

DECREASED ENERGY EXPENDITURE IS CAUSED BY ABNORMAL BODY COMPOSITION IN INFANTS WITH PRADER-WILLI SYNDROME

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Objective Infants with Prader-Willi syndrome (PWS) are hypotonic and underweight before the onset of childhood obesity. This study evaluates body composition in the PWS infant and its relationship to energy expenditure.

Study design Sixteen infants and toddlers with PWS (mean age, 12.4 ± 6 months; eight female subjects) underwent analysis of body composition with dual-energy x-ray absorptiometry and deuterium dilution, and energy expenditure with both doubly labeled water and indirect calorimetry.

Results Percent body fat was significantly increased (male subjects, $P < .001$; female subjects, $P < .001$) and fat-free mass (FFM) was significantly decreased (male subjects, $P < .001$; female subjects, $P = .04$) in infants with PWS when compared with age-matched published data for normal infants. Meanwhile, total energy expenditure was significantly decreased (male subjects, $P = .025$; female subjects, $P < .001$) in infants with PWS when compared with published normative data. There was a normal relationship between FFM and total energy expenditure in infants with PWS.

Conclusion Compared with published data for infants without PWS, infants with PWS demonstrate increased percent body fat, decreased FFM, and decreased energy expenditure. Importantly, total energy expenditure per kilogram of FFM appears similar in infants with and without PWS. We conclude that lower energy expenditure in infants with PWS is caused by decreased FFM. (*J Pediatr* 2003;143:372-6)

Prader-Willi Syndrome (PWS), described in 1956,¹ is characterized by obesity, hypotonia, short stature, mental retardation, hypothalamic dysfunction, and hypogonadism.¹⁻⁴ The genetic abnormality is located on chromosome 15 (q11-13), specifically a deletion of the paternal allele or presence of maternal disomy.²⁻⁴ Other defining features include abnormal body composition with increased fat mass and decreased lean body mass seen even in young children with PWS.^{2,5} Growth hormone therapy improves but does not normalize body composition^{2,6} and abnormally low energy expenditure in children with PWS.²

Infants with PWS typically demonstrate failure to thrive before the onset of hyperphagia and obesity. Body fat estimates, determined by skin fold thickness, are nevertheless increased in these underweight infants.⁷ Direct measurement of body composition by dual-energy x-ray absorptiometry (DXA) or by deuterium dilution (DD) has not been previously reported in infants and toddlers with PWS.

Energy expenditure (EE) plays an important role in the development of obesity and is closely related to fat-free mass (FFM). Previous studies of children with PWS using indirect calorimetry (IC) reported markedly reduced resting EE in these subjects.² To measure total energy expenditure (TEE), the use of doubly labeled water (DLW) integrates expenditure over a more representative time and is recommended. This procedure requires ingestion of water labeled with deuterium and ¹⁸O with subsequent urine collection in free-living conditions. The difference in the two isotope elimination rates is used to calculate carbon dioxide production and, thus, EE.⁸ To understand better

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DD	Deuterium dilution	FFM	Fat-free mass
DXA	Dual-energy x-ray absorptiometry	IC	Indirect calorimetry
DLW	Doubly labeled water	PWS	Prader-Willi syndrome
EE	Energy expenditure	TEE	Total energy expenditure
GH	Growth hormone		

the propensity for obesity in persons with PWS, we measured TEE by DLW and resting EE by IC, and determined body composition by both DXA and DD in 16 infants and toddlers with PWS.

PATIENTS AND METHODS

Sixteen infants and toddlers with PWS age 5 to 23 months (eight female subjects, eight male subjects) were enrolled after informed consent was obtained from a parent or legal guardian. Institutional Review Board approval was obtained before study enrollment. All had a diagnosis of PWS confirmed by high-resolution cytogenetics, fluorescent in situ hybridization, methylation studies, or more than one of these methods. Ten subjects had deletion of chromosome 15q11-13, five had uniparental disomy, and one patient was diagnosed by an abnormal methylation test. Anthropometric measurements were obtained by a single observer by using a Harpenden stadiometer (Holtain, Crymch, UK). Stat GrowthCharts version 2.01 software was used to determine weight, height, and weight for height *z* scores. Subjects underwent clonidine-stimulated growth hormone (GH) testing (0.15 mg/m²) after a 6-hour fast with blood drawn at 60 minutes for GH (*n* = 14) and insulin-like growth factor I (*n* = 13) levels. GH was measured by chemiluminescent sandwich method at Associated Regional and University Pathologists, Salt Lake City, Utah. Insulin-like growth factor I levels were measured by radioimmunoassay by Nichols Laboratory (San Juan Capistrano, Calif).

Percent body fat and FFM were measured by DXA (Lunar Prodigy by GE Medical Systems, Madison, Wisc). FFM was also determined by DD (*n* = 14) calculated from the percent hydration expected for age and gender.⁹ From this, percent body fat was calculated.

Fourteen patients underwent DLW studies to determine TEE. A loading dose of 0.15 g/kg ¹⁸O (Isotec, Miamisburg, OH) and 0.1 g/kg deuterium-labeled water (Cambridge Isotope Laboratory, Andover, MA) was given on day 1. Urine samples collected in cotton diapers were obtained at 24 hours before dosing, then 12, 24, 120, and 180 hours after dosing. Isotope analysis was performed at the Schoeller Energy Expenditure Laboratory, Department of Nutritional Sciences, University of Wisconsin, Madison, Wisconsin by using all data points. The difference in elimination rates of the isotopes was used to calculate CO₂ production and EE by using established equations assuming a respiratory quotient of 0.89.¹⁰ The average isotope dilution space ratio was 0.989, and the average difference in measured elimination rates per subjects was 0.05.

Resting EE using IC (Medgraphics, Minneapolis, MN) was measured in 14 patients after a 6-hour fast and during provocative growth hormone testing using clonidine. All measurements were made by using body, temperature, pressure, saturated correction, with room temperature, humidity, and barometric pressure, which were entered before testing. Measurements were over a period of 20 minutes and were dependent on sleep state. Before testing, an external

Table I. Anthropometric data and GH axis results in infants and toddlers with PWS

Age (mo)	12.4 ± 6
Weight (kg)	8.3 ± 2
Weight Z score	-1.7 ± 1.3
Length Z score	-1.5 ± 0.9
Weight/Height Z score	-0.6 ± 1.7
Body mass index (kg/m ²)	16.4 ± 2
GH (μg/L)	11.4 ± 6
IGF-I (ng/dL)	29 ± 14
IGF-I SDS male	-2.1
IGF-I SDS female	-3.1

IGF-I, Insulin-like growth factor I; SDS, standard deviation score.

calibration was performed after a 30-minute warm-up. The current barometric pressure, temperature, and humidity were entered. A syringe calibration was performed by using a 3-L syringe and multiple flow rates. This was followed by a gas calibration by using zero, room air, and 7% CO₂ and 16% O₂ references. Last, a phase delay was performed to ensure that the analyzer response time was within range.

Statistical Methods

Pearson correlation coefficients were used to evaluate the correlation of body mass index versus percent body fat by DD, percent body fat by DD versus DXA, FFM by DD versus DXA, and EE by DLW versus IC. In addition, Bland-Altman plots and Tukey studentized range tests were performed to compare techniques of DXA with DD. PWS infant data for body composition and EE were compared with published data for normal infants (to age 24 months) by using two independent sample Student *t* tests, where pooled variances were calculated and *t* statistics were produced and compared with the standard table.¹¹⁻¹³ Because the normative data were presented by gender, our data were also stratified by gender for these *t* tests; otherwise, the data were pooled for genders. Comparison of male and female subjects with PWS demonstrated no difference in percent body fat, FFM, and TEE (*P* > .25, *P* > 0.1, and *P* > 0.1), respectively.

The relation of TEE to FFM between infants with and without PWS was evaluated with comparison of the quadratic polynomial regression slopes and intercepts by using a generalized hypothesis test approach.¹³ Analysis of covariance was used to define the relationship between changes in TEE and the changes in FFM and in percent body fat, adjusting for gender effects.

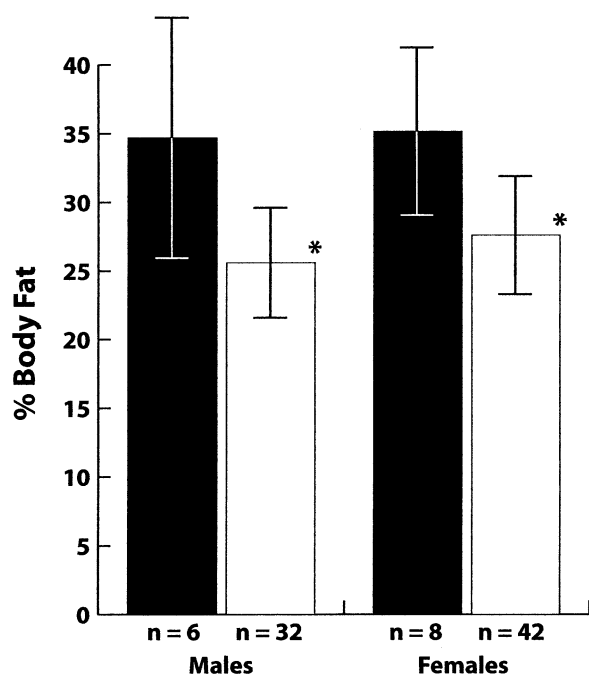
RESULTS

Anthropometric data and GH results are presented in Table I. Mean age of the study group was 12.4 ± 6 months. Subjects were short in length (mean *z* score, -1.5 ± 0.9) and underweight (mean *z* score, -1.7 ± 1.3). Mean stimulated peak GH levels were 11.4 ± 6 μg/L.

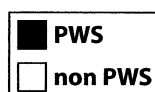
Body composition and EE data are listed in Table II. Measurements of percent body fat (*r* = 0.74, *P* < .001) and

Table II. Body composition and EE in infants and toddlers with PWS

	DXA (n = 19)	DLW (n = 17)	IC (n = 16)	r (P)
% Body fat	28.8 ± 6	34.9 ± 7	—	0.74 (.001)
FFM (kg)	5.7 ± 1.4	5.6 ± 1.2	—	0.92 (<.001)
EE (kcal/d)	—	587 ± 189	758 ± 477	0.38 (.19)
Respiratory quotient	—	—	0.81 ± 0.07	—

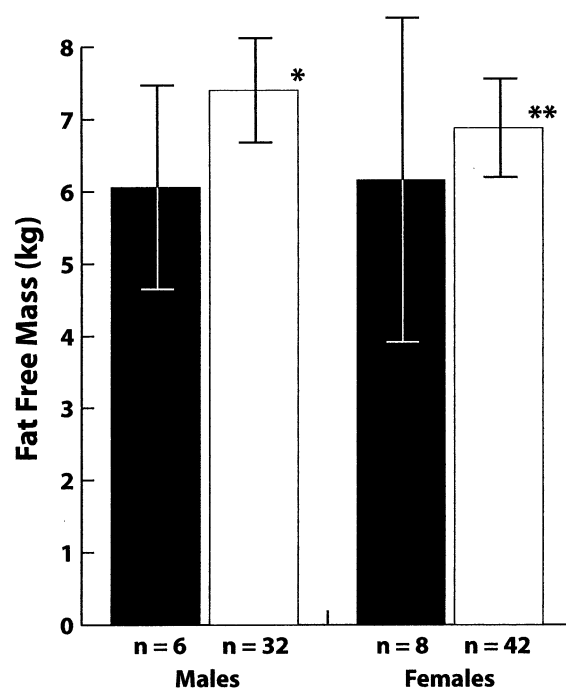


* p < .001

**Fig 1.** Body fat: PWS versus non-PWS.¹¹

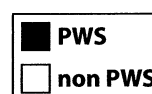
FFM ($r = 0.92$, $P < .001$) by DXA and calculated from DD were comparable. By using Bland-Altman plots, biases of 4.5 and -0.21 were seen for percent body fat and FFM, respectively. Statistically, there was no difference between the DD and DXA techniques for FFM ($P = .98$), but there was a difference for percent body fat ($P = .03$). Body mass index correlated with percent body fat calculated from DD ($r = 0.74$, $P < .001$).

Percent body fat by DD was significantly increased (male subjects, $P < .001$; female subjects; $P < .001$) compared with age-matched published data in healthy infants (Fig 1). FFM was significantly decreased in male ($P < .001$) and female ($P = .04$) infants with PWS (Fig 2). Compared with same-sex published norms, TEE in infants with PWS was significantly decreased (male subjects, $P = .025$; female subjects, $P < .001$) (Fig 3).



* p < .001

** p = .04

**Fig 2.** Lean body mass: PWS versus non-PWS.¹¹

There was no difference in the slope of the relationship between TEE and FFM between infants with and without PWS ($P = .12$) (Fig 4). The decrease in TEE was significantly associated with the decrease in FFM ($P < .001$) and was not related to percent body fat ($P = .56$).

There was no correlation between TEE measured by DLW and resting EE measured by IC. TEE measured by DLW in all infants with PWS (mean, 587 ± 189 kcal/d; 71 kcal/kg/d) was ~30% less than energy requirements per kilogram recommended by the World Health Organization (104 kcal/kg/d).

DISCUSSION

Infants with PWS typically fail to thrive, whereas children with PWS demonstrate progressive obesity with

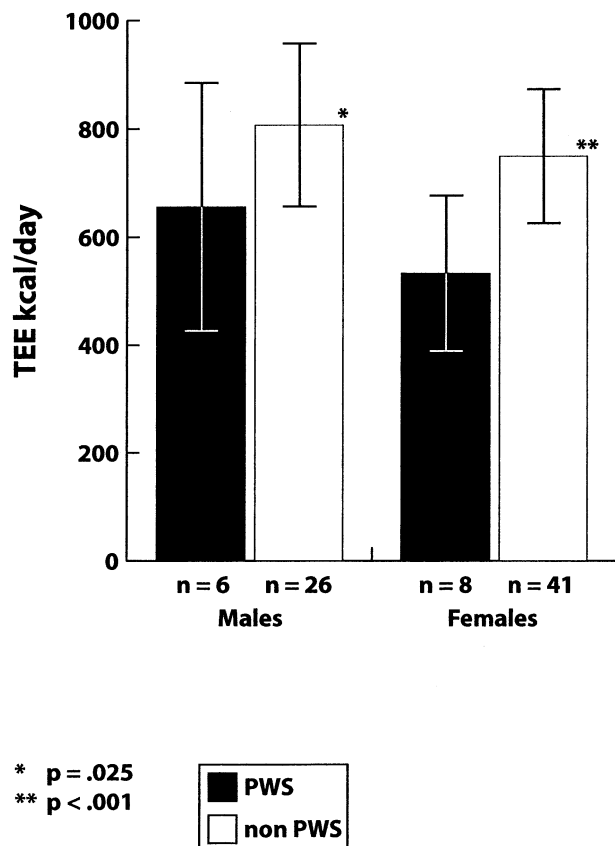


Fig 3. TEE: PWS versus non-PWS.¹²

abnormal body composition. Interestingly, previous studies using skinfold measurement reported increased subcutaneous fat in underweight infants with PWS. By using more accurate methods of DD, data confirm and extend these observations by showing significant differences in both percent body fat and FFM for male and female infants with PWS when compared with published data for healthy infants without PWS.¹¹ Abnormalities in FFM appeared more severe in male subjects than in female subjects with PWS. Typically, in healthy infants, age-matched male subjects have greater FFM than female subjects, whereas the male and female infants with PWS had similar FFM results. The relatively low FFM in male infants with PWS may reflect early infancy androgen deficiency caused by hypogonadotropic hypogonadism.

Abnormal body composition found in PWS is similar to that observed in patients with GH deficiency (increased percent body fat and decreased FFM). Interestingly, and in contrast with older children with PWS, our subjects did not demonstrate low GH levels after clonidine provocation. Possible explanations for this include the following: (1) clonidine is a poor provocative agent for assessing GH secretion in this very young population; (2) some manifestations of hypothalamic dysfunction present in PWS worsen with advancing age; and (3) increasing obesity during childhood has an additive, and perhaps exaggerated, suppressing effect on GH secretion in children with PWS.

Dual-energy x-ray absorptiometry offers a convenient method of determining body composition, and its application

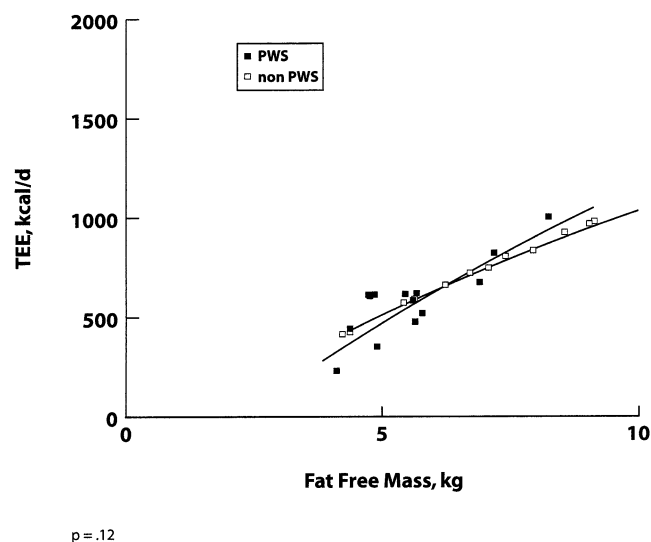


Fig 4. TEE per lean body mass: PWS versus non-PWS.¹²

in the pediatric population has been increasing. The correlation between DXA and DD was actually stronger than that seen in earlier studies,¹⁴ ie, we did not observe the overestimation of fat mass by DXA previously reported in children without PWS. Explanations for this discrepancy are unclear; however, there are modest (0%-6%) differences in FFM as determined by using different DXA models and software packages, and this may have been a contributing factor. It should also be pointed out that the comparative values for normal infants were obtained by using yet a different body composition assessment methodology. We acknowledge the difference in methods used to determine body composition in our study and the reference study of normal infants (ie, DD vs multicomponent model) and the fact that the methods are not identical for this age group; however, the assumptions with each method and expected group differences are small. Thus, results should compare within 3% to 4% body fat, which is much less than the difference we report.

Energy expenditure is composed of resting EE, physical activity, and the thermic effect of food, and can be challenging to quantify.¹⁵ The current gold standard is by DLW. Previous studies have successfully measured resting EE in older children by using IC. In this study, both total energy (DLW) and resting energy (IC) were measured. As expected, these values differed, but not in the expected direction. In fact, resting EE was greater than TEE, which cannot be explained physiologically. This discrepancy indicates that the technical difficulties in performing IC in infants and toddlers significantly undermine the validity of this methodology in this very young age group.

Several studies evaluating EE in infants by using DLW suggest that recommended energy requirements may be excessive.¹⁶⁻¹⁹ When compared with World Health Organization-recommended energy requirements and with measured EE in healthy infants (by using DLW),¹² we observed a significant decrease in EE before the onset of hyperphagia and obesity. TEE is closely related to resting EE and FFM

and is often expressed as a ratio of TEE:FFM.¹⁵ Importantly, these data show that this ratio is similar in children with and without PWS. Consequently, decreased TEE observed in infants with PWS with a normal TEE:FFM ratio suggests that reduced EE in infants with PWS is caused by abnormal body composition, namely reduced lean mass when compared with normal infants. Further, when energy requirements are determined on weight alone, these data demonstrate that caloric needs may be overestimated. Early life interventions that could increase FFM (eg, GH therapy alone) and decrease fat mass (eg, GH therapy combined with physiologically appropriate caloric restriction) may reduce the severity of obesity and body composition abnormalities seen later in the life of these patients.²⁰

REFERENCES

1. Prader A, Labhart A, Willi H. Ein Syndrom von Adipositas Kleinwuchs, Kryptorchismus und Oligophrenie nach myatonieartigen Zustand im Neugeborenenalter. *Schweiz Med Wochenschr* 1956;86:1260-1.
2. Carrel AL, Myers SE, Whitman BY, Allen DB. Growth hormone improves body composition, fat utilization, physical strength and agility, and growth in Prader-Willi syndrome: a controlled study. *J Pediatr* 1999;134:215-21.
3. Myers SE, Carrel AL, Whitman BY, Allen DB. Sustained benefit after 2 years of growth hormone on body composition, fat utilization, physical strength and agility, and growth in Prader-Willi syndrome. *J Pediatr* 2000;137:42-9.
4. van Mil Edgar, Westerterp KR, Gerver WJM, Lichtenbelt WDM, Kester ADM, Saris WHM. Body composition in Prader-Willi syndrome compared with nonsyndromal obesity: relationship to physical activity and growth hormone function. *J Pediatr* 2001;139:708-14.
5. Brambilla P, Bosio L, Manzoni P, Pietrobelli A, Beccaria L, Chiumello G. Peculiar body composition in patients with Prader-Labhart-Willi syndrome. *Am J Clin Nutr* 1997;65:1369-74.
6. Lindgren AC, Ritzen EM. Five years of growth hormone treatment in children with Prader-Willi syndrome. Swedish Growth Hormone Advisory Group. *Acta Paediatr Suppl* 1999;88:109-11.
7. Eiholzer U, Blum WF, Molinari L. Body fat determined by skinfold measurements is elevated despite underweight in infants with Prader-Willi syndrome. *J Pediatr* 1999;134:222-5.
8. Schoeller DA. Recent advances from application of doubly labeled water to measurement of human energy expenditure. *J Nutr* 1999;129:1765-8.
9. Schoeller DS. Human body composition. In: Roche AF, Heymsfield SB, Lohman TG, et al, eds. *Hydrometry*. Champaign (IL): Human Kinetics; 1996. p. 25-44.
10. Racette SB, Schoeller DA, Luke AH, Shay K, Hnilicka J, Kushner RF. Relative dilution spaces of 2H and 18-O-labeled water in humans. *Am J Physiol* 1994;267:E585-90.
11. Butte NF, Hopkinson JM, Wong WW, Smith EO, Ellis KJ. Body composition during the first 2 years of life: an updated reference. *Pediatr Res* 2000;47:578-85.
12. Butte NF, Wong WW, Hopkinson JM, Heintz CJ, Nehta NR, Smith EO. Energy requirements derived from total energy expenditure and energy deposition during the first 2 y of life. *Am J Clin Nutr* 2000;72:1558-69.
13. Freund RJ, Wilson WJ. *Statistical methods*. San Diego (CA): Academic Press; 1997:184-212.
14. Butte N, Heinz C, Hopkinson J, Wong W, Shypailo R, Ellis K. Fat mass in infants and toddlers: comparability of total body water, total body potassium, total body electrical conductivity, and dual-energy x-ray absorptiometry. *J Pediatr Gastroenterol Nutr* 1999;29:184-9.
15. Goran M. Energy metabolism and obesity. *Med Clin North Am* 2000;84:347-53.
16. Wells JCK, Davies PSW. Estimation of the energy cost of physical activity in infancy. *Arch Dis Child* 1998;78:131-6.
17. Stunkard AJ, Berkowitz JI, Stallings VA, Schoeller DA. Energy intake, not energy output, is a determinant of body size in infants. *Am J Clin Nutr* 1999;69:524-30.
18. Davies PSW. Energy requirements and energy expenditure in infancy. *Eur J Clin Nutr* 1992;46(suppl 4):S29-35.
19. Davies PSW. Energy requirements for growth and development in infancy. *Am J Clin Nutr* 1998;68(suppl):S939-43.
20. Schoeller DS, Levitisky LL, Bandhi LG, Dietz WW, Walczak A. Energy expenditure and body composition in Prader-Willi syndrome. *Metabolism* 1988;37:115-20.