Klinik Çalışma - Araştırma / *Original Article*

Evaluation of Serum Ghrelin and Neuropeptide Y Levels in Epileptic Children Under Valproate Treatment

Valproat Tedavisi Alan Çocuklarda Serum Ghrelin ve Nöropeptid Y Düzeylerinin Değerlendirilmesi

Ahmet GÜZEL, Serap KARASALİHOĞLU, Yasemin KÜÇÜKUĞURLUOĞLU, Esra SAYAR, Hakan KUNDURACILAR[1]

Departments of Pediatrics and [1]Clinical Microbiology, Medical Faculty of Trakya University, Edirne

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Objectives: Weight gain and obesity are also among the side effects of long-term valproate treatment, whose etiology remains unclear. The aim of this study is to investigate the relationship between weight gain, serum neuropeptide Y and ghrelin levels in children taking valproate treatment.

Patients and Methods: Our study included 25 epileptic children (14 males, 11 females; mean age 7.7±3.8 years; range 4 to 12 years) who were taking only valproate monotherapy at the outpatient clinics. All study participants were analyzed in terms of body mass index, serum neuropeptide Y and ghrelin levels before treatment and after a follow-up period of one year.

Results: At the end of follow-up, the mean body mass index measurements increased, whereas the mean serum neuropeptide Y and ghrelin levels decreased; twelve patients (48%) gained obesity.

Conclusion: This study demonstrated low serum ghrelin and neuropeptide Y levels in children receiving valproate treatment. New studies with larger sample sizes and longer follow-up periods are needed to evaluate the mechanism of weight gain due to valproate treatment in this population.

Key words: Valproate; ghrelin; neuropeptide Y; children; obesity; weight gain.

Amaç: Tartı alımı ve obezite uzun süreli valproik asit tedavisi alan epileptik hastalarda oldukça sık rastlanan ve etyolojisi net olarak bilinmeyen bir yan etkidir. Bu çalışmada valproik asit tedavisi alan çocuklarda meydana gelen tartı alınımı ile serum ghrelin ve nöropeptid Y düzeylerinin ilişkisi araştırılmıştır.

Hastalar ve Yöntemler: Bu çalışmaya epilepsi tanısıyla takip edilen, ilk defa çocuk polikliniğimize başvuran ve sadece valproik asit tedavisi alan 25 çocuk hasta (14 erkek, 11 kız; ort. yaş 7.7±3.8; dağılım 4-12) alınmıştır. Hastalar tedavi öncesi ve tedavi başlangıcından bir yıl sonra vücut kitle indeksi, serum ghrelin ve nöropeptid Y düzeyleri bakılarak değerlendirilmeye alınmıştır.

Bulgular: Çalışma sonunda olguların %48'inde obezite gelişirken, ortalama vücut kitle indeksi ölçümlerinde artma, serum ghrelin ve nöropeptid Y düzeylerinde ise azalma gözlenmiştir.

Sonuç: Bu çalışma valproik asit tedavisi gören çocuklarda serum ghrelin ve nöropeptid Y düzeylerinde azalma olduğunu göstermiştir. Valproik asit tedavisi ile ilişkili tartı alımında etkili faktörlerin değerlendirilmesinde daha uzun süreli, daha fazla hastanın katılımında kontrollü çalışmalara ihtiyaç vardır.

Anahtar sözcükler: Valproik asit; ghrelin; nöropeptid Y; çocuk; obezite; tartı alımı.

Correspondence (İletişim adresi): Dr. Ahmet Güzel. Trakya Üniversitesi Tıp Fakültesi Çocuk Sağlığı ve Hastalıkları Anabilim Dalı, 22030 Edirne.
Tel: 0284 - 235 76 41 Fax (Faks): 0284 - 235 23 38 e-mail (e-posta): ahmetgzl@yahoo.com

Valproate (VPA) is commonly used in the treatment of epilepsy in childhood. Although regarded as safe, the use of VPA can cause noticeable increase in body weight.[1] In previous studies, the rates of weight gain due to VPA treatment was reported as 14-71% in adult studies.[2-4] The pathophysiology of weight gain in patients taking VPA treatment remains unclear. Possible ways of weight gain in VPA treatment are *i)* VPA inhibition of β-oxidation, *ii)* VPA competition with fatty acids for albumin binding, *iii)* the role of VPA in regulation of energy expenditure and appetite in hypothalamus, *iv)* VPA leads to hyperinsulinemia and hyperleptinemia.[3,5-6]

The discovery of ghrelin and neuropeptide Y (NPY) has added new perspective to the pathophysiology of weight gain.

Ghrelin is an orexigenic peptide predominantly secreted by endocrine cells in the stomach mucosa and hypothalamus, but its expression has also been exhibited in many other tissues.[7-10] Ghrelin stimulates food intake and regulate energy homeostasis through activating the expression of the orexigenic neuropeptides NPY and agouti-related protein in hypothalamic neurons, by which it plays a key role in obesity pathogenesis. It is known that serum ghrelin levels increase before meal and decrease after feeding.[7-10]

Serum ghrelin levels decrease after feeding. However, ghrelin has other actions including stimulation of lactotroph and corticotroph function, influence on the pituitary-gonadal axis, control of energy balance, influence on sleep and behavior, control of digestive system, influence on pancreatic function as well as on glucose metabolism.[11-12] Neuropeptide Y is primarily secreted from central and peripheral neurons and plays an important role in the stimulation of feeding behavior.[13-14] However, the roles of ghrelin and NPY in antiepileptic-related weight gain or obesity are largely unknown.

The aim of present study is to investigate the relationship between weight gain in children who under VPA treatment and serum ghrelin and NPY levels.

PATIENTS AND METHODS

The study was conducted in randomly selected patients who were diagnosed as epilepsy at the outpatient clinic of the Department of Pediatrics, Trakya University Medical Faculty, Edirne, Turkey. The study protocol was approved by the local ethical committee.

Twenty-five newly diagnosed epileptic patients (14 males, 11 females) aged between 4 and 12 years were included in the study. Twenty-four patients had generalized epilepsy, and one patient had idiopathic partial epilepsy (Table 1). The diagnosis of epilepsy was made after an appropriate personal history, clinical examination, neuroradiologic evaluation and electroencephalography. All patients began VPA (Depakin syrup, Sanofi Aventis İlaç A.Ş., İstanbul, Turkey) orally twice daily after two unprovoked seizures.

Patients with pathology in cranial imaging, any syndrome, chrosomal abnormality, endocrinopathies, diabetes mellitus, obesity in family history, multi-antiepileptic drug usage, liver and kidney diseases or pathology in neurological examination were not recruited according to the exclusion criterias. All the patients were evaluated before the beginning of therapy and after one year of VPA treatment. All patients' weights, heights, body mass index (BMI) percentiles, ages, and genders were recorded, and none of them had obesity before the beginning of the VPA treatment. All patients were evalu-

Table 1. The clinical features of patients studied

Number of patients	Age (years)	Gender (F/M)	Type of epilepsy		VPA usage dose	Serum level of VPA
			G	P/PG	(mg/kg/day)	(μg/ml)
25	7.72±3.75†	11/14	24	1	21.96±7.81†	73.68±14.47†

†Values are given as mean±SD. G: Primarily generalized; P: Partial; PG: Partial secondarily generalized; VPA: Valproic acid.

Table 2. Demographic and biochemical characteristics of the study population

Characteristics	Before treatment (n=25)	After one year (n=25)	p^*
Weight (kg)	25.86±14.16	33±17.07	<0.01
Height (m)	121.88±24.88	126±24.52	<0.01
BMI (kg/m^2)	16.02±2.59	19.52±4.02	<0.01
Ghrelin (pg/mL)	93.50±60.21	67.35±56.97	<0.05
Neuropeptide Y (pg/mL)	66.28±12.86	60.96±16.50	>0.05

Values are given as mean±SD. *Wilcoxon matched-pairs test.

ated after at least one year of treatment with clinical follow-up for any seizures, weight gain, drug usage and daily dosage. After follow-up period, all cases were reexamined and recorded weights, heights, BMI percentiles, drug daily dosages that patients were taking and serum levels of VPA were recorded. Daily activities, and nutritional behavior of the patients was not changed during the period of study.

Heights and weights were measured, and the Quetelet index BMI was then calculated as weight (kg) divided by height (m^2). Body mass index over 95% for age was accepted as obese.[15] Blood samples were taken promptly and collected before the treatment and at the end of follow-up, and stored at –80 °C until assayed. Samples were taken at 8.00 am for ghrelin and NPY after 12 hours fasting period without taking VPA in the morning.

Serum ghrelin was determined with a commercial ELISA kit (Active Ghrelin ELISA Kit, Linco Research, Inc, St. Charles, Missouri, USA). Serum NPY levels were measured by a commercial ELISA kit (NPY ELISA kit, Phoenix Pharmaceuticals, Inc, USA). Serum VPA concentrations were determined in duplicate by a capillary gas chromotography method. Normal serum VPA levels were accepted as 50-100 μg/mL.

Statistical analyses

All data are reported as the mean ± SD. The normal distribution of variables was checked with the Kolmogorov-Smirnov test. The differences between the values before and after treatment were tested with the Wilcoxon matched-pairs test. The Mann-Whitney U-test was used to compare the mean values between the groups. The correlation between variables were tested with the Spearman test. A p value less than 0.05 was accepted as the level of significance.

RESULTS

We followed up all patients from the beginning of treatment for at least one year; at the end of follow-up, there were 12 patients (48%) in whom obesity was developed. The seizures were controlled in all patients, and their mean ages were 7.7±3.8 years (Table 1). None required additional antiepileptic drugs during follow-up. The baseline weights, heights, and BMI were within normal limits.

Table 3. Demographic and biochemical characteristics of obese and nonobese patients

	Obese (n=12) Mean±SD			Nonobese (n=13) Mean±SD		
Characteristics	Before treatment	After one year	p	Before treatment	After one year	p
BMI (kg/m^2)	16.21±2.43	21.90±3.79	0.002	15.83±2.81	16.33±2.88	0.916
Ghrelin (pg/mL)	68.06±47.66	65.80±67.23	0.388	116.99±62.63	78.78±48.40	0.066
NPY (pg/mL)	64.25±21.05	60.41±12.21	0.209	60.46±20.74	61.46±21.19	0.814

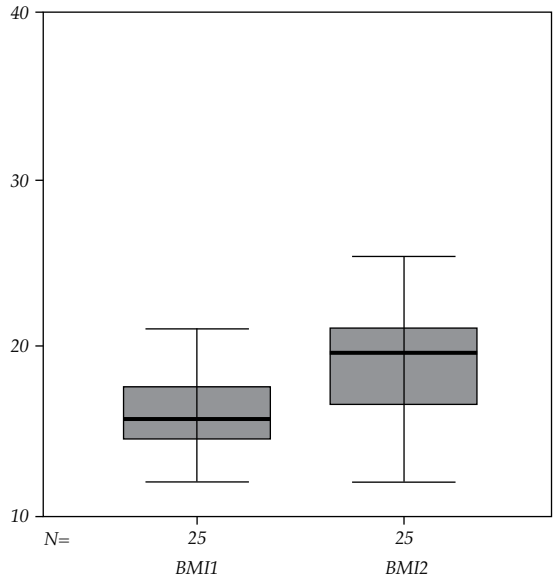

Fig. 1. *The mean body mass index values of patients were significantly increased at the end of one year (16.02±2.59 versus 19.52±4.02, p<0.01).*

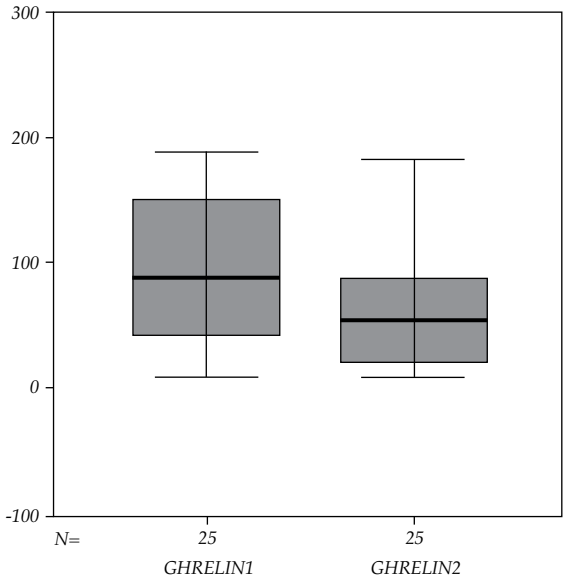

Fig. 2. *Serum ghrelin levels were significantly decreased at the end of one year (93.50±60.21 versus 67.35±56.97, p<0.05).*

After one year of VPA treatment, BMI values increased, whereas the mean serum ghrelin and NPY levels decreased (Figs. 1-3, Table 2). The mean baseline BMI of the patients was 16.02±2.59 and the mean BMI was changed to 19.52±4.02 at the end of the one year. The BMI change was found statistically significant ($p<0.05$) (Fig. 1, Table 2). The mean initial serum ghrelin levels was 93.50±60.21 and it was 67.35±56.97 at the end of one year period (Fig. 2, Table 2). The mean initial serum NPY levels was 66.28±12.86 and it was 60.96±16.50 at the end of one year period (Fig. 3, Table 2). Although the decrease in the ghrelin levels were significant ($p<0.05$), differences in serum NPY levels between before and after treatment were not statistically significant ($p>0.05$) (Table 2). Differences in serum NPY and ghrelin levels between before and after treatment were not statistically significant in obese and nonobese patients ($p>0.05$). Demographic and biochemical characteristics of obese and nonobese patients are shown in Table 3.

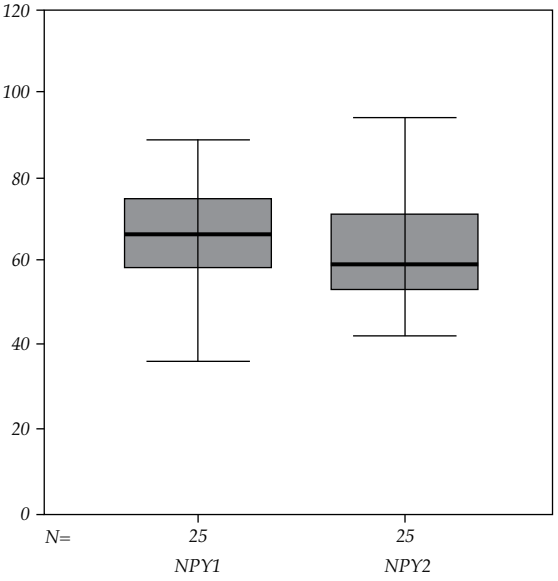

Fig. 3. *Serum neuropeptide Y levels were not significantly decreased at the end of one year (66.28±12.86 versus 60.96±16.50, p>0.05).*

The mean serum VPA levels of all patients were within therapeutic limits (73.68±14.47 μg/ml) and the mean VPA daily dosage of patients were 21.96±7.81 mg/kg/day (Table 1). We obtained no significant correlation between serum VPA levels and serum ghrelin, serum neuropeptide Y levels and BMI, whereas a negative correlation between VPA daily dosage and serum ghrelin level ($r=-0.456$, $p<0.05$) was determined.

DISCUSSION

The results of the present study demonstrated an association between VPA treatment, weight gain, and decrease of serum ghrelin and NPY levels.

After the discovery of new antiepileptic drugs in recent years, long-term side effects of these drugs were investigated with new scientific researches.

Weight gain and obesity are among the long-term side effects of the antiepileptic treatment. Especially VPA, carbamazepine, vigabatrin, lamotrigin and gabapentin are found in relation with weight gain and obesity.[5,16-18] Although pathogenesis of weight gain in patients treated with these antiepileptics remains unclear.

Valproate is a branched fatty acid unrelated to any other antiepileptic drug.[19] The intake of VPA may be associated with adverse effects which weight gain is one of the most common. The rate of weight gain due to VPA treatments was reported as 14-71% in adult studies.[2-4] However, there are a few studies about weight gain and obesity due to VPA treatment in children.[19-21] The frequency of VPA-related obesity observed in our study was close to that reported in previous studies.

Valproate-related obesity and weight gain are assumed to be owing to an increased feeding or a decreased expenditure of energy by a direct GABAergic agonist effect in the hypothalamus.[16] On the other hand, several other possible ways have also been proposed. An impairment of β-oxidation of fatty acids, a competition with fatty acids for albumin binding, an increase in lipogenesis, hyperinsulinemia, and hyperleptinemia are some of the factors.[3,5-6] However, the pathogenetic mechanisms leading to weight gain in long-term VPA treatment are still uncertain. Our study aimed to evaluate the role of ghrelin and NPY in VPA-related weight gain.

Ghrelin is a peptide hormone produced by endocrine cells in the gastrointestinal system.[7-10] Ghrelin regulates the eating behavior with central effect. Recent studies indicate the fact that ghrelin levels in systemic circulation reflect mainly nutritional status and are predominantly involved in the regulation of energy homeostasis. Ghrelin, which increases with hunger and is been suppressed by feeding, modulates NPY and agouti-related peptide and leads energy and adipose lipid storage.[7-10] Adipose lipid storage stimulates leptin and leptin makes NPY inhibition.[22] Thus the status associated with weight gain is accompanied by a decrease in serum ghrelin levels. We have confirmed these facts and detected a decrease in ghrelin serum levels in pediatric patients treated with VPA.

Gungor et al.[21] investigated the effect of VPA on ghrelin and its potential effects on weight gain. They found significantly increased ghrelin levels in their study group. However, they did not examine NPY levels. They suggest that VPA may activate the NPY pathway by increasing ghrelin levels, stimulating the food intake, thereby leading to weight gain.[21] However, in the present study, we examined the ghrelin and NPY levels in pediatric patients taking VPA treatment, and we found significant decrease in ghrelin and serum levels. In addition, Greco et al.[23] also found decreased ghrelin levels in adult epileptic patients taking VPA treatment for two years and suggested that ghrelin is down-regulated in human VPA-induced obesity. They reported that this down-regulation may be a result of increased insulin levels. Our data were in accordance with the result of Greco et al.[23]

In conclusion, this study demonstrated that serum ghrelin and NPY levels decrease in children receiving VPA treatment. Since VPA is the most commonly used antiepileptic agent in childhood epilepsy, new studies with larger sample sizes and longer follow-up periods are needed to evaluate the mechanism of weight gain due to VPA treatment in this population.

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