

Whole-body protein turnover and resting energy expenditure in obese, prepubertal children<sup>1-3</sup>

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**ABSTRACT**

**Background:** Obesity is becoming more frequent in children; understanding the extent to which this condition affects not only carbohydrate and lipid metabolism but also protein metabolism is of paramount importance.

**Objective:** We evaluated the kinetics of protein metabolism in obese, prepubertal children in the static phase of obesity.

**Design:** In this cross-sectional study, 9 obese children ( $\bar{x} \pm \text{SE}$ :  $44 \pm 4$  kg,  $30.9 \pm 1.5\%$  body fat) were compared with 8 lean ( $28 \pm 2$  kg,  $16.8 \pm 1.2\%$  body fat), age-matched ( $8.5 \pm 0.2$  y) control children. Whole-body nitrogen flux, protein synthesis, and protein breakdown were calculated postprandially over 9 h from <sup>15</sup>N abundance in urinary ammonia by using a single oral dose of [<sup>15</sup>N]glycine; resting energy expenditure (REE) was assessed by indirect calorimetry (canopy) and body composition by multiple skinfold-thickness measurements.

**Results:** Absolute rates of protein synthesis and breakdown were significantly greater in obese children than in control children ( $\bar{x} \pm \text{SE}$ :  $208 \pm 24$  compared with  $137 \pm 14$  g/d,  $P < 0.05$ , and  $149 \pm 20$  compared with  $89 \pm 13$  g/d,  $P < 0.05$ , respectively). When these variables were adjusted for fat-free mass by analysis of covariance, however, the differences between groups disappeared. There was a significant relation between protein synthesis and fat-free mass ( $r = 0.83$ ,  $P < 0.001$ ) as well as between protein synthesis and REE ( $r = 0.79$ ,  $P < 0.005$ ).

**Conclusions:** Obesity in prepubertal children is associated with an absolute increase in whole-body protein turnover that is consistent with an absolute increase in fat-free mass, both of which contribute to explaining the greater absolute REE in obese children than in control children. *Am J Clin Nutr* 1999;69:857–62.

**KEY WORDS** Energy metabolism, resting metabolic rate, protein metabolism, whole-body protein turnover, protein synthesis, fat-free mass, obesity, children

**INTRODUCTION**

Although much research has been conducted on human obesity in the past decades, more investigations have been performed of adult obesity than of childhood onset obesity. It is well known, however, that many obese adults were obese when they were young (1–5). Therefore, important issues to explore are whether the metabolic disturbances encountered in adult obesity (blunted postprandial thermogenesis, increased lipid oxidation, impaired glucose tolerance, and insulin resistance) can be

observed at younger ages and to assess the magnitude of these disturbances and the age of onset.

The postabsorptive resting metabolic rate of obese children was explored previously by several investigators (6–8). When expressed as an absolute value, the rate of resting energy expenditure (REE) was found to be greater in obese than in nonobese children or adolescents (6–8), confirming results observed in obese adolescents (9) and obese adults (10). The small increase in fat-free mass (FFM) accompanying the large inflation in adipose tissue in obese children explains the greater REE in these children than in lean children. However, this constitutes an explanation based on a purely static concept. Whole-body protein turnover is a dynamic process and an important energy-requiring process because of the high ATP consumption involved in the formation of peptide bonds during protein synthesis. For example, the process of whole-body protein turnover in infants aged 1 y may explain more than one-third of their REE; in adults, whole-body protein turnover may explain about one-fifth of the REE (11). The extent to which this dynamic, high-energy-cost process contributes to the higher REE of obese children compared with lean children remains to be investigated. As far as we are aware, little information exists on the magnitude of whole-body protein turnover in obese children and its relation to FFM and REE. To provide a more thorough picture of the extent to which the metabolic changes associated with adult obesity appear early in life, we studied energy and protein metabolism in prepubertal, obese children and compared these results with those obtained in nonobese children of the same mean age and height.

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## SUBJECTS AND METHODS

### Subjects

Investigations were carried out in 17 prepubertal (male and female), white children aged 7–10 y who were divided into 2 groups: 9 obese and 8 nonobese children. Age and height were not significantly different between the 2 groups, whereas weight, body mass index (BMI), fat mass, and FFM were significantly higher in the obese group (Table 1). Obesity was defined as a BMI higher than the 97th percentile of the reference value for age and sex based on a French sample of children (12). Nonobese children were selected on the basis of a BMI less than or equal to the 97th percentile of the reference values for age and sex (range: 5th to 92nd percentile).

A physical examination excluded health problems other than obesity. Pubertal stage was assessed according to Tanner (13). None of the subjects reported significant changes in body weight during the month preceding the study and none of the obese children had followed a hypoenergetic diet immediately before the study. No child was taking any medication. The experimental protocol was in accordance with the Declaration of Helsinki of 1975, as revised in 1983, and was approved by the ethical committee of the University Hospital of Verona (Italy).

### Physical characteristics

Anthropometric measurements (weight, height, and skinfold thicknesses) were made by the same investigator. Height was measured to the nearest 0.5 cm with a standardized, wall-mounted height board. Weight was measured to the nearest 0.1 kg with a standard physician's beam scale with the child dressed only in light underwear and without shoes. BMI was calculated as weight divided by height squared ( $\text{kg}/\text{m}^2$ ). Skinfold thicknesses were measured at 4 sites (biceps, triceps, suprailium, and subscapula) in triplicate to the nearest millimeter with a Harpenden skinfold caliper (CMS Weighing Equipment Ltd, London). The formula derived by Deurenberg et al (14) was used to calculate percentage body fat from the sum of the 4 skinfold thicknesses. Body fat mass was obtained by multiplying body weight by percentage body fat. FFM was calculated by subtracting body fat from body weight.

### Experimental design

The study lasted for 10 h, during which time the children were under constant medical supervision. During this period, REE was measured 5 times: once before the first meal (preprandial) and 4 times postprandially.

On the days preceding the study, the children consumed unrestricted diets. The day before the test, they did not perform any intense physical exercise. On the day of the test, the children arrived by car at the Department of Pediatrics at  $\approx 0730$  in a fasting state, having not consumed a meal after 2000 the day before. After the children had rested for 30 min on a hospital bed in a comfortable, temperature-controlled environment, continuous respiratory exchange measurement was initiated by indirect calorimetry. During each of the five 30-min indirect calorimetry measurement periods [ie, at 0800 (before the first meal) and at 1030, 1300, 1530, and 1800 (postprandially)], the children rested quietly and watched cartoons. Throughout the REE measurement, an investigator observed the child to make sure that he or she remained motionless and was relaxed during the entire respiratory exchange measurement. Special efforts were made to prevent extra body movements or hyperventilation because the

TABLE 1  
Physical characteristics and resting energy expenditure (REE) of the nonobese and obese prepubertal children<sup>1</sup>

	Nonobese (n = 8)	Obese (n = 9)
Age (y)	8.6 ± 0.4	8.3 ± 0.2
Height (cm)	133.4 ± 3.1	135.7 ± 2.8
Weight (kg)	28.5 ± 1.6	44.2 ± 3.6 <sup>2</sup>
BMI ( $\text{kg}/\text{m}^2$ )	16.0 ± 0.6	23.8 ± 1.3 <sup>3</sup>
Fat-free mass (kg)	23.6 ± 1.1	29.9 ± 1.7 <sup>4</sup>
Fat mass (% of body wt)	16.8 ± 1.2	30.9 ± 1.5 <sup>3</sup>
REE		
(kJ/d)	4405 ± 184	5548 ± 225 <sup>2</sup>
(kcal/d)	1053 ± 44	1326 ± 54 <sup>2</sup>

<sup>1</sup> $\bar{x} \pm \text{SEM}$ .

<sup>2-4</sup>Significantly different from nonobese children: <sup>2</sup> $P < 0.005$ , <sup>3</sup> $P < 0.0001$ , <sup>4</sup> $P < 0.05$ .

former increase REE and the latter engenders a rise in respiratory quotient. One child was excluded from the study because of hyperventilation that led to respiratory quotients systematically  $> 1.0$  in all measurement periods.

### Resting energy expenditure

REE was assessed by respiratory gas exchange over sequential periods of 30 min as described previously (6). The average REE value was calculated in the postprandial phase (4 nonconsecutive measurements) because protein metabolism was also assessed postprandially. An open-circuit, computerized indirect calorimeter (Deltatrac TM; Datex Division, Helsinki) connected to a transparent hood system was used. Before each measurement, the instrument was calibrated with a mixture of 2 gases (95.2%  $\text{O}_2$  and 4.8%  $\text{CO}_2$ ). Rates of oxygen consumption ( $\dot{V}\text{O}_2$ ) and carbon dioxide production ( $\dot{V}\text{CO}_2$ ) were calculated and printed out at 1-min intervals. Energy expenditure was derived from  $\dot{V}\text{O}_2$  and  $\dot{V}\text{CO}_2$  by using classic equations (15). To ensure a steady state in REE, the mean of the last 20 min of measurements was taken.

### Test meals

Four small meals were provided to the children on the day of the study and were eaten under supervision at 0830, 1100, 1330, and 1600. Each meal consisted of cheese, popcorn, a protein concentrate (Power Play; Wander-Novartis, Bern, Switzerland), French biscuits (Petit Beurre; Christie & Brown and Co, Paris), and a drink with sugar added. Total energy intake during the experimental period was equivalent to the baseline postabsorptive REE. The total calculated metabolizable energy value of the 4 meals averaged  $5509 \pm 223$  kJ ( $1317 \pm 53$  kcal) in obese and  $4406 \pm 192$  kJ ( $1053 \pm 46$  kcal) in nonobese children. Fifteen percent of energy was derived from protein ( $49.0 \pm 2.0$  g in obese children and  $39.1 \pm 1.7$  g in nonobese children), 30% from fat ( $44.1 \pm 1.8$  g in obese children and  $35.0 \pm 1.5$  g in nonobese children), and 55% from carbohydrate ( $182.2 \pm 7.4$  in obese children and  $146.4 \pm 6.5$  g in nonobese children).

### Assessment of protein turnover

A single dose of [<sup>15</sup>N]glycine [a nonradioactive isotope, 100 mg, 99 atom% (one); Amersham International, Buckinghamshire, United Kingdom] was given orally (diluted in water and added to the drink provided with the first meal). Urine was collected before

the first meal, before the [<sup>15</sup>N]glycine dose to assess background isotopic abundance, and during the 9-h period after administration of [<sup>15</sup>N]glycine. The volume of each urine sample was measured and the sample was immediately acidified with a solution of 6 mol HCl/L to a pH of 1.5 and frozen at -20°C.

The rate of protein turnover was estimated from the isotopic enrichment of urinary ammonia after the single oral dose of [<sup>15</sup>N]glycine. Details of the method were described previously by Fern et al (16, 17). The <sup>15</sup>N enrichment of ammonia was corrected for background isotopic <sup>15</sup>N abundance in urine (typically 0.366 atom%) as measured in the first predose urine sample.

The rate of whole-body nitrogen flux (Q) was calculated from the equation given by Waterlow et al (18), with use of ammonia as the end product:

$$Q = E \times d/e \quad (1)$$

where Q is the rate of whole-body nitrogen flux postprandially (g/9 h), E is the amount of nitrogen excreted in urine as ammonia postprandially (g/9 h), d is the dose of <sup>15</sup>N given orally (g), and e is the total amount of isotope excreted in urine as ammonia postprandially (g/9 h) during the pooled collection period. The use of ammonia alone (as the end product) was justified because the use of urea as the end product requires blood sampling at the beginning and end of the test to correct for the <sup>15</sup>N remaining in the urea pool.

Protein synthesis and breakdown were derived from the following expression:

$$Q = I + B = S + E \quad (2)$$

where Q is the amount of nitrogen entering the pool from the diet (I) and from protein breakdown (B) and the latter terms are the amounts of nitrogen leaving the pool through urinary excretion (E) or protein synthesis (S). The rates of whole-body protein synthesis and protein breakdown can thus be calculated by simple arithmetic difference.

$$S = Q - E \quad (3)$$

and

$$B = Q - I \quad (4)$$

Note that the difference between S and B is mathematically equivalent to nitrogen balance and does not depend on the administration of the <sup>15</sup>N tracer. Nitrogen balance, however, gives no information about rates of protein synthesis and breakdown. A factor of 6.25 was used to convert amount of nitrogen to amount of protein. Miscellaneous nitrogen losses (eg, through sweat and feces) were considered to be negligible.

### Biochemical analysis

Urinary ammonia was measured by an enzymatic method as described by Kun and Kearney (19). Total urinary nitrogen excretion was determined by the Kjeldahl method (Autoanalyzer; Technicon, Tarrytown, NY).

### Stable-isotope analysis

The stable-isotope method was based on trials by Preston and McMillan (20) with minor modifications. Briefly, after each urine sample (10–15 mL) was neutralized to a pH of 6.0 ± 0.1 with 5 mol NaOH/L, ammonia nitrogen was isolated by using a cationic exchange resin (Ag 50W × 8; Bio-Rad Laboratories, Richmond, CA). After the supernate was discarded and after the

resin was washed several times with purified water, ammonia was extracted with 1.5 mL of 2.5 mol KHSO<sub>4</sub>/L and analyzed for <sup>15</sup>N enrichment after combustion (Roboprep CN; Europa Scientific, Crewe, United Kingdom) with a continuous-flow isotope ratio mass spectrometer (Tracermass; Europa Scientific). A 5%-urea solution was used as an internal standard.

### Statistical analysis

Results are expressed as means ± SEMs. Unpaired t tests were used to compare the physical characteristics of obese and nonobese children. Statistical differences between the 2 groups as well as between postabsorptive and postprandial states within each group were assessed by analysis of variance. Adjusted values were obtained by analysis of covariance (ANCOVA) with FFM as the covariate. Correlations between variables were determined by Pearson product-moment linear correlation analysis. The SAS statistical package (version 6.0; SAS Institute Inc, Cary, NC) was used for statistical analyses.

## RESULTS

### Postabsorptive and postprandial energy expenditure

The absolute postabsorptive metabolic rate was significantly higher in obese than in nonobese children (5548 ± 225 compared with 4405 ± 184 kJ/d; P < 0.0005). Postabsorptive energy expenditure normalized for FFM (or adjusted for FFM by ANCOVA) was not significantly different between the 2 groups (187.1 ± 5.3 compared with 187.5 ± 4.8 kJ·kg FFM<sup>-1</sup>·d<sup>-1</sup> in obese and nonobese children, respectively).

After meal ingestion, REE increased in both obese and nonobese children: in absolute values, it was higher in the obese group than in the nonobese group (5820 ± 52 compared with 4732 ± 10 kJ/d; P < 0.005). When the values were normalized per kg FFM, however, postprandial energy expenditure was not significantly different between groups (196.4 ± 5 compared with 201.8 ± 0.4 kJ·kg FFM<sup>-1</sup>·d<sup>-1</sup>). Expressed as a percentage increase over postabsorptive energy expenditure (premeal baseline), the postprandial rise in energy expenditure in obese children (5.0 ± 1.0%) was slightly lower than that in nonobese children (7.6 ± 1.4%), but not significantly so.

### Nitrogen flux, whole-body protein synthesis, and whole-body protein breakdown

The rate of whole-body protein turnover, expressed as an absolute value, was significantly higher in obese children than in nonobese children: the rate of nitrogen flux averaged 42.7 ± 3.8 g/d in the obese children compared with 29.4 ± 2.5 g/d in the nonobese children (Figure 1). Similarly, rates of whole-body protein synthesis and breakdown were significantly greater in obese children (208 ± 24 and 149 ± 20 g/d, respectively) than in nonobese children (137 ± 14 and 89 ± 13 g/d, respectively). When the values were expressed per kg FFM, the rate of protein breakdown remained significantly greater in obese children than in nonobese children (5.0 ± 0.5 compared with 3.7 ± 0.4 kJ·kg FFM<sup>-1</sup>·d<sup>-1</sup>; P < 0.05). However, this mode of expression assumes that the relation between whole-body protein synthesis (or protein breakdown) and FFM has a zero intercept, which was not the case. When the values were adjusted for FFM, with FFM as a covariate, no significant differences in either whole-body protein synthesis (176 compared with 172 g/d in obese and nonobese children, respectively) or whole-body protein break-

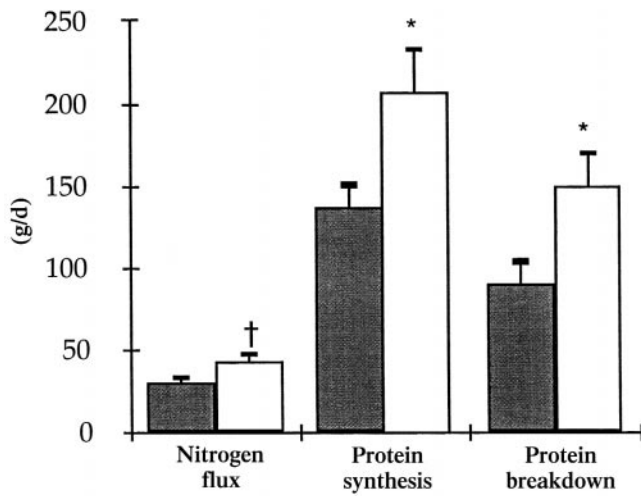


FIGURE 1. Mean ( $\pm$ SEM) whole-body nitrogen flux, whole-body protein synthesis, and whole-body protein breakdown expressed as absolute values in nonobese (■) and obese (□) prepubertal children. \* $\dagger$  Significantly different from nonobese children: \* $P < 0.05$ ,  $\dagger P < 0.005$ .

down (127 compared with 114 g/d in obese and nonobese children, respectively) were observed.

There was a significant positive correlation between whole-body nitrogen flux and FFM ( $r = 0.73$ ,  $P < 0.01$ ) and between whole-body nitrogen flux and REE ( $r = 0.75$ ,  $P < 0.001$ ):

$$Q \text{ (g/d)} = 1.78 \times \text{FFM (kg)} - 9.7 \quad (5)$$

$$Q \text{ (g/d)} = 0.012 \times \text{REE (kJ/d)} - 24.1 \quad (6)$$

The magnitude of whole-body protein synthesis was also associated with both FFM ( $r = 0.83$ ,  $P < 0.001$ ) and REE ( $r = 0.79$ ,  $P < 0.005$ ):

$$S \text{ (g/d)} = 10.75 \times \text{FFM (kg)} - 115 \quad (7)$$

$$S \text{ (g/d)} = 0.067 \times \text{REE (kJ/d)} - 173 \quad (8)$$

## DISCUSSION

The present study confirms previous findings of a significantly greater absolute REE in obese children and adolescents than in their lean counterparts (6–8). The higher REE in obese children in the present study could be accounted for almost entirely by an increased FFM because both variables were highly correlated ( $r = 0.92$ ,  $P < 0.001$ ), confirming previous results in prepubertal children (6).

Some methodologic aspects of the present study require comment. The accuracy of the assessment of body composition in children may be challenged because the equation used to estimate percentage body fat from skinfold thicknesses (although intended for prepubertal children) may be subject to error in obese children. To address this issue, body-composition differences between groups were recalculated on the basis of triceps skinfold thickness (in mm). As expected, the differences between the groups were maintained.

The method we used to estimate protein turnover also requires comment. As in our previous studies of children (21), pregnant women (22), and the elderly (23), we used the  $^{15}\text{N}$  end product method based on [ $^{15}\text{N}$ ]ammonia to assess protein turnover because this allowed us to obtain an estimate without blood sam-

pling (an advantage when investigating children). The basic assumption of the end product method is that the fraction of the isotope dose excreted in ammonia is the same as the excretion of unlabeled nitrogen in the end product when the end product is expressed as a fraction of the flux.

The rate of protein turnover derived from urea is generally higher than that from ammonia. These differences have been attributed to differences in the site of synthesis: urea is synthesized in the liver, whereas ammonia is synthesized in the kidney (17). An important issue is whether the end product method can accurately detect small differences in turnover rates among individuals. Using a single dose of [ $^{15}\text{N}$ ]glycine, as in the present study, Fern et al (16) reported an average reproducibility of the method in adults of 6.7%; this is 10 times less than the difference in whole-body protein breakdown between the 2 groups in the present study.

The results of this study show that the absolute rates of whole-body protein turnover (nitrogen flux), whole-body protein synthesis, and whole-body protein breakdown were significantly higher in obese, prepubertal children than in their lean counterparts. The difference between obese and nonobese children in nitrogen flux (45%) and protein synthesis (52%) was substantially greater than the difference in REE (26%) or the difference in FFM (27%). Note that protein synthesis largely exceeded protein breakdown in the present study (Figure 1) because the measurements were made under postprandial conditions, during which there is apparent protein storage. The reverse would have been observed in the postabsorptive phase.


We are not aware of any previous studies in prepubertal children for comparison, although several studies that used different methodologic approaches (24) were performed in obese adults who were in the static phase of obesity (25–28), who were dieting (29–32), or who had concomitant type 2 diabetes with or without exogenous insulin administration (33, 34). The effect of insulin resistance in obese subjects has also been studied (35). Only one study was performed in obese adolescents (36). Taken together, these experimental studies showed that whole-body nitrogen flux, protein synthesis, and protein breakdown were greater in obese persons (including those with type 2 diabetes) than in nonobese, nondiabetic persons. Our study confirms these results in prepubertal children. Note, however, that the mode of expression of protein turnover used in the above studies varied; in some studies, values were reported in absolute terms (g/d), whereas in others they were expressed per unit body weight (g/kg) or per unit FFM (g/kg FFM) or were statistically adjusted for FFM by ANCOVA.

As for energy expenditure, the type of normalization (ie, per kg FFM or per kg body wt) will affect the conclusions of studies when obese persons are compared with a nonobese group. For example, in our study, when protein metabolism values were expressed per kg FFM, only protein breakdown was significantly greater in obese than in nonobese children. This confirms the results of experimental studies performed previously in adults (26, 27). When the values were adjusted for FFM by ANCOVA, however, the difference in protein breakdown disappeared. The small sample size in the present study as well as the heterogeneity of the groups associated with the methodologic issues raised above may have resulted in insufficient statistical power to detect a difference between the groups.

Rates of REE and the size of the FFM were the 2 major variables significantly associated with whole-body protein flux, whole-body protein synthesis, and whole-body protein break-

down. Our interpretation of these results is that the absolute increase in FFM (including muscle mass) in obese children may engender a greater absolute protein turnover, although the protein turnover of the adipose tissue, still unknown at the present time, may somehow contribute to this value. Protein turnover is rapid in visceral tissues (such as the liver and gastrointestinal tract); thus, it is likely that the greater amount of substrates ingested by obese children to maintain their obesity (in the static phase) as well as the general elevation in substrate flux engender greater protein turnover in the splanchnic bed in particular.

In a previous study of carbohydrate metabolism in obese, prepubertal children (37), we reported a net decrease in glycogen breakdown that indicated a sparing of endogenous glycogen. We hypothesized that this was due to decreased glycogen turnover at an early age. The presence of an absolute increase in whole-body protein synthesis and breakdown concomitant with a decrease in glycogen breakdown constitutes indirect evidence of a dissociative effect in the disturbance of substrate metabolism. Whether a differential sensitivity of the action of insulin on protein compared with carbohydrate metabolism is operative in children, as elegantly shown in adults (35, 38, 39), remains to be seen.

In conclusion, the salient feature of the present study was that an absolute increase in protein metabolism, in particular whole-body protein synthesis and breakdown, was observed in obese children concomitant with an absolute rise in energy expenditure. This corroborates results described previously in obese adults. However, when protein and energy metabolism were adjusted for differences in FFM between the obese and nonobese children, the difference disappeared. It is hoped that subsequent studies will explore protein turnover in individual tissues to better understand which tissues contribute to explaining the net absolute increase in whole-body protein synthesis and breakdown in obesity. 

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