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Decreased Bone Mineral Density in Prader-Willi Syndrome: Comparison With Obese Subjects

Merlin G. Butler^{1,*}, Lawrence Haber², Ray Mernaugh³, Michael G. Carlson³, Ron Price³, and Irene D. Feurer³

¹Children's Mercy Hospitals and Clinics and University of Missouri-Kansas City School of Medicine, Kansas City, Missouri

²Ohio State University, Columbus, Ohio

³Vanderbilt University, Nashville, Tennessee

Abstract

Bone density, anthropometric data, and markers of bone turnover were collected on 21 subjects diagnosed with Prader-Willi syndrome (PWS) and compared with 9 subjects with obesity of unknown cause. In addition, urinary N-telopeptide levels were obtained in all subjects. N-telopeptides are the peptide fragments of type I collagen, the major bone matrix material. During periods of active bone degradation or high bone turnover, high levels of N-telopeptides are excreted in the urine. However, no significant difference was detected in the urinary N-telopeptide levels when corrected for creatinine excretion (raw or transformed data) between our subjects with obesity or PWS and the observed effect size of the between-group difference was small. Although N-telopeptide levels were higher but not significantly different in the subjects with PWS compared with obese controls, the subjects with PWS had significantly decreased total bone and spine mineral density and total bone mineral content (all $P < 0.001$). No differences in N-telopeptide levels or bone mineral density were observed between subjects with PWS and chromosome 15q deletion or maternal disomy. Thus, decreased bone mineral density in subjects with PWS may relate to the lack of depositing bone mineral during growth when bones are becoming more dense (e.g., during adolescence), possibly because of decreased production of sex or growth hormones and/or long-standing hypotonia. It may not be caused by loss, or active degradation, of bone matrix measurable by the methods described in this study further supporting the possible need for hormone therapy during adolescence.

Keywords

Prader-Willi syndrome; bone mineral density; DEXA; bone mineral content; N-telopeptides; obesity

*Correspondence to: Merlin G. Butler, Section of Medical Genetics and Molecular Medicine, Children's Mercy Hospitals and Clinics, 2401 Gillham Road, Kansas City, MO 64108. mgbutler@cmh.edu.

INTRODUCTION

Prader-Willi syndrome (PWS) is characterized by short stature, hypogonadism, infantile hypotonia, mental deficiency, small hands and feet, behavior problems, early-onset childhood obesity, and a characteristic facial appearance [Cassidy, 1984, 1997; Butler et al., 1986; Butler, 1990; Holm et al., 1993; Butler and Thompson, 2000]. About 60% of subjects with PWS have a de novo paternally derived chromosome 15q11–q13 deletion, while the remaining subjects have uniparental maternal disomy of chromosome 15 or genetic imprinting defects. PWS occurs in about one in 15,000 individuals and is recognized as the most common genetic cause of marked obesity in humans [Butler, 1990; Butler and Thompson, 2000]. Recently, growth hormone deficiency has been documented in the majority of patients with PWS and this may impact negatively on bone mineral density (BMD) [Cassidy, 1997; Butler and Thompson, 2000].

Osteoporosis is reported to occur in 60% to 90% of patients with PWS and affects an estimated 25 million persons in the United States [Butler, 1990; Cassidy, 1997]. Osteoporosis is usually an asymptomatic condition until, or unless complications occur, such as fractures. Although the asymptomatic phase of osteoporosis can be recognized by direct measurement of bone mass from the spine or total body using dual-energy X-ray absorptiometry (DEXA) [Mazess et al., 1990], osteoporosis is defined as the microarchitectural deterioration of bone tissue and a consequent increase in fracture risk. Bone formation and resorption (or bone turnover) are reflected by high levels in blood and urine of various enzymes from bone cells, by-products of bone matrix synthesis or breakdown products of bone. Additional biochemical markers provide adjunctive information for bone density measurements as determined by DEXA.

Bone mineral density is a function of a dynamic process involving continuous resorption and production of mineralized bone matrix, with type I collagen accounting for more than 90% of the organic matrix [Schneider and Barrett-Conner, 1997]. The degradation of collagen during the process of osteoclastic bone resorption can be determined by measuring the level of urinary cross-linked N-telopeptides of type I collagen. N-telopeptides are small, amino-terminal peptide fragments containing the pyridinium cross-linked region of type I collagen and are released in circulation and excreted in the urine when the bone matrix is degraded. The metabolism of bone is influenced by several factors throughout the lifespan of an individual including sex hormone changes, gender, and age. Aging and estrogen deficiency are major factors in increased bone remodeling, with higher resorption activity resulting in bone loss and decreased BMD leading to osteoporosis. In addition, hypogonadism seen in the majority of patients with PWS may contribute to adult osteoporosis. Other reported orthopedic complications in PWS include scoliosis, hip dislocations, and fractures [Butler, 1990; Cassidy, 1997; Butler and Thompson, 2000].

Herein, we report our experience with PWS and obese subjects for comparison to determine whether bone matrix is actively degrading in this obesity disorder by measuring urinary N-telopeptides, bone density, and endocrine levels.

MATERIALS AND METHODS

Twenty-one subjects with PWS (14 females, 7 males; mean age $\pm$ SD = 21.3 ± 8.7 years; age range = 10–44 years) and 9 subjects with comparable intelligence and obesity of unknown cause (7 females, 2 males; mean age $\pm$ SD = 35.3 ± 11.8 years; age range = 16–50 years) were selected for comparison. All subjects were examined by one of us (M.G.B.), and the clinical diagnosis of PWS was made and confirmed by genetic testing (e.g., chromosome studies with fluorescence in situ hybridization, DNA methylation analysis), whereas the subjects with obesity were nonsyndromic and had normal genetic testing. All subjects had extensive endocrine studies including insulin, C-peptide, glucose, cortisol, sex hormone, thyroid, and leptin levels. The cause of the obesity in the obese subjects was not found. Written informed consent was obtained from each subject or from his or her parent or legal guardian before study participation. Subjects were selected from a large study on genotype/phenotype correlations in PWS and matched obese subjects recruited to Vanderbilt University and admitted to the Vanderbilt University Clinical Research Center. Twelve subjects with PWS had a 15q11–q13 deletion, and 9 were found to have maternal disomy 15 (both 15s from the mother). Both groups of subjects were matched for IQ (both subject groups had IQs in the mild mental retardation range) and had comparable obesity status.

Height, weight, and body mass index (BMI) (kg/m^2) were obtained on each subject. DEXA (Model DPX, Lunar Corporation, Madison, WI) was performed routinely in the radiology department while the subject was in the supine position to determine total BMD (g/cm^2), total bone mineral content (kg), lumbar spine BMD (g/cm^2), and body composition (percent fat). In addition, bone density data were compared with standards for age and sex. The demographic and anthropometric data are shown in Table I.

Urine samples were collected during a 12-hr period (7:00 p.m.–7:00 a.m.) from each subject while the subject was an inpatient at the research center. Samples were frozen at -70°C until analyzed. Fasting blood samples were collected at 7:00 a.m. for electrolytes, alkaline phosphatase, and endocrine studies. No patient was treated with growth hormone, a common treatment currently in use with many subjects with PWS.

A competitive inhibition enzyme-linked immunoabsorption assay (ELISA) was performed to measure N-telopeptide levels using a kit available from Osteomark (Seattle, WA) following manufacturer's protocols. This kit was standardized by the company using a multicenter cross-sectional study to determine the reference range for N-telopeptide levels from healthy premenopausal women and comparable aged males. The mean $\pm$ SD for the females was 35 ± 15 nmol bone collagen equivalent (BCE)/mmol of creatinine and 27 ± 12 for males (personal communication, Osteomark, Seattle, WA). Using the commercial kit and following manufacturer's recommendations, quantitation of N-telopeptide levels in each urine specimen was determined spectrophotometrically and calculated from a standard calibration curve in the Molecular Recognition Unit at Vanderbilt University. Assay values were corrected for urinary dilution by urine creatinine levels and expressed in nanomoles of BCE per millimole of creatinine. Statistical methods included standard exploratory techniques, logarithmic transformation of the N-telopeptide data (to a normal distribution), parametric

and nonparametric comparisons of group means, and bivariate correlation. Table II shows data on bone density and markers of bone turnover for the PWS and obese subjects.

RESULTS AND DISCUSSION

Scatterplots of subjects with PWS and obesity showing body mass index, percent fat, total bone mineral and spine mineral density, and N-telopeptide levels with age are shown in Figures 1–5. There was no significant difference in percent fat by DEXA (mean percent fat of 46.3 for obese controls and 53.6 for PWS subjects) or BMI (mean BMI of 38.9 for obese controls and 36.4 for subjects with PWS—generally subjects with PWS are shorter than obese controls at comparable ages if untreated with growth hormone) between the subjects with obesity and PWS. In addition, no difference was found in calcium or phosphorus levels. However, there were significant between-group differences (obese control vs. PWS) in spine (and total) BMD (g/cm^2) determined by DEXA (e.g., for total BMD mean $\pm$ SD = 1.25 ± 0.06 , and 1.10 ± 0.08 ; $P < 0.001$) and for alkaline phosphatase (72.4 ± 26.3 vs. 126.3 ± 72.3 IU/L; $P = 0.01$). No significant difference was detected in N-telopeptide levels when corrected for creatinine between obese control and PWS subjects (mean $\pm$ SD = 46.8 ± 46.4 , and 75.4 ± 80.3 ; t -test, $P = 0.33$ —the high variation may indicate a large age range for both PWS and obese subjects). Eight PWS subjects (3 males; 5 females) and one obese male had N-telopeptide levels above the upper limits of normal ($+2$ SD) established by Osteomark (Seattle, WA) in the use of their ELISA assay. Because the N-telopeptide data were significantly skewed (Shapiro-Wilk test of normality; $P = 0.01$), a logarithmic transformation was used to achieve a symmetric distribution in both groups (Shapiro-Wilk; $P = 0.39$). A t -test of the transformed data and nonparametric comparison of group means (PWS vs. obese controls) were not statistically significant (both $P = 0.43$). Although N-telopeptide levels were higher but not significantly different in PWS subjects compared with controls with obesity, the subjects with PWS had decreased BMD. Generally, subjects with obesity have increased bone density compared with controls of normal weight. No significant correlation was found between N-telopeptide and estrogen levels among the obese or PWS females. The sample precluded evaluation of the relationship between testosterone and N-telopeptide levels in obese males, and the relationship was not significant in the PWS males. A significant association between N-telopeptide and alkaline phosphatase was observed in these obese subjects ($r = 0.86$; $P < 0.01$) but not in the PWS group ($r = 0.06$; $P = 0.80$). An effect of gender on N-telopeptide was not observed in either the obese control or PWS groups. No difference in N-telopeptide or BMD data were observed in either deletion or maternal disomy PWS subjects. Total and spine BMD and total bone content were greater ($P < 0.001$) in obese controls compared with PWS subjects. Seven of 9 (78%) obese subjects and 1 of 21 subjects with PWS (5%) had total percent BMD greater than or equal to 100% for age-matched standards.

The World Health Organization diagnostic criteria for osteoporosis [Hurley and Khosla, 1997] uses BMD to define the status of an individual [normal: BMD within 1 SD of reference mean for young adults; low bone mass (osteopenia): BMD between -1.0 and -2.5 SD lower than reference mean for young adults; osteoporosis: BMD -2.5 or more SD lower than reference mean for young adults; severe osteoporosis: osteoporosis (as defined above) with one or more fragility fractures]. The BMD of all 9 of our subjects with obesity would

be classified as normal regardless of age. Twelve of the 21 PWS subjects were less than 20 years of age and could not be classified using the previously mentioned BMD criteria. Five of the remaining 9 subjects with PWS and greater than 20 years of age would be classified as normal, whereas 4 of the 9 subjects with PWS were classified as having low bone mass or osteopenia.

In summary, we detected significantly decreased BMD in the subjects with PWS compared to obese controls, but we did not identify significantly higher N-telopeptide levels in the subjects with PWS. Given that the absence of an effect of group on N-telopeptide data could be influenced by small sample size (and limited power), the difference was computed and expressed as the effect size [Cohen, 1998]. The observed group means, SDs, and sample sizes reflect a mean difference (in means) of 28.6 and an estimated population standard deviation of 154.4 [Borenstein et al., 1997]. Thus, the observed effect size (mean difference/population SD) was 0.19, which is small and provides further support of the absence of an effect of the subject group on N-telopeptide data. The decreased BMD in PWS subjects may be related to the lack of depositing bone mineral content in the developing bones of subjects with PWS and not because of loss or active degradation of bone matrix measurable by the method used in this study. Whether the decreased bone mass is related to sex hormone imbalance during puberty, hypotonia, or inactivity (common features in PWS) is unknown. In addition, patients with PWS generally have short stature and growth hormone deficiency [Cassidy, 1997; Butler and Thompson, 2000], and whether this hormone deficiency along with other endocrine imbalances further contributes to bone density or bone metabolism disturbances (e.g., alkaline phosphatase levels were higher in subjects with PWS compared with subjects with obesity and may reflect increased osteoblastic activity in the subjects with PWS in the absence of liver disease) is unclear but should be considered in the management of subjects with PWS. The combination of the above factors may contribute to decreased BMD and not directly relate to bone loss as detectable by the methods described in this study. Our study further supports the importance of early diagnosis of PWS and DEXA measurements in early childhood to determine the bone density status of these subjects. Possible hormone replacement (sex, growth, and/or thyroid) may be needed if studies show hormone deficiency, and calcium supplements may be needed in the diet. Thus, the decreased bone density in subjects with PWS may be alleviated and risk lowered for possible fractures in this subject population.

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References

- Borenstein, M.; Rothstein, H.; Cohen, J. Power and precision. Teaneck, NJ: Biostat; 1997.
- Butler MG. Prader-Willi syndrome: Current understanding of cause and diagnosis. *Am J Med Genet.* 1990; 35:319–332. [PubMed: 2309779]

- Butler MG, Thompson T. Prader-Willi syndrome: Clinical and genetic findings. *The Endocrinologist*. 2000; 10(Suppl):3S–16S. [PubMed: 27570435]
- Butler MG, Meaney FJ, Palmer CG. Clinical and cytogenetic survey of 39 individuals with the Prader-Labhart-Willi syndrome. *Am J Med Genet*. 1986; 23:793–809. [PubMed: 3953677]
- Butler MG, Hedges L, Hovis CL, Feurer ID. Genetic variants of the human obesity (OB) gene in subjects with and without Prader-Willi syndrome: comparison with body mass index and weight. *Clin Genet*. 1998; 54:385–393. [PubMed: 9842990]
- Cassidy SB. Prader-Willi syndrome. *Current Problems in Pediatrics*. 1984; 14:1–55.
- Cassidy SB. Prader-Willi syndrome. *J Med Genet*. 1997; 34:917–923. [PubMed: 9391886]
- Cohen, J. *Statistical power analysis for the social sciences*. Hillsdale, NJ: Erlbaum; 1998.
- Holm VA, Cassidy SB, Butler MG, Hanchett JM, Greenberg F. Prader-Willi syndrome: consensus diagnostic criteria. *Pediatrics*. 1993; 91:398–402. [PubMed: 8424017]
- Hurley DL, Khosla S. Update on primary osteoporosis. *Mayo Clinic Proc*. 1997; 72:943–949.
- Mazess RB, Barden HS, Bisek JP, Hanson J. Dual-energy X-ray absorptiometry for total-body and regional bone-mineral and soft-tissue composition. *Am J Clin Nutr*. 1990; 51:1106–1112. [PubMed: 2349926]
- Schneider DL, Barrett-Conner EL. Urinary N-telopeptide levels discriminate normal, osteopenia and osteoporotic bone mineral density. *Arch Intern Med*. 1997; 157:1241–1243. [PubMed: 9183236]

Fig. 1.

Scatterplot data from obese (2 males; 7 females) and Prader-Willi syndrome (7 males; 14 females) subjects showing age in years and body mass index (BMI). Obesity was defined as BMI ≥ 27 for subjects older than or equal to 15 years of age and ≥ 23 for ages 10–14 years [Butler et al., 1998] as represented on the scatterplot by the small broken lines. The number of subjects, correlations, and *P* values are presented for each subject group. Arrows by symbols represent males in each group.

Fig. 2. Scatterplot data from obese (2 males; 7 females) and Prader-Willi syndrome (7 males; 14 females) subjects showing age in years and total percent fat. The number of subjects, correlations, and *P* values are presented for each subject group. All subjects were in the obese range (greater than 35% body fat) excluding one 46-year-old female judged to have regional obesity with 35% fat in her lower extremities identified by dual-energy X-ray absorptiometry (DEXA). Arrows by symbols represent males in each group.

Fig. 3. Scatterplot data from obese (2 males; 7 females) and Prader-Willi syndrome (7 males; 14 females) subjects showing age in years and total bone mineral density. A representative upper and lower range for bone mineral density from premenopausal women is designated by the upper and lower small broken lines. The number of subjects, correlations, and P values are presented for each subject group. Arrows by symbols represent males in each group.

Fig. 4.

Scatterplot data from obese (2 males; 7 females) and Prader-Willi syndrome (7 males; 14 females) subjects showing age in years and spine bone mineral density. The number of subjects, correlations, and *P* values are presented for each subject group. Arrows by symbols represent males in each group.

Fig. 5.

Scatterplot data from obese (2 males; 7 females) and Prader-Willi syndrome (PWS) (7 males; 14 females) subjects showing age in years and N-telopeptide levels. The number of subjects, correlations, and P values are presented for each subject group. Upper limit of normal (+2 SD) for N-telopeptides of 65 nmol bone collagen equivalent (BCE)/mmol creatinine in premenopausal women is represented by the small broken line. Levels for males are generally lower. N-telopeptide levels for eight PWS subjects (3 males; 5 females) and one obese male were above this upper limit representing high levels. An N-telopeptide value of 67 or greater is associated with significant risk of bone loss as measured by bone mineral density. The relative risk for bone loss for values greater than 67 is 17.3 (personal communication, Osteomark, Seattle, WA). Arrows by symbols represent males in each group.

TABLE I

Anthropometric Data[†]

Group	N	Age range (yr)	Age (yr)	Height (cm)	Weight (kg)	BMI (kg/m ²)	% Fat (DEXA)
PWS	21	10–44	21.3 ± 8.7 ^{**}	149.7 ± 8.7 [*]	81.9 ± 22.4	36.4 ± 9.1	53.6 ± 5.4
Obese control	9	16–50	36.3 ± 11.8 ^{**}	157.1 ± 8.6 [*]	96.3 ± 29.7	38.9 ± 11.5	46.3 ± 10.8

* $P < 0.05$ [may be influenced by wide age range in both Prader-Willi syndrome (PWS) and obese subjects but generally PWS subjects without growth hormone treatment have short stature. Our patients were not treated with growth hormone].

^{**} $P < 0.01$.

[†] Entries (unless otherwise noted) are mean ± SD. BMI, body mass index; DEXA, dual-energy x-ray absorptiometry.

TABLE II

Bone Density and Markers of Bone Turnover[‡]

Group	N	Sex M:F	Total BMD (g/cm ²)	Spine BMD (g/cm ²)	Total BMC (kg)	Alkaline phosphatase (IU/L)	N-telopeptide (nmol BCE/mmol)
Prader-Willi	21	7:14	1.10 ± 0.08 ^{**}	0.97 ± 0.16 ^{**}	1.90 ± 0.45 ^{**}	126.3 ± 72.3 [*]	75.4 ± 80.3
Obese control	9	2:7	1.25 ± 0.06 ^{**}	1.27 ± 0.21 ^{**}	2.65 ± 0.51 ^{**}	72.4 ± 26.3 [*]	46.8 ± 46.4

^{*} *P* = 0.01.

^{**} *P* < 0.001.

[‡] Entries (unless otherwise noted) are mean ± SD. BMD, bone mineral density; BCE, bone collagen equivalent.