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Body composition and bioelectrical impedance vector analysis in children on valproate treatment: a pilot study.

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Key words: epilepsy; pediatrics; valproic acid; body composition; bioimpedance; BIVA.

Abstract. Valproate treatment seems to be associated with significant weight gain and related metabolic disorders; however, body composition assessment among epileptic patients has not been frequently reported. We aimed to evaluate valproic acid-associated changes in body composition in pediatric epileptic patients. Twenty eight epileptic children aged from 3-14 years, receiving valproic acid (VPA) therapy for at least 6 months were included in this pilot cross sectional study. A control group was matched in a 1:1 pattern by age, sex, and body mass index (BMI). Body composition was measured by bioimpedance (BIA). Prediction BIA-equations, Phase Angle (PA) and Bioimpedance Vector Analysis (BIVA) were calculated. We did not find statistically significant differences in body composition determined by BIA equations, PA and BIVA among pediatric epileptic patients treated with VPA and healthy children. Although the results of this study show no statistically significant differences, there was a trend toward higher body fat mass in VPA treated children. Since BIA and BIVA may detect early changes in body compartments, it would be advisable to assess them in pretherapy and monitoring of patients with epilepsy, in order to detect changes before adverse consequences could happen. It would be necessary to increase the sample size in the future.

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Composición corporal y análisis vectorial de bioimpedancia en niños en tratamiento con ácido valproico: estudio piloto.

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Palabras clave: epilepsia; pediatría; ácido valproico; composición corporal; bioimpedancia; BIVA.

Resumen. El tratamiento con ácido valproico (VPA) ha sido frecuentemente relacionado con un incremento significativo del peso corporal, así como con diversos trastornos metabólicos; sin embargo, existen pocos estudios publicados de evaluación de composición corporal en pacientes epilépticos. El objetivo del presente estudio fue valorar los cambios en la composición corporal en pacientes pediátricos epilépticos en tratamiento con VPA. Para ello se realizó un estudio observacional, transversal, incluyendo a veintiocho pacientes epilépticos con edades comprendidas entre 3 y 14 años, en tratamiento con VPA durante al menos 6 meses. Como controles sanos fueron incluidos 28 niños, reclutados en un patrón de 1:1 por edad, sexo e índice de masa corporal. El estudio de composición corporal se determinó por bioimpedancia (BIA). Se calcularon las ecuaciones de predicción de BIA, el ángulo de fase (PA) y el análisis vectorial de bioimpedancia (BIVA). No se encontraron diferencias estadísticamente significativas en la composición corporal determinada por las ecuaciones de BIA, PA y BIVA entre los pacientes epilépticos tratados con VPA y los niños sanos. Aunque los resultados de este estudio no muestran diferencias significativas, se observó una tendencia hacia un mayor porcentaje de masa grasa en los niños tratados con VPA. Dado que BIA y BIVA pueden detectar alteraciones precoces en los compartimientos corporales, sería recomendable realizar una evaluación y seguimiento en los pacientes epilépticos en tratamiento con VPA, con el fin de detectar cambios antes de que pudieran aparecer potenciales complicaciones. Sería necesario aumentar el tamaño de la muestra en el futuro.

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INTRODUCTION

Chronic epilepsy and long-term use of antiepileptic drugs may be associated with adverse metabolic consequences, such as weight changes. In the last years, a growing body of literature indicates an association between valproic acid (VPA) therapy and weight gain (1-3). However, evidence regarding effects on body composition in epileptic patients is deficient (4). The pathophysiology of the weight gain remains uncertain. Decreased blood glucose level, impairment of beta-oxidation of fatty acids, and increased insulin levels are some of the possible mechanisms (1-5). Few studies indicated that epilepsy itself could also lead to weight gain (5). It has been reported that long-term kindled seizures induced higher serum concentrations of leptin and significant weight gain. Furthermore, people with epilepsy tend to be less physically active, have poorer self-esteem, and higher levels of anxiety and depression than the general

population, which can result in increased weight (5). Even, certain epilepsy genetic syndromes are related to obesity (6-7).

Body mass index (BMI) is commonly used to classify overweight and obesity. However, BMI cannot distinguish between weight change due to a variation in fat mass, lean mass or water (8). Estimation of body composition may detect early changes in body compartments. Body compartments can be predicted and analyzed using bioimpedance measurement techniques and equations. Bioelectrical impedance analysis (BIA) is a simple method of estimating body composition (9). Body composition measured by BIA has been reported both in Spanish adults (10) and children (11). Many BIA prediction equations have been proposed to estimate total body water (TBW), fat free mass (FFM) and fat mass (FM) as a function of impedance, weight, height, sex, and age (12, 13). Recommended equations are shown in Table I (13-19). These equations make predictions of body compartments in subjects with fixed and normal 73% hydration of soft tissues. In abnormal hydration conditions, these algorithms can produce biased estimates of body compartments. The use of raw bioelectrical impedance measurements may be useful in situations in which BIA assumptions are not valid. They can be expressed as a ratio (phase angle) or as a plot (bioelectrical impedance vector analysis) (20,21).

The aim of this study was to evaluate VPA-associated body composition changes in pediatric epileptic patients and to compare them with healthy children.

PATIENTS AND METHODS

The present cross-sectional study included ambulatory Caucasian children of both genders, aged, 3 to 14 years old. Participants were recruited from a clinical convenience sample. All of the subjects resided in the health district of the province of Cáceres, Spain. Parental consent and the child's assent were required from all subjects. The Office for Protection against Research Risks of the University of Extremadura approved the study.

Cases: Children clinically diagnosed with epilepsy, receiving VPA as monotherapy for at least 6 months. The participants were recruited from the Neuropediatric Department of San Pedro de Alcántara Hospital in Cáceres, Spain. The diagnosis of epilepsy was based on the International League Against Epilepsy criteria (22). A total of 60 patients with epilepsy were screened for enrolment. Those who were receiving another treatment, children diagnosed with cerebral palsy or feeding disorders were excluded from the study. Thirty-two subjects did not meet the eligibility criteria, and a total of 28 were finally enrolled in the study.

Control Group: 28 healthy children aged between 3–14 years were enrolled control group. They were age, gender and BMI matched (1:1). They were recruited from a previous study released in a total of 245 healthy children, aged 3-16 years, attending primary schools in the Health district of Cáceres, Spain.

A complete medical history and a physical examination were performed before each candidate was included in the study. Nutrient intake was quantified by using dietary scales, measuring cups and spoons, as previously described elsewhere, based on 7 days of dietary records (23-25). Physical activity status was assessed on the answer to the following question: "How much do you exercise or physically exert yourself in your leisure time?" The categories were: nonambulation; sedentary (reading, watching television); moderate (walking, cycling and exercising in other ways for at least 4 hours per week); active (fitness-improving sport at least three times per week); and competitive sport (26). None of the participants chose competitive sport.

A trained anthropometrist used standard techniques to take anthropometric measurements for all of the children. We calculated body mass index (weight/height^2)

and used the World Health Organization reference to estimate age- and sex-specific BMI z-scores (27, 28).

Body composition was measured by bioelectrical impedance using a Holtain body composition analyzer (Holtain Ltd). It was determined by single and multiple-frequency BIA. BIA was made according to standard protocol. TBW, FFM and FM were calculated from BIA measurements and anthropometric variables with specific BIA recommended equations (table I) (13-19). TBW was also obtained from FFM using a hydration fraction of 0.732 ml/g (14, 29). Body fat content was determined from predicted FFM and body weight (FM=body weight-FFM; body fat %=FM/body weight) and also with recommended equations (Table I) (13, 30).

Due to the possible lack of accuracy of BIA in clinical setting, phase angle (PA) and bioelectrical impedance vector analysis (BIVA) were also analyzed. PA was obtained from multiple-frequency BIA. Vector BIA was performed with BIVA software (31). We normalized resistance (R) and reactance (Xc) measurements to the height of the subjects and plotted individual vectors as a point on the RXc graph, according to the RXc graph method (32). The points were subsequently joined to form a tolerance ellipse, corresponding to the 95th percentile for the impedance vector distribution.

TABLE I
BIA RECOMMENDED EQUATIONS.

	Reference	Equation
Total Body Water	Danford et al. (15) (5-10 y)	0.45 Ht^2/R50 + 0.11Wt + 1.84 R2 0.98 SEE 0.62 kg
	Kushner et al. (17) (0.02-67 y)	0.59 Ht^2/R50 + 0.065Wt + 0.04 R2 0.99 SEE 1.41 kg
	Bray et al. (14)	0.40x(Ht^2/R50)+ 0:148Wt - 3.32 R2 = 0.86
Fat Free Mass	Houtkooper et al. (13) (10-19 y)	0.61 (Ht^2/R50) + 0.25Wt + 1.31 R2 0.95 SEE 2.1 kg
	Easton et al. (30) (10-14 y)	0.52 (Ht^2/R50) + 0.28Wt + 3.25 R2 0.93 SEE 2.2 kg
	Deurenberg et al. (19) (7-15 y)	0.406 x10^4 (Ht^2(m)/ R50) + 0.360Wt + 5.580Ht (m) + +0.56 gender – 6.48 (Gender: female 0, male 1) R2 0.97 SEE 1.7 kg
	Ramírez et al. (29)	0.661 (Ht^2/R50) + 0.200Wt − 0.32
Percentage Body Fat Mass	Houtkooper et al. (13) (10-14 y)	-1.11 (Ht^2/R50) + 1.04Wt + 15.16 R2 0.74 SEE 4.2%
	Easton et al. (30) (11-17 y)	-1.00 (Ht^2/R50) + 1.03Wt + 12.04 R2 0.60 SEE 4.7%

Ht: height (cm); Wt: weight (kg); y: years; R50: resistance measured at 50 kHz (Ω); R2: correlation coefficient; SEE: standard estimation error.

Normal distribution and homogeneity of variances were assessed using the Kolmogorov–Smirnov and Levene tests respectively. Variables were not normally distributed, thus, comparisons were performed with a non-parametric Mann-Whitney U test. Since the cases and control group were 1:1 matched, a Wilcoxon test was also performed. A minimum p-value of <0.05 was the necessary condition for statistical significance. All values were expressed as the median and interquartile range. These studies were performed using SPSS 21 (SPSS Inc., Chicago, IL, USA).

Hotelling's T2 test was performed for BIVA analysis. Separate 95% confidence ellipses correspond to statistically significant difference between mean vector displacements on the R-Xc plane ($p<0.05$), which is equivalent to a significant difference in R, Xc or both parameters. Mahalanobis D distance (D) among mean vectors was also calculated.

RESULTS

Twenty-eight epileptic children, 9 girls and 19 boys, aged from 3-14 years were enrolled in this study. All the patients were on treatment with VPA as monotherapy for at least 6 months, with a mean duration in treatment of 53 months. The 28 healthy children in the control group were matched by age, sex and BMI; therefore, there were no significant differences in the demographics data between the epileptic children and the control group. There were also no statistical differences in the analysis by gender. Anthropometric characteristics are shown in Table II.

Nutrient intake was quantified using a dietetic scale. There were no significant differences in kcal/kg/day and in macronutrients intakes between the groups studied (Table III). Physical activity status assessment also showed no significance differences.

Body composition was determined by single and multiple-frequency BIA. Table IV shows the electrical bioimpedance data measured at 50 Hz frequency. There were no significant differences in body composition parameters between both groups ($p>0.05$). PA data did not either reflect statistically significant differences among epileptic children and control group (Table IV). Body compartments were calculated by recommended prediction equations. Results are displayed on Table V. No significant differences were found; neither in the whole sample nor in the analysis by gender.

Fig. 1 shows the 95% confidence ellipses of the mean impedance vectors in cases and control group. The mean vector positions did not differ significantly among the studied groups. Statistical comparison of groups with Hotelling's T2 test, with the corresponding p value and Mahalanobis distance D was also analyzed. The 95% confidence ellipses did not differ significantly among epileptic patients and healthy children (Hotelling's T2 test= 3.9; $p=0.1558$) (Mahalanobis $D=0.53$).

TABLE II
ANTHROPOMETRIC CHARACTERISTICS

	Cases (n=28)	Controls (n=28)	
	Median (IQR)	Median (IQR)	p
Sex ratio (girls/boys)	9/19	9/19	0.61
Age (y)	8.15 (6.25-11)	7.50 (6.25-11)	0.80
Weight (kg)	31.25 (23.43-48.75)	32 (26.25-42.50)	0.96
Height (cm)	132.5 (118.08-148.75)	130 (125-147.25)	0.65
BMI (kg/m^2)	19.31 (16.08-22.85)	18.31 (16.12-21)	0.65
BMI z score	1.24 (0.17-2.58)	1.16 (0.31-1.79)	0.52

IQR: interquartile range. Y: years.

TABLE III
NUTRIENTS INTAKE

	Cases (n=28)	Controls (n=28)	
	Median (IQR)	Median (IQR)	p
Kcal/day	2447.20 (1867.05-2930.53)	2220 (1795-2476.75)	0.12
Carbohydrates (g/d)	252.05 (193.52-305.80)	238 (193.42-291.30)	0.62
Fat (g/d)	116.17 (85.39-138.92)	98.58 (80.92-112.12)	0.08
Protein (g/d)	104.93 (78.15-131.80)	94.30 (80.28-112.57)	0.35

IQR: interquartile range; g/d: grams/day.

TABLE IV
BIOIMPEDANCE TOTAL DATA

	Cases (n=28)	Controls (n=28)	
	Median (IQR)	Median (IQR)	p
Impedance	716 (616- 794)	691 (655.75-722.25)	0.36
Reactance	90.5 (76.05-100.45)	92.2 (81.25-104.42)	0.50
Resistance	711 (619.25-787.75)	686 (649.00-717.00)	0.23
Phase Angle	7.60 (6-8.68)	7.75 (7-8.58)	0.43

IQR: interquartile range. Impedance, Reactance, Resistance and Phase Angle at 50Hz. Phase angle in degrees.

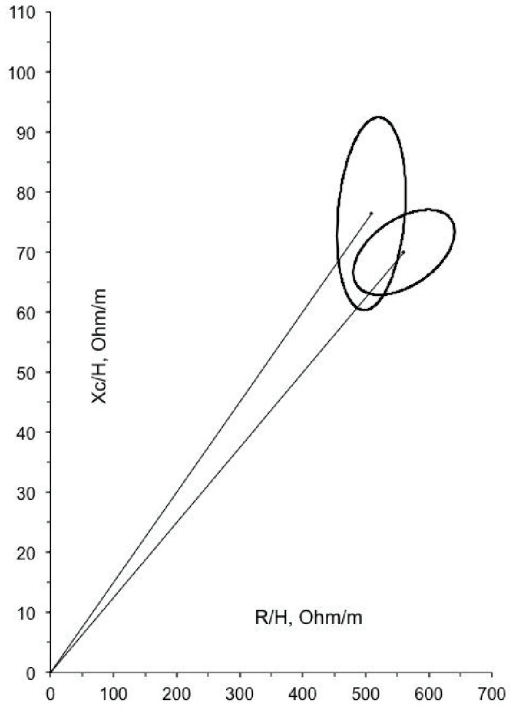

Fig. 1. Z-score graph. 95% confidence intervals of mean vectors in the two groups of children and reference values.

TABLE V
BODY COMPOSITION PARAMETERS DETERMINED BY BIA PREDICTION EQUATIONS

		Cases (n=28)	Controls (n=28)	
	Reference	Median (IQR)	Median (IQR.)	p-value
Fat Free Mass (Kg)	Houtkooper et al. (13)	26.15 (18.94-33.32)	24.93 (21.18-32.93)	0.60
	Easton et al. (30)	26.68 (19.95-34.28)	25.69 (21.85-33.35)	0.66
	Deurenberg et al. (19)	24.59(16.82-33.98)	23.87(19.10-31.68)	0.69
	Ramírez et al. (29)	23.90 (17.01-30.50)	22.71 (19.46-30.52)	0.55
Fat Mass	Houtkooper et al. (%) (13)	21.47 (18.31-26.21)	19.04 (16.34-23.84)	0.22
	Easton et al. (%) (30)	21.60 (16.20-27.49)	18.44 (15.50-23.42)	0.32
	Deurenberg et al. (Kg) (19)	9.95(5.79-14.60)	8.70(6.31-11.88)	0.51
	Ramírez et al. (Kg) (29)	10.77 (5.19-16.95)	8.52 (6.06-12.68)	0.49
		Cases (n=28)	Controls (n=28)	
	Reference	Median (IQR)	Median (IQR.)	p-value
Total Body Water (Kg)	Houtkooper et al. (13)	19.14 (13.87-24.39)	18.25 (15.50-24.10)	0.60
	Eston et al. (30)	19.53 (14.61-25.09)	18.81 (15.99-24.41)	0.66
	Deurenberg et al. (19)	17.95(12.28-24.80)	17.43(13.94-23.12)	0.69
	Ramírez et al. (30)	17.50 (12.45-22.33)	16.62 (14.24-22.34)	0.61
	Kushner et al. (17)	17.34 (12.64-21.36)	16.70 (14.84-22.29)	0.54
	Danford et al. (15)	17.33 (12.98-21.40)	16,57 (14.66-21.57)	0.57
	Bray et al. (14)	19.00 (14.48-23.44)	18.25 (15.96-23.28)	0.63

M: Median; IQR: interquartile range.

DISCUSSION

Weight gain is a common side effect associated with the use of anticonvulsant drugs such as VPA, carbamazepine, vigabatrin or gabapentin (4,5,33,34). VPA therapy and weight gain association have been frequently reported; however, this fact is not always observed (35). The exact mechanisms of weight gain in patients taking VPA remain to be established, although several potential mechanisms have been suggested: dysregulation of the hypothalamic system, valproate-induced hyperinsulinemia, valproate-induced hyperleptinemia, interaction between valproate and adiponectin, and influence of genetic factors on body weight during VPA therapy (33,34).

Studies about body composition in epileptic patients are scarce (4). Body composition assessment can detect early body compartments changes (9). BIA is a simple method of estimating body composition. Compared to BMI, anthropometric and skin fold methods, BIA offers trustable results about fatness across human tissues estimation (36).

In this study, we have compared body composition estimated by BIA among epileptic pediatric patients on VPA treatment and healthy children. Although there was a trend toward higher body fat mass in VPA treated children, no statistical differences between both groups were found.

At the time of the study, 40% of the sample was obese. We compared cases with

controls matched by age, sex and BMI; therefore, no anthropometrical differences could be detected. Several researchers have investigated VPA and weight gain association in the pediatric population (2,3,33-40). Most of them do not have a BMI-matched control group, so their reported differences cannot be properly considered.

VPA-effects on weight gain can be observed within the first three months of treatment (3,40). In our study, children were on VPA monotherapy for at least six months, with a median time of 44.5 months (range 21.5-72); hence, weight gain should have been detected.

Grosso *et al*. (2) observed an increase BMI z-score among pediatric patients on VPA treatment, but the percentage of overweight patients detected in their series at the end of the study overlapped with those found in epidemiologic studies of healthy Italian children. Consequently, they could not rule out the possibility that a group of patients would have shown weight increased independently of VPA treatment.

Information about tissue hydration and integrity can be obtained from raw BIA measurements using two indicators, PA and BIVA. They are independent of regression equations or weight, and can be carried out in situations in which BIA assumptions are not valid (20,21). PA has been reported to be a parameter reflecting the body cell mass and a general health indicator (41). PA has been used as an indicator of nutritional status, considering that all membrane electrical properties are influenced by changes in cell mass (42,43). It is possible to distinguish between weight gain caused by an increase in body cell mass and that caused by edema, through analysis of changes in body weight and PA (44). To our knowledge, there are no Spanish reports where PA has been studied in children; therefore, no cut-off-PA values are available. Data published by Bosy-Westphal *et al.* (45), found in a German Pediatric group aged 6 to 17 years old a PA mean of 5.14-6.09º in girls and 4.79-6.35º in boys. De Palo *et al*. (46) reported in a group of Italian children a PA value from 4º at the age of 2 till 6.2º in 15 years old boys. We have analyzed PA in both groups of our study. The mean value in our study was 7.6º, which is higher than the above-mentioned data reported by German and Italian populations. This result can be interpreted as better nutritional status. In our study, we did not find statistical differences between PA values of cases and controls.

Piccoli et al established BIVA method to estimate the hydration status using height indexed resistance and reactance data (R-Xc graph) from bioimpedance measurements. BIVA can give a semi-quantitative evaluation of body composition from BIA measurements, once it combines information from fluids and soft tissues. Part of its prognostic power is derived from PA, but the additional information about tissue hydration could increase its usefulness in clinical practice (31,32,47,48). BIVA allows individual and group comparisons. It has shown its utility in situations in which fluid imbalances are present and BIA equations are not valid. It has also been useful as a prognostic tool in other clinical situations (20,21). Because vector distribution patterns differ between sex, race, ethnicity and are dependent on BMI and age, national reference distributions have been stratified (49). Redondo del Río et al have recently reported BIVA reference values in children and adolescents from Castilla y León, Spain (50). In our pilot study, BIVA data differ from these Spanish values in both groups. Redondo del Río et al reported BMI data slightly lower than ours, which might partially explain BIVA differences.

Regarding the limitations of the present study, its cross-sectional nature precludes definite conclusions and requires larger prospective studies. Moreover, the limited sample size could influence the results. We are aware that we have probably not reached a minimum statistical power to detect subtle FM differences between groups, thus we could be under a type II error frame.

In conclusion, since estimation of body composition may detect early changes in body compartments, it would be advisable to assess it in pre-therapy and monitoring of patients with epilepsy, in order to detect changes before adverse consequences could happen. The results of this study show no significant statistical differences in body composition among pediatric epileptic patients on VPA-treatment and healthy children, although there was a trend toward higher body fat mass in VPA treated group. It would be necessary to increase the sample size in future studies to be able to detect more subtle differences, if present.

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