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Vitamin D and Calcium-Sensing Receptor polymorphisms differentially associate with resting energy expenditure in peri-pubertal children

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Abstract

Given that calcium metabolism is influenced by genes and is tightly linked to energy-utilizing pathways, this study evaluated the association of single nucleotide polymorphisms (SNPs) in the vitamin D receptor (VDR) and calcium-sensing receptor (CASR) with resting energy expenditure (REE). In 273 boys and girls, 7-12y, cross-sectional REE was measured via indirect calorimetry, body composition by DXA, and dietary measures by 24-hour recall. SNPs for VDR Cdx-2 (rs11568820) and CASR A986S (rs1801725) were genotyped using the Illumina Golden Gate assay. Multiple linear regression models were used to determine the association between SNPs and REE. African American carriers of the 'A' VDR Cdx2 allele had increased levels of REE in the overall sample and this association was apparent among participants with an adiposity level of <25% and 30% body fat in males and females, respectively. For CASR, an association between carriers of the 'A' allele and REE was observed only in those in the upper median of calcium intake. VDR and CASR variants are associated with REE in children, and are influenced by levels of calcium intake and adiposity. Our results bring awareness to mechanisms underlying the regulation of REE and biological and dietary influential factors.

Keywords

calcium homeostasis; resting energy expenditure; calcium-sensing receptor; vitamin D receptor; single nucleotide polymorphisms; adolescents

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Conflict of interest.

The authors declare no conflict of interest.

Introduction

Resting energy expenditure (REE), the largest constituent of overall energy output, is a dynamic host of regulatory processes required for obligatory physiological function, and may be mediated (at least in part) by serum calcium homeostasis. Calcium is critical for cellular physiology, involved in a vast number of energy-dependent physiological processes (such as bone mineralization, muscle contraction, neuronal excitability and blood coagulation) which impact the storage and utilization of molecular, cellular and physiological resources [18;52].

Due to its pivotal role in energy regulation, calcium levels are tightly maintained within the extracellular fluid, however, variation in calcium handling can be influenced by genetic factors. Single nucleotide polymorphisms (SNPs) in the vitamin D (VDR) [43;51] and calcium-sensing (CASR) receptors [33] have been associated with regulation of calcium absorption, transport and excretion by potentially altering the binding ability of specific metabolites. It might be speculated that SNPs in these regions could, in part, explain variability in REE through altered calcium handling capacity. The *Cdx-2* rs11568820 polymorphism of VDR has been associated with alterations in transcriptional activity of its promoter, which may affect calcium absorption [2]. Additionally, CASR variation at rs1801725 has been suggested to influence circulating calcium concentrations by modulating signal transduction, intracellular trafficking and cell surface receptor expression [25]. It is plausible that intrinsic aspects of REE are driven by respective VDR and CASR mediation of calcium absorptive capacity and bone calcium resorption.

A limited number of studies have explored the potential contribution of genetic factors on the calcium-REE relationship. This is especially warranted in the context of peri-puberty, a period when modifiable factors (such as dietary behavior) prominently contribute to long-term body composition patterning. The objective of this study was to evaluate if VDR *Cdx-2* and CASR SNPs are associated with REE, and how their involvement is mediated by inherent (i.e., adiposity, ethnicity) and/or dietary (i.e., energy, calcium, and/or vitamin D intake) factors.

Materials and Methods

Participants

A sample of 273 (52% male, 48% female) European American (EA), African American (AA) and Hispanic American (HA) children, 7-12 years of age, were recruited to study the influence of genetic and environmental parameters on racial/ethnic differences in metabolic outcomes. The participants were early pubertal (Tanner stage 3), healthy, and not on medications known to affect body composition. Parents and children provided consent/assent, respectively, after reviewing the protocol by study personnel. The protocol was approved by the Institutional Review Board for human participants at the University of Alabama at Birmingham (UAB). All measurements were performed between 2005 and 2009.

Protocol

Participants required two visits that were no more than thirty days apart. On the first visit, pubertal stage, anthropometrics and body composition were measured, and a 24-hour dietary recall was obtained. On the second visit, participants were admitted to the General Clinical Research Center for an overnight stay (ensuring ~10-hour fast) and a second 24-hour dietary recall was obtained. Upon completion of the overnight fast, indirect calorimetry was performed and serum was obtained for metabolite analyses.

Indirect Calorimetry

REE was measured via computerized, open-circuit, indirect calorimetry system with a ventilated canopy (Delta Trac II; Sensor Medics, Yorba Linda, CA). One-minute average intervals of O₂ uptake and CO₂ production were measured continuously for 30 minutes, in which the last 20 minutes were used to calculate energy expenditure.

Genotyping

DNA was obtained from fasting blood samples of the study participants and genotyping of VDR and CASR SNPs at rs11568820 and rs1801725, respectively, was performed at the UAB Heflin Genotyping Core using the Illumina GoldenGate assay on the BeadXpress system (Illumina, Inc.). Briefly, the GoldenGate assay involves biotin-labeling of genomic DNA followed by capture of the labeled DNA onto streptavidin-coated sepharose beads. An artificial nucleotide-based molecule that contains universal priming sequences on either end and is complimentary to the target DNA sequence of interest interrogated. Once the array has been visualized with the BeadXpress reader, wavelength and intensity values of the fluorescence are used to determine genotype. A custom LIMS is used to track both samples and laboratory throughput. Allele detection and genotype calling were performed using the GenomeStudio software v3 (Illumina, Inc.). Each marker (SNP) was genotyped using a fluorescent allele-specific polymerase chain reaction (AS-PCR)-based assay [20]. Reaction components were assembled on an array tape platform (<http://www.douglasscientific.com>) using nanolitre volumes (500-1000 nL). PCRs were carried out in a water bath thermocycler using a standard three-stage parameter (denaturation, primer annealing, primer extension). The specific parameters of each PCR vary depending on the nature of the primers and the SNP being genotyped. The array tape was scanned post-PCR and the ratio of fluorescent signals was used to determine the genotype (homozygous or heterozygous for one allele).

In addition to the SNPs of interest, ancestry informative markers (AIMs) were genotyped to obtain estimates of genetic admixture. AIMs genotyping was performed at Prevention Genetics (Marshfield, WI) using the Chemicon Amplifluor SNPs Genotyping System coupled with ArrayTape technology as previously described [17]. A panel of 142 AIMs was used to estimate the genetic admixture proportion of each subject. Information about the AIMs along with previously described parental population frequencies have been recently provided as supplemental materials by Klimentidis et al [26]. Molecular techniques and methodology for marker genotyping have been described elsewhere [36]. Genotypic information was translated into estimates of African, European, and Native American admixture for each subject using maximum likelihood estimation based on the algorithm described by Hanis et al. [19].

Anthropometric Measures

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) were measured in minimal clothing without shoes. BMI percentile was calculated using age- and sex-specific growth charts [11].

Body Composition

Body composition (i.e., bone mineral content, BMC; lean mass; and total and percent fat mass) was measured by dual-energy x-ray absorptiometry (DXA) using a GE Lunar Prodigy densitometer (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. Because excess fat has been associated with an imbalance of calcium homeostasis, categorization according to adiposity level was performed such that females

and males with > 30 and 25% fat mass, respectively, were characterized as having an excess adiposity level [8;49]. To account for height differences, a fat mass index (FMI) variable was derived by dividing total body fat by height-squared.

Diet

Dietary measures were obtained from two averaged 24-hour recalls using the “multiple pass” method, in which cup and bowl sizes were provided 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, Minnesota). Total energy (kcal/d), calcium, and vitamin D intake were generated as variables from the analyses.

Pubertal Status

The Tanner stages have been demonstrated as reliable indicators of pubertal development. Assessment of pubertal stage was by direct observation by a pediatrician, the ‘gold standard’ for differentiating among the five stages of maturity [12;21]. The staging based on the criteria of Marshall and Tanner [29;30] is according to both breast and pubic hair development in girls and genitalia and pubic hair development in boys. One composite number was assigned for Tanner staging, representing the higher of the two values defined by breast development and/or pubic hair [28].

Physical Activity by Accelerometer

The MTI Actigraph accelerometer (Actigraph GT1M – Standard Model 198-0100-02, ActiGraph LLC, Pensacola, FL and accompanying software) was used to measure physical activity levels and patterns for seven days prior to participant’s inpatient visit at the GCRC. Epoch length was set at one minute and data expressed as counts per minute. Children were instructed to wear the monitor on an elastic belt at the waist above the right hip, removing only for sleeping, bathing or swimming. Actigraph monitors have previously demonstrated a high degree of inter-instrument reliability [6]. Daily and total counts per minute were summed and averaged.

Insulin/Glucose Dynamics

Insulin/glucose homeostasis is essential for maintaining energy balance. In addition, vitamin D has recently emerged as a potential mediator in this relationship [13;39;42;47;48]. Measures of fasting insulin and glucose, along with insulin sensitivity, were obtained via intravenous glucose tolerance test. Following the overnight fast, a topical anesthetic (Emla cream, AstraZeneca, Wilmington, DE) was applied to the antecubital space of both arms, where flexible intravenous catheters were placed. Subsequently, an intravenous glucose tolerance test was performed as previously described [9]. The acute insulin response to glucose, an approximation of first-phase insulin secretion, was calculated as the incremental area under the curve for insulin during the first ten minutes after glucose injection using trapezoidal methodology [45]. Values for fasting insulin and glucose were obtained from the average of the two baseline values, and were entered into the MINMOD computer program for determination of insulin sensitivity as described elsewhere [34]. Fasting samples of glucose and insulin were analyzed using a SIRRUS analyzer (Standio Laboratory, Boeme, Texas). All analyses were performed in the Core Laboratory Nutrition Obesity Research Center at UAB.

Statistical Analyses

ANOVA was used to assess sex- and ethnic-specific differences in descriptive statistics. Hardy Weinberg Equilibrium (HWE) was evaluated for each SNP (rs11568820 and

rs1801725) for the overall sample and by ethnicity, in which a p value of <0.05 indicated deviation in goodness of fit. Allele and genotype frequency for the overall sample and between groups (i.e. sex, ethnicity) were performed using the χ^2 test. Orthogonal codes to represent presence of two copies of the 'A' allele, presence of only one 'A' allele, and absence of the 'A' allele, were constructed in an additive (coded as 0, no 'A' allele; 1, one copy of 'A' allele; or 2, two copies of the 'A' allele; further orthogonally coded, using 2 as reference group) and non-additive (dominant model coded as: 0, two copies of the 'A' allele OR 1, zero or one copy of the 'A' allele; recessive model coded as 0, zero or one copy of the 'A' allele OR 1, two copies of the 'A' allele) manner. For all regression models, studentized residuals were evaluated for normality and logarithmic transformations of the dependent variable was performed when necessary. In accordance with the assumptions of regression, the observations for which the residuals of the association models were above and below three standard deviations were removed from the analyses. Two individuals fitting this criteria were removed as the recorded REE was not reflective of physiologic range in children (<650 kcal/d).

Step-wise multiple linear regression analysis was employed to guide inclusion of covariates in the association models, in which sex, fat mass index, total lean mass, BMC, dietary variables, and physical activity were investigated, with either VDR or CASR SNPs as the independent variable, and with entrance and stay criteria set at $p=0.15$ and <0.010 , respectively. Insulin dynamics, reported to influence vitamin D metabolism [3] as well as energy utilization [41], was included in step-wise regression with VDR as the independent variable. Based on the stepwise exploratory analysis, the variables sex, fat mass index, total lean mass, fasting insulin and dietary calcium were considered as covariates for the association with VDR and sex, fat mass index, total lean mass, and dietary calcium were considered as covariates for CASR. Given that meeting the recommended levels of dietary calcium would drive effective calcium sensing and regulatory responses, analyses for CASR were further stratified by high and low intake of calcium. The frequently reported relationship between vitamin D status and adiposity served as a justification to the exploration of the VDR association by adiposity levels. In addition, to control for population stratification and to reduce Type I error rate in association tests [5;10;14], genetic admixture was included in all analyses, as was Tanner stage to account for the potential physiological contributors of puberty on the outcome of interest [22]. All analyses were performed using SAS version 9.2 (SAS Institute, Cary, NC). Significance level was set at $p = 0.05$. To account for multiple comparisons, Bonferroni corrections and permutation tests were performed. Calculations for considering Bonferroni corrections resulted in a p -value of 0.005, and the sample size was not sufficient to attain significance at this level. Permutation tests resulted in statistical significance identified at $p<0.04$ and a trend towards significance at <0.08 .

Results

Overall and sex- and ethnic-specific sample characteristics are described in Table 1. AA were reproductively the most mature (i.e., advanced Tanner staging), with the highest BMC and lean mass. HA were categorized as being in the highest BMI percentile. EA had the lowest levels of fasting insulin. HA had the greatest level of calcium intake, followed by EA, who in turn exceeded that of AA. Table 2 shows the genotypic frequency of VDR and CASR for the entire sample and by sex and ethnicity. The 'A' allele presence (A/A or A/G) of VDR was greatest in African Americans (75.9%) compared to the other groups. EA had a significantly greater presence of the 'A' allele (14.4%) of CASR than AA and HA. Significant interactions were observed between VDR and adiposity ($p=0.01$), as well as between CASR and dietary calcium ($p=0.03$). Preliminary analyses involving evaluation of additive and non-additive models for VDR were not significant, thus only the non-additive model was used for final analyses. For CASR, only one subject (European American

female) was homozygous for the 'A' allele, therefore only the non-additive model was evaluated for genotype analysis.

Table 3 illustrates the findings for analyses of REE and VDR. HWE was not apparent in the overall sample, yet was among ethnic groups; thus analyses regarding VDR were run according to ethnicity. A significant association was identified of 'A' allele presence and REE in African Americans ($p < 0.05$), which showed a trend towards significance in European Americans ($p < 0.07$), and was not significant in Hispanic Americans. Due to a significant interaction observed between VDR and adiposity ($p = 0.006$), the VDR model was stratified accordingly. There was a significant association between 'A' allele carriers and REE only among those characterized as having normal body fat.

Table 4 illustrates the findings for analyses of REE and CASR. HWE was apparent in the sample, yet only one subject (European American female) was homozygous for the 'A' allele; therefore only the non-additive model was evaluated for analysis. There was no genotypic association with REE in the overall model. Due to a significant interaction with calcium intake ($p < 0.05$), the model was stratified by sample median (838mg). In those with higher calcium intake, presence of the 'A' allele of CASR was associated with REE ($p < 0.05$), which was not significant in those with lower calcium intake.

Discussion

The multitude of metabolic processes involved in calcium regulation supports contribution to REE [44]. REE itself, as well as the capacity for maintaining, sensing and absorbing calcium, is at least in part under genetic control [43], with variation evident across certain populations. Although reports are equivocal, differences in calcium intake across pediatric groups have also been observed [27;35]. This effort sought to investigate genetic variants involved in calcium regulation and potential influence on REE, which to date has not been investigated. Specifically, we evaluated the association of VDR *Cdx-2* and CASR *A986S* variants with REE in a multi-ethnic sample of children and investigated the impact of body composition and diet. We observed VDR 'A' allele presence was associated with greater REE in AA. In addition, an association of the CASR 'A' allele presence with REE was only apparent in those with upper reported intakes of calcium (though not exceeding current recommendations). Given the integrative role of vitamin D in calcium handling, as well as documented superior calcium conservation among AA relative to other groups, heightened calcium availability intersects mechanisms driving REE, explaining to some degree modulation based on inherent capacity for calcium utilization [1;7].

There was an observed association between the presence of the 'A' allele of the VDR *Cdx-2* polymorphism and REE, which was stronger in individuals of African American descent. In theory, calcium concentration increases in response to VDR-induced greater intestinal absorptive capacity [2;43;51]. In general, AA have better ability to conserve calcium via various pathways, displaying greater calcium retention in comparison to other groups [1;7;46], which may drive higher REE. Scientific literature indicates that AA adults have lower REE [15], an observation that was not apparent in our sample, but could be explained as part of the multidimensional changes occurring during growth and development. Our results support a potential genetic influence on REE linked with calcium and vitamin D metabolism in peri-pubertal children.

The observed association of the 'A' allele of VDR with REE in individuals characterized as having normal adiposity could be due to the capacity of adipose tissue to sequester circulating vitamin D [40]. An inverse relationship between vitamin D status and adiposity has been frequently reported [24;37;50], and excess fat has been associated with an

imbalance of calcium regulation [16]. Thus excess fat accrual may impair the potential for greater calcium-absorptive capacity by the 'A' allele of VDR genotype. Further studies would be required to confirm this speculation and to explore how changes in physiological function related to increased adiposity may plausibly alter gene expression.

A potential environmentally-induced contribution to calcium homeostasis may also be present in the context of CASR. Though not apparent in the overall sample, association between CASR A986S polymorphism and REE demonstrated specificity in terms of dietary calcium. The observation of REE being greater among those with the 'A' allele only in those with higher calcium intake is particularly relevant in this developmental stage. These early pubertal children, with mean pubertal stage halfway between Tanner 1 and 2, represents proximity to the completion of longitudinal bone growth, peak growth velocity, as well as that of substantial bone mineralization [4], and the CASR is central to mechanisms driving these processes. Biologic differences in terms of sex must also be considered given that both males and females were included in this sample. Females, closer to completing longitudinal bone growth, were mostly in their peak growth velocity, as well as that of calcium deposition into bone and thus findings of CASR and REE may have been pulled in different directions. Exploratory analysis (data not shown) indicated a positive relationship of CASR and REE in females, which was inverse among male subjects. Research has reported that CASR activation increases its own expression as well as that of VDR [38], the latter of which serves to enhance vitamin D action, which further increases CASR expression and action, amplifying the cycle. In view of this evidence, we performed exploratory analyses to consider a gene-gene interaction between the studied VDR and CASR SNPs; however, this was not apparent in this cohort (data not shown).

Elucidation of factors contributing to calcium homeostasis is important for optimal body composition during this critical period of growth and development, peri-puberty. This is the first study to our knowledge investigating the association of genetic variants involved in calcium regulation with REE, particularly in growing children. Our experimental design considered the evaluation of physiologic dietary response to calcium ingested as part of diet, overcoming the limitations of other investigations centered on absolute calcium intake [23;31;44;52]. The robust methodology used to measure the physiologic variables of interest, the strong physiological rationale supporting our hypothesis and our results, along with the specific peri-pubertal age group in which they were measured, serve as strengths of this study. The comprehensive phenotyping offers a unique capacity to evaluate the influence of the alleles to biologic and non-biologic contributors to growth and developmental changes in this critical period. However, limitations of the study are acknowledged, with sample size a limiting factor in confirming the association with strong statistical significance. Given the relatively modest sample size of our study we only evaluated two polymorphisms and reported associations that were not adjusted by multiple testing. Taking into account that the Bonferroni approach is known to be a stringent approach to address multiple testing [32] which can challenge the delicate balance between reducing Type I errors and increasing Type II errors in genetic studies with moderate sample size, we reported our results without such adjustment. Additionally we used a less conservative approach, permutation tests for multiple comparisons and statistical significance ($p = 0.08$) was maintained. Accordingly, findings serve as a basis of investigation, indicating a much larger study is required for substantiation. The nature of these receptors is also important to note. Responses may vary according to tissue type, as expression is not limited to an individual organ, and any given set of circumstances may impact REE. Moreover, the mechanism(s) driving REE may be saturable, such that derived effects may be limited in terms of overall gene expression, as well as gene-by-gene and gene-by-diet interactions. Additionally, assessments of metabolites integral to calcium handling (i.e., parathyroid hormone, circulating vitamin D metabolites, calcitonin) were not available in this sample.

Future studies of longitudinal design utilizing hormones involved in calcium regulation should facilitate further understanding the relationships described herein.

In conclusion, our findings suggest that the VDR *Cdx-2* and CASR *A986S* variants are associated with REE in early pubertal children, and the mechanisms underlying these associations are influenced by genetic and environmental variables. Association with REE is likely adiposity- and ethnic-specific for VDR genotype, whereas calcium intake seems to influence the association of REE and CASR genotype. Metabolically active processes associated with reproductive onset may impact genes involved in calcium regulation, reflected by differential findings of associations with REE. Further studies are warranted to facilitate reliable extrapolation of gene involvement in calcium regulation on REE during pre-puberty to larger populations.

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Table 1Sample characteristics (overall and by sex and ethnicity; mean $\pm$ SE)

Variable	Overall (n=273)	Boys(n=142)	Girls (n=131)	EA (n=108)	AA (n=87)	HA (n=78)
Age (yr)	9.56 $\pm$ 0.09	9.78 $\pm$ 0.13 ^a	9.31 $\pm$ 0.013 ^b	9.60 $\pm$ 0.15	9.62 $\pm$ 0.16	9.43 $\pm$ 0.18
Pubertal stage	1.49 $\pm$ 0.04	1.38 $\pm$ 0.05 ^a	1.62 $\pm$ 0.07 ^b	1.35 $\pm$ 0.06 ^a	1.76 $\pm$ 0.09 ^b	1.40 $\pm$ 0.07 ^a
Height (in)	54.9 $\pm$ 0.2	55.2 $\pm$ 0.3	54.6 $\pm$ 0.4	55.02 $\pm$ 0.39 ^{ab}	55.57 $\pm$ 0.41 ^a	54.06 $\pm$ 0.46 ^b
Weight (kg)	36.8 $\pm$ 0.6	37.3 $\pm$ 0.8	36.1 $\pm$ 0.7	35.35 $\pm$ 0.81	37.69 $\pm$ 1.04	37.67 $\pm$ 1.10
BMI percentile	66.8 $\pm$ 1.5	66.8 $\pm$ 2.1	66.8 $\pm$ 2.2	66.6 $\pm$ 2.5 ^a	64.6 $\pm$ 2.9 ^a	77.9 $\pm$ 2.1 ^b
Total % Fat	23.5 $\pm$ 0.6	21.1 $\pm$ 0.8 ^a	26.2 $\pm$ 0.7 ^b	22.3 $\pm$ 0.8 ^a	20.8 $\pm$ 1.0 ^a	28.0 $\pm$ 0.9 ^b
Fat mass (kg)	9.0 $\pm$.3	8.4 $\pm$ 0.5 ^a	9.6 $\pm$ 0.4 ^b	8.13 $\pm$ 4.7 ^a	8.38 $\pm$ 6.7 ^b	10.84 $\pm$ 6.4 ^a
BMC (g)	1287.4 $\pm$ 18.9	1319.3 $\pm$ 25.7 [*]	* 1252.0 $\pm$ 27.6 [*]	1230.0 $\pm$ 25.9 ^a	1395.8 $\pm$ 38.0 ^b	1248.6 $\pm$ 33.1 ^a
Lean mass (kg)	25.7 $\pm$ 0.3	26.7 $\pm$ 0.4 ^a	24.5 $\pm$ 0.4 ^b	25.3 $\pm$ 0.5 ^a	27.2 $\pm$ 0.6 ^b	24.8 $\pm$ 0.6 ^a
European admixture	0.54 $\pm$ 0.02	0.54 $\pm$ 0.03	0.53 $\pm$ 0.03	0.96 $\pm$ <.01 ^a	0.14 $\pm$ 0.01 ^b	0.39 $\pm$ 0.02 ^c
Fasting insulin	12.6 $\pm$ 0.4	11.7 $\pm$ 0.5 ^a	13.6 $\pm$ 0.6 ^b	10.7 $\pm$ 0.4 ^a	12.9 $\pm$ 0.6 ^b	14.7 $\pm$ 0.9 ^b
REE (kcal/d)	1192.7 $\pm$ 13.7	1239.8 $\pm$ 19.8 ^a	1140.9 $\pm$ 17.8 ^b	1181.7 $\pm$ 22.3	1190.2 $\pm$ 21.4	1210.7 $\pm$ 27.9
Calcium intake (mg/d) [†]	860 $\pm$ 19	862 $\pm$ 24	855 $\pm$ 25	874 $\pm$ 26 ^a	745 $\pm$ 28 ^b	968 $\pm$ 30 ^c
Energy intake (kcal/d)	1898 $\pm$ 27	1956 $\pm$ 37 ^a	1835 $\pm$ 39 ^b	1880 $\pm$ 43	1891 $\pm$ 47	1932 $\pm$ 50

EA=European American AA=African American, HA=Hispanic American, BMC=bone mineral content, REE=resting energy expenditure

^{a,b,c} p<.05 for difference among groups,^{*} p=0.07 for difference between groups,[†] Controlled for overall energy intake

Table 2

Allele and genotype frequency of Vitamin D receptor (VDR) and calcium-sensing receptor (CASR) polymorphisms in the total sample and according to sex and ethnicity

	Overall (n=273)	Boys (n=145)	Girls (n=128)	EA (n=108)	AA (n=87)	HA (n=78)
Genotype frequency						
VDR						
A/A	67 (22.6%)	26 (9.5%)	33 (12.0%)	8 (13.6%)	50 (84.8%)	1 (1.7%)
G/G	133 (44.8%)	68 (24.7%)	58 (21.1%)	67 (53.2%)	5 (4.0%)	54 (42.9)
CASR						
A/A	1 (0.3%)	0 (0%)	1 (0.3%)	1 (100%)	0 (0%)	0 (0%)
C/C	244 (82.7%)	121 (44.3%)	107 (39.2%)	78 (34.2%)	81 (35.5%)	69 (30.3%)
Allele frequency						
VDR						
A	38.9%	35.6%	40.3%	22.9% ^a	75.9% ^b	16.5% ^a
CASR						
A	8.8%	9.3%	8.6%	14.4% ^a	3.4% ^b	5.8% ^b

EA=European Americans, AA=African Americans, HA=Hispanic Americans;

VDR=Vitamin D receptor, CASR=Calcium-sensing receptor

^{a,b} p<0.05 for difference between groups;

Table 3

Association between VDR recessive genotype and resting energy expenditure (REE)

Model [†]	Group	N	PE	p-value
By Ethnicity	EA	97	-0.060	0.0679
	AA	77	-0.111	0.0425
	HA	78	-0.037	0.2836
By Adiposity [‡]	Normal	172	-0.039	0.0481
	Excess	76	0.029	0.5174

PE=Parameter Estimate;

*A/A and A/G genotypes coded as 0 and G/G genotype coded as 1

[†] controNed for sex, pubertal stage, European admixture, fasting insulin, fat mass index (fat in kg divided by height in m²), lean mass, and calcium[‡] normal: <25% for males and <30% for females

Table 4Association between CASR recessive genotype^{*} and resting energy expenditure (REE)

Model [†]	Group	N	β	p-value
Overall		261	-0.011	0.6273
By Median Calcium [‡]	Low	129	0.023	0.4310
	High	134	-0.076	0.0578

^{*} A/A and A/C genotypes coded as 0 and C/C genotype coded as 1

[†] controlled for sex, pubertal stage, European admixture, fat mass index (fat in kg divided by height in m²), lean mass, and calcium intake;

[‡] < / 838mg, model also controlled for overall energy intake