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Associations among calcium intake, resting energy expenditure, and body fat in a multiethnic sample of children

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Abstract

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 (DXA), 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 ($p < 0.01$) and between REE and total body fat ($p < 0.0001$). There was indirect effect of calcium intake on total body fat ($p < 0.01$). There were positive associations between calcium intake and REE ($p < 0.01$), and a trend towards an association of calcium intake and total body fat ($p = 0.065$) among males only; whereas, the only significant relationship among females was an association of REE on total body fat ($p < 0.0001$).

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 males.

Keywords

calcium intake; resting energy expenditure; body fat; genetic admixture; peripubertal children; structural equations modeling

Efforts to combat the increasing rates of obesity have led to the development of a variety of dietary management programs which generally incorporate a combination of macronutrient manipulation coupled with caloric restriction¹⁻³. 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^{4,5,6}. Calcium, a

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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 REE⁷, 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 composition¹³, REE^{14,15,16} and dietary intake¹⁷ have been previously observed, utilizing 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 are likely established during this period²⁰. 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.

Methods

A sample (n=315; 53% male) of European- (n=122) African- (n=107) and Hispanic-American (n=86) children, 7-12 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 (UAB). All measurements were performed between 2004 and 2008.

Subjects participated in two 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 non-caloric, decaffeinated beverages were permitted after 2000h until after the morning testing.

Anthropometric measures were obtained by the same registered dietitian. Height (Heightronic 235; Measurement Concepts, Snoqualmie, WA) and weight (Scale-tronix 6702W; Scale-tronix, Carol Stream IL) was obtained in minimal clothing without shoes. BMI percentile was calculated using age- and sex-specific growth charts²².

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 one parent. A registered dietitian coded and entered the data into Nutrition Data System for Research version 2006 (Nutrition

Coordinating Center, University of Minnesota, Minneapolis, MN). Total energy (kcal/d) and calcium intake (g/d) were generated as variables from the analyses. Total energy intake was included because calcium intake most likely increases with increasing caloric intake, and positive energy balance is known to have an effect on body fat ¹⁰.

Body composition was measured by DXA using a GE Lunar Prodigy densitometer (DXA; GE Lunar Radiation corp., Madison, WI) 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 utilized. The staging based on the criteria of Marshall and Tanner ^{25,26} 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 two values defined by breast/genitalia and pubic hair ²⁷.

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, CA) 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 (VO₂) and carbon dioxide production (CO₂) were measured continuously for thirty 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 multi-collinearity 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 ²⁸. The information from the AIMs was translated into estimates of African, European, and Native American admixture for each subject using maximum likelihood estimation based on the maximum likelihood (ML) algorithm described by Hanis et al. ²⁹.

Because socioeconomic status (SES) has been reported as an environmental factor influencing dietary intake and adiposity ^{30,31}, a measure of SES was included in analyses. SES was determined according to the Hollingshead four factor index of social status ³². 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, NC) with statistical significance level was 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, CA) with ML estimation was employed 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 regression coefficients. The estimates control for correlations among multiple presumed causes of the same variable. Indirect effects are estimated statistically as the product of the direct effects which comprise them, and are also interpreted as path coefficients.

The following measures of fit for SEM³³ were employed: chi-square (χ^2) test of model fit, its p-value and degrees of freedom (df); CFI (comparative fit index); and RMSEA (root mean square error of approximation). The χ^2 test signified how well the models fit the data, whereas small, non-significant χ^2 values indicated little divergence between the structure of the observed data and hypothesized model. The CFI compared the hypothesized model with the null model, taking model complexity into account. A well-fitting model had CFI values >0.90. The RMSEA indicated how closely the model fit approximated an acceptable model, with values <0.10 representing good model fit. Total body fat was modeled as a single indicator of adiposity as a dependent variable influenced by REE. Initial analyses indicated that overall model fit would be substantially improved by including this measure as opposed to BMI percentile or percent body fat. REE was modeled as a determinant of total adiposity. Because REE is highly dependent upon lean mass, lean mass was controlled for in modeling REE. Reported calcium intake was modeled as a determinant of both adiposity and REE^{16,34}. The hypothesized causal paths in the determination of total body fat and REE were estimated by linear regression coefficients (shown using single-headed arrows).

The base model (combined analysis; Figure 1) was adjusted for sex, pubertal status, height, SES, race/ethnicity, total energy intake, and genetic admixture. Race/ethnicity was dummy-coded with European Americans being the reference group (European Americans=0, African Americans=1, Hispanic Americans=2). Sex was coded: males=0 and females=1. Because the measured value for each of the three genetic admixture components adds to one, only European and African admixture (as the two admixtures with the widest variation among our sample) were included as covariates to avoid overspecification of the statistical models. Specifically, the models tested: (1) if calcium intake significantly affected REE (2) if calcium intake significantly affected total body fat, (3) if REE had a significant effect on total body fat, and (4) if the relationship of calcium intake and total body fat was mediated by REE indirectly. We further analyzed these relationships without the variable REE included in the model to assess the degree of mediation.

Results

The Table represents participant characteristics for the total sample and stratified by sex. Males were significantly older than females ($p<0.05$), had higher total lean mass and had higher REE ($p<0.01$), whereas females tended to have higher total fat mass ($p=0.0678$). However, there was no difference in BMI percentile between the sexes. Males had higher energy intake than females ($p<0.05$), but no difference in calcium intake.

Figure 1 illustrates the overall relationships between calcium intake, REE, and total body fat. All model fit indices indicated a good model fit. Specifically, the χ^2 which tested the hypothesis that the model implied variances and covariances were equal to those of the observed data was not rejected for our proposed model (Figure 1). Other fit indices were included to support that this was a well-fitting model. The CFI and the RSMEA were also indicative that this was a good fitting model with values above 0.95 and below 0.05, respectively.

The total amount of variation in REE and total body fat explained by the SEM base model was $R^2=0.455$ and $R^2=0.401$, respectively (both $p<0.0001$). There was a direct association between calcium intake and REE ($p<0.01$), but the observed relationship between calcium intake and total body fat was not statistically significant. REE had a direct effect on total body fat ($p<0.0001$). Furthermore, there was a significant indirect effect of calcium intake on total body fat, suggesting that REE mediated the influence of calcium intake on total body fat ($p<0.01$). In the model excluding REE, the effect of calcium intake on total body fat was larger and showed a trend ($p=0.056$), thus indicating mediation.

The multigroup model (Figure 2), in which the sexes were stratified, yielded the following values of selected fit indices: $\chi^2=18.04$, $p=0.45$, $df=18$, $CFI=1.000$, and $RMSEA=0.004$. These values were indicative of a good fitting model, and standardized effects were equal across groups. There was somewhat greater predictive power for the males than for the females, such that the proportions of explained variance for calcium intake on total body fat were 0.470 and 0.362, respectively. Consistent with the combined analysis, the total amount of variation in REE and total body fat explained by this model for males was $R^2=0.415$ and $R^2=0.470$, and for females was $R^2=0.474$ and $R^2=0.362$, respectively (all $p<0.0001$).

For males, calcium intake was directly associated with REE ($p<0.01$), there was a trend toward an association of calcium intake and total body fat ($p=0.065$), and REE was directly associated with total body fat ($p<0.0001$). There was an indirect effect of calcium intake on total body fat, which was mediated by REE ($p<0.05$). In a model excluding REE, the direct effect of calcium intake on total body fat was significant ($p<0.05$), further indicating mediation in males.

For females, the only significant association was between REE and total body fat ($p<0.0001$). Therefore, unlike in males, there is not sufficient evidence for REE mediating the effect of calcium intake on total body fat in females.

Discussion

The relationships observed herein contribute insight into the inconsistencies reported by other studies investigating the relationships among dietary calcium, REE and body fat. Consistent with our results, a randomized, controlled crossover study of 9-10 year-old children reported that milk consumption induced greater REE and thermic effect of food after six days of supplementation relative to supplementation with a sugar-only beverage¹⁰. Conversely, in a study evaluating calcium intake and total energy expenditure no effects of 24h energy expenditure was observed in diet groups with varying levels of dietary calcium⁵. In an adult weight-loss trial, there was no difference in total energy expenditure among various groups consisting of low calcium intake, calcium supplementation, and high dairy³⁵. Whereas our analysis indicated a positive indirect relationship between calcium intake and body fat, in an analysis of NHANES III data and in randomized trials, an inverse association between calcium intake and relative risk of obesity (suggestive of lower body fat) among adults has been observed³⁶. Although the inconsistencies existing in the literature indicate a need for greater understanding of the role of calcium intake on obesity-related phenotypes (particularly across age groups), our results support the notion that the relationship between calcium and obesity traits is mediated by other aspects of energy balance that deserve careful consideration in future studies. Further, as the illustrated inherent virtues of SEM, evaluation using more sophisticated approaches that resolve issues of collinearity should be employed.

Exploring differences based on utilization of the race/ethnicity categorical variable could be an additional influential factor in the inconsistencies reported across studies. A uniqueness

of our study is that we were able to evaluate the genetic contribution (assessed by ancestral genetic admixture) to our dependent variables. In exploratory analyses, the overall model and the model representing stratification by sex were also run without the inclusion of genetic admixture (data not shown). A trend towards significance directly relating calcium intake with total body fat for the overall sample was identified in this model. This may suggest that ancestral genetic background contributes, at least in part, to the relationship. Thus, genetic admixture as a tool to scientifically assess the heterogeneity of human populations allows for a more accurate assessment of individual variability and clearer understanding of the relationships among calcium, REE, and body fat.

Our findings related to the contribution of genetic admixture were of particular interest. In the overall model, neither European nor African admixtures were significant contributors to body fat. However, when investigating the model by sex, African admixture was a negative predictor of total body fat in females, whereas European admixture was a positive predictor of total body fat in males. These findings suggest a differential contribution of ancestral genetic background in boys and girls that deserves further exploration in studies evaluating the role of genetic admixture in measures of sex differences in body composition among children.

The disparate findings in the relationships among calcium intake, REE, and body fat between males and females indicate inherent differential underlying physiology between the sexes. Inclusion of pubertal status and lean mass in the models, two factors which have been identified as contributors to the sexual dimorphism in REE, did not account for such differences. As such, the involvement of factors in addition to those evaluated here is evident. A plausible determinant of differential mediation could be the difference in hormonal milieu among males and females; estrogen is known to drive fat deposition³⁷, whereas testosterone is known to drive lean mass³⁸. However, measurements of hormones were not available for this study. The evaluation of hormonal differences in explaining the relationship between calcium, REE and body composition in boys and girls deserves further exploration.

Children may be an ideal model system in which to explore the relationships between REE, dietary intake and body composition due to active growth and development, particularly during the peripubertal period. REE is known to be relatively high in children compared with adults, likely due to differences in oxidative requirements of the tissues needed for growth and development³⁴. Increased REE translates into increased energy requirement. Theoretically, metabolic alterations that minimize positive energy flux by creating a greater caloric need have the potential to result in less fat accumulation over time. By this theory, the positive association found between dietary calcium and REE could have a positive impact on long-term weight maintenance. However, among children there were opposing findings. In addition, other metabolic factors associated with body composition and energy substrate utilization could be contributing. For example, regulation of intracellular calcium levels by parathyroid hormone (PTH), further regulated by the circulating active metabolite of vitamin D, has been found to be positively associated with changes in fat mass and fat oxidation³⁶. The impact of calcium levels and vitamin D status on PTH may in turn mediate the systemic effects of these dietary nutrients, but potential relationships with energy metabolism have not been well examined. Thus investigation of independently- and/or interactively-acting contributing factors is warranted.

Strengths of our study are that we had a large cohort of racially/ethnically diverse subjects and had robust measures of body composition. We also employed SEM which allowed for the simultaneous evaluation of multiple regression equations. There were, however, limitations. The data expressed are statistically significant, yet explain less than 50% of the

variance. It is likely that unconsidered factors also impact the relationships evaluated. For example, calcium/dairy intake level has been shown to possibly affect fat oxidation^{5,35,39}, a measure we were unable to attain. Further, since serum PTH and vitamin D status are both proposed to have a role in the mediation of calcium and REE, inclusion of these measures would have likely enhanced our understanding of potential mechanisms³⁹. Additionally, although considered an acceptable and appropriate tool for describing mean intakes of a large group of subjects, dietary assessment via 24-hour recall has limitations, particularly in the assessment of micronutrients. However, it is the most commonly used method for dietary surveys in the US⁴⁰. The cross-sectional design of the study prevents the inference of long-term relationships; thus, longitudinal data would be necessary to investigate the effects of calcium intake over time on our dependent variables.

Calcium intake may play a role in body fat accumulation and energy balance through its effects on REE in children. Future investigations evaluating mechanisms in which calcium, and possibly other key nutrients, affects energy balance and body composition are warranted.

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List of abbreviations

REE	resting energy expenditure
EA	European Americans
AA	African Americans
HA	Hispanic Americans
DXA	dual energy x-ray absorptiometry
GCRC	General Clinical Research Center
BMI	body mass index
AIMs	ancestry informative markers
ML	maximum likelihood
SES	socioeconomic status
ANOVA	analysis of variance
SEM	structural equations modeling
CFI	comparative fit index
RMSEA	root mean square error of approximation
PTH	parathyroid hormone

Figure 1. Relationships between calcium intake, REE, and total body fat

The arrows represent the causal paths; specifically the arrowhead points to the presumed effect, and the line stems from the presumed cause.

HA = Hispanic American; AA = African American; Af Adm = African American Admixture; Eur Adm= European American Admixture; SES = Socioeconomic Status; Dark Boxes = Dependent Variables; Light Boxes = Independent Variables; White Boxes = Parameter Estimate (Numbers Represent β -Coefficients); * <0.05 , ** $0.01 < p < 0.10$; $\chi^2=15.13$; p-value=0.127; df=10; CFI=0.984; RMSEA=0.040

Figure 2. (Supplement) Relationships between calcium intake, REE, and total body fat stratified by sex

The arrows represent the causal paths; specifically the arrowhead points to the presumed effect, and the line stems from the presumed cause.

HA = Hispanic American; AA = African American; Af Adm = African American Admixture; Eur Adm = European American Admixture; SES = Socioeconomic Status; Dark Voxes = Dependent Variables; Light Voxes = Independent Variables; White boxes = Parameter Estimate (Numbers Represent β -Coefficients); * <0.05 , ** $0.01 < p < 0.10$; $\chi^2=18.04$; p-value=0.450; df=10; CFI=1.000; RMSEA=0.004

Table 1

Descriptive statistics of total sample according to sex

	Total Sample	Sex	
	Overall (n=315)	M (n=167)	F (n=148)
Age (yrs)	9.6 ± 1.6	9.8 ± 1.6 ^a	9.3 ± 1.5 ^b
Tanner stage	1.5 ± 0.7	1.4 ± 0.6 ^b	1.6 ± 0.8 ^a
Height (cm)	139.5 ± 10.6	140.1 ± 10.6	138.9 ± 10.6
Weight (kg)	36.7 ± 9.5	37.1 ± 10.2	36.1 ± 8.7
BMI percentile	66.3 ± 26.1	66.5 ± 26.1	66.1 ± 26.3
Race/Ethnicity (%)			
EA	38.7	20.0	18.7
AA	34.0	18.7	15.2
HA	27.3	14.3	13.0
SES	38.7 ± 14.5	38.6 ± 14.0	38.8 ± 15.0
REE (kcal/day)	1,192.33 ± 234.8	1,240.48 ± 248.3	1,139.6 ± 207.3 ^b
Total lean mass (kg)	25.6 ± 5.3	26.6 ± 5.3 ^a	24.4 ± 5.0 ^b
Total fat mass (kg)	8.9 ± 5.7	8.3 ± 6.3 ^b	9.5 ± 5.0 ^a
Energy (kcal/d)	1,886.5 ± 469.6	1,943.3 ± 486.7 ^a	1,823.6 ± 443.0 ^b
Calcium Intake (mg/d)	859.0 ± 333.8	872.8 ± 329.9	843.6 ± 338.5
European American admixture (%)	54.6 ± 38.8	54.2 ± 40.1	54.9 ± 37.5
African American admixture (%)	31.3 ± 38.4	31.7 ± 39.5 ^{a*}	30.9 ± 37.4 ^{b*}

^{a, b} superscripts represent differences between groups

* represents trend for difference (p<0.10)