Associations among Calcium Intake, Resting Energy Expenditure, and Body Fat in a Multiethnic Sample of Children

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Objective The objective was to determine if calcium intake was associated with resting energy expenditure (REE) and body fat in children after accounting for ancestral genetic background.

Study design Participants included 315 children. REE, body composition, and dietary calcium were assessed by indirect calorimetry, dual-energy x-ray absorptiometry, and 24-hour recalls, respectively. Structural equations modeling assessed the relationships among REE, calcium intake, and body fat.

Results There were positive associations between calcium intake and REE ($P < .01$) and between REE and total body fat ($P < .0001$). There was indirect effect of calcium intake on total body fat ($P < .01$). There were positive associations between calcium intake and REE ($P < .01$), and a trend toward an association of calcium intake and total body fat ($P = .065$) among boys only, whereas the only significant relationship among girls was an association of REE on total body fat ($P < .0001$).

Conclusions REE was associated with calcium intake and mediated a relationship between calcium intake and total body fat. These findings suggest calcium intake may play a role in fat accumulation and energy balance through its effects on REE, especially in boys. *(J Pediatr 2010;157:473-8)*.

Efforts to combat the increasing rates of obesity have led to the development of a variety of dietary management programs that generally incorporate a combination of macronutrient manipulation coupled with caloric restriction.[1-3] More recent investigations have explored how the consumption of specific dietary factors influence weight loss/maintenance and overall health, including the role of calcium as a functional micronutrient.[4-6] Calcium, a key regulator of metabolism, may influence body fat levels through its effects on resting energy expenditure (REE). The largest fraction of total daily energy expenditure is accounted for by REE,[7] and alterations in energy expenditure can predict weight changes.[8,9] In the growing child, studies have indicated inadequate levels of dietary calcium can interfere with metabolism, possibly contributing to fat accumulation.[4,10,11,12] However, the underlying mechanism driving the relationship of dietary calcium and body fat is complex and has yet to be fully understood. The relationship between calcium and body fat is further complicated when considering inherent differences in physiology and metabolism observed between racial/ethnic groups.

Differences in body composition,[13] REE,[14-16] and dietary intake[17] have been previously observed, using traditionally racial/ethnic classification as the unit of comparison. However, disentangling the etiology of these differences, particularly among intermixed individuals, becomes challenging because race/ethnicity represents a unique social construct characterized by autochthonous cultural differences, behavioral practices, and dietary preferences. Genetic admixture elucidates biological rather than environmental variance within individuals, which may also have a mediating effect on metabolic pathways.[18,19] Thus, further investigation into the relationship of specific nutrients with REE that influence body composition, while taking factors depicting this admixture of populations into account, are warranted to capture the complex etiology of population differences.

Investigations including the associations of etiological factors may be particularly critical in childhood, as body fat trajectories probably are established during this period.[20] This study was conducted to investigate relationships among calcium intake, REE, and body fat in peripubertal children while accounting for differences in body composition as well as using genetic admixture as a control for genetic variability.

AIMS	Ancestry informative markers
BMI	Body mass Index
CFI	Comparative fit index
DXA	Dual-energy x-ray absorptiometry
ML	Maximum likelihood
REE	Resting energy expenditure
RMSEA	Root mean square error of approximation
SEM	Structural equations modeling
SES	Socioeconomic status

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Methods

A sample (n = 315; 53% male) of European American (n =122), African American (n = 107) and Hispanic American (n = 86) children, 7 to 12 years of age, were recruited. The children were pubertal stage ≤3 as assessed by a pediatrician (according to Marshall and Tanner),[21] healthy, and not taking medications known to affect body composition. Parents and children provided consent/assent, respectively, after receiving the protocol by study personnel. The protocol was approved by the Institutional Review Board for human subjects at the University of Alabama at Birmingham. All measurements were performed between 2004 and 2008.

Subjects participated in 2 visits. On the first visit, pubertal status, anthropometric assessment, and body composition were measured and a 24-hour dietary recall was obtained. On the second visit, subjects were admitted for an overnight stay and a second 24-hour dietary recall was obtained. All participants received the same meal and snack foods. Only water and/or noncaloric, decaffeinated beverages were permitted after 8 PM until after the morning testing.

Anthropometric measures were obtained by the same registered dietitian. Height (Heightronic 235; Measurement Concepts, Snoqualmie, Washington) and weight (Scaletronix 6702W; Scale-tronix, Carol Stream, Illinois) was obtained in minimal clothing without shoes. Body mass index (BMI) percentile was calculated using age- and sex-specific growth charts.[22]

Dietary composition was assessed using the average of the two 24-hour dietary recalls using the "multiple pass" method, providing cup and bowl sizes to help estimate portion sizes. Each recall was performed in the presence of at least 1 parent. A registered dietitian coded and entered the data into Nutrition Data System for Research version 2006 (Nutrition Coordinating Center, University of Minnesota, Minneapolis, Minnesota). Total energy (kcal/d) and calcium intake (g/d) were generated as variables from the analyses. Total energy intake was included because calcium intake probably increases with increasing caloric intake, and positive energy balance is known to have an effect on body fat.[10]

Body composition was measured by dual-energy x-ray absorptiometry (DXA) using a GE Lunar Prodigy densitometer (DXA; GE Lunar Radiation Corp., Madison, Wisconsin) with pediatric software (version 1.5e). Subjects were scanned in light clothing, lying flat on their back with arms at their sides.

Pubertal Status

Direct observation for the assessment of pubertal stage by a pediatrician was used. The staging based on the criteria of Marshall and Tanner[23,24] is according to both breast and pubic hair development in girls and genitalia and pubic hair development in boys. One composite number is assigned for Tanner staging, representing the higher of the 2 values defined by breast/genitalia and pubic hair.[25]

REE was measured in the morning immediately after awakening during the overnight visit. A computerized, open-circuit, indirect calorimetry system with a ventilated canopy (Delta Trac II; Sensor Medics, Yorba Linda, California) was used. While lying supine on a bed, the head of the subject was enclosed in a plexiglass canopy. Subjects were instructed not to sleep and remain quiet and still, breathing normally. One-minute average intervals of oxygen uptake (VO_2) and carbon dioxide production (CO_2) were measured continuously for 30 minutes.

Parental self-report was used for classification of subjects into racial/ethnic categories. Scientific evaluation of the uniqueness of population-based differences is challenging, in particular because in many contexts, delineation between biology and environment in the variable "race/ethnicity" is not clearly defined. Further, race/ethnicity changes according to historical periods, social structure, and as individuals become more admixed. In our analysis, statistical models include race/ethnicity as a control variable for social and cultural characteristics. Although there is multicolinearity between the admixture variables and race/ethnicity, it is accounted for using the structural equations modeling (SEM) approach.

Genotyping of the ancestry informative markers (AIMs) for the measurement of genetic admixture was performed at Prevention Genetics using the Chemicon Amplifluor SNPs Genotyping System coupled with ArrayTape technology. as previously described.[17] A panel of 140 AIMs was used to estimate the genetic admixture proportion of each subject. Molecular techniques for the allelic identification and methodology for genetic admixture application have been described elsewhere.[26] The information from the AIMs was translated into estimates of African American, European American, and Native American admixture for each subject using maximum likelihood estimation based on the maximum likelihood (ML) algorithm described by Hanis et al.[27]

Because socioeconomic status (SES) has been reported as an environmental factor influencing dietary intake and adiposity,[28,29] a measure of SES was included in analyses. SES was determined according to the Hollingshead 4-factor index of social status.[30] This scale (ranging from 8 to 66) combines the education level and occupational prestige for the working parents in each child's family, with higher values representing higher SES.

Descriptive statistics evaluating sex differences were determined using ANOVA (SAS version 9.2 software; SAS Institute, Cary, North Carolina) with statistical significance level set at $\alpha = 0.05$. Our objective of identifying the relationships among dependent and independent variables was evaluated using a SEM approach. Specifically, Mplus software (Muthen and Muthen, Los Angeles, California) with ML estimation was used to test models that describe the relationship between calcium intake and REE and how these measures predict total body fat. SEM allows for simultaneous evaluation of multiple regression equations with the inclusion of covariates, providing estimates of the direct and indirect effects while accounting for colinearity among all variables. Specifically, the direct effects refer to paths, and statistical estimates representing path coefficients are interpreted as