done as described by Tanner (7). The Kaiser Permanente nurse practitioner (Exam 1) and the Kapiolani study coordinator (Exam 2) performed the staging using standardized methods.

Data were double entered using a database program in Microsoft Access 97. Statistical analysis was performed using the SAS statistical package, version 8.2 (SAS Institute). The data are summarized as means ± SD unless stated otherwise. Paired sample t tests were performed to identify significant changes in means from Exam 1 to Exam 2. In some analyses, data from both exams were used (2 records per girls, i.e., one from Exam 1, the other from exam 2); sample size varies because of the missing values for the variables used in the analysis. To account for the observations being correlated between exams, the standard error of regression coefficients was estimated using the method of generalized estimating equations (GEE) (8). Multiple regression analysis was used to identify factors influencing trunk:peripheral fat ratio, body shape, size, maturation, and energy intake and expenditure. Our analysis centers around the question of how fat deposition varies with ethnicity and is primarily a cross-sectional analysis, with body fat variables adjusted for skeletal variables and Tanner pubic hair stage measured at the same time (the exception to this was that we used mean energy intake and physical activity from Exams 1 and 2 in the multivariate analysis, thus increasing reliability). Differences were regarded as significant and marginally significant if the corresponding P-value was ≤ 0.05 or ≤ 0.10, respectively.

We examined the residuals from the regression model for skewness and kurtosis and compared the results using a log (and sometimes square root) transformation of all of the dependent variables. Transformation did not change any conclusions. Results are presented in the tables in the original scales except for subscapular skinfold, for which we used the log scale.

RESULTS

Using multiple logistic regression, a variable that reflected nonattendance at Exam 2 was regressed on the girl's age at Exam 1, her proportion of Asian ethnicity, and her BMI at Exam 1 to test for potential bias caused by differential attrition. The model was not significant ($\chi^2 = 0.34$ with df = 3, $P > 0.90$); thus, age, ethnicity, and BMI were not related to attrition rate.

Characteristics of the study population are presented in **Table 1** by ethnic group and Exam. All measures increased between the 2 Exams, except triceps skinfold thickness and waist:hip ratio, which decreased. At Exam 2, 8% of girls were in Tanner pubic hair stage 1, 14% were in Stage 2, 37% of girls were in each of Tanner pubic hair stage 3 and 4, and 2% were in stage 5.

DXA body fat and lean mass characteristics of the study population at Exam 2 are presented in **Table 2**. Only the trunk:peripheral fat ratio and arm lean mass varied significantly among the 3 ethnic groups ($P = 0.0002$ and 0.026, respectively). Asians had higher trunk:peripheral values than Whites and lower arm lean mass values, whereas girls of Mixed Asian and White ethnicity had intermediate values for both measures.

After adjusting for Tanner pubic hair stage, energy intake, physical activity, and age, the proportion of Asian ethnicity remained associated with the DXA trunk:peripheral fat ratio **(Table 3)**. There were no differences in waist, hip circumference, waist:hip ratio, or subcutaneous fat distribution. The proportion of Asian ethnicity was inversely associated with height ($P = 0.01$). This shorter stature in Asian ethnicity compared with White ethnicity was due to shorter legs ($P < 0.001$) since trunk length was not associated with Asian ethnicity ($P = 0.77$). After adjusting for Tanner pubic hair stage and age, the proportion of

TABLE 1

Characteristics of the study population[1]

	Asian (*n* = 40)			White (*n* = 26)			Mixed (*n* = 41)		
	Exam 1	Exam 2	Difference	Exam 1	Exam 2	Difference	Exam 1	Exam 2	Difference
Age, *y*	11.84 ± 1.47	13.88 ± 1.47	2.04 ± 0.07	11.57 ± 1.41	13.63 ± 1.39	2.06 ± 0.07	11.041 ± 1.23	13.12 ± 1.23	2.08 ± 0.13
Tanner pubic hair stage, 1–5	2.38 ± 1.19	2.85 ± 0.89	0.48 ± 0.85	2.46 ± 1.39	3.38 ± 0.90	0.92 ± 1.13	2.10 ± 1.30	3.12 ± 1.08	1.02 ± 0.99
Physical activity, *(MET·h)/wk*	38.55 ± 36.78	39.46 ± 47.65	0.91 ± 43.56	53.07 ± 42.46	58.40 ± 66.06	5.33 ± 71.53	32.42 ± 26.06	38.39 ± 31.52[†]	6.71 ± 28.66
Energy intake, *kJ/d*	7826 ± 1673*	8011 ± 1974	217 ± 1929	7628 ± 1817	7434 ± 1783	−193 ± 1901	7160 ± 2023*	7403 ± 1943	243 ± 2527
Birth weight, *kg*	3.08 ± 0.38[‡]	—	—	3.12[Ω] ± 0.61	—	—	3.48 ± 0.56[†]	—	—
Weight, *kg*	40.45 ± 9.22	47.04 ± 7.90	6.59 ± 4.46	42.11 ± 9.00	50.01 ± 7.33	7.90 ± 4.97	43.83 ± 11.96	51.32 ± 12.68	8.48 ± 5.16
Height, *m*	1.49 ± 0.10	1.56 ± 0.07	0.07 ± 0.05	1.49 ± 0.10	1.59 ± 0.08	0.10 ± 0.04	1.45 ± 0.13	1.56 ± 0.10	0.12 ± 0.11
Sitting height, *cm*	77.57 ± 5.88	82.98 ± 4.53	5.41 ± 2.56	76.46 ± 5.07	81.98 ± 6.89	5.53 ± 6.21	75.30 ± 9.26	82.89 ± 5.66	7.63 ± 8.19
Leg length, *cm*	71.39 ± 4.76	73.22 ± 3.27	1.83 ± 3.27	72.91 ± 5.24	77.36 ± 7.19	4.45 ± 5.99	70.12 ± 7.44	74.02 ± 4.88	3.90 ± 5.75
BMI, *kg/m^2*	18.05 ± 2.52	19.19 ± 2.43	1.15 ± 1.43	18.77 ± 2.88	19.63 ± 2.03	0.86 ± 1.99	20.49 ± 7.07	20.66 ± 4.00	0.16 ± 5.60
Biacromial breadth, *cm*	34.11 ± 2.61	34.97 ± 1.92	0.87 ± 1.43	33.96 ± 2.52	34.92 ± 2.07	0.96 ± 1.43	33.90 ± 2.88	34.86 ± 2.65	0.96 ± 1.77
Bi-iliac breadth, *cm*	25.28 ± 2.58	26.69 ± 1.73	1.40 ± 1.79	25.35 ± 2.34	27.36 ± 2.00	2.01 ± 1.59	26.05 ± 2.95	27.77 ± 2.59	1.72 ± 1.70
Waist circumference, *cm*	61.07 ± 5.89	64.28 ± 5.10	3.21 ± 4.05	62.25 ± 6.79	65.9 ± 4.54	3.65 ± 4.35	63.88 ± 9.17	67.29 ± 9.21	3.41 ± 4.42
Hip circumference, *cm*	79.32 ± 8.33	87.10 ± 6.88	7.78 ± 4.17	80.66 ± 8.37	89.89 ± 6.43	9.24 ± 4.51	80.44 ± 13.72	90.08 ± 9.53	9.64 ± 9.62
Waist:hip ratio	0.77 ± 0.06	0.74 ± 0.04	−0.03 ± 0.04	0.77 ± 0.06	0.73 ± 0.03	−0.04 ± 0.04	0.82 ± 0.29	0.75 ± 0.05	−0.08 ± 0.30
Subscapular skinfold, *mm*	9.44 ± 3.47	13.9 ± 3.92	4.46 ± 3.03	9.23 ± 4.81	13.08 ± 5.07[Ω]	3.84 ± 4.23	11.83 ± 7.39	15.67 ± 8.11	3.84 ± 6.02
Triceps skinfold, *mm*	13.65 ± 5.11	10.33 ± 4.49	−3.33 ± 4.93	15.52 ± 5.61	10.87 ± 3.94	−4.65 ± 6.49	16.12 ± 7.05	11.50 ± 4.90	−4.61 ± 5.231
Bicep skinfold, *mm*	6.79 ± 3.30	14.50 ± 6.28	7.71 ± 5.50	7.41 ± 3.16	16.00 ± 5.58	8.59 ± 3.78	8.59 ± 4.90	16.40 ± 8.20	7.81 ± 5.97
Iliac skinfold, *mm*	11.25 ± 5.00	16.6 ± 6.10	5.35 ± 5.30	10.62 ± 4.97	16.23 ± 6.67	5.61 ± 4.74	13.03 ± 6.99	19.61 ± 9.72	6.58 ± 7.04
Calf skinfold, *mm*	13.10 ± 4.17	15.46 ± 5.59	2.36 ± 4.65	15.42 ± 4.25	17.60 ± 3.56	2.17 ± 3.81	15.17 ± 6.93	16.43 ± 7.52	1.26 ± 4.61

[1] Values are means ± SD: *n = 39; [‡]n = 38; [Ω]n = 25; [†]n = 40.

TABLE 2

DXA body fat and lean mass characteristics of the study population at Exam 2[1]

	Asian (n = 40)	White (n = 26)	Mixed (n = 41)	ANOVA P-value
Arm fat mass, kg	0.98 ± 0.49	1.17 ± 0.48	1.27 ± 0.89	0.16
Arm lean mass, kg	2.98 ± 0.47	3.36 ± 0.57	3.24 ± 0.75	0.04
Leg fat mass, kg	4.95 ± 1.82	6.15 ± 1.70	6.06 ± 3.21	0.07
Leg lean mass, kg	10.94 ± 1.57	11.52 ± 1.57	11.52 ± 2.12	0.27
Trunk fat mass, kg	6.03 ± 2.69	6.21 ± 2.10	7.32 ± 4.34	0.18
Trunk lean mass, kg	15.02 ± 2.01	15.51 ± 2.18	15.68 ± 2.68	0.42
Total body fat mass, kg	12.54 ± 5.01	14.11 ± 4.16	15.26 ± 8.55	0.17
Total body fat,[2] %	27.27 ± 7.60	29.21 ± 6.03	29.53 ± 9.44	0.41
Total body lean mass, kg	32.72 ± 4.59	33.40 ± 4.33	33.55 ± 5.57	0.73
Peripheral fat mass,[3] kg	5.94 ± 2.28	7.32 ± 2.13	7.33 ± 4.08	0.08
Peripheral lean mass,[4] kg	13.91 ± 1.97	14.88 ± 2.08	14.76 ± 2.80	0.17
Trunk:peripheral fat ratio[5]	1.00 ± 0.15	0.84 ± 0.15	0.98 ± 0.15	0.0001
Trunk:peripheral lean ratio[6]	1.08 ± 0.07	1.04 ± 0.06	1.07 ± 0.08	0.12

[1] Values are means ± SD.
[2] Total percentage of body fat = [total body fat mass/(total body fat mass + total body lean mass)] × 100.
[3] Peripheral fat mass = DXA arm fat mass + DXA leg fat mass.
[4] Peripheral lean mass = DXA arm lean mass + DXA leg lean mass.
[5] Trunk:peripheral fat ratio = DXA trunk fat mass/[DXA arm fat mass + DXA leg fat mass].
[6] Trunk:peripheral lean ratio = DXA trunk lean mass/[DXA arm fat mass + DXA leg fat mass].

Asian ethnicity was inversely associated with physical activity ($P = 0.005$), but not with dietary energy intake. Finally, after adjusting for age, energy intake, and physical activity, the proportion of Asian ethnicity was associated with an earlier (Tanner) stage of pubic hair maturation. It is remarkable that despite associations of ethnicity with birth weight and adolescent leg length, there was no association with skeletal breadth (either biacromial or bi-iliac), or total fat percentage in adolescence. Overall, 7% of girls had a low birth weight (<2.5 kg) and 5% a high birth weight (>4 kg).

The predictors of the DXA trunk:peripheral fat ratio are examined in more detail in **Table 4**. This model includes Asian ethnicity, age, Tanner pubic hair stage, physical activity, dietary energy intake, birth weight, height, and biacromial and bi-iliac breadths. Asian ethnicity remained an important predictor of the trunk:peripheral fat ratio. Interestingly, bi-iliac breadth (but not biacromial breadth or height) was significantly positively associated, and birth weight was negatively associated, with the trunk:peripheral fat ratio.

DISCUSSION

In this study, we found that Asian ethnicity, compared with White ethnicity, was directly associated with the DXA trunk:peripheral fat ratio and inversely associated with leg length. Moreover, the trunk:peripheral fat ratio was also associated with bi-iliac breath (directly) and birth weight (inversely).

Trunk fat has been associated with chronic diseases such as diabetes and heart disease (9). The NIH recommendation for waist circumference is <88 cm for women (10), although cut-off points have not been established for adolescents. Central adiposity appears to contribute more to the development of cardiovascular risk than general adiposity. For a given level of BMI, subscapular skinfold (central body fat measure) remains a significant and independent predictor of definite coronary heart disease in men (11). In Hong Kong Chinese, overall obesity increased the levels of the risk factors, but central adiposity contributed to a greater extent to adverse HDL cholesterol, triglyceride, and insulin resistance levels (12).

Our study showed that, although Asian adolescent girls have total body fat similar to that of White adolescent girls, they have a higher trunk:peripheral fat ratio and thus carry more fat in the trunk region. In a recent study among women (25–35 y old) by Novotny et al. (13), Asian women in Hawaii had a significantly higher percentage of body fat and higher measures of central adiposity than Whites as measured by subscapular skinfold thickness and the waist:hip ratio. This trend appears to have begun in adolescence, as evidenced by findings of the current study.

A pattern of fat on the trunk, independently of general body fatness, was also related to high levels of LDL and VLDL and low levels of HDL cholesterol in 6- to 18-y-old youth (14). Excess trunk subcutaneous fat and abdominal adiposity were also shown to be important determinants of insulin resistance in adult Asian Indians (15). In a cross-sectional analysis among elderly Japanese American men, 3 measures of adiposity (BMI, waist circumference, and subscapular skinfold thickness) were independently related to hyperinsulinemia (16).

In this study, Asians reported significantly less physical activity than Whites. Although our measure of physical activity was not associated with central obesity in Table 4, because it is measured with error, it still might partially explain the greater trunk:peripheral fat ratio in Asians. Ross et al. (17), in an exercise intervention study, reported a greater reduction in total abdominal and abdominal subcutaneous fat in the exercise weight loss group than in the diet weight loss group, exercise without weight loss group, or weight-stable control group. In another study, mean BMI, the percentage of body fat, and the waist:hip ratio were significantly lower for each increasing physical activity level (18).

Most adolescents do not achieve ≥5 bouts of moderate physical activity per week, and continue to fail to achieve this amount of activity into adulthood. Of those achieving ≥5 weekly sessions of moderate-to-vigorous physical activity and ≤14 h of weekly screen time (TV, video viewing, computer/video game use) as adolescents, few continued to achieve these favorable amounts of activity (4.4%) and screen time (31.1%) when they become adults (19).

TABLE 3

Effect of Asian vs. White ethnicity for various variables, adjusted for covariates using multiple regression[1]

Dependent variables	n	Regression coefficient[2]	SE	P
Exam 2 measures				
DXA measures				
Trunk:peripheral fat ratio, *kg*	102	0.170	0.040	< 0.0001
Trunk:peripheral lean ratio, *kg*	102	0.030	0.020	0.14
Total body fat, %	102	−0.796	2.166	0.71
Total body lean mass, *kg*	102	193.076	1176.322	0.87
Trunk fat mass, *kg*	102	435.182	894.147	0.623
Trunk lean mass, *kg*	102	2.286	495.951	0.99
Peripheral fat mass, *kg*	102	−826.052	534.681	0.32
Peripheral lean mass, *kg*	102	−368.471	533.646	0.49
Tanner pubic hair stage, 1–5	102	−0.615	0.839	0.004
Birth weight, *kg*	102	−1.039	0.3000	0.001
Exams 1 and 2 combined (2 measures per girl)[3]				
Waist circumference, cm	303	−0.643	1.161	0.51
Hip circumference, cm	303	−1.299	1.286	0.31
Waist:Hip ratio, *cm*	303	0.003	0.007	0.69
Log of subscapular skinfold, *mm*	302[4]	0.530	0.293	0.07
Triceps skinfold, *mm*	303	0.277	0.809	0.73
Biceps skinfold, *mm*	303	−1.158	1.340	0.39
Iliac skinfold, *mm*	303	1.295	1.1810	0.27
Calf skinfold, *mm*	303	−0.867	0.842	0.30
Height, *m*	303	−2.813	1.1026	0.01
Weight, *kg*	303	−2.891	3.241	0.37
Sitting height, *cm*	299[5]	−0.181	0.608	0.77
Leg length, *cm*	303	−3.075	0.747	< 0.0001
Biacromial breadth, *cm*	303	0.080	0.333	0.81
Biiliac breadth, *cm*	303	−0.078	0.323	0.81
Energy intake, *kJ/d*	303	−31.620	71.954	0.66
Physical activity, *(MET·h)/wk*	303	−19.399	− 33.048	0.005

[1] All dependent variables, except for energy intake, physical activity, and Tanner pubic hair stage were adjusted for the independent variables: age, Tanner pubic hair stage, physical activity, and dietary energy intake. When energy intake and physical activity were treated as dependent variables, they were adjusted for age and Tanner pubic hair stage only, and when Tanner pubic hair stage was treated as a dependent variable it was adjusted for age, energy intake, and physical activity.
[2] Effect of the proportion of Asian ethnicity vs. the proportion of White ethnicity.
[3] Values are means ± SE, adjusted using the method of Generalized Estimating Equations (GEE).
[4] One girl had a missing value at exam 2.
[5] Two girls with outlier values were not included for sitting height analysis (2 measures per girl).

TABLE 4

Multiple regression of the DXA trunk:peripheral fat ratio on various variables (n = 102)[1,2]

	Regression coefficient	SE	P-value
Intercept	0.67	0.319	
Age, *y*	0.004	0.013	0.741
Asian ethnicity, %	0.138	0.041	0.001
Tanner pubic hair stage, 1–5	0.018	0.021	0.384
Physical activity, *(MET·h)/wk*	−0.117	0.406	0.773
Energy intake, *kJ/d*	−0.015	0.010	0.120
Biacromial breadth, *cm*	0.003	0.011	0.802
Biiliac breadth, *cm*	0.034	0.011	0.002
Height, *m*	−0.004	0.003	0.189
Birth weight, *kg*	−0.068	0.029	0.021

[1] Trunk:Peripheral fat ratio = DXA trunk fat mass/[DXA arm fat mass + DXA leg fat mass].
[2] All independent variables were entered in the multiple regression. Independent variables were from Exam 2 except that physical activity and energy values for Exam 1 and 2 were averaged to increase reliability. Adjusted $R^2 = 29\%$.

develop a more central adipose tissue distribution. This suggests that circulating testosterone favors an increase in trunk adipose tissue (23). Thus, the higher trunk:peripheral fat ratio of Asians compared with Whites suggests that Asian girls may have a higher testosterone:estrogen ratio than White girls at the same stage of pubertal development.

In this study, greater bi-iliac breadth was associated with more placement of fat in the trunk region. Based on the first exam, Asian girls in this cohort reached menarche earlier than White girls (24); this resulted in shorter legs, although trunk length was the same as that of White girls. Two years later, however, White girls had surpassed Asian girls in Tanner pubic hair maturation, according to the findings presented in this paper. This suggests a longer period of late pubescent growth among Asian girls, or perhaps they are not reaching Tanner stages 4 or 5, reference stages that were initially characterized in a population of White girls. As we continue to follow the girls in the present study into late pubescence, we can clarify this point.

Lower birth weights were associated with a higher trunk: peripheral ratio. This may reflect early metabolic programming, as was found by Barker and others (25), in which early undernutrition predisposes to later obesity and chronic disease.

The limitations of our study include a 54% attrition rate and a classification scheme for ethnicity that may lack biologic specificity. Nevertheless, we found no evidence that differential attrition may explain our results, and this ethnic classification is commonly used in health policy and public health, including descriptions of obesity.

In conclusion, a greater proportion of Asian vs. White ethnicity is associated with shorter legs and relatively more placement of fat on the trunk vs. periphery among adolescent girls.

Fat cells in the abdominal region have been thought to be more sensitive to nutritional and/or hormonal factors than fat cells in other regions (20). In the presence of sex steroid hormones, a normal gynoid distribution of body fat exists in women. With a decrease in sex steroid hormones, as occurs with aging, there is a tendency for central obesity to increase (21), (22), as is seen in postmenopausal women. Further, women given exogenous androgens or suffering from virilizing tumors or disorders such as congenital adrenal hyperplasia,

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ASIAN ETHNICITY IS POSITIVELY ASSOCIATED WITH BONE MINERAL CONTENT OF FEMALE ADOLESCENTS.

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Objectives: To study the effect of Asian ethnicity on bone mineral content during adolescents. Dietary, physical activity, pubertal stage and body size parameters were investigated among 152 female adolescents in Hawaii for their association with bone mineral content and bone mass. Baseline and follow-up data are presented from the Female Adolescent Maturation study (FAM). ***Methods:*** Subjects were 9 - 14 years of age at baseline visit and 13 to 16 by visit 2. They were recruited from Kaiser Permanente Oahu in a cohort study. Mean dietary intakes from Visits 1 and 2 were measured by a 3-day food record and physical activity with a questionnaire. Pubertal stage was measured at visit 2 by Tanner scales, body size with anthropometry and bone mineral content with DXA (Lunar Prodigy). ***Results:*** 81% of the variability in DXA bone mineral content was positively explained by age, weight, pubic hair Tanner, % Asian ethnicity, and biacromial breadth in a multiple regression model. Physical activity, calcium, energy intake, macronutrients intake, and isoflavones did not significantly contribute to the regression model. ***Conclusions***: Asian ethnicity is a known risk factor for osteoporosis in late adulthood. Further research is needed to investigate lifestyle changes between adolescents and adulthood among Asians.

Birth weight is negatively associated with DXA trunk to periphery fat ratio of multiethnic adolescent girls.<u>Daida Y</u>, Novotny R, Grove J, Le Marchand L, Vijayadeva V, 1955 East-West Rd. University of Hawaii at Manoa, Honolulu, Hawaii, 96822. A group of 160 girls of predominantly Asian and Caucasian ancestry identified from an HMO membership were examined for predictors of body fat. Girls were measured twice at a 2-year interval during puberty. At each exam, physical activity was measured with a validated questionnaire of past year activity, dietary intake with a 3-day dietary record, and Tanner stage was assessed clinically. A model predicting DXA trunk to periphery fat ratio (exam 2), was adjusted for METs of physical activity, kj/day (mean of exams 1 & 2), Tanner public hair stage (exam 2) and height (exam 2). Age (exam 2) and birth weight negatively predicted trunk to periphery ratio while Asian ethnicity positively predicted trunk to periphery ratio. A hundred gram of birth weight was associated with a 3 unit decrease in DXA trunk to periphery fat ratio during adolescence. Thus, infant birth weight may be important in influencing body fat distribution during adolescence and, possibly, later in life.

Protein intake is positively associated with waist circumference in female adolescents
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There is recent interest in the role of dietary protein in management of weight and body fat, with central body fat an important health indicator in adulthood that may track from early years. We examined adolescent girls (n = 106) of Asian and White ethnicity for predictors of waist circumference. Girls were identified from an HMO membership and measured at a 2-year interval during puberty. At each exam, physical activity was measured with a validated questionnaire of past year activity, dietary intake by 3-day dietary record (two weekdays and one weekend day, completed by the girls with parental assistance), and Tanner stage was assessed clinically. Nutrient analysis was done at the Cancer Research Center of on Hawaii. Waist circumference was measured with an inextensible measuring tape at Exam 2. Mean protein intakes (67gm, Exam 1 and 65 g, Exam 2) were well above the RDA. Mean waist circumference was 66 cm. In a multiple regression model, after adjustment for age, ethnicity, pubic hair Tanner stage, height, bi-iliac breadth (Exam 2), physical activity, mean dietary energy, and mean dietary fat (mean of Exam 1 and 2), mean dietary protein intake (mean of Exam 1 & 2) was positively associated with waist circumference ($p<0.03$). Similar results were produced with weight as the outcome variable. Thus, higher dietary protein intake may contribute to increased central body fatness among adolescent girls.