

## Research article

# Serum Ghrelin and Glukagon-like Peptid 1 Levels in Children with Prader-Willi and Bardet-Biedl Syndromes

Turkkahraman D et al. Ghrelin and GLP-1 Levels in PWS and BBS

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## what is already known about this topic

Ghrelin stimulates appetite and secretion of growth hormone, and induces a positive energy balance and leads to weight gain. GLP-1 exerts its central effects through the GLP-1 receptor in central nervous system reducing the rate of absorption of food into the blood via suppressing appetite reducing the rate of gastric emptying and inhibiting glucagon secretion.

## what this study adds to the literature

We found no definite role for ghrelin and GLP-1 in the pathogenesis of PWS. There is only one study in which the plasma ghrelin levels are not different between the BBS and control groups, and there is no study evaluating serum GLP-1 levels in BBS patients. However, similar studies with larger series are needed.

## Abstract

**Objective:** Prader-Willi Syndrome (PWS) and Bardet-Biedl syndrome (BBS) are common cause of pediatric syndromic obesity. We aim to investigate a possible role of ghrelin and glukagon-like peptid-1 (GLP-1) in pathophysiology of Prader-Willi Syndrome (PWS) and Bardet-Biedl syndrome (BBS).

**Methods:** We recruited 12 subjects with PWS, 12 subjects with BBS, 13 obese controls (OC) and 12 lean controls (LC). Fasting serum ghrelin and GLP-1 levels were measured by ELISA method.

**Results:** In PWS group, no significant difference was detected for ghrelin when compared with OC and LC; 0.96 (0.69-1.15), 0.92 (0.72-1.20) and 1.13 (0.84-1.29 ng/ml), respectively. Similarly, no significant difference was detected for GLP-1 when compared with OC and LC; 1.86 (1.5-2.94), 2.24 (1.62-2.78) and 2.06 (1.8-3.41) ng/ml, respectively.

In BBS group, no significant difference was detected for ghrelin when compared with OC and SC; 1.05 (0.87-1.51), 0.92 (0.72-1.20) and 1.13 (0.84-1.29) ng/ml, respectively. Similarly, no significant difference was found for GLP-1 when compared with OC and SC; 2.46 (1.91 to 4.17), 2.24 (1.62-2.78) and 2.06 (1.8-3.41) ng/ml, respectively.

**Conclusion:** We found no definite role for ghrelin and GLP-1 in the pathogenesis of obesity in PWS and BBS. However, similar studies with larger series are needed.

**Keywords:** BBS, PWS, Ghrelin, GLP-1

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## Introduction

Prader-Willi Syndrome (PWS) is the most common cause of pediatric syndromic obesity. The clinical characteristics of PWS consist of hyperphagic obesity in early childhood, decreased fetal movement, neonatal hypotonia, developmental delay, cognitive deficits, short stature, and hypogonadotropic hypogonadism (1). Subjects with PWS developed uncontrolled appetite resulting in weight gain after 2 years of age, although they had poor feeding and appetite in infancy. PWS is seen in all races regardless of sex with a frequency of approximately 1 in 10,000 to 1 in 15,000 live births, and results mostly from the absence of functionally active paternal inheritance in 15q11.2-13 chromosome region (2, 3).

On the other hand, Bardet-Biedl syndrome (BBS) can be described as a genetically heterogeneous ciliopathy with an autosomal recessive inheritance. The characteristics of BBS is described by six primary attributes: progressive rod-cone dystrophy, early-onset obesity, postaxial polydactyly, hypogonadism, cognitive impairment, and genitourinary tract malformations and progressive renal dysfunction. Its prevalence is about 1 to 9 in 1,000,000. Mutations in 24 different genes have been detected to date to be responsible for this phenotype (4).

Ghrelin is a 28-aminoacid peptide that is the natural ligand for the growth hormone secretagogue receptor (GHS-R) and produced mainly by stomach and increases in fasting. Ghrelin stimulates appetite and secretion of growth hormone, and induces a positive energy balance and leads to weight gain. Ghrelin levels are low after meals or in hyperglycemia, and also in obesity (5). Hyperghrelinemia has been stated in older children and adults with PWS in many studies but its correlation with obesity is still unclear (6). However, there is only one study evaluating ghrelin levels in BBS (7).

Glucagon-like peptide-1 (GLP-1) is synthesized mainly by L-cells in duodenum, small intestine and in small quantities by pancreas and hypothalamus. Its secretion in gastrointestinal tract is influenced by glucose and fatty acids after food intake or as a result of stimulation of the vagus nerve. The main mechanisms of action of GLP-1 include stimulating insulin secretion by  $\beta$ -cells in the islets of Langerhans and inhibiting glucagon secretion by  $\alpha$ -cells. GLP-1 also exerts its central effects through the GLP-1 receptor in central nervous system reducing the rate of absorption of food into the blood via suppressing appetite, and reducing the rate of gastric emptying (8). The relationship between GLP-1 and obesity is not certain and controversial in terms of underlying pathophysiology (9). There are only two studies evaluating fasting plasma GLP-1

levels; in the first one, fasting GLP-1 concentrations in adults with PWS were similar in individuals with obesity and lean control groups. In the second one, fasting GLP-1 concentrations in adults with PWS were higher than obese control and lean control groups, but GLP-1 concentrations in obese and lean group were found to be similar (10,11). However, there was no study evaluating fasting plasma GLP-1 levels in BBS patients. In this study, we aimed to investigate the role of ghrelin and GLP-1 in the pathophysiology of syndromic obesity.

#### Material and methods

The patients with diagnosis of BBS and PWS between the ages of 3 and 18 years were selected among the patients followed in our pediatric endocrinology and clinical genetics clinics. We recruited 12 subjects with PWS (all genetically confirmed) and 12 subjects with BBS (9 genetically confirmed, 3 clinically confirmed). The clinical diagnosis of BBS was made according to criteria published by Beales et al (12). The control subjects matched for age, sex and puberty were selected from our pediatric clinics, and those with any endocrine (diabetes mellitus, hypothyroidism, and adrenal deficiency etc.), systemic or infectious diseases, and those taking any kind of medication were excluded. The study protocol was approved by the Local Institutional Review Board (no: 2022-164). Informed written consents from the subjects (> 8-year-old) and their parents were obtained. The study was conducted according to the principles of the Declaration of Helsinki.

#### Anthropometric measurements

Body weight of subjects were measured using a pre-calibrated digital SECA scale. Height was measured with a 0.1 cm sensitivity in a Harpenden Stadiometer. The obesity was defined as a BMI-SDS greater or equal to 2 using national BMI data defined according to age and gender (13). Pubertal developmental stage was evaluated using Marshall and Tanner's standards (14).

#### Assays

Venous blood samples were taken from the subjects in the morning after a 12-h fast. After centrifugation for 20 min at 2000 RPM, serum samples were stored at -20 °C. Serum ghrelin and GLP-1 levels were measured by the ELISA method (Shanghai Korain Biotech (BT-LAB) Co., Ltd., Shanghai, China). The detection range of ghrelin assay (by competitive inhibition method) was 0.05–10 ng/ml with a sensitivity of 0.01 ng/ml. The detection range of GLP-1 assay (by competitive inhibition method) was 0.05–30 ng/ml with a sensitivity of 0.026 ng/ml. The inter-assay coefficient of variation (CV) and intra-assay CV for both 2 assays was the same; <10% and <8%, respectively.

#### Statistical analysis

The sample size calculation was performed using the DSS statistical software package (DSS, Locke Ave, Fort Worth, USA) for research sample size calculations. It was calculated that a minimum of 12 participants in each group would be required to demonstrate a difference of at least 10% for GLP-1 between the groups, with a power of 80% at the 5% significance level. The statistical analyses were performed using SPSS 15.0 for Windows (SPSS, Chicago, IL, USA). The Shapiro-Wilk-test was used for examining the continuous variables with normal and abnormal distributions while continuous variables with parametric distribution was analyzed using Analysis of Variance and if the differences were significant, a post-hoc Tukeys test performed, and it was presented as the mean±standard deviation (SD). The Kruskal-Wallis test was used for the abnormally distributed continuous variables and it was presented as median and IQR (inter quartil range, 25th–75th percentile). When the Kruskal-Wallis test indicated statistically significant differences, the causes of those differences were determined using a posthoc Dunn's test. The nominal variables were analyzed using the Pearson's chi-square or Fisher's exact test when applicable. The categorical variables were presented as the number of cases. Statistical significance was set at  $p < 0.05$ .

#### Results

We studied 12 subjects with PWS (mean age;  $10.6 \pm 4.8$  years), 12 subjects with BBS (mean age:  $10.3 \pm 4.7$  years), 13 obese controls (OC), (mean age:  $11.1 \pm 3.8$  years) and 12 lean controls (LC), (mean age:  $10.9 \pm 4.2$  years). There is no statistically significant difference between PWS, BBS, OC and LC in terms of age, gender and pubertal status ( $p > 0.05$ ). BMI-SDS was similar in PWS, BBS and OC ( $p > 0.05$ ), whereas BMI-SDS of LC was lower than that of PWS, BBS and OC ( $p < 0.001$ ). Furthermore, in PWS group, 3 patients have been on both L-thyroxine and recombinant growth hormone, and one patient has been on L-thyroxine and hydrocortisone treatments. In BBS group, only one patient has been on L-thyroxine because of central hypothyroidism.

The median ghrelin level was 0.96 (0.69-1.15) in PWS group, 0.92 (0.72-1.20) in OC group, and 1.13 (0.84-1.29) ng/ml in LC group. There was no statistically significant difference between the three groups ( $p > 0.05$ ). Additionally, median GLP-1 level was 1.86 (1.5-2.94) in PWS group, 2.24 (1.62-2.78) in OC group, and 2.06 (1.8-3.41) ng/ml in LC group. There was no statistically significant difference between the three groups ( $p > 0.05$ ), **Figure 1**.

The median ghrelin level was 1.05 (0.87-1.51) in BBS group, 0.92 (0.72-1.20) in OC group, and 1.13 (0.84-1.29) ng/ml in LC group. There was no statistically significant difference between the three groups ( $p > 0.05$ ). Additionally, the median GLP-1 level was 2.46 (1.91-4.17) in BBS group, 2.24 (1.62-2.78) in OC group, and 2.06 (1.8-3.41) ng/ml in LC group. There was no statistically significant difference between the three groups ( $p > 0.05$ ), **Figure 1**. Furthermore, when PWS and BBS groups were compared regarding ghrelin and GLP-1 levels, no statistically significant difference was found between the two groups ( $p > 0.05$ ). Clinical characteristics and laboratory data of the subjects are shown in **Table 1**.

#### Discussion

Early onset obesity and hyperphagia are the main characteristic features of PWS. In many studies, hyperghrelinemia has been reported in these patients (15). In large a review by Tauber M et al, hyperghrelinemia was detected in PWS patients and the authors linked obesity and hyperphagia to hyperghrelinemia (16). On the other hand, several groups reported that total ghrelin levels were not elevated in young children with PWS compared to control groups. Among these studies, Haqq et al found that plasma ghrelin values are similar to the control group in a study with 33 infants with PWS (17). Buttler et al divided PWS patients into groups of patients under and over 3 years of age, but they did not detect hyperghrelinemia in these groups compared to controls (18). Lastly, Erdic-Lalena et al found that ghrelin values were similar in PWS patients under 5 years of age compared to controls (19). According to these studies obesity in PWS develops after the initial phase of poor feeding, starting in the second year of life and hyperghrelinemia does not precede or coincide with the development of hyperphagia. An increased number of ghrelin-producing cells in the stomach of PWS patients is supposed to cause ghrelin levels to rise (20). In our study, we did not find hyperghrelinemia in PWS compared to obese controls. We found no definite role for ghrelin in the pathogenesis of obesity in PWS. This shows that obesity in PWS patients may not be due to hyperghrelinemia. In contrast, in a study by Turkkahraman et al, it was found that the mean  $\alpha$ -MSH level in PWS group is significantly lower than in obese controls and therefore they suggested that obesity in PWS might be due to MC4R upstream pathologies (21). As a result, in literature there is no consensus regarding the ghrelin levels especially in children with PWS.

On the other hand, there are only two studies in literature investigating serum GLP-1 levels in PWS patients. In the first adult study, fasting GLP-1 concentrations in PWS subjects were similar in individuals with obesity and lean control groups (10). In the second adult study, fasting GLP-1 concentrations in PWS were higher than obese control and lean control groups, but GLP-1 concentrations in obese and lean group were found to be similar (11). Our results consistent with the results of the first study. In the present study, we did not find a statistically significant difference in GLP-1 concentrations in subjects with PWS compared to control groups, and in obese controls compared to lean controls. Similarly, in literature there is no consensus regarding GLP-1 levels especially in children with PWS.

The BBSome is a critical regulator of cilia function. Primary cilia are important signaling organelles including neuronal trafficking (22). Deng-Fu Guo et al. showed that selective disruption of BBSome via BBS1 gene deletion leads to a significant increase in body weight and adiposity and its lead to leptin resistance and hyperleptinemia (23). In literature, there is only one study in which the plasma ghrelin levels are not different between the BBS and control groups (7). On the other hand, there was no study evaluating serum GLP-1 levels in BBS patients. In the present study, we compared both ghrelin and GLP-1 concentrations of BBS patients with control groups, and we found no definite role for ghrelin and GLP-1 in the pathogenesis of obesity in BBS. The possible limitation of our study is the small number of PWS and BBS patients.

In conclusion, there are conflicting results regarding ghrelin levels, and no enough data for GLP-1 levels in children with PWS. Similarly, there are no enough data regarding ghrelin and GLP-1 levels in BBS patients. Even though we could not detect any relation between the ghrelin and GLP-1 levels and these two syndromes, we hopefully think that our study will contribute to the pathogenesis of PWS and BBS. However, multicenter studies with larger patient groups are needed.

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#### Conflict of interest

The authors declare that they have no competing interests.

#### Authors' contributions

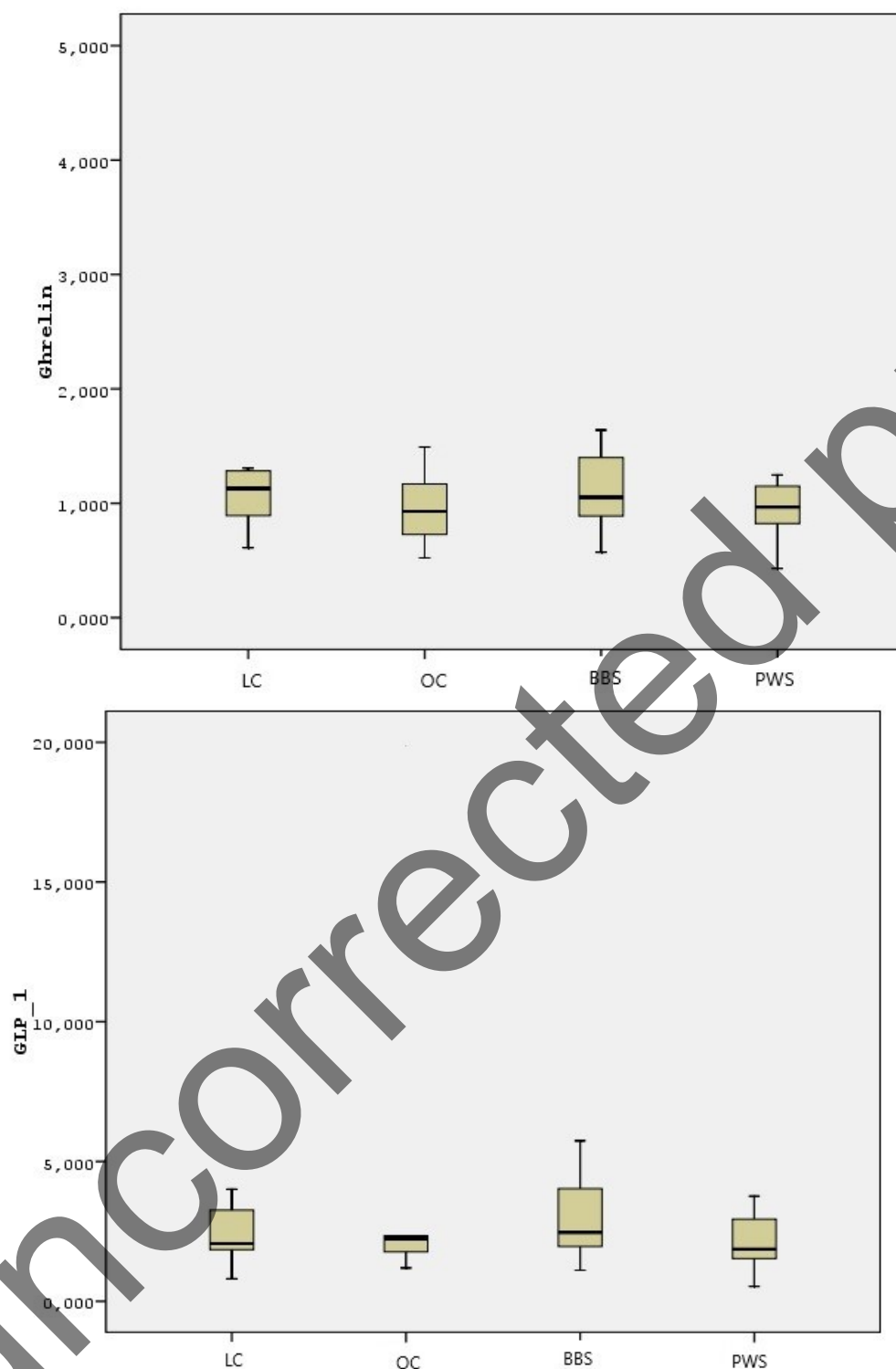
Concept and design: DT, ST, MG Data collection or Processing: ST, Analysis and Interpretation: GA, ST, MG Literature Search: ST, DT, MG Writing: DT, ST, MG, GA. All authors critically revised the manuscript and approved the final version.

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**Fig 1.** Box-plot presentation of serum ghrelin (ng/ml) and GLP-1 (ng/ml) levels in subjects with Prader-Willi syndrome (PWS) and Bardet-Biedl syndrome (BBS), and in obese controls (OC) and lean controls (LC). The lower and upper limits of the boxes represent 25<sup>th</sup> and 75<sup>th</sup> percentiles, and middle lines in each box represent 50<sup>th</sup> percentile, while the bottom and top end of the whiskers represent the min. and max. values, respectively. Dots represent the outlier data.

**Table 1** Clinical characteristics and laboratory findings of the patients and the control subjects

	<b>PWS (n=12)</b>	<b>BBS (n=12)</b>	<b>OC (n=13)</b>	<b>LC (n=12)</b>	<b>P</b>
Age (Years) (Min-max)	10.6 ±4.8 (3-17.6)	10.3±4.7 (5.3-19)	11.1±3.8 (6.8-17.2)	10.9±4.2 (2.8-16.4)	0.970
Female/male	6/6	4/8	6/7	8/4	0.412
Pubertal/prepubertal	4/8	6/6	4/8	6/6	0.136
BMI-SDS	2.7±1.6 <sup>c</sup>	3.13±0.5 <sup>b</sup>	2.81±0.5 <sup>a</sup>	1.18±0.9 <sup>a,b,c</sup>	<b>&lt;0.001</b>
Ghrelin (ng/ml)	0.96 (0.69-1.15)	1.05 (0.87-1.51)	0.92 (0.72-1.20)	1.13 (0.84-1.29)	0.485
GLP-1 (ng/ml)	1.86 (1.5-2.94)	2.46 (1.91-4.17)	2.24 (1.62-2.78)	2.06 (1.8-3.41)	0.460

PWS; Prader-Willi Syndrome, BBS; Bardet-Biedl Syndrome, OC; obese controls, LC; lean controls

Laboratory data were given as median for ghrelin and GLP-1 (inter-quartile range, 25<sup>th</sup>-75<sup>th</sup> percentile). One-way ANOVA (mean ± SD) was used for the age and BMI-SDS, Kruskal Wallis was used for the rest.

a: OC vs LC p< 0.05

b: BBS vs LC p< 0.05

c: PWS vs LC p< 0.05