

# Energy expenditure and substrate oxidation predict changes in body fat in children<sup>1-3</sup>

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## ABSTRACT

**Background:** The hypothesis that alterations in energy metabolism predict body fat gain is controversial.

**Objective:** The aim of this study was to determine which components of energy metabolism were most important in predicting fat gain in children aged  $10.8 \pm 0.6$  y.

**Design:** A 2-y longitudinal study to examine whether components of energy metabolism are predictors of body fat gain was conducted in 114 preadolescent African American and white children aged 9–11 y by measuring total daily energy expenditure on the basis of doubly labeled water (DLW), resting metabolic rate, the thermic effect of food, energy expended in physical activity, and substrate oxidation after a meal. The primary endpoint was the 2-y change in percentage body fat (%BF).

**Results:** Individual variables of energy metabolism predicted up to 7% of the variance in changes in %BF over the 2-y interval in the whole group. Predictors of change in body fatness tended to be sex and race specific. Protein oxidation during a test meal explained a significant portion of the variance in change in %BF in the overall group and in nearly all of the subgroups. Multivariate prediction models accounted for 10–41% of the variance in change in %BF. Tanner stage at 2-y follow-up was highly predictive of change in body fatness and improved the overall prediction, accounting for 24–62% of the variance in change in %BF in those groups in which Tanner entered the model.

**Conclusion:** This study provides evidence that total daily energy expenditure, resting metabolic rate, substrate oxidation, and total energy intake are predictors of gain in body fatness during late childhood in boys and girls. *Am J Clin Nutr* 2006;84:862–70.

**KEY WORDS** Metabolic rate, ethnicity, boys, girls, thermic effect of food, respiratory quotient

## INTRODUCTION

Obesity in early childhood is a predictor of obesity in adolescence and adulthood (1–5). In addition, childhood obesity predicts insulin resistance and total and HDL cholesterol in young adulthood (3). These findings, combined with the increasing prevalence of childhood obesity (6, 7), provide an impetus for identifying the predictors of weight gain in childhood so as to help prevent childhood and adult obesity and associated diseases. Although childhood obesity significantly tracks into adult obesity, 30–60% of obese children do not remain obese, so it is important to study obese children to examine predictors of loss in percentage body fat (%BF), rather than just gain in %BF.

Studies in Pima Indians have shown that a low resting metabolic rate (RMR), a low spontaneous physical activity, and a high 24-h respiratory quotient (RQ) predict weight gain over 2–3 y (8, 9). In one study, low energy expenditure (EE) predicted weight gain in infants (10), but other investigators did not confirm that finding (11). On the other hand, physical activity, but not total daily EE (TDEE) of 9–12-mo-old infants was related to skinfold thickness at 2 y of age (12). In an early study (13), low EE in children predicted the development of obesity, but, in a more recent, 4-y, longitudinal study in 75 white preadolescent children (14), no relation was found between any measure of total EE and change in fat mass. In contrast to the ambiguity of data on resting or total EE and fat gain, several studies indicated that reduced physical activity may predict fat gain in children (2, 15, 16). In addition, a lower thermic effect of food (TEF) has been observed in obese women than in nonobese women (17).

Thus, the question of whether reduced EE is a predictor of weight gain in infants and children is unresolved. The current investigation was designed to test the hypothesis that component(s) of energy metabolism predict change in body fatness in children as they go through the pubertal growth spurt. This report presents data on the relation of initial energy metabolism to body fat gain over a 2-y period.

## SUBJECTS AND METHODS

### Subjects

The baseline data for the 131 children enrolled in the Baton Rouge Children's (BAROC) Study were described previously (18). To enroll in the study, children had to be healthy and not taking any medication that would affect growth or energy metabolism. In addition to these criteria, we enrolled equal numbers of black and white children, girls and boys, and lean and obese children. The children also agreed to participate in all aspects of the study, including a 2-y follow-up study. After 2 y ( $2.00 \pm 0.05$  y), the children participated in follow-up studies. Information regarding the study was sent to the parents, along with new

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consent forms. Of the initial 131 children, 114 completed the follow-up examinations (19, 20).

Each child who met the selection criteria was provided detailed information about the study, and a parent or guardian and the child gave written informed consent for the longitudinal study. The study was approved by the Louisiana State University Institutional Review Board.

### Protocol

To initiate the protocol, the child came to the Pennington Center on a weekend for measurement of body composition and familiarization with the RMR procedure. All other testing was performed in a mobile metabolic laboratory at the child's school (18).

Within a few days of body-composition measurements, after resting quietly for 30 min, children underwent a 30-min measurement of RMR, which was followed by the ingestion of a meal and then a 3-h measurement of metabolic rate. Within 5 d of the RMR measurements, children were dosed with doubly labeled water (DLW;  $^2\text{H}_2^{18}\text{O}$ ) for measurement of TDEE (18).

### Doubly labeled water measurement of total energy expenditure

During the week when RMR was measured, a urine sample was collected after an overnight fast to measure baseline isotopic abundance. Children then drank a dose of DLW containing 0.3 g  $\text{H}_2^{18}\text{O}$ /kg total body water (TBW) and 0.14 g  $^2\text{H}_2\text{O}$ /kg TBW. The container was washed with an additional 50 mL tap water, which was also given to the subject to drink. Saliva samples were taken at 2 and 3 h for TBW measurements (21). Morning urine samples were obtained on days 1, 8, and 9 after administration of the dose. The  $^{18}\text{O}$  and  $^2\text{H}$  isotope abundances were measured as described previously (22). The mean daily carbon dioxide production ( $\text{rCO}_2$ ; in mol/d) was obtained from isotope elimination rates calculated by using regression analysis of all points and from updated isotope dilution space ratios (23). EE was calculated by multiplying  $\text{rCO}_2$  by the energy equivalent of carbon dioxide for an estimated RQ of 0.86, which corresponds to a food quotient equivalent to a diet with 35% of energy as fat. In a double-blind study of between-laboratory variability in the DLW measurement of TDEE, our laboratory performed very well in isotope analyses and was 1 of only 4 labs to obtain a difference in oxygen and deuterium elimination rates within 2% of the theoretical value (*see* Code HSDL in Table 2, reference 24).

### Body composition

The %BF used for all predictions was obtained from the average of 3 independent measures of body fat, dual-energy X-ray absorptiometry (DXA), underwater weighing, and determination of fat-free mass (FFM) by isotope dilution. In our hands, each of these methods was shown to perform well in relation to a 4-compartment model and to compare well with each other (20).

The DXA scans were performed by using a Hologic QDR-2000 whole-body scanner (Hologic, Waltham, MA) in array mode. Scans were analyzed with WHOLE BODY software (version 6.0; Hologic). The CV of repeat measures in 38 adults with this technique in our laboratory is 0.3%, 1.0%, 1.6%, and 1.5% for body weight, lean body mass, fat mass, and %BF, respectively. Underwater weight was measured while subjects wore a

bathing suit and by using a chair suspended on 4 "force cube" transducers similar to those of Akers and Buskirk (25). Residual lung volume was measured by using a helium dilution technique while the children were submerged in the tank (Sensormedics PFT; Sensormedics, Fullerton, CA). The precision of this procedure, as ascertained in 10 college-age students, was 2.6% for underwater weight and 5.2% for residual volume; the CV for the %BF measurement was 5.1%. As part of the DLW procedure, TBW was measured by using  $\text{H}_2^{18}\text{O}$  dilution. FFM was measured by using appropriate hydration values (20, 26), and %BF was calculated.

### Indirect calorimetry

RMR and TEF were measured at the children's schools in our mobile metabolic laboratory (18). Metabolic carts with canopies (Sensormedics 2900Z; Sensormedics, Yorba Linda, CA), were used to measure oxygen consumption and carbon dioxide production. Urine samples were collected during the measurement of the TEF for measurement of nitrogen by chemiluminescence by using a Model 703C pyrochemiluminescent nitrogen system (Antek Instruments Inc, Houston, TX), equipped with an automatic sample injector and a Spectra Physics computing integrator. The Weir equation was used to calculate metabolic rate (27).

Children arrived at the laboratory fasting and rested for 30 min before the hood was put on. After measurement of RMR for 30 min, each child emptied his or her bladder and received the meal consisting of Ensure (Ross Labs, Columbus, OH; 10.9% of energy as protein, 54.3% of energy as carbohydrate, and 34.9% of energy as fat) at 35% of each subject's measured RMR (28). Metabolic rate was measured for 3 h after completion of the meal. At the end of the 3-h period, a complete urine void was collected for measurement of urinary nitrogen. EE during the RMR measurement was subtracted from the EE after the meal to measure the TEF. Protein oxidation was calculated from urinary nitrogen excretion, and carbohydrate and fat oxidation were calculated from the nonprotein RQ (29). The energy expended in physical activity was calculated by subtracting RMR and TEF [ $(\text{TEF}\% / 100) \times \text{TDEE}$ ] from the TDEE.

### Statistical analysis

The primary outcome variable is the change in %BF over a 2-y period. The %BF was calculated for each of the 2 y as an average of 3 independent measures of body fat (ie, obtained by DXA, calculated from isotope dilution, and measured by underwater weighing). Of the 114 children, 2 did not undergo an underwater weighing measurement in year 1, and 2 did not undergo the measurement in year 2. For these children, body fat was calculated as the average of the remaining 2 independent measures of body fat. The criteria used to stratify the children as lean or obese during the initial examination were used for this analysis. Children were classified as lean if they had  $< 25\%$ BF (DXA) and obese if they had  $\geq 25\%$ BF (18).

As the first step, differences between groups (ie, sex, race, and body fat group) were investigated for all measures at baseline and changes from baseline. This was carried out by using an ANOVA containing all factors and their interactions up to the third order. For multiple comparisons, Tukey's adjustment was used.

The primary interest in the analysis was to establish the relation between the change in %BF and the measures of energy metabolism and anthropometric measures in year 1. All 114



**TABLE 1**  
Subject characteristics<sup>1</sup>

	Girls		Boys		Significance <sup>2</sup>
	Black	White	Black	White	
Baseline					
<i>n</i>	28	25	31	29	
Age (y)	10.7 ± 0.7	10.6 ± 0.4	10.9 ± 0.8	10.9 ± 0.6	—
Height (cm)	145.3 ± 7.1	143.2 ± 7.5	145.5 ± 7.0	147.3 ± 6.1	—
Weight (kg)	37.9 ± 9.1	39.4 ± 11.3	45.0 ± 12.2	42.6 ± 10.7	S
BMI (kg/m <sup>2</sup> )	17.9 ± 3.6	18.9 ± 4.0	21.0 ± 4.3	19.5 ± 3.7	S
Body fat (%)	24.8 ± 9.3	28.5 ± 9.5	27.0 ± 10.3	26.4 ± 9.2	R
Fat mass (kg)	10.0 ± 6.1	12.1 ± 7.7	13.2 ± 7.8	12.1 ± 7.1	—
FFM (kg)	27.9 ± 4.4	27.2 ± 4.4	31.8 ± 5.2	30.5 ± 4.1	—
TDEE (MJ/d)	9.13 ± 1.03	9.68 ± 1.47	10.76 ± 1.60	10.78 ± 1.38	S
RMR (MJ/d)	5.38 ± 1.01	5.55 ± 1.04	5.83 ± 0.94	6.42 ± 0.82	S, R
TEF (%)	7.0 ± 3.6	5.7 ± 2.8	6.9 ± 2.9	5.1 ± 3.3	R
EEA (MJ/d)	3.1 ± 0.7	3.6 ± 1.0	4.2 ± 1.2	3.9 ± 1.0	S, S × R interaction
PAL <sup>3</sup>	1.74 ± 0.32	1.77 ± 0.29	1.87 ± 0.26	1.69 ± 0.23	—
2-Y measures					
Tanner stage	3.79 ± 0.95	3.04 ± 0.96	3.27 ± 1.18	2.40 ± 0.94	S, R
Weight gain (kg)	10.7 ± 4.3	10.8 ± 4.7	12.8 ± 5.2	9.7 ± 6.1	—
Fat gain (kg)	2.6 ± 3.6	3.8 ± 3.3	3.5 ± 5.0	2.2 ± 5.0	—
FFM gain (kg)	8.1 ± 1.6	7.0 ± 2.3	9.2 ± 4.3	7.5 ± 4.3	R
Percentage change in fat (%)	-0.04 ± 5.04	1.18 ± 3.67	-0.19 ± 6.47	-0.76 ± 6.89	—

<sup>1</sup> All values are  $\bar{x} \pm SD$ . S, sex; R, race; FFM, fat-free mass; TDEE, total daily energy expenditure; RMR, resting metabolic rate; TEF, thermic effect of food; EEA, energy expended in physical activity; PAL, physical activity level. Analysis of variance conducted by using the full model including race, sex, and obesity group.

<sup>2</sup> No interaction terms were significant except EEA, for which the sex × race interaction was significant.

<sup>3</sup> PAL = TEE/RMR.

children had RMR and TDEE measures, but 3 children did not have TEF measures, and therefore, they did not have an EE for activity (EEA) measure in year 1. To provide data for the analysis that were as complete as possible, we imputed the missing TEF values for these 3 children as group means calculated as sex by race by body fat group means of the group to which each child belonged, so that EEA could be calculated.

To establish the associations between the change in %BF and both the anthropometric measures and measures of energy metabolism, simple correlation analysis was carried out. This was done for the whole sample as well as for the subgroups such as sex, race, body fat groups, and Tanner categories. In addition, regression analyses were performed to ascertain whether the slopes and intercepts were significantly different from 0 (independent tests and combined slope and intercept tests), and whether slopes were significantly different for subgroups.

Finally, with the use of a multiple regression analysis and the “all possible submodels” variable selection method ( $R^2$  option in SAS software, version 8.2), optimal linear prediction equations were created for all children as well as for subgroups. The full set of variables contained protein oxidation, RQ, RMR, TDEE, EEA, and TEF, all measured in year 1, and Tanner stage measured in year 2. The “best” model was the model that provided the highest  $R^2$  with the lowest mean square error. One restriction on the models was that TDEE, RMR and energy expended in activity could not all be included in a model, because they are so highly correlated (TDEE includes both RMR and energy expended in activity, and energy expended in activity is calculated by difference). All values are given as means ± SDs. All statistical analyses were carried out by using SAS software (version 8.2; SAS Institute, Cary, NC).

## RESULTS

Subject characteristics of children stratified by sex and race are shown in **Table 1**. At baseline, boys were heavier and had a higher TDEE and RMR and more EEA than did girls. Black children had a lower %BF and RMR but higher TEF than did white children. At the 2-y follow-up, girls had a significantly higher Tanner stage score than boys, and black children had a significantly higher Tanner stage score than whites. Over the 2-y period, significant differences were found in FM and FFM between the racial groups, but no difference was found in the change in body weight or fat gain between groups. There was a wide range in change in %BF—a reduction of >2% (eg, body fat decreased from 25% to 23%) in 36 children and a gain of >2% in 46 children—whereas 32 children remained within 2% of original body fat. There was no significant difference between groups in the change in %BF over 2 y (Table 1).

The relation between subject characteristics and change in %BF is shown in **Table 2**. All measures of initial body size and composition were negatively correlated with changes in body fat. In other words, %BF was likely to decrease in the heavier children. For boys but not girls, height was negatively associated with change in %BF. When the subjects were stratified by sex, body fatness was negatively correlated with change in %BF in girls. Overall and for boys, the Tanner staging at the 2-y follow-up was negatively associated with change in %BF, which meant that boys who became more sexually mature became less fat. The change in %BF was positively correlated with the change in body fat gain (kg). In boys and in the whole group, FFM gain was negatively correlated with change in %BF—that is, in the children who gained more FFM, %BF increased less or actually decrease.

**TABLE 2**

Subject characteristics at baseline as predictors of change in percentage body fat in a 2-y study<sup>1</sup>

	Overall (n = 114)		Subgroups with significantly different slopes and intercepts <sup>3</sup>	Significantly different groups <sup>4</sup>	Girls (n = 53)		Boys (n = 61)	
	r <sup>2</sup>	P			r	P	r	P
Height	-0.234	0.012	M, W	S			-0.347	0.006
Weight	-0.302	0.001	M, W, B <sup>5</sup>	—	-0.255	0.066	-0.312	0.014
BMI	-0.254	0.006	M, W	—	-0.280	0.042	-0.225	0.082
Percentage body fat	-0.292	0.002	M <sup>6</sup> , F, B, W	—	-0.431	0.001	-0.221	0.087
Tanner stage, year 2 of study	-0.427	0.0001	M, B, W	S, R × S interaction			-0.634	0.0001
Fat gain (kg)	0.806	0.0001	M, F, B, W	—	0.741	0.0001	0.833	0.0001
FFM gain (kg)	-0.544	0.0001	M, B, W	S <sup>5</sup>			-0.655	0.0001

<sup>1</sup> M, males; F, females; W, white; B, black; FFM, fat free mass.

<sup>2</sup> Simple correlation coefficients between changes in percentage body fat and selected initial subject characteristics.

<sup>3</sup> Slope and intercept are significantly different from 0 (independent tests) for each group as listed, *P* < 0.05 (unless noted otherwise).

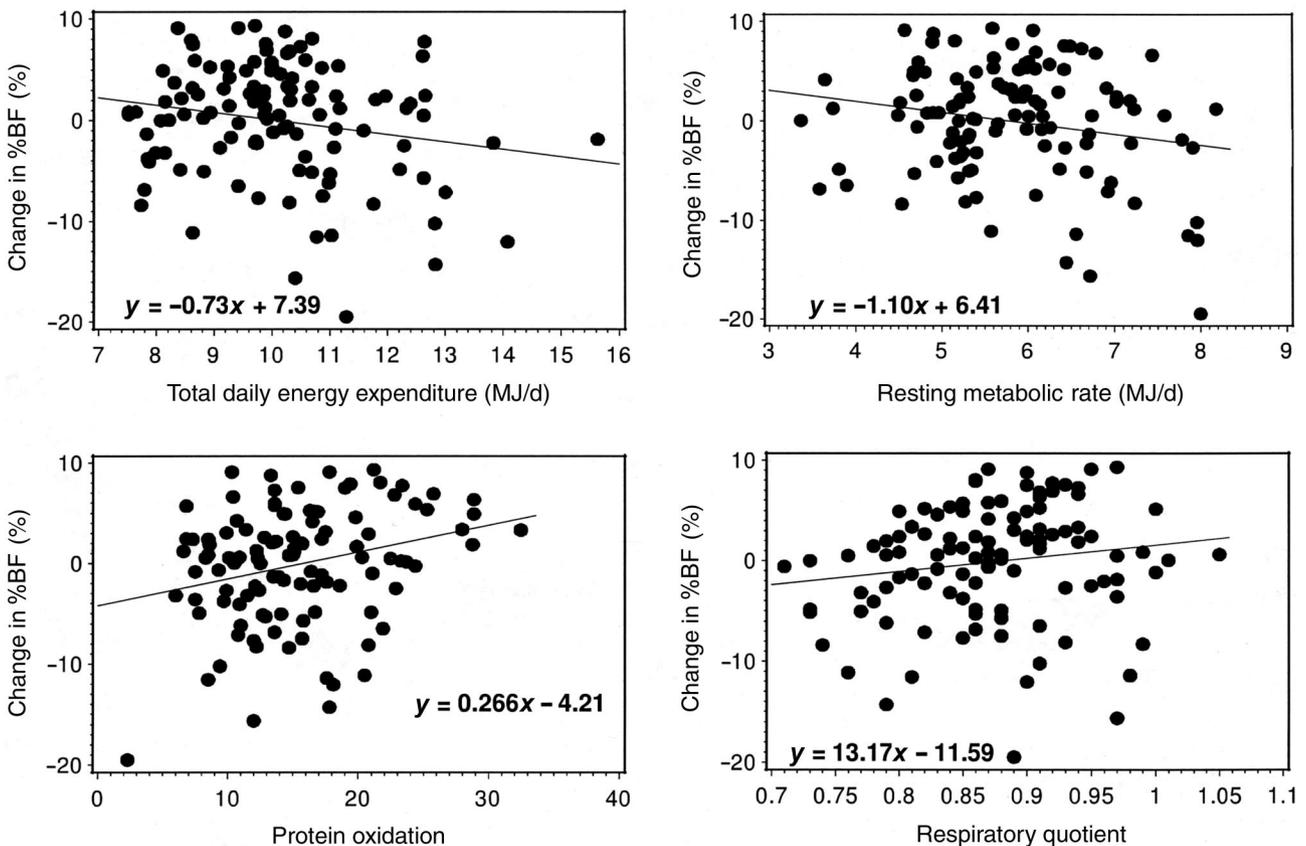
<sup>4</sup> Slope of line was significantly different by sex (S) or race (R), *P* < 0.05.

<sup>5</sup> Slope was significantly different from 0 but not from intercept, *P* < 0.09.

<sup>6</sup> *P* < 0.08.

Several metabolic variables were significantly correlated with changes in %BF. For the overall group, TDEE and RMR were negatively associated with change in %BF—that is, children with lower EE gained more fat. In contrast, protein oxidation and RQ (*P* < 0.10) were positively associated with changes in %BF (Figure 1).

Because of the relation between initial body weight and change in %BF, the EE variables had to be adjusted for body size before the relation between metabolic variables and changes in %BF was examined. We chose to normalize for height by calculating the residuals between each EE variable and height.



**FIGURE 1.** Relation between metabolic variables and changes in percentage body fat (%BF) in the entire cohort. Total daily energy expenditure: *R*<sup>2</sup> = 0.039, *P* < 0.04; resting metabolic rate: *R*<sup>2</sup> = 0.039, *P* < 0.04; protein oxidation: *R*<sup>2</sup> = 0.071, *P* < 0.005; and respiratory quotient: *R*<sup>2</sup> = 0.025, NS.

**TABLE 3**Body-composition changes by Tanner stage at follow-up<sup>1</sup>

	Low Tanner stage (≤ 2.5) (n = 46)	High Tanner stage (>2.5) (n = 66)
Weight gain (kg)	10.20 ± 4.76 <sup>a</sup>	11.56 ± 5.56 <sup>b</sup>
Fat gain (kg)	4.17 ± 4.17 <sup>a</sup>	1.85 ± 4.62 <sup>b</sup>
FFM gain (kg)	5.85 ± 2.37 <sup>a</sup>	9.51 ± 3.37 <sup>b</sup>
Change in fat over 2 y (%)	2.58 ± 4.04 <sup>a</sup>	-2.45 ± 5.92 <sup>b</sup>

<sup>1</sup> FFM, fat-free mass. Means within rows with different superscript letters are significantly different,  $P < 0.05$  (Tukey multiple comparison adjustment). ANOVA was conducted by using the full model including race, sex, and obesity group. Significant ( $P < 0.05$ ) interactions were observed for FFM gain (sex × Tanner stage) and percentage change in fat (sex × Tanner stage and race × Tanner stage).

One confounding factor in the determination of metabolic predictors of body fat gain of these children is the Tanner stage attained at the 2-y follow-up. We chose to study children during the pubertal growth spurt because this is a time of great metabolic change. As expected, the girls were at a higher Tanner stage than were the boys, and blacks were at a higher Tanner stage than were whites (Table 1). Tanner stage was significantly associated with body fat change only in the boys, and there was a race × sex interaction between Tanner stage at follow-up and change in %BF (Table 2). As shown in **Table 3**, the children who were more sexually mature—ie, those at a higher Tanner stage (3.78 ± 0.72)—gained less fat and more FFM, which led to a loss of %BF, whereas %BF increased in those children who had a low Tanner stage (2.00 ± 0.40). Because of these Tanner stage differences, Tanner stage was included in the correlation analyses and prediction models.

When the race and sex subgroups were examined, even after adjustment for Tanner stage, more metabolic variables were generally associated with change in %BF (**Table 4**). Substrate oxidation was associated with change in %BF in girls and in the black children. In addition, TEF was negatively associated with change in %BF in girls. Protein oxidation was positively and RMR was negatively associated with change in %BF in boys.

**TABLE 4**Baseline metabolic variables predicting change in percentage body fat over 2 y<sup>1</sup>

	Sex				Race			
	Girls (n = 53)		Boys (n = 61)		Black (n = 59)		White (n = 55)	
	r	P	r	P	r	P	r	P
Protein oxidation during TEF	—	—	0.31	0.02	—	—	—	—
RQ during TEF	0.28	0.05	—	—	0.30	0.03	—	—
Fat oxidation (% of TEF)	-0.29	0.04	—	—	-0.30	0.03	—	—
Carbohydrate oxidation (% of TEF)	0.25	0.09	—	—	0.27	0.05	—	—
Total daily energy expenditure	—	—	—	—	—	—	-0.30	0.03
Total daily energy expenditure residual <sup>2</sup>	—	—	—	—	—	—	-0.29	0.04
Resting metabolic rate	—	—	-0.27	0.04	—	—	-0.27	0.05
Resting metabolic rate residual <sup>2</sup>	—	—	—	—	—	—	-0.29	0.04
TEF (% of calories in a meal)	-0.26	0.07	—	—	—	—	—	—

<sup>1</sup> Partial correlation coefficients between change in percentage body fat and selected initial subject characteristics after adjustment for Tanner stage. RQ, respiratory quotient; TEF, thermic effect of food; EEA, energy expended in physical activity.

<sup>2</sup> Adjusted for height.

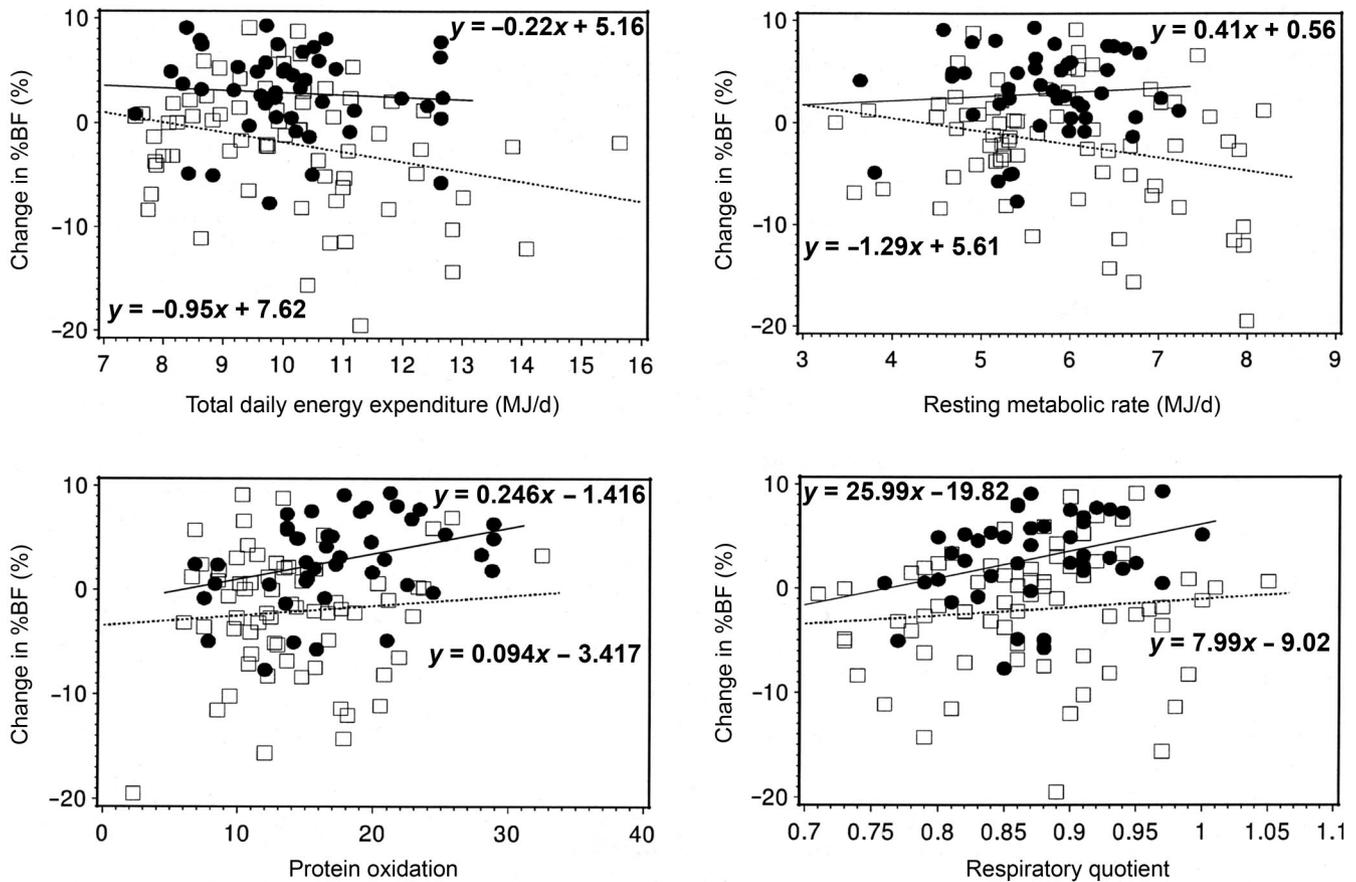
When we did not adjust for Tanner stage, all of the above relations were observed, except for TDEE residual in white children. In addition, TDEE ( $r = -0.35$ ,  $P = 0.05$ ) was associated with changes in %BF in boys.

The relation between the metabolic variables and change in %BF was also different between the 2 Tanner stage groups (**Figure 2**). In the high Tanner stage group, TDEE and RMR were negatively associated with change in %BF. In the low Tanner stage group, protein oxidation and RQ during the measurement of the TEF were positively associated with changes in %BF.

Multiple regression analysis was employed to ascertain whether combinations of the metabolic variables could improve the prediction of change in %BF over 2 y (**Table 5**). In the overall group of children, 3 variables entered the model to account for a total of 10% of the variance in body fat gain. Protein oxidation and RQ during the measurement of the TEF were positively associated and TDEE residuals were negatively associated with fat gain. When the children were subdivided by sex or race, the variance of the change in %BF explained by the metabolic variables increased to ≈20% (Table 5). With the exception of a positive association between protein oxidation and %BF, the variables associated with change in %BF were different for girls and boys. In boys there was a negative association between EEA residuals and change in %BF, whereas for girls, there was a surprising positive association between EEA and change in %BF. Protein oxidation entered the model for both black and white children. Whereas RQ and RMR residuals entered the model for both black and white children, the relation between the 2 races was opposite.

When the children were further stratified by race and sex subgroups, the metabolic variables explained up to 41% of the variance of change in %BF (Table 5). However, we did not obtain a significant prediction model for black boys ( $P = 0.15$ ). Variables in the model for the 4 subgroups generally were different, although protein oxidation entered the model for all but black girls, and at least one variable of EE, either TDEE, RMR, or EEA residuals or TEF entered all models.

When Tanner stage was included in the multivariate analyses, the Tanner stage at year 2 was the first variable to enter the model



**FIGURE 2.** Relation (regression) between metabolic variables and changes in percentage body fat (%BF) in the groups with low (●) and high (□) Tanner status. Total daily energy expenditure:  $R^2 = 0.22$ ,  $P < 0.0001$  [slopes not different from each other ( $P < 0.30$ ), although lines significantly different from each other ( $P < 0.0001$ )]; resting metabolic rate:  $R^2 = 0.217$ ,  $P < 0.0001$  [slopes not different from each other ( $P < 0.14$ ), although lines significantly different from each other ( $P < 0.0001$ )]; protein oxidation:  $R^2 = 0.207$ ,  $P < 0.0001$  [slopes not different from each other ( $P < 0.35$ ), although lines significantly different from each other ( $P < 0.0001$ )]; respiratory quotient:  $R^2 = 0.209$ ,  $P < 0.0001$  [slopes not different from each other ( $P < 0.28$ ), although lines significantly different from each other ( $P < 0.0001$ )].

in all subgroups except girls and black girls (Table 5). The variance in change in %BF explained by Tanner stage was high and in most cases higher than that explained by the best model of metabolic variables. However, even when Tanner stage was included, many of the same metabolic variables that entered the models without Tanner stage still entered the models and explained up to 62% of the variance in the change in %BF (Table 5). In all cases, inclusion of metabolic variables in addition to Tanner stage increased the explained variance, from an increase of 4% (overall) to an increase of 24% (black girls). Another interesting observation was that although Tanner stage did not enter the model for change in %BF in girls overall, Tanner stage was the first variable to enter the model for white girls and did enter the model for black girls. In addition, the direction of the relation was negative in white girls but positive in black girls.

## DISCUSSION

This longitudinal study of energy metabolism in children going through the pubertal growth spurt identified several variables of energy metabolism that predicted change in percentage body fatness over 2 y. Individual variables accounted for a small proportion of the variance in change in %BF, although multiple regression models could explain up to 41% of the variance in

black girls. Variables that predicted change in body fat included TDEE residuals, RMR residuals, TEF, RQ during the measurement of the TEF, and protein oxidation during the test meal. Significant predictors of change in %BF were, for the most part, sex and race specific, although tests for equality of slopes indicated that only some of the relations by race and sex were significantly different. In boys, protein oxidation and EEA predicted changes in %BF. In girls, RQ, TEF, protein oxidation during the test meal, and EEA were associated with fat gain.

This study has several significant strengths. First, it is a longitudinal study in children in which several components of EE were carefully measured. Second, TDEE was directly determined by use of DLW. This technique has been validated in infants and adults, whether they are in positive, zero, or negative energy balance, sedentary ( $1.4 \times$  sleeping MR) or highly active ( $2.61 \times$  sleeping MR), or receiving parenteral nutrition (10, 30, 31). Third, RMR and TEF were measured within 5 d of the TDEE measurements. Fourth, body fat was assessed by using the average of 3 independent measures of body fat (20, 26). And, finally, this study had a nearly 90% follow-up over 2 y.

Among the published metabolic predictors of obesity are a low metabolic rate, high RQ, high carbohydrate oxidation, insulin sensitivity, and low sympathetic activity (9, 32). In the current study, we confirmed that metabolic rate is a predictor of fat gain,

**TABLE 5**  
Prediction models of change in percentage body fat<sup>1</sup>

Group	Without Tanner stage			With Tanner stage					
	Best model			First variable			Best model		
	R <sup>2</sup>	P	Variables	R <sup>2</sup>	P	Variables	R <sup>2</sup>	P	Variables
Overall	0.10	0.002	Protein oxidation <sup>2,3</sup> TDEE residuals <sup>2,4</sup> RQ <sup>3</sup>	0.20	0.0001	Tanner <sup>4</sup>	0.24	0.0001	Tanner <sup>2,4</sup> TDEE residuals <sup>2,4</sup> RQ <sup>3</sup> Protein oxidation <sup>3</sup>
Sex									
Girls	0.25	0.013	RQ <sup>2,3</sup> TEF <sup>2,4</sup> EEA residuals <sup>3</sup> Protein oxidation <sup>3</sup>	0.092	0.03	RQ <sup>3</sup>	0.25	0.02	RQ <sup>2,3</sup> TEF <sup>2,4</sup> EEA residuals <sup>3</sup> Protein oxidation <sup>3</sup>
Boys	0.16	0.002	Protein oxidation <sup>2,3</sup> EEA residuals <sup>4</sup>	0.40	0.0001	Tanner <sup>4</sup>	0.46	0.0001	Tanner <sup>2,4</sup> Protein oxidation <sup>2,3</sup>
Race									
Black	0.17	0.03	RQ <sup>2,3</sup> Protein oxidation <sup>3</sup> RMR residuals <sup>3</sup>	0.18	0.0001	Tanner <sup>4</sup>	0.28	0.001	Tanner <sup>2,4</sup> RQ <sup>2,3</sup> RMR residuals <sup>3</sup>
White	0.19	0.006	RMR residuals <sup>2,4</sup> Protein oxidation <sup>3</sup> TEF <sup>4</sup> RQ <sup>4</sup>	0.28	0.0001	Tanner <sup>4</sup>	0.34	0.0001	Tanner <sup>2,4</sup> RMR residuals <sup>2,4</sup>
Race and sex									
Black girls	0.41	0.03	RQ <sup>2,3</sup> TEF <sup>4</sup> TDEE residuals <sup>3</sup>	0.24	0.013	RQ <sup>3</sup>	0.48	0.009	RQ <sup>2,3</sup> Tanner <sup>2,3</sup> Protein oxidation <sup>3</sup> TDEE residuals <sup>3</sup>
White girls	0.30	0.04	TEF <sup>2,4</sup> Protein oxidation <sup>3</sup> EEA residuals <sup>3</sup>	0.32	0.005	Tanner <sup>4</sup>	0.45	0.001	Tanner <sup>2,4</sup> TEF <sup>2,4</sup>
Black boys	0.15	0.15	Protein oxidation <sup>3</sup> RQ <sup>3</sup> RMR residuals <sup>3</sup>	0.53	0.0001	Tanner <sup>4</sup>	0.58	0.0001	Tanner <sup>2,4</sup> Protein oxidation <sup>3</sup> RMR residuals <sup>3</sup>
White boys	0.27	0.01	Protein oxidation <sup>2,3</sup> TDEE <sup>4</sup>	0.49	0.0001	Tanner <sup>4</sup>	0.62	0.0001	Tanner <sup>2,4</sup> Protein oxidation <sup>3</sup> RQ <sup>3</sup> RMR residuals <sup>4</sup>

<sup>1</sup> TDEE, total daily energy expenditure; RMR, resting metabolic rate; RQ, respiratory quotient; TEF, thermic effect of food; EEA, energy expended in physical activity. Multiple regression models used the "all possible submodels" variable selection method ( $R^2$  option in SAS software, version 8.2). The Best model was selected as the model with the lowest mean squared error. Energy metabolism variables are baseline values, whereas the Tanner rating is the follow-up value.

<sup>2</sup> Slopes significantly different from 0,  $P < 0.05$ .

<sup>3</sup> Positive relation with percentage body fat.

<sup>4</sup> Negative relation with percentage body fat.

but the direction of the effect differed between boys and girls. In boys, a low TDEE along with a low RMR predicted fat gain. This is consistent with the findings of Ravussin et al (9) and Roberts et al (10). The finding in girls that EEA was associated with fat gain was unexpected and implies that girls who gain more fat have an even higher intake of energy to provide for higher metabolism and accumulation of additional fat. In support of this conclusion, a higher weight gain with higher energy intake has been reported in women but not men (33). That study found that women who reported the highest caloric intake had the highest weight gain at follow-up. A surprising positive association between sleeping EE and change in body fat also was observed in prepubertal girls (34). However, in the current study, the positive association between EEA and change in %BF was not that strong, because EEA was the third variable entering the model that

predicted change in %BF (Table 5), and, whereas the simple correlation was positive ( $r = 0.20$ ), it was not significant ( $P = 0.14$ ; Table 3).

One confounding factor in this study was that many children, particularly the lean children, did not reach sexual maturity over the 2-y period. This fact is an important confounder, because many endocrine changes occur during puberty that have important effects on body composition (35). As a whole, both boys and girls were still in transition from prepubescence to full sexual maturity. Seventy-eight percent of boys and fifty-seven percent of girls were at a Tanner stage of  $\leq 3$ . Only 6 of the girls and 4 of the boys in the sample achieved a Tanner stage of 5, which indicates complete sexual maturity. All girls averaged a Tanner stage of  $\geq 2$ , whereas 2 boys remained at stage 1, which indicated that they had not yet entered pubescence. However, this variability in Tanner

staging also allowed us to examine the role that sexual maturation played in the change in %BF. One limitation of this study is that the initial metabolic variables are only a momentary record, and they provide no information on any changes that may have occurred during the intervening 2 y.

The relation between physical activity and current body fat or longitudinal body fat gain differs between male and female adults (36–39). Although each of these studies used different methods of measuring physical activity, the studies show similar findings. In a study of 106 children aged  $7.8 \pm 0.9$  y, EEA and physical activity level were inversely associated with %BF in boys but not in girls (36). In the current study, the correlation between TDEE and body fat change was  $-0.31$  ( $P < 0.05$ ) in boys and  $0.08$  ( $P = 0.54$ ) in girls. Leisure sport activity in the past year correlated negatively with %BF in Pima Indian boys but not girls (39). In adult Pima Indians, spontaneous physical activity measured in a metabolic chamber correlated inversely with subsequent body fat change in males only (38). A decrease in physical activity and TDEE has been observed before puberty in girls, but not boys (37). Furthermore, time spent in moderate and vigorous physical activity has been shown to be related to body fatness, and boys spent more time in such activity than did girls (40).

In the multiple regression analysis, a high RQ, indicating a higher carbohydrate oxidation and lower fat oxidation, predicted fat gain in several subgroups. This is consistent with the observations of Zurlo et al (41) in Pima Indians, in whom a high RQ and high carbohydrate oxidation predicted weight gain. Fasting RQ has also been shown to be a predictor of weight gain in the Baltimore Longitudinal Study on Aging (42) and of weight regain in obese women after the period of low energy intake (43). Furthermore, in a 10-y longitudinal study of 775 men, initial RQ was positively related to weight gain (42).

Protein oxidation during the test meal predicted fat gain in the whole group, and in boys in particular, and it entered multiple regression models for all but 3 of the subgroups (Table 5). Several studies have found differences in protein metabolism between lean and obese subjects, as well as differences between subjects with central or visceral fat distribution (44–46). These findings indicate that alterations in protein metabolism may be involved in fat gain. Rolland-Cachera et al (47) provides support for a role of protein intake in weight gain. These investigators showed that protein intake at 2 y of age is positively related to the development of obesity at follow-up 6 y later, even after adjustment for energy intake at 2 y and for parental BMI. Several studies support the idea that high protein oxidation reflects high protein intake (48–51). Furthermore, nitrogen balance has been shown to increase in children involved in an exercise program and to improve in obese children who participated in a walking program while undergoing a weight-loss program (52, 53). These findings also suggest that physical activity is important not only in avoiding positive energy balance but also in improving protein retention and deposition.

In summary, this longitudinal study of energy metabolism in 114 black and white children during the pubertal growth spurt identified several variables of energy metabolism that could account for a small percentage of the variation in change in %BF over 2 y. Variables that predicted change in %BF included TDEE as measured by DLW, RMR, and RQ during the measurement of the TEF (measure of carbohydrate and fat oxidation) and by protein oxidation during the measurement of the TEF. The metabolic predictors of change in body fatness varied by subgroup.

When Tanner stage was included in the model, variables of energy metabolism still accounted for 4–24% of the variance in change in %BF. Furthermore, we provide indirect evidence that protein or total energy intake or both are predictors for gain in %BF during late childhood in boys and girls. 

GAB, JPD, and DWH designed the study; JPD and DWH collected the data; JV performed the statistical analysis; and JPD and GAB wrote the manuscript. None of the authors had a personal or financial conflict of interest.

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