



Do hormonal indices of maturation explain energy expenditure differences in African American and Caucasian prepubertal children?

M Sun¹, BA Gower¹, TR Nagy¹, AA Bartolucci¹ and MI Goran^{1*}

¹Division of Physiology and Metabolism, Department of Nutrition Sciences and Department of Biostatistics, University of Alabama at Birmingham, Birmingham, AL, USA

OBJECTIVE: To examine the relationships between hormonal indices of maturation and total, resting and physical activity-related energy expenditure (TEE, REE and AEE) in African American and Caucasian prepubertal children.

DESIGN: Cross-sectional study.

SUBJECTS: Sixty-four African American and 48 Caucasian prepubertal children.

MEASUREMENTS: TEE (by doubly labeled water), REE (by indirect calorimetry), fat mass and fat-free mass (by dual-energy X-ray absorptiometry), fasting serum dehydroepiandrosterone-sulfate (DHEAS), androstenedione, and estrone-sulfate (by radioimmunoassay).

RESULTS: Serum concentrations of hormones correlated significantly with REE and TEE (r values range from 0.33 to 0.76, $P < 0.001$). Only androstenedione correlated significantly with AEE ($r = 0.23$, $P < 0.05$). However, these correlations were no longer significant after adjusting energy expenditure components for fat-free mass. In multiple regression models, ethnicity was not a significant determinant of any energy expenditure component after adjusting for body composition and hormone concentrations.

CONCLUSION: Hormonal indices of maturation do not influence energy expenditure in this group of African American and Caucasian prepubertal children.

Keywords: energy expenditure; hormones; ethnicity; pubertal maturation

Introduction

Previous studies have shown a lower resting energy expenditure in African Americans compared with Caucasians. These findings have been shown in children,^{1–4} adult women,^{5,6} and the elderly.⁷ However, a previous study from our laboratory in prepubertal children did not observe differences in total, resting or physical activity-related energy expenditure between African Americans and Caucasians, after adjusting for gender and body composition.⁸

One possible explanation for inconsistent findings among studies is potential differences in the maturation status of children. Maturation may influence energy expenditure through its related changes in fat-free mass⁹ or effects on hormones.^{10,11} At age 3, 7, 8, 9 and 10, African American children are more mature.^{12,13} Therefore, differences between studies regarding ethnic difference in energy expenditure could be due to population differences in maturation, which may obscure the actual relationship between ethnicity and energy expenditure previously observed. However, after adjusting for pubertal stage (defined

by physical examination) and lean body mass, Morrison *et al*² still observed a lower resting energy expenditure in African American than in Caucasian girls (6–16 y). Therefore, we hypothesized that there could be an ethnic difference in energy expenditure after adjusting for differences in maturation in our prepubertal cohort.

Because the rate of maturation differs between African American and Caucasian children, differences between studies in the presence or absence of an ethnic difference in resting energy expenditure may be due to differences in the concentrations of circulating hormones that increase with maturation. Our previous study⁸ was conducted in prepubertal children, as defined by physical examination (Tanner stage 1). Even within stages of maturation defined by physical examination, there may be more subtle differences in maturation, which could be reflected by differences in hormones including dehydroepiandrosterone-sulfate (DHEAS) and androstenedione.^{14,15}

The androgens DHEAS and androstenedione have been shown to be positively correlated with total energy expenditure in adult women.^{10,11} The relationships between androgen concentrations and components of energy expenditure have never been examined in children. Estradiol has been shown to increase energy expenditure in rats.¹⁶ However, the relationship of estradiol and energy expenditure has never been examined in humans. Estrone-sulfate, the

*Correspondence: MI Goran, Division of Physiology and Metabolism, Department of Nutrition Sciences, University of Alabama, Birmingham, AL 35294, USA. E-mail: MIG@uab.edu.
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form of estrogen that circulates in the highest concentration, could be locally converted to estradiol, which is undetectable in the systemic circulation in prepubertal children. Therefore, estrone-sulfate may serve as a marker for estrogen status in prepubertal children. In addition, adrenal androgens could be converted to estrogens. Because of wide ranges of hormone concentrations in prepubertal children,¹⁷ the influence of hormones on energy expenditure needs to be further examined when considering ethnic differences in energy expenditure. We hypothesized that adrenal androgens and estrogen are positively associated with energy expenditure in prepubertal children and could possibly explain reported ethnic differences in energy expenditure. The main purpose of this study is to examine whether concentrations of hormones are related to total, resting and activity-related energy expenditure after adjustment for body composition in African American and Caucasian prepubertal children.

Methods

Subjects

Our study included 48 Caucasian children (30 boys and 18 girls) and 64 African American children (34 boys and 30 girls) from Birmingham, Alabama. The children were recruited using newspaper advertisements and by word of mouth. Children included in this study were at prepubertal stage based on breast stage and pubic hair development in girls¹⁸ and genitalia development in boys,¹⁹ according to the criteria of Tanner. We further confirmed prepubertal status by absence of detectable concentrations of testosterone and estradiol. Two subjects had marginally detectable estradiol concentrations of 4.90 and 4.96 pg/ml and six subjects had low testosterone concentrations ranging from 12.0 to 21.9 ng/dl. These hormone values are below those associated with pubertal maturation.^{20,21} Ethnicity was defined based on the self-ascribed ethnicity status of children's parents and grandparents derived by questionnaire. The eligibility criteria were discussed in detail in a previous paper.⁸ We have also previously reported energy expenditure,⁸ body composition,²² and aerobic fitness²³ data in these children. The nature, purpose and possible risks of the study were carefully explained to the parents before obtaining consent. This study was approved by the Institutional Review Board at the University of Alabama at Birmingham, and parents provided informed consent before testing commenced. All measurements were performed at the General Clinical Research Center (GCRC) or in The Department of Nutrition Sciences at the University of Alabama at Birmingham between 1994 and 1997.

General outline of protocol

Children were admitted to the GCRC in the late afternoon for an overnight visit. Upon arrival, a baseline

urine sample was collected, and subjects were dosed with the doubly labeled water. Anthropometric measurements were obtained, and dinner was served (~17:00 h). An evening snack was allowed, and after 20:00 h only water and energy-free, non-caffeinated beverages were permitted until after the morning testing. On the following morning after the overnight fast, resting metabolic rate was assessed by indirect calorimetry upon awakening at 5 a.m. The subjects were allowed to urinate if necessary. If the subjects urinated, they were asked to rest for 15 min before resting metabolic rate measurement was taken. At 7 a.m., blood samples were collected for hormone analyses. Two times urine samples were collected for the doubly labeled water analysis. Two weeks later, the children arrived at the Department of Nutrition Sciences at 07:00 h in the fasted state and body composition was determined by dual-energy X-ray absorptiometry (DXA). Two additional timed urine samples were collected for the doubly labeled water analysis.

Measurement of energy expenditure components

Total energy expenditure was measured over 14 days under free-living conditions with the doubly labeled water technique, using a protocol with a theoretical error of less than 5%, as previously described.²⁴ Samples were analyzed in triplicate for H₂¹⁸O and ²H₂O by isotope ratio mass spectrometry at the University of Alabama at Birmingham as previously described.²⁴ The mean dilution space ratios (DSR) were not significantly different between gender or ethnic groups. CO₂ production rate was determined using equation (R2) of Speakman *et al*²⁵ and assuming a fixed DSR of 1.0427; energy expenditure was calculated using equation (12) of De V. Weir,²⁶ and the mean value for the food quotient of the children's diet (0.90 in Caucasian and 0.87 in African American) was determined by 24 h recall performed in duplicate.

Resting energy expenditure was measured in the early morning after an overnight fast in the GCRC using a Deltatrac Metabolic Monitor (Sensormedics Corp., Yorba Linda, CA). During testing, all subjects were instructed to lie as still as possible and remain awake. An adult-size canopy hood was used to collect the expired air. After a 10-min equilibration period, data on oxygen consumption and carbon dioxide production were collected continuously for 20 min. Energy expenditure was calculated using the equation (12) of De V. Weir.²⁶

Physical activity-related energy expenditure was estimated from the difference between total and resting energy expenditure after reducing total energy expenditure of 10% to account for the thermic effect of feeding. Seventy-six subjects had complete analyses for total and activity-related energy expenditure.

Assessment of body composition and anthropometry

Total and regional body compositions were measured by DXA using a Lunar DPX-L densitometer that we

have previously validated in the pediatric body weight range.^{27,28} Subjects were scanned in light clothing while lying flat on their backs with arms by their sides. DXA scans were performed and analyzed using pediatric software (version 1.5e), as previously described.^{27,28} On the day of each test, the DPX-L was calibrated using the procedures provided by the manufacturer. Height was measured without shoes using a stadiometer. Weight was measured in light clothing on an electronic scale.

Hormone assays

After overnight fasting, three samples of blood were collected over a period of 40 min. The blood samples were centrifuged at 1800 *g* for 10 min at 4°C. Sera were separated, pooled and placed in cryovials, and all samples were stored at -85°C until assayed for hormones. DHEAS, androstenedione and estrone-sulfate were analyzed in duplicate by radioimmunoassay and estradiol was analyzed in duplicate by double antibody radioimmunoassay (Diagnostic System Laboratories Inc., Webster, TX). Testosterone was analyzed in duplicate by radioimmunoassay (Coat-A-Count Total Testosterone, Diagnostic Products Corporation, Los Angeles, CA). In our laboratory, the limit of detectability and intra-assay coefficients of variation (CV) are as follows: DHEAS, 2.3 µg/dl and 11.7% at 63% bound (23.5 µg/dl); androstenedione, 0.03 ng/ml and 11.0% at 55% bound (1.14 ng/ml); estrone-sulfate, 0.05 ng/ml and 6.2% at 62% bound (0.46 ng/ml); estradiol, 4.2 pg/ml and 3.6% at 51% bound (52.0 pg/ml); and testosterone, 11.8 ng/dl and 2.7% at 62% bound (119.3 ng/dl). The mean inter-assay coefficients of variation are as follows: DHEAS, 10% at 54% bound (36.0 µg/dl), 9.1% at 26% bound (191.1 µg/dl), and 2.5% at 13% bound (630.0 µg/dl); androstenedione, 7.1% at 53% bound (1.05 ng/ml) and 5.1% at 19% bound (5.98 ng/ml); estrone-sulfate, 2.7% at 58% bound (0.59 ng/ml); estradiol, 7.2% at 48% bound (60.3 pg/ml) and 3.1% at 31% bound (159.1 pg/ml); and testosterone, 11.4% at 70% bound (65.4 ng/dl), 6.8% at 40% bound (344.4 ng/dl), and 7.5% at 30% bound (634.5 ng/dl).

Statistics

Differences in physical characteristics, body composition, energy expenditure, and serum concentrations of hormones among African American and Caucasian boys and girls were examined using a two-way (gender and ethnic groups) analysis of variance (ANOVA). Student *t*-tests were used to explore possible interaction effects between gender and ethnicity. Fat mass and hormone variables were log transformed in order to achieve normality. The correlations between energy expenditure variables (total, resting and activity-related energy expenditure) and hormone concentrations were examined with Pearson correlation analysis. A first-order partial correlation was conducted for energy expenditure and hormone con-

centrations after controlling for fat-free mass. Pearson correlations among hormone variables were also examined. Multicollinearity was tested by Pearson correlation analysis for all the independent variables ($r < 0.95$) before proceeding with multiple regression analyses. In multiple regression models, each component of energy expenditure was first examined as a dependent variable with fat-free mass, ethnicity and gender as independent variables. Serum concentrations of DHEAS, androstenedione, and estrone-sulfate were then included in each model. Data were analyzed using SAS software version 6.10 (Carey, NC), with a significance level set at $P < 0.05$ for all tests.

Results

Subject characteristics

The characteristics of the children, including energy expenditure components and hormone concentrations, are presented in Table 1. Caucasian children were older than African American children ($P = 0.01$). The girls had greater fat mass ($P = 0.03$). There were significant interaction effects between gender and ethnicity for fat-free mass ($P = 0.01$) and resting energy expenditure ($P = 0.03$). This indicates that the gender differences for fat-free mass and resting energy expenditure were not consistent for Caucasians and African Americans, and ethnic differences for fat-free mass and resting energy expenditure were not consistent for boys and girls. Student *t*-tests (data not shown) showed that gender difference in fat-free mass was significant for African Americans ($P = 0.003$), but not for Caucasians ($P = 0.5$). Ethnic differences in fat-free mass was significant for girls ($P = 0.04$), but not for boys ($P = 0.4$). The same pattern was observed for resting energy expenditure (data not shown). There was no significant effect of gender or ethnicity in serum concentrations of DHEAS, androstenedione and estrone-sulfate. This effect remained insignificant after adjusting for age (data not shown).

Correlations

Pearson correlation and first-order partial correlation coefficients for energy expenditure and hormones with and without adjustment for fat-free mass are presented in Table 2. Resting energy expenditure was significantly correlated with serum concentrations of DHEAS ($r = 0.50$, $P = 0.0001$), androstenedione ($r = 0.33$, $P = 0.0005$), and estrone-sulfate ($r = 0.50$, $P = 0.0001$). Total energy expenditure was also significantly correlated with serum concentrations of DHEAS ($r = 0.45$, $P = 0.0001$), androstenedione ($r = 0.41$, $P = 0.0001$), and estrone-sulfate ($r = 0.50$, $P = 0.0001$). Only androstenedione was significantly correlated with activity-related energy expenditure ($r = 0.23$, $P = 0.04$). After controlling for fat-free

Table 1 Subject characteristics, energy expenditure and hormone levels

	Caucasian boys (n = 30)	Caucasian girls (n = 18)	African American boys (n = 34)	African American girls (n = 30)	Two-way ANOVA (P value)
Age (y)	8.5 ± 1.4 (5.6, 11.0)	8.7 ± 1.4 (5.9, 11.0)	8.0 ± 1.6 (5.1, 10.9)	7.7 ± 1.6 (4.7, 10.2)	Ethnicity (0.01), gender (NS)
Weight (kg)	34.4 ± 10.6 (17.0, 63.9)	40.5 ± 17.4 (22.2, 84.4)	34.9 ± 12.4 (19.8, 68.4)	31.6 ± 11.2 (14.1, 58.0)	NS
Height (cm)	132.1 ± 10.8 (110, 156)	133.7 ± 12.7 (117.0, 162.5)	133.0 ± 10.6 (113.0, 153.0)	128.4 ± 12.1 (104.8, 155.0)	NS
Body mass index (kg/cm ²)	19.3 ± 3.7 (13.9, 26.2)	19.2 ± 4.2 (11.8, 29.2)	21.8 ± 5.2 (15.1, 32.0)	18.7 ± 4.3 (12.8, 28.3)	NS
Fat free mass (kg)	22.4 ± 4.7 (12.4, 35.6)	23.6 ± 6.9 (14.5, 41.3)	23.5 ± 5.3 (14.6, 35.5)	19.6 ± 4.6 (10.6, 29.1)	Ethnicity, gender (NS), interaction (0.01)
Fat mass (kg)	9.4 ± 6.2 (2.6, 24.0)	14.8 ± 10.7 (4.2, 44.7)	9.6 ± 7.3 (1.8, 30.8)	10.5 ± 7.0 (1.9, 26.3)	Ethnicity, interaction (NS), gender (0.03)
Total energy expenditure (kcal/d)	1697 ± 408 (1178, 2975)	1853 ± 418 (1235, 2393)	1741 ± 385 (990, 2721)	1680 ± 445 (940, 2585)	NS
Resting energy expenditure (kcal/d)	1335 ± 243 (919, 1915)	1351 ± 252 (1027, 2088)	1343 ± 282 (930, 2116)	1156 ± 191 (869, 1610)	Ethnicity (0.05), gender (NS), interaction (0.03)
Activity-related energy expenditure (kcal/d)	289 ± 324 (-304, 1216)	330 ± 283 (55, 784)	312 ± 238 (-213, 744)	353 ± 247 (-23, 1007)	NS
DHEAS (µg/dl)	44.9 ± 40.0 (5.6, 178.5)	45.9 ± 31.7 (10.0, 110.8)	53.5 ± 39.3 (5.9, 178.4)	37.7 ± 28.5 (5.6, 110.2)	NS
Androstenedione (ng/ml)	0.5 ± 0.4 (0.04, 1.5)	0.6 ± 0.3 (0.1, 1.2)	0.6 ± 0.3 (0.1, 1.5)	0.6 ± 0.4 (0.1, 1.5)	NS
Estrone-sulfate (ng/ml)	0.5 ± 0.3 (0.1, 1.7)	0.6 ± 0.4 (0.1, 1.5)	0.5 ± 0.3 (0.1, 1.1)	0.5 ± 0.3 (0.2, 1.3)	NS

Data are mean ± standard deviation with range in parentheses. NS, not significant.

mass, all these correlations became non-significant (Table 2). DHEAS, androstenedione and estrone-sulfate were significantly correlated with each other ($P = 0.0001$, not shown).

Associations between hormones and energy expenditure

The relationships between hormones and components of energy expenditure are presented in Table 3. There was no ethnic difference in any component of energy expenditure, after adjusting for hormone concentrations and body composition. Serum concentrations of DHEAS, androstenedione and estrone-sulfate were not independently related to any component of energy expenditure after adjusting for ethnicity, gender, fat-free mass and fat mass.

Discussion

In our current study, we did not observe ethnic differences in any component of energy expenditure

in African American and Caucasian prepubertal children, before or after adjusting for concentrations of hormones and body composition. Therefore, unlike in other published studies, energy expenditure does not differ with ethnicity in our group of children.

Maturation may be related to changes of energy expenditure through changes in the quality and composition of fat-free mass.⁹ Therefore, it is conceivable that subtle and continuous changes in maturation, not detected by physical examination, may influence the quality of fat-free mass and thereby energy expenditure. In this study, fat-free mass explained the largest portion of the variance in energy expenditure. Hormones had strong correlations with energy expenditure ($P < 0.001$); however, after adjusting for fat-free mass, all significant associations disappeared. These findings indicate that the possible association between energy expenditure and ethnicity, if influenced by hormonal indices, could be potentially masked by fat-free mass. In contrast to the present results, previous studies in adult women have shown positive associations between DHEAS and androstenedione and energy expenditure, after adjusting for fat-free

Table 2 Pearson and first-order correlation coefficients for energy expenditure components and hormones^a with and without adjustment for fat-free mass

(n = 112)	Resting energy expenditure (n = 112)		Total energy expenditure (n = 76)		Activity-related energy expenditure (n = 76)	
	r ^b	Partial ^c	r	Partial r	r	Partial r
Fat-free mass	0.76***		0.72***		0.33**	
DHEAS	0.50***	-0.10	0.45***	-0.06	0.19	-0.05
Androstenedione	0.33***	-0.06	0.41***	0.06	0.23*	0.07
Estrone-sulfate	0.56***	-0.02	0.50***	0.05	0.21	-0.02

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. ^aAll hormone variables are log transformed. ^br is the correlation coefficient. ^cPartial r is the correlation coefficient after adjusting for fat-free mass.

Table 3 Multiple regression models of the relationship between components of energy expenditure and ethnicity, gender, fat-free mass, fat mass and various hormones

	Independent variables		
	REE	TEE	AEE
Intercept	941.7 ± 217.7***	1031.7 ± 570.3	-105.8 ± 529.4
Ethnicity ^a	-27.7 ± 24.4	33.3 ± 65.4	54.9 ± 60.7
Gender ^b	-100.9 ± 27.5***	-124.2 ± 82.6	-40.3 ± 76.7
Fat-free mass (kg)	26.4 ± 3.8***	37.0 ± 3.8*	8.1 ± 10.9
Log(fat mass) (kg)	292.0 ± 57.5***	619.5 ± 196.2**	340.0 ± 182.1
Log (DHEAS) (µg/dl)	-151.2 ± 93.6	-254.3 ± 242.6	-53.1 ± 225.2
Log(androstenedione) (ng/ml)	32.9 ± 55.5	183.5 ± 144.5	133.4 ± 134.1
Log(estrone-sulfate) (ng/ml)	197.3 ± 118.7	175.7 ± 320.9	-122.4 ± 297.9
Model R ² (P value)	0.76 (0.0001)	0.55 (0.0001)	0.10 (0.05)

Data are expressed as parameter estimate ± s.e.. TEE, total energy expenditure; REE, resting energy expenditure; AEE, activity-related energy expenditure. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Fat mass and hormone variables are log transformed. ^aEthnicity is coded as 1 = Caucasian, 2 = African-American. ^bGender is coded as 1 = boys, 2 = girls.

mass.^{10,11} In the present study, even after obese children (defined as $\geq 30\%$ body fat) were excluded from the analyses, there was no significant ethnic difference in any component of energy expenditure after adjusting for body composition and hormone concentrations (data not shown). The reason we did not detect an ethnic difference in any component of energy expenditure or an independent association between hormone concentration and energy expenditure might have been due to the low concentrations of hormones in prepubertal children. Therefore, future longitudinal studies are warranted to examine the associations between changes in energy expenditure, hormone concentrations and body composition.

One of the reasons that hormones need to be considered when examining energy expenditure in children is the timing difference of maturation between African American and Caucasian children. Both African American boys and girls were reported to enter puberty earlier than their Caucasian counterparts.¹⁴ In a pediatric office setting,¹² 27.2% African American and 6.7% Caucasian girls had evidence of pubertal development at age 7; 48.3% African Americans and 14.7% Caucasians had breast and pubic hair development at age 8. On average, African American girls began puberty at 8–9 y old, about 1–2 y earlier than did Caucasian girls.

In the prepubertal stage, the ethnic difference in maturation may be apparent in different adrenal hormone concentrations in African American compared with Caucasian children. If African American children are more mature at any given age, higher concentrations of hormones may be expected in African American than Caucasian prepubertal children. A previous study of children through puberty found estradiol was significantly higher in African American than Caucasian boys after adjusting for pubertal stage, age, height, weight and ponderosity index (weight/height³).¹⁴ However, androstenedione concentrations were found to be 29.4% and 20% lower in African American boys and girls than Caucasian counterparts, respectively.¹⁴ Because changes in fat-

free mass and the association between changes of maturation stage and body composition were not considered in this earlier study,¹⁴ we cannot rule out the possibility of higher hormone concentrations in African American children. No differences in concentration of DHEAS, androstenedione or estrone-sulfate between African American and Caucasian boys and girls were found, even after adjusting for age (data not shown). Therefore, present results suggest that more advanced maturation may not necessarily be reflected in different or higher hormone concentrations in African American prepubertal children.

DHEAS and androstenedione were examined because they are strong hormone correlates of pubertal development.^{14,15} The relationship between these hormones and energy expenditure, particularly with respect to potential ethnic differences, has not been previously examined in children. DHEAS was positively correlated with resting energy expenditure ($P < 0.01$) in women.¹¹ Androstenedione explained 4% of the variance in sleeping energy expenditure,¹¹ and 3% of the variance in resting energy expenditure after adjusting for body composition in women.¹⁰ These results suggest that androgens may affect processes that ultimately increase energy expenditure. However, this effect is not shown in this group of prepubertal children. The absence of association between total or resting energy expenditure and hormone concentration cannot be explained by an insufficient sample size. Power analyses (SPSS Sample Power 1.0, New York, 1997) show that our multiple regression models had sufficient power to detect 99% of the variance, which is more than the observed variance in total (55%) and resting energy expenditure (76%) given the sample size and number of variables. However, the absence of association between activity-related energy expenditure and hormone concentrations may have suffered from an insufficient sample size ($n = 76$, $r^2 = 0.49$).

The mechanism of the influence of adrenal hormone action on energy expenditure observed previously is not well understood. Previous studies have

shown that testosterone has a protein anabolic effect.^{29,30} Testosterone deficiency is related to decreased whole body protein anabolism and reduced resting energy expenditure, which results from decreased lipid oxidation and fat-free mass in males.²⁹ On the other hand, testosterone administration has been shown to increase muscle mass and muscle protein synthesis in prepubertal boys.³⁰ These data suggest that adrenal androgens, such as DHEAS and androstenedione, may be related to growth and deposition of lean mass, which may in turn influence energy expenditure.

Another possible explanation for ethnic differences in energy expenditure is socioeconomic status (SES). Lower SES has been hypothesized to be associated with psychosocial stress,³¹ which is associated with elevated cortisol concentrations.³² In our study, African American children had significantly lower SES status than Caucasian children (unpublished data). However, in multi-variate analyses, SES was not related to any component of energy expenditure. The lack of association between SES and energy expenditure may have suffered from an insufficient power due to the small sample size ($n = 44$) in SES multiple regression models.

Conclusions

Hormonal indices of maturation did not influence energy expenditure or the relationship between ethnicity and energy expenditure in this group of prepubertal African American and Caucasian children. Ethnic differences in hormone concentrations may be more apparent when children go through puberty. Future longitudinal investigation on maturation-related changes in energy expenditure is warranted.

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