RESEARCH ARTICLE

New adipocytokines (vaspin, apelin, visfatin, adiponectin) levels in children treated with valproic acid

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ABSTRACT. ***Aim.*** **To investigate the relationship between the newly discovered adipocytokines and increasing body weight (paralleled by increased insulin resistance), and antiepileptic drug therapy with valproic acid (VPA).** ***Design and methods.*** **44 children with idiopathic, generalized epilepsy treated with valproic acid (VPA), and 40 control group children were included in this study.** ***Results.*** **Both the VPA-treated group and the control group showed no significant difference in terms of age, total cholesterol and LDL-cholesterol. Subjects in the VPA group had significantly higher BMI-SDS than control subjects (2.3±0.15 *vs* -0.04±0.8, p<0.001). HOMA-IR, apelin and visfatin levels were significantly increased (4.95±2.07 *vs* 1.46 *vs* 0.6, p<0.001; 2.21±1.14 *vs* 0.57±0.15, p<0.001; 31±12 *vs* 18.4±10.4, p<0.001; respectively), and adiponectin levels were significantly lower in the VPA group (2.02±1.03 *vs* 12.4±6.1, p<0.001). Triglyceride levels were significantly increased (126±70 *vs* 80±40 mg/dL, p=0.001), and HDL-cholesterol levels were significantly lower in the VPA group. Vaspin levels were higher in the VPA group than the control group, but the difference was not significant.** ***Conclusion.*** **Based on the findings of this study, apelin, visfatin and adiponectin levels may be considered as potential regulators of glucose and fat metabolism during valproic acid therapy**

Keywords: valproate, apelin, visfatin, adiponectin, vaspin

Antiepileptic drug therapy with valproic acid (VPA), an effective anticonvulsant, has been found to be associated with an increase in body weight, paralleled by increased serum insulin levels [1, 2]. Verrotti *et al*. found that the extent of obesity in a group of 40 epileptic patients without mental retardation was significantly higher in VPA-treated patients (37.5%) than in a group of 40 epileptics treated with other anticonvulsant drugs (10%) [3]. Corman *et al*. reported 71% percent of the VPA-treated group were weight gainers versus 43% in the carbamazepine-treated group. However, in a study of children aged 2-8 years, VPA-associated weight gain occurred in only 16% of patients [4, 5].

All VPA-induced obesity studies confirm the side-effect of VPA on insulin metabolism, with consequent insulin resistance [3]. However, the exact pathogenesis is still not completely understood. Several mechanisms have been proposed. A condition of increased free fatty acid levels, such as occurs with VPA therapy, plays an important role in the development of insulin resistance [6]. Pylvanen *et al*. showed that both obese and lean patients taking VPA had hyperinsulinemia, suggesting the development of insulin resistance as the leading factor for weight gain during VPA treatment [7].

Verrotti *et al*. found no significant correlation between serum VPA concentrations and leptin levels, or between serum VPA and insulin concentrations [8]. However, more recent studies indicate an important role for adipose tissue hormones or "adipokines", with the exception of leptin, in obesity-associated complications. Adiponectin is exclusively expressed and secreted by adipose tissue, and is involved in glucose and lipid metabolism. Hypoadiponectinemia has been shown to be associated with insulin resistance in animal and human studies [9, 10]. Plasma adiponectin levels are decreased in subjects with obesity and insulin resistance or type 2 diabetes mellitus, and correlate inversely with visfatin and fasting insulin levels [11, 12]. Both tissue expression and plasma levels of visfatin increase in parallel with obesity. It has insulin-mimetic effects and lowers plasma glucose levels [13]. Apelin synthesis in adipocytes is stimulated by insulin, and plasma apelin levels markedly increase in obesity associated with insulin resistance and hyperinsulinemia [14]. Vaspin was identified as an adipokine with insulin-sensitizing effects, and is predominantly secreted from visceral adipose tissue in a rat model of type 2 diabetes [15]. Youn *et al*. have recently shown that vaspin mRNA expression in adipose tissue is

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related to parameters of obesity and glucose metabolism in adults [16].

The mechanism of VPA-induced weight gain remains to be determined, but the association between VPA therapy and hyperinsulinemia has generated great interest. We hypothesized that the four circulating adipokines mentioned above, are linked to markers of insulin sensitivity and obesity induced by VPA therapy.

DONORS AND METHODS

The study protocols were approved by the institutional review board of GATA Medical Faculty Ethical Committee. Signed, informed consent forms were obtained from the parents of the children.

Forty children [epilepsy group; 20 girls and 20 boys, mean age: 10.91 $\pm$ 2.65 y, mean BMI standard deviation score (BMI-SDS): 2.31$\pm$0.14] were recruited from among the children who attended the outpatient clinic of the Department of Pediatric Neurology Service for obesity, between 2007 and 2008. Control subjects (20 girls and 20 boys, mean age: 11$\pm$3 y, mean BMI-SDS: -0.04$\pm$0.8) were enrolled the study from among healthy children who attended the hospital for minor illnesses such as the common cold. All of the 274 children treated with VPA had the diagnosis of idiopathic generalized epilepsy. Treatment lasted from 3 to 10 years and all had normal BMI-SDS before the VPA therapy. Forty four of them that had higher BMI-SDS following the VPA therapy were included in the study. The VPA was prescribed at the usual dosage, and all patients showed plasma drug levels within a stable, therapeutic range. Managed in the primary care setting, we collected the blood samples from the control group at the time of the control examination.

Anthropometric measurements were performed for all patients. Height and weight were measured with an empty bladder, in post-absorptive conditions. Height was measured to the nearest 0.5 cm on a standard height board, and weight was determined to the nearest 0.1 kg on a standard physician's beam scale with the subject dressed only in light underwear and without shoes. BMI was calculated as weight (in kilograms) divided by height (in meters) squared. Patients with a body mass index of $\geq 95^{th}$ percentile according to reference curves for Turkish children and adolescents were accepted as obese. The degree of obesity was quantified using Cole's least mean-square method, which normalizes BMI-skewed distribution and expresses BMI as an SD score (BMI-SDS) [17]. This measure gives age- and sex-specific estimates of the distribution median, the coefficient of variation and the degree of skewness by a maximum-likelihood fitting technique. Obesity was defined as a BMI-SDS $\geq$1.64. Their pubertal development stages were assessed by a pediatric endocrinologist using the criteria of Tanner stages. Staging for sexual maturation was greater than 2 in all patients (Tanner stages 2-4). After the child had rested for at least five minutes and in a sitting position, diastolic and systolic pressure (mmHg) measurements were taken, using a mercury-gravity manometer and a cuff appropriate for body size. The appropriate-sized cuff for a child was long enough to completely encircle the circumference of the arm (with or without overlap), and wide enough to cover approximately 75% of the upper arm between the top of the shoulder and the olecranon, leaving sufficient room both at the antecubital fossa to comfortably place the bell of the stethoscope, and at the upper edge of the cuff to prevent obstruction of the axilla. The onset of a clear tapping sound (Korotkoff sounds), defined as phase I, corresponded to systolic blood pressure. Phase IV was used as the diastolic blood pressure.

Children were excluded if they had had prior major illness, including type 1 or type 2 diabetes, taken medication, or had a condition known to influence body composition, insulin action, or insulin secretion (*e.g.* glucocorticoid therapy, hypothyroidism, Cushing's disease). All patients with secondary epilepsy syndromes and acute illnesses were excluded from the study. None of the patients had a family history of diabetes.

Blood samples

Venous blood samples were obtained by venipuncture, at 08:00 am, following overnight fasting, in order to measure plasma glucose and insulin levels. After clotting, the serum was separated and immediately analysed. Plasma glucose was determined by the glucose oxidase method. Plasma insulin was measured using an IMMULITE immunoassay (IMMULITE Diagnostic Products Corporation, Los Angeles, CA, USA). Plasma concentrations of total cholesterol, triglycerides and high-density lipoprotein-cholesterol (HDL-cholesterol) were measured using routine enzymatic methods with an Olympus 2700 Analyzer. Low density lipoprotein cholesterol (LDL-cholesterol) levels were calculated using the Friedewald formula. Plasma adiponectin and vaspin levels were determined by radioimmunoassay (Linco Research, St. Charles, MO, USA). Determination of visfatin and apelin levels was performed using enzyme immunoassay (visfatin C-terminal [human] EIA; Phoenix Pharmaceuticals, Belmont, CA, USA).

Insulin sensitivity indices

The "gold standard" for measuring insulin sensitivity is the euglycemic-hyperinsulinemic clamp technique. Another common method is to use a frequently sampled, intravenous glucose tolerance test, performing the minimal model assessment of insulin sensitivity. Unfortunately, neither of these two methods is feasible in the pediatric age group. Previous studies have evaluated simple indices for assessing insulin sensitivity in a wide range of conditions associated with insulin resistance. In a study of prepubertal and pubertal, obese children and adolescents, HOMA-IR and QUICKI were significantly correlated with indices derived from the glycemic and insulinemic responses to an OGTT [18]. Vaccaro *et al.* recently reported that QUICKI performed less well than HOMA-IR and fasting insulin as surrogate measures of insulin resistance in the adult population [19]. We previously reported that HOMA-IR was more reliable than FGIR and QUICKI as a measure of insulin resistance among children and adolescents [20]. In present study, we estimated insulin resistance using the homeostasis model assessment for insulin resistance (HOMA-IR; fasting insulin X fasting glucose/22.5) [21]. Insulin resistance is defined as levels of the homeostasis model assessment for insulin resistance (HOMA-IR) greater than 3.16 [22].

Statistical analysis

Data were expressed as mean±SD. Differences in the means of variables were tested using both parametric and non-parametric tests depending on the distribution of the variables. Correlation analyses were conducted using Spearman or Pearson correlation coefficients depending, once again, on the distribution of the variables. A probability value of less than 0.05 was considered significant. SPSS version 10.1 (SPSS, Chicago, IL, USA) was used for analysis.

RESULTS

The characteristics of the study population are shown in *table 1*. There were no differences between the groups as regards gender ratio, age and height. The characteristics of the 44 VPA-treated children and the 40 control subjects are summarized in *table 1*. We found no significant differences in parameters between girls and boys of the same age (10-14 years).

Both the VPA group and control group showed no significant difference in terms of age, total cholesterol and LDL-cholesterol. Subjects in the VPA group had significantly higher BMI-SDS than control subjects (2.3±0.15 *vs* -0.04±0.8; p<0.001). Triglyceride levels were significantly increased (126±70 *vs* 80±40 mg/dL; p=0.001) and HDL-cholesterol levels were significantly decreased in the VPA group. HOMA-IR, apelin and visfatin levels were significantly elevated (4.95±2.07 *vs* 1.46 *vs* 0.6; p<0.001; 2.21±1.14 *vs* 0.57±0.15; p<0.001; 31±12 *vs* 18.4±10.4; p<0.001, respectively), and adiponectin levels were significantly lower in the VPA group (2.02±1.03 *vs* 12.4±6.1; p<0.001). Vaspin levels were higher in the VPA group than in the control group, but this was not significant. *Table 2* shows a correlation between adipocytokines and lipids and BMI-SDS and HOMA-IR. Apelin correlated positively with BMI-SDS and HOMA-IR (r: 0.33, p: 0.02 and r:0.86, p<0.001), visfatin correlated positively with BMI-SDS and HOMA-IR (r: 0.6, p<0.001 and r: 0.63, p<0.001); adiponectin correlated negatively with BMI-SDS and HOMA-IR (r: -0.45, p: 0.002 and r:-0.44, p: 0.004).There was no correlation between lipid profiles and BMI-SDS and HOMA-IR.

DISCUSSION

In this study, we analyzed the adipokine levels in children with VPA-induced obesity. We found higher BMI-SDS and increased adipokine levels (apelin and visfatin) [related to lower insulin sensitivity (HOMA-IR)] in patients, than in the control children. Moreover, the patients had significantly lower adiponectin values, with no difference between the groups as regards vaspin levels.

Studies performed during the last decade have indicated that adipose tissue is not only a triglyceride storage site, but also a source of several, biologically-active mediators, including leptin, tumor necrosis factor-α (TNF-α), adiponectin, apelin, visfatin, vaspin, acylation-stimulating protein (ASP), resistin, interleukin-6 (IL-6), plasminogen activator inhibitor-1 (PAI-1), and transforming growth factor-β (TGF-β) [23, 24]. They modulate insulin sensitivity and are new therapeutic targets in metabolic syndrome. Adipokines are an exciting new link not only between

Table 1
Clinical and laboratory characteristics of the study population.

	VPA-treated children	Controls	p
N (f/m)	25/19	20/20	
Age (years)	10.9±2.7	11±3	0.8
BMI-SDS	2.3±0.15	-0.04±0.8	<0.001
Systolic blood pressure (mmHg)	118.9±13	104.7±9	<0.001
Diastolic blood pressure (mmHg)	77.1±12	73.2±9.6	ns
Total cholesterol (mg/dL)	172±35	160±23	0.17
Triglycerides (mg/dL)	126±70	80±40	0.001
HDL-cholesterol (mg/dL)	43±8	54±12	<0.001
LDL-cholesterol (mg/dL)	102±31	91±22	0.059
HOMA-IR	4.95±2.07	1.46±0.6	<0.001
Fasting glucose (mg/dL)	87±11	85,2±7,47	ns
Fasting insulin (μU/mL)	31±21	11±2,6	<0.001
FGIR	3.8±2.0	8.4±2.3	<0.05
QUICKI	0.29±0.02	0.33±0.01	<0.05
Vaspin (μg/L)	0.74±1.03	0.55±0.32	0.25
Apelin	2.21±1.14	0.57±0.15	<0.001
Adiponectin (μg/mL)	2.02±1.03	12.4±6.1	<0.001
Visfatin	31±12	18.4±10.4	<0.001

Data are given as means±SD. Difference at p<0.05 level.
HOMA-IR: homeostasis model assessment for insulin resistance (fasting insulin (μU/mL) x fasting glucose (mg/dL)/22.5.
FGIR: fasting glucose to insulin ratio; QUICKI: quantitative insulin-sensitivity check index; BMI-SDS: body mass index-standard deviation score.

Table 2
Correlation between adipocytokines and lipids with BMI-SDS and HOMA-IR in VPA-treated children.

	BMI-SDS		HOMAIR	
	r	**p**	**r**	**p**
Lipids				
Total Cholesterol (mg/dL)	-0.68	0.66	-0.09	0.53
Triglycerides (mg/dL)	0.06	0.71	0.18	0.26
HDL-cholesterol (mg/dL)	-0.14	0.36	-0.13	0.4
LDL-cholesterol (mg/dL)	-0.06	0.69	-0.11	0.48
Adipocytokines				
Vaspin (μg/L)	0.22	0.2	0.06	0.71
Apelin	0.33	0.02	0.86	<0.001
Adiponectin (μg/mL)	-0.45	0.002	-0.44	0.004
Visfatin	0.6	<0.001	0.63	<0.001

obesity and insulin resistance, but also obesity and cardiovascular disease, hypertension, as well as hyperlipidemia [25]. Our results, showing an increased BMI in VPA-treated patients, are in accordance with several previously published studies [1]. The precise mechanism underlying the VPA-associated weight gain however remains unclear. Insulin resistance is paralleled by an increase in plasma levels of FFA [19]. VPA is a fatty acid derivative [20] and fatty acids also modulate pancreatic insulin secretion [21]. In the present study, significantly higher visfatin, apelin and HOMA-IR levels and lower adiponectin levels were found in the VPA group compared to the control group.

Hypoadiponectinemia has been shown to be associated with insulin resistance in animal and human studies [10]. Plasma adiponectin levels are decreased in subjects with obesity and insulin resistance or type 2 diabetes mellitus, and correlate inversely with visfatin and fasting insulin levels [11, 12]. Berndt *et al*. showed that plasma visfatin levels correlate significantly with percentage body fat, body mass index, and visfatin mRNA level in visceral adipose tissue [26]. In a recent study [27], plasma visfatin levels were higher in patients with type 2 diabetes mellitus than in normoglycemic controls. Rauchenzauner *et al*. showed lower adiponectin in patients during VPA-therapy, however, they found no relationship with visfatin levels in the control group [28]. Qiao *et al*. showed in their adipocyte study that VPA inhibits adiponectin gene expression in a dose- and time-dependent manner [29]. The present study supported their findings, as significantly lower adiponectin levels were found in the VPA group than in the control group.

Recently, apelin mRNA and protein were identified in adipocytes and vascular stromal fractions isolated from mouse and human subcutaneous adipose tissue [30]. Apelin transcripts and immunoreactivity are expressed in the central nervous system and in various peripheral tissues, including the heart, lung, and mammary gland [31, 32]. Widespread expression of apelin in peripheral tissues is associated with its synthesis by endothelial cells [33]. Hosoya *et al*. showed that plasma apelin levels markedly increase in obesity associated with insulin resistance and hyperinsulinemia [14]. In this study, there were higher apelin levels in the VPA group than in the control group. Additionaly, apelin and visfatin levels were positively and adiponectin levels negatively correlated with HOMA-IR levels.

Vaspin is a newly-described adipocytokine expressed predominantly in visceral, white adipose tissues. In several studies, serum vaspin levels have been shown to decrease with worsening of diabetes and body weight loss, although these serum vaspin levels could be normalized by insulin or pioglitazone treatment [34]. In the present study, there was no significant difference in vaspin levels between controls and the VPA-treated children.

Increased body weight is closely associated with insulin resistance and type 2 diabetes mellitus [20, 35]. The role of various adipokines as connectors between obesity and diabetes mellitus has been elucidated in recent years. VPA is a very important drug in neurology, but VPA-associated weight gain is still not well-understood, and further studies in this area are of clinical importance with respect to weight-reduction and weight-maintaining therapeutic procedures. Based on the findings of this study, apelin, visfatin and adiponectin levels may be considered as potential regulators of glucose and fat metabolism during VPA therapy

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