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ORIGINAL ARTICLE

The effect of the ketogenic diet on leptin, chemerin and resistin levels in children with epilepsy

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Abstract

OBJECTIVES: Recently, inflammation have been proposed as one of the mechanisms underlying the patology of drug-resistant epilepsy (DRE). Ketogenic diet (KD) is one of the therapeutic methods used in DRE. There are some data that adipokines may modulate inflammatory processes and their concentrations are influenced by KD. Therefore, the aim of this study was to evaluate the influence of KD on serum leptin, chemerin and resistin in children with DRE.
DESIGN: A cross-sectional observational study performed on 72 subjects aged 3-9 years, divided into 3 groups: 24 children with DRE treated with KD, 26 treated with valproic acid (VPA), and a control group of 22 children.
MATERIAL AND METHODS: Anthropometric measurements (weight, heigth, BMI, waist to hip circumerences ratio) were performed in all participants. Biochemical tests included serum fasting glucose, insulin, beta-hydroxybutyric acid, lipid profile, alanine aminotransferase and aspartate aminotransferase activities and blood gasometry. Serum levels of leptin, chemerin and resistin were assayed using commercially available ELISA tests.
RESULTS: Serum levels of leptin and chemerin in the KD group were significantly lower and resistin – higher in comparison to patients receiving VPA and the control group. In children treated with the KD, leptin concentrations correlate with insulin levels and HOMA-IR scores. Chemerin levels in this group, in contrast, show negative correlation with body mass and height expressed as standard deviation scores from the mean for age and sex.
CONCLUSIONS: Modification of pro-inflammatory adipocytokine levels is potentially one of the mechanisms of anticonvulsant effects of KD in children with refractory epilepsy.

Abbreviations:

ALT	- alanine aminotransferase
ANOVA	- analysis of variance
AST	- aspartate aminotransferase
ATP	- adenosine triphosphate
BHBA	- beta-hydroxybutyrate
BMI	- body mass inde
CCRL2	- chemokine C-C motif receptor-like 2
CMKLR1	- chemokine-like receptor 1
CNS	- central nervous system
CRP	- C-reactive protein
ELISA	- enzyme-linked immunosorbent assay
FIZZ3	- found in inflammatory zone 3
GABA	- gamma aminobutyric acid
GPR1	- G protein-couple receptor 1
HOMA-IR	- homeostatic model assessment of insulin resistance
IFNχ	- interferon χ
IL-1	- interleukin 1
IL-10	- interleukin 10
IL-12	- interleukin 12
IL-1β	- interleukin 1β
IL-6-	- interleukin 6
KD	- ketogenic diet
PPARγ	- peroxisome proliferator-activated receptor gamma
SDS	- standard deviation score
TLE	- temporal lobe epilepsy
TNF-α	- tumor necrosis factor α
VPA	- valproate
WHR	- waist to hip ratio

INTRODUCTION

The ketogenic diet (KD) is one of the nonpharmacological methods of treating epilepsy. It is based on switching a person's metabolism to a metabolic state that mimics that observed during chronic fasting. This goal can be achieved by changing the proportions of particular nutrients to a high proportion of fat, protein content appropriate for one's age, and a reduced proportion of carbohydrates. The target weight ratio of fat to combined protein and carbohydrates (the ketogenic ratio) depends on the age and is most often 4:1 (or 3:1 in children under 2 years old). In the KD, around 90% of the daily energy requirement is provided by fats. Ketone bodies are produced in the process of beta-oxidation of free fatty acids (acetone, acetoacetate, beta-hydroxybutyric acid). They are incorporated into the Krebs cycle and thus become a direct source of energy for cells. In comparison, as much as 49% of the energy in a conventional diet comes from carbohydrates, while only 35% comes from fat (Kossoff *et al.* 2018; Van Der Louw *et al.* 2016; Dudzińska, 2014).

Fat tissue is the body's largest energy reservoir. For many centuries, until the beginning of the twentieth century, it was considered to be a simple thermal insulation layer, metabolically neutral, and to protect organs from mechanical damage (Hauner & Hochberg, 2002; Sperling *et al.* 2012). A great variety of adipose tissue hormones called adipokines have been described, and currently, adipose tissue is considered as an integrated endocrine organ that is involved in metabolic homeostasis processes with effects on appetite, angiogenesis, neuroendocrine functions, and immunological functions. It plays a role in both the metabolism of steroid hormones and the production and secretion of biologically active protein substances—adipokines—which can have local (auto- and paracrine) or systemic (endocrine) impacts (Kershaw & Flier, 2004).

Leptin is the best known adipokine (Zhang *et al.* 1994), and its secretion is directly proportional to fat tissue mass and nutritional status. In addition to adipose tissue, it is also produced in areas such as the hypothalamus, pituitary gland, cerebral cortex, and limbic system (Ahima & Lazar, 2008). Leptin acts through 5 types of receptors: OB-Ra, OB-Rb, OB-Rc, OB-Rd, and OB-Re. The first four of these are membrane receptors, while OB-Re is a soluble receptor that circulates in the blood (Ziora *et al.* 2003). Factors that stimulate the secretion of this hormone include TNF-α and other pro-inflammatory cytokines, whereas inhibitory agents include PPARγ receptor agonists (Kershaw & Flier, 2004).

Leptin is a protein with multidirectional (pleiotropic) action. It regulates lipid metabolism by inhibiting hepatic and muscle lipogenesis and by stimulating the beta-oxidation of fatty acids. It also increases glucose uptake and metabolism in peripheral tissues and improves insulin sensitivity in the muscles and liver. Moreover, it participates in the processes of osteogenesis, haematopoiesis, angiogenesis, and reproductive and immunological processes. In the central nervous system, it affects hormonal activity in the hypothalamus and the pituitary gland and is also involved in the regulation of neuronal excitability, displaying neuroprotective properties (Obeid *et al.* 2010). Leptin is considered an adipokine with pro-inflammatory activity as it increases the synthesis of eicosanoids, nitric oxide, and pro-inflammatory cytokines such as TNF-α, IL-6, and IL-12, while decreasing IL-10 levels. In addition, it improves phagocytic functions, induces chemotaxis and the release of oxygen free radicals by neutrophils, activates and enhances the cytotoxicity of natural killer T-cells, and promotes a TH1-type response (Fantuzzi, 2005; Lago *et al.* 2007; Tilg & Moschen, 2006).

A few reports point to a potential role of leptin in the process of epileptogenesis. It may be directly involved in the etiopathogenesis of epilepsy by modulating the excitability of the nervous tissue, as well as indirectly by influencing the course of inflammatory and metabolic processes (Mora-Muñoz *et al.* 2018).

Also known as TIG2 (tazarotene-induced gene 2 protein) or RARRES2 (retinoid acid receptor responder 2), chemerin is mainly secreted by adipose tissue, which is regulated by pro-inflammatory cytokines (TNF-α, IL-1β and IFNχ) (Kralisch *et al.* 2009; Lehrke *et al.* 2009; Landgraf *et al.* 2012). It acts through three types of receptors: CMKLR1 (chemokine-like receptor 1), CCRL2 (chemokine C-C motif receptor-like 2), and GPR1 (G protein-couple receptor 1), which are all expressed in the central nervous system (CNS) (Meder *et al.* 2003; Rourke *et al.* 2013). This protein is involved

Tab. 1. Results of anthropometric measurements and biochemical assays performed on the examined children

Parameter	Group			p
	KD (n=24) [1]	VPA (n=26) [2]	C (n=22) [3]	
	mean ± SD (min-max)			
age (years)	6.2 ± 2.2 (3.0-9.9)	6.7 ± 2.3 (3.1-9.9)	7.7 ± 1.6 (3.6-9.7)	0.84
body mass (kg)	19.73 ± 7.59 (9.1-39.0)	24.76 ± 9.43 (15.5-54.1)	28.25 ±6.7 (17.0-40.7)	0.08
body mass SDS	0.15 ± 1,03 (-1.30-3.50)	-0.03 ± 1.19 (-1.94-2.53)	-0.82 ± 0.91 (-3.04-0.72)	**1/3 0.02**
height (cm)	112.65 ± 15.82 (86.0-138.0)	120.4 ± 16.41 (95.0-157.5)	129.17 ± 9.83 (103.0-141.5)	0.07
height SDS	-1.08 ± 1.43 (-5.12-1.11)	-0.27 ± 1.3 (-2.26-2.35)	0.17 ± -0.96 (-1.54-2.53)	**1/3 0.006**
BMI (kg/m^2)	15.05 ± 2.27 (11.23-21.84)	16.55 ± 2.57 (11.36-21.81)	16.74 ± 2.46 (13.73-23.71)	0.11
BMI SDS	-1.08 ± 1.43 (-5.12-1.11)	0.14 ± 1.11 (-2.11-2.9)	0.1 ± 1.01 (-1.06-3.52)	**1/3 0.04**
waist circ. (cm)	50.58 ± 7.76 (39.5-67.0)	54.98 ± 6.65 (45.0-74.0)	58.15 ± 6.45 (48.0-74.0)	0.05
waist circ. SDS	-0.96 ± 1,17 (-3.08-0.98)	0.01 ± 1.06 (-1.38-2.7)	0.26 ± 1.19 (-1.43-3.73)	**1/3 0.01**
hip circ. (cm)	56.87 ± 10.12 (40.5-79.0)	62.55 ± 8.32 (51.0-89.0)	68.67 ± 8.34 (55.0-84.0)	**1/3 0.01**
hip circ. SDS	-1.19 ± 1.09 (-3.82-0.74)	-0.29 ± 1.18 (-2.43-2.09)	0.25 ± 1.3 (-2.21-3.20)	**1/3 0.003**
WHR	0.89 ± 0.05 (0.74-0.98)	0.88 ± 0.05 (0.78-1.00)	0.85 ± 0.06 (0.71-0.98)	0.1
total cholesterol (mg/dl)	171.6 ± 42.12 (87.0-279.0)	162.02 ± 29.92 (112.0-235.0)	171.07 ± 27.54 (127.0-257.0)	0.81
LDL-cholesterol (mg/dl)	102.27 ± 34.34 (38.0-180.6)	96.54 ± 28.03 (58.3-161.3)	101.76 ± 23.51 (54.3-159.6)	0.51
HDL-cholesterol (mg/dl)	53.10 ± 17.04 (26.0-102.0)	51.74 ± 8.5 (35.5-65.0)	57.28 ± 12.8 (36.6-88.2)	0.16
triglycerides (mg/dl)	103.1 ± 53.34 (29.0-302.0)	67.03 ± 43.79 (26.0-279.0)	67.63 ± 37.52 (31.0-204.0)	**1/2; 1/3 0.001**
AST (IU/l)	30.2 ± 40.61 (6.0-219.0)	27.2 ± 7.37 (18.0-43.0)	26.8 ± 4.57 (19.0-37.0)	0.19
ALT (IU/l)	35.37 ± 22.27 (11.0-120.0)	15.43 ± 8.8 (6.0-54.0)	15.77 ± 3.86 (9.0-22.0)	**1/2 0.04**
fasting glucose (mg/dl)	72.03 ± 8.98 (53.0-90.0)	86.47 ± 7.94 (64.0-101.0)	88.5 ± 7.05 (74.0-101.0)	**1/2; 1/3 0.001**
insulin (mU/ml)	2.48 ± 0.64 (1.34-3.75)	4.19 ± 0.64 (3.13-5.3)	4.55 ± 0.92 (3.09-5.98)	**1/2; 1/3 0.001**
HOMA-IR	0.44 ± 0.13 (0.22-0.7)	0.91 ± 0.18 (0.66-1.29)	1.0 ± 0.23 (0.65-1.51)	**1/2; 1/3 0.001**
CRP (mg/l)	0.9 ± 1.44 (0.2-5.6)	0.56 ± 0.72 (0.2-3.8)	1.87 ± 3.16 (0.1-5.7)	0.42
HCO^{3-} (mmol/l)	19.95 ± 2.03 (16.5-24.1)	22.62 ± 1.68 (19.5-25.5)	21.95 ± 1.08 (19.1-24.3)	**1/2; 1/3 0.001**
BE (mEq/l)	-4.39 ± 2.14 (-7.9-0.4)	-1.68 ± 1.75 (-4.9-0.9)	-2.35 ± 1.05 (-4.2- (-0.5))	**1/2; 1/3 0.001**
BHBA (mmol/l)	4.31 ± 1.62 (1.0-7.3)	0.29 ± 0.23 (0.1-1.3)	0.29 ± 0.25 (0.1-1.3)	**1/2; 1/3 0.001**

KD – patients with epilepsy treated with the ketogenic diet and pharmacotherapy; VPA – group of patients with epilepsy treated with valproates; C – control group; SD – standard deviation; BMI – body mass index; WHR – waist/hip ratio; SDS – standard deviation score; AST – aspartate aminotransferase; ALT – alanine aminotransferase; HOMA-IR – insulin resistance score; CRP – C-reactive protein; HCO3- – bicarbonate level; BE – base excess; BHBA – beta-hydroxybutyrate; significant differences are marked in bold

in the regulation of metabolic processes by reducing glucose uptake by myocytes and increasing insulin resistance. It is also postulated to have a stimulating effect on nervous tissue's excitability by inhibiting the activity of adenylyl cyclase (Zhao *et al.* 2015). Moreover, chemerin plays a role in the regulation of adaptive and innate cellular immunity and modulates the functioning of the immune system. It is considered an adipokine with a pro-inflammatory effect, but due to its multifunctional nature, it may also exert anti-inflammatory properties under certain conditions (Shimamura *et al.* 2009).

Resistin is a pro-inflammatory peptide that was initially called FIZZ3 (found in inflammatory zone 3) (Holcomb *et al.* 2000). This hormone is believed to be secreted by mainly stromal cells in human adipose tissue, with trace amounts derived from preadipocytes and adipocytes (Oświęcimska, 2010). The expression of resistin mRNA is enhanced by pro-inflammatory cytokines such as IL-1, IL-6, TNF-α and lipopolysaccharides, while PPARγ receptor agonists inhibit it (Tilg & Moschen, 2006; Steppan & Lazar, 2004). In mice, resistin has been shown to increase blood glucose and insulin levels and to inhibit the hypoglycaemic effect of insulin (Steppan *et al.* 2001). Nevertheless, there are conflicting results in studies on the impact of this adipokine on the development of insulin resistance in humans (Steppan & Lazar, 2004). Elevated concentrations of resistin show positive correlations with inflammatory markers such as CRP, IL-6, soluble TNF-α receptor-2, and lipoprotein-associated phospholipase A2. Furthermore, they are related to an increased risk of cardiovascular disease and metabolic syndrome (Oświęcimska, 2010).

The KD is linked to a significant shift in the proportions of delivered nutrients due to an increase in the supply of lipids combined with restricted carbohydrate intake. The diet modifies endocrine activity in adipose tissue by changing the profile of secreted adipokines (Paoli *et al.* 2015; De Amicis *et al.* 2019). The vast majority of the adipose tissue's hormones affect the course of inflammatory processes (Trayhurn & Wood, 2004). This aspect of their activity seems particularly interesting in the context of hypotheses regarding the role of inflammatory processes in the development of epilepsy and its complications. Many studies have demonstrated that a number of substances secreted in inflammatory states of the CNS or peripheral tissues induce changes in the expression and function of neurotransmitter receptors, leading to a decrease in the seizure threshold. In the longer term, recurrent epileptic seizures cause damage to neurons and the blood-brain barrier, which promotes further activation and expansion of inflammatory processes. By increasing the secretion of pro-inflammatory cytokines, low-grade inflammation can increase the frequency of seizures and their generalization, as well as contribute to the development of drug resistance and epilepsy complications (Paudel *et al.* 2018).

The KD has proven clinical efficacy in the treatment of refractory epilepsy in children, but there is more to explore in regard to its mechanism of action and potential new therapeutic possibilities associated with the function of adipokines or substances modulating their secretion/activity. This compelled us to study the serum concentrations of selected pro-inflammatory adipokines (leptin, chemerin, and resistin) in children with epilepsy who are being treated with the KD. We compared the results with those obtained from children receiving valproates (VPA) and a control group (children diagnosed with headaches) and analysed the correlations with anthropometric and metabolic parameters.

MATERIAL AND METHODS

The study involved 72 patients from the Department of Paediatric Neurology at the Independent Public Healthcare Centre – Municipal Hospital Complex in Chorzów. There were 40 girls and 32 boys aged 3-9 years without signs of puberty (at Tanner stage I). The participants were divided into 3 groups. The study group (KD) consisted of 24 patients (11 girls, 13 boys) aged 3-9 years (average age 6.2 ± 2.2 years) who were diagnosed with drug-resistant epilepsy and treated for >3 months with the KD. In this period, no modifications in the pharmacotherapy were introduced. Generalized seizures were observed in 14 children from this group (6 girls and 8 boys). There were polymorphic seizures in 9 of them (5 girls, 4 boys), and 1 boy experienced focal seizures.

The KD was the only treatment administered to 3 patients, while 4 children on the KD also received 1 medication (of these, 3 patients received VPA, and 1 received levetiracetam). There were 14 patients on the KD who also received 2 antiepileptic drugs (10 patients received VPA in combination with topiramate, levetiracetam, ethosuximide, or clonazepam, while 4 patients were given levetiracetam in combination with clonazepam or vigabatrin). In the 3 remaining patients, triple pharmacotherapy was used in addition to the KD (with the use of VPA in each case).

The second study group consisted of children diagnosed with epilepsy who were treated with only VPA. This group included 26 patients (16 girls, 10 boys) aged 3-9 years (average age 6.7 ± 2.3 years). In this group, generalized seizures were observed in 17 children (10 girls, 7 boys), while 9 (5 girls, 4 boys) presented with focal seizures. The antiepileptic treatment prior to biochemical assays lasted at least 3 months.

The control group (C) was composed of 22 patients (13 girls, 9 boys) aged 3-9 years (average age 7.7 ± 1.6 years) who were admitted to the hospital to determine the cause of headaches. Laboratory and imaging tests in these patients did not reveal any abnormalities.

For each patient, a detailed medical history was obtained, and a meticulous physical examination was performed, which took into account anthropometric measurements [weight, height, waist and hip circumferences, and sexual maturity according to the Tanner scale (Marshall & Tanner, 1969)] . The results of the measurements were used to calculate the body mass index (BMI) according to the formula BMI = body weight (kg)/height (m^2). The waist/hip ratio (WHR) was calculated according to the formula WHR = waist circumference (cm)/hip circumference (cm). Anthropometric measurements were also expressed as standard deviations from the mean values (standard deviation score, SDS) for age and sex. All parameters were matched against percentile growth charts from the OLAF PL0080 research project (Świąder-Leśniak *et al.* 2015; Kułaga *et al.* 2015).

Venous blood samples of 10 ml were drawn in the morning between 8 AM and 10 AM in the fasted state at the same time as routine laboratory tests during hospitalization. Levels of high-sensitivity C-reactive protein (CRP), fasting blood glucose, beta-hydroxybutyrate (BHBA), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities, lipid profile, and gasometry were determined at the Laboratory of the Municipal Hospital Complex in Chorzów (formerly the Chorzów Centre of Paediatrics and Oncology). Hormonal tests were performed at the Institute and Department of Medical and Molecular Biology, Faculty of Medical Sciences, Medical University of Silesia in Katowice.

Leptin, chemerin, resistin, and vaspin concentrations were determined via the enzyme-linked immunosorbent assay (ELISA) method using commercial tests (Bio-Vendor LLC Laboratorní Medicína a.s., Prague, Czech Republic). For insulin levels, the same method was applied using a Mercordia Ultrasensitive Insulin ELISA kit (Mercordia AB, Uppsala, Sweden). The HOMA-IR (homeostasis assessment model - insulin resistance) score was calculated according to the formula:

$$\text{HOMA-IR} = \frac{\text{fasting insulin } (\mu\text{IU/ml}) \times \text{fasting glucose (mmol/l)}}{22.5}$$

The research was approved by the Bioethics Committee of the Medical University of Silesia in Katowice (Resolution No. KNW/0022/KB1/155/15 of 15.12.2015). Additionally, we received written consent from the patients' parents or legal guardians.

Statistical Analysis

A database was created in a Microsoft Excel spreadsheet. Statistical calculations were done using the software Statistica 10.0 (StatSoft Inc., USA). The Kolmogorov-Smirnov test was used to check whether the tested parameters had a normal distribution. The homogeneity of variances was assessed with Levene's test. For variables with a normal distribution, group comparisons were performed using a one-way analysis of variance (one-way ANOVA) and Tukey's HSD post-hoc tests. The Kruskal-Wallis test was used if the distribution of variables differed significantly from a normal distribution and Levene's test showed no homogeneity of variances.

For variables with a normal distribution, linear correlations were established by determining Pearson coefficients. In the case of variables with a distribution significantly deviating from normal, Spearman coefficients were used. A significance level of $\alpha<0.05$ was adopted in statistical calculations. Values in the tables and text are presented as the mean ± SD (minimum-maximum).

RESULTS

The results of the anthropometric measurements and biochemical assays are shown in Table I. Leptin levels in the KD group (2.71 ± 0.93 ng/ml, range 1.28-4.32 ng/ml) were significantly lower (p=0.001) than in the VPA group (5.85 ± 1.79 ng/ml, range 3.45-10.48 ng/ml) and C group (6.14 ± 2.07 ng/ml, range 3.31-10.89 ng/ml) (Fig. 1).

Chemerin levels in children treated with the KD (144.24 ± 23.47 ng/ml, range 104.88-197.80 ng/ml) were also significantly lower (p=0.001) than in the VPA group (180.79 ± 12.87 ng/ml, range 157.86-208.68 ng/ml) and C group (182.80 ± 12.71 ng/ml, range 155.37-199.20 ng/ml) (Fig. 2).

Conversely, resistin concentrations in the KD group (3.53 ± 0.44 ng/ml, range 2.56-4.26 ng/ml) were significantly higher (p=0.003) than in the VPA group (3.16 ± 0.31 ng/ml, range 2.58-4.02 ng ml) and C group (3.15 ± 0.32 ng/ml, range 2.67-4.26 ng/ml) (Fig. 3).

The analysis of correlations established that in children treated with the KD, serum leptin concentrations correlated with insulin levels (r=0.60, p<0.01) and HOMA-IR scores (r=0.55; p<0.01). In contrast, chemerin levels in this group showed a negative correlation with body mass (r=-0.49; p=0.02) and height (r =-0.47; p=0.02) expressed as SDS. In the VPA group, there was a positive relationship between chemerin concentrations and WHR (r=0.49; p=0.02). Resistin levels and fasting blood glucose (r=0.50; p=0.02) also correlated positively, while in the case of AST, this relationship was inversed (r =-0.47; p=0.02).

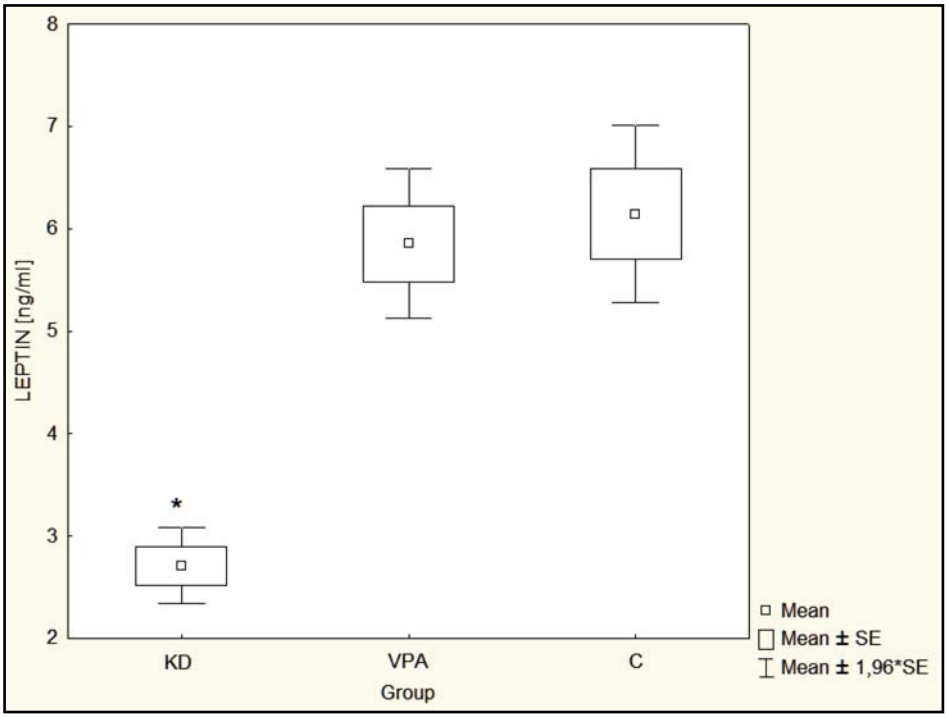

Fig. 1. Serum leptin concentrations in the examined groups of children (n=72)
KD – ketogenic diet; VPA – valproates; C – controls; *p=0.001 KD vs. VPA and KD vs. C

In the control group, leptin concentrations correlated with body mass (r=0.10; p=0.04), insulin concentrations (r =0.51, p=0.02), and HOMA-IR scores (r=0.52; p=0.01). Furthermore, an inverse relationship was found between chemerin concentrations and age (r =-0.43; p=0.04), height (r=-0.46; p=0.03), and ALT activity (r=0.60; p<0.01). Table II shows the results of the correlation analysis between anthropometric parameters, biochemical assays, and serum adipokine levels.

DISCUSSION

Reports published over the past two decades provide a growing body of evidence regarding the role played by a dysfunctional immune system and inflammation in the process of epileptogenesis (Vezzani & Granata, 2005; Vezzani, 2008; Vezzani *et al.* 2011). It is believed that the brain is not a privileged organ in terms of immunity, despite the existence of the blood-brain barrier. Nevertheless, it does have its own local immune system, where glial cells play a primary role. These cells protect and nourish the neurons and also participate in initiating inflammatory processes (Engelhardt, 2008).

Glial dysfunction might be a potential factor causing a chronic inflammatory conditions. Such conditions predispose a patient to the occurrence or aggravation of various central nervous system disorders and manifest at the cellular level. One example is the overexpression of pro-inflammatory cytokines or intercellular communication disorders (Damani *et al.* 2011; Vezzani & Friedman, 2011; Aronica & Crino, 2011). Additionally, a process with the characteristics of peripheral or systemic inflammation may provoke an inflammatory response in the CNS, causing exacerbation of ictal activity due to modulation of the activity of drug-metabolising enzymes and systems that transport drugs across the brain ((Riazi *et al.* 2010; Nielsen *et al.* 2007; Yu *et al.* 2011).

The KD is one of the non-pharmacological methods used to treat drug-resistant epilepsy. Like the pathomechanism of epileptogenesis, the anticonvulsant mechanism of the KD is very complex. Despite its many years of use and numerous studies, its mechanism has not been fully understood (Simeone *et al.* 2017; Maalouf *et al.* 2009; Giordano *et al.* 2014). It is believed that the diet has a direct anticonvulsant and neuroprotective effect thanks to ketone bodies. It is also thought to provoke changes in neurotransmission (increases in GABA production, inhibition of the glutaminergic system, and stimulation of the noradrenergic system), inhibition of voltage-gated ion channels (sodium and calcium), increases in ATP synthesis and adenosine accumulation, reductions in oxidative stress, and modulation of hormonal processes and of the immune response (Maalouf *et al.* 2009; Stafstrom & Rho, 2012; Gasior *et al.* 2006).

The anti-inflammatory effect of fasting and the KD has been well documented (Maalouf *et al.* 2009; Gasior *et al.* 2006). By inhibiting the microglia's pro-inflammatory response, ketone bodies inhibit transmission related to the NFκB pathway and the associated production of pro-inflammatory cytokines (Maalouf *et al.* 2009; Ghosh *et al.* 2018).). The gene expression of the NFκB transcription factor (nuclear factor κB) is also

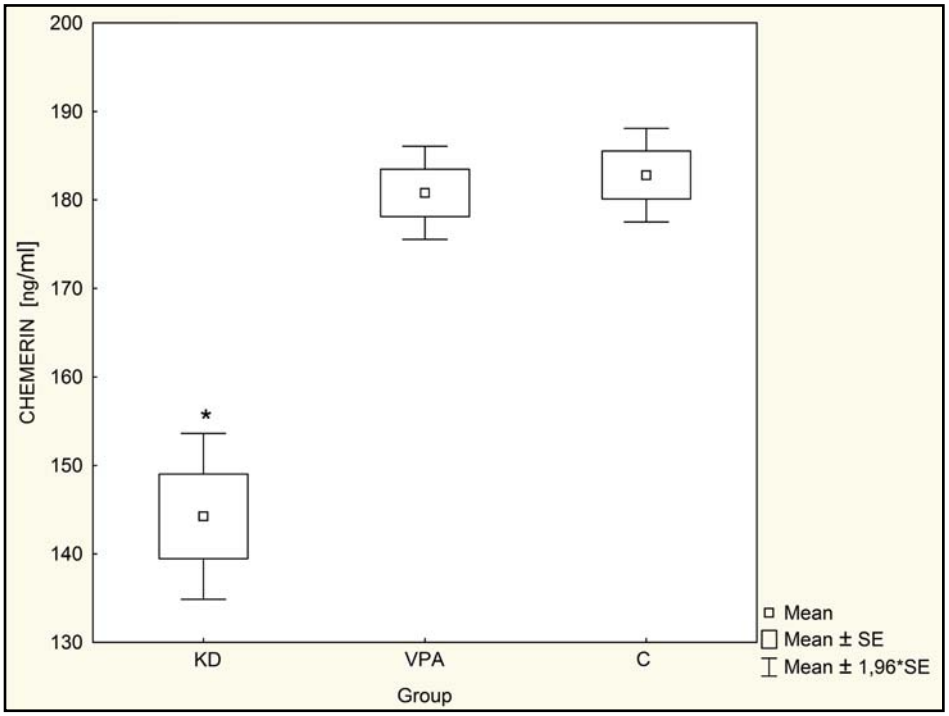

Fig. 2. Serum chemerin concentrations in the examined groups of children (n=72)
KD – ketogenic diet; VPA – valproates; C – controls; *p<0.001 DK vs. VPA and KD vs. C

affected by receptors that are activated by peroxisome proliferator-activated receptor (PPAR), which is present in various body tissues (including microglia). They are involved in the regulation of carbohydrate and lipid metabolism, adipogenesis, and immunomodulation, and ketone bodies modulate their levels (Maalouf *et al.* 2009; Badman *et al.* 2007).

Changes in serum adipokine levels in patients using KD therapy would seem extremely interesting when considering the links between low-grade inflammation within the CNS or peripheral tissues and the phenomenon of drug resistance in epilepsy, along with the anti-inflammatory effect discovered in the anti-epileptic mechanism of the KD. Adipose tissue hormones indeed participate in the regulation of the body's metabolism and energy homeostasis, as well as the modulation of inflammatory processes. Our results indicate that one of the potential mechanisms behind the KD's action in patients with drug-resistant epilepsy may be a decrease in the concentration of the pro-inflammatory adipokines leptin and chemerin.

The research by de Brito Toscano *et al.* suggests that the development of temporal lobe epilepsy (TLE) in adults is associated with local and systemic activation of low-grade inflammation due to constant stimulation of CNS cells (especially astrocytes and microglia) and leukocytes, which manifests as elevated serum leptin levels. They are a very good predictor of TLE diagnosis in a logistic regression model. They may also be responsible for the somatic complications of epilepsy in the form of hypertension and disorders of the digestive, cardiovascular, and respiratory systems (de Brito Toscano *et al.* 2019). Interestingly, another study observed a negative correlation between leptin levels and the frequency of generalized seizures observed. Concentrations of this adipokine were reduced within 24 hours of a seizure, which can be explained by the body's anti-inflammatory compensatory response to convulsions (Palmio *et al.* 2016).

A small number of studies on animals indicate bi-directional effects of leptin in epileptogenesis. It can exert both pro-seizure and anticonvulsant effects. In some types of epilepsy, leptin can inhibit epileptogenesis by activating potassium channels (Bentzen *et al.* 2014). Xu *et al.* found elevated plasma and brain concentrations of leptin accompanied by a reduction in seizures when researching the effect of this adipokine on seizures induced by the administration of 4-aminopyridine or pentylenetetrazole in mouse models. This result is associated with the inhibitory effect of leptin on synaptic transmission and the selective inhibition of AMPA receptors (Xu *et al.* 2008). On the other hand, a positive correlation was observed between leptin levels and seizure activity in mouse models with epilepsy induced by intraventricular administration of penicillin (Ayyildiz *et al.* 2006). Lynch *et al.* described a similar effect in mouse models for leptin supplied together with NMDA and kainate receptor agonists (Lynch *et al.* 2010).

Our results are consistent with those presented by Rauchenzauner *et al.* who examined 10 prepubertal children with epilepsy and Glut 1 deficiency syndrome treated with the KD for 6 months (ketogenic ratio 3:1). They noted lower levels of insulin,

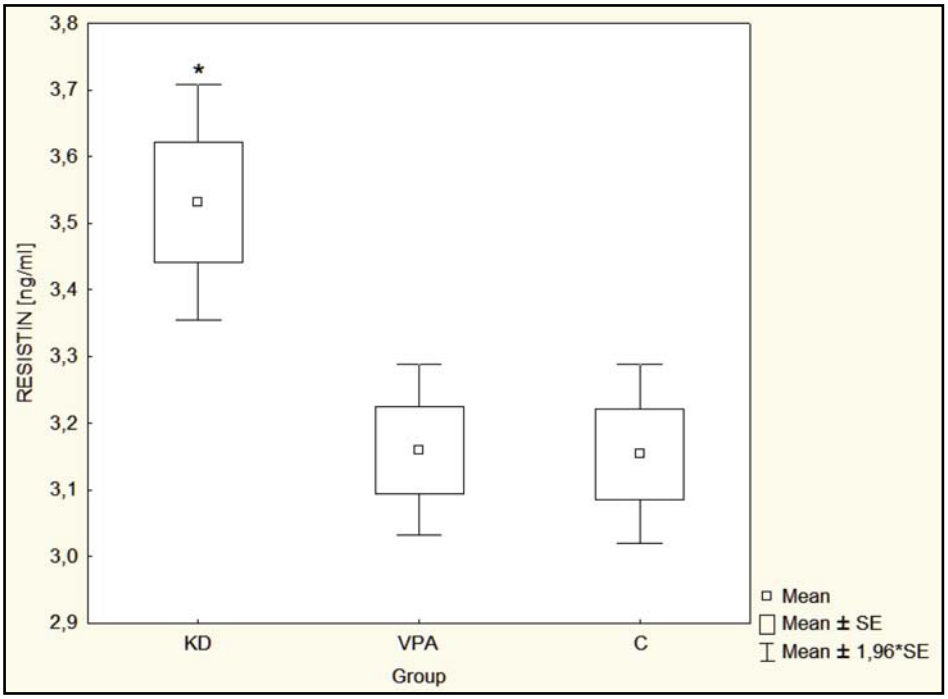

Fig. 3. Serum resistin concentrations in the examined groups of children (n=72)
KD – ketogenic diet; VPA – valproates; C – controls; *p=0.003 KD vs. VPA and KD vs. C

leptin, and HOMA-IR scores than the control group, which consisted of children with benign childhood epilepsy (Rauchenzauner *et al.* 2008). In a prospective uncontrolled study, Lambrechts *et al.* demonstrated a decrease in leptin levels following a 12-month period of using different variants of the KD in 1 to 40-year-old patients with refractory epilepsy (Lambrechts *et al.* 2016). Other studies, however, have not found changes in the concentration of this hormone in the course of KD treatments lasting 3 (Rho & Stafstrom, 2012), 6, and 12 months (De Amicis *et al.* 2019). This may have resulted from methodological differences in the type of KD used, study protocol, age, diagnosis, and multi-drug pharmacotherapy administered in parallel to the diet.

It is also hypothesized that the decrease in leptin levels caused by the KD is associated with body mass loss (De Amicis *et al.* 2019). Our results point to the existence of such a dependence since the body mass and BMI in the KD group were significantly lower than in the children receiving VPA and healthy children. Moreover, we confirmed that elevated leptin levels in children treated with VPA, as reported by a number of studies, are associated with an increased appetite, the development of obesity, and insulin resistance rather than with the direct effect of this drug (Tokgoz *et al.* 2012; Cansu *et al.* 2011; Hamed *et al.* 2009; Aydin *et al.* 2005; Rauchenzauner *et al.* 2008; Sonmez *et al.* 2013). Leptin levels in the VPA group were indeed similar to those in the control group, but there were no significant differences in body mass, BMI, insulin levels, or HOMA-IR scores. Amongst other antiepileptic drugs, carbamazepine, oxcarbazepine, and lamotrigine do not affect leptin levels (Hamed *et al.* 2009; Uludag *et al.* 2009; Cansu *et al.* 2011). In the case of topiramate in the paediatric population, an inverse relationship (a reduction of plasma leptin levels) was detected (Sonmez *et al.* 2013, Ozcelik *et al.* 2014). However, these observations have not been confirmed in adult populations using topiramate as both a monotherapy and an add-on drug (Genc *et al.* 2010; Theisen *et al.* 2008).

There is only one report about the concentration of chemerin in the sera of children with idiopathic epilepsy aged 5-10 years. Concentrations of this adipokine were significantly higher in a group of 50 patients aged 5-10 years than a control group of healthy children. Furthermore, they correlated with the severity of the disease and its duration. They have also been proven to be a valuable predictor of poor response to pharmacotherapy with 80% sensitivity and 88% specificity. It was suggested that elevated levels of chemerin in epileptic patients may be a manifestation of the activation of inflammatory processes, especially since previous studies also indicated pro-inflammatory effects of this adipokine in other neurological conditions, such as strokes, multiple sclerosis, and autoimmune encephalitis (Elhady *et al.* 2018). In light of the scant data, the significantly lower levels of chemerin observed in children treated with the KD seem to confirm its anti-inflammatory mechanism of action.

For resistin, we obtained slightly different results than in the case of leptin and chemerin since the levels of this adipokine were significantly higher in children treated with the KD than in the other groups.

The results of the few studies on resistin concentrations in patients with epilepsy have been very divergent (de Brito Toscano *et al.* 2019; Sonmez *et al.* 2013; Tomoum & El-Hadidi, 2009). De Brito Toscano *et al.* demonstrated that resistin levels in patients with TLE are significantly lower than in a control group (de Brito Toscano *et al.* 2019). In prospective studies, Tomoum *et al.* did not demonstrate any significant difference regarding resistin levels during VPA therapy in children, although none of the patients developed obesity, which is consistent with our observations (Tomoum & El-Hadidi, 2009). On the other hand, Sonmez *et al.* found a significant increase in its levels over 12 months of VPA treatment (Sonmez *et al.* 2013). The current state of knowledge does not allow us to explain these discrepancies. Perhaps the key to interpreting them is that resistin is not a typical adipokine as its main source is mononuclear stromal cells from adipose tissue and not adipocytes (Oświęcimska, 2010).

There are a few limitations to this study. It is cross-sectional study, and the groups were relatively small. In the majority of cases, children from the KD group also received additional pharmacotherapy. However, for clinical reasons, standardization of the KD group in terms of the anti-epileptic drugs administered turned out not to be feasible.

In our research, we evaluated serum concentrations of chemerin and resistin in children treated with the KD for the first time ever. We did not find any reports on this subject in the literature. Another merit of the study is its wide assessment of metabolic parameters, which cover carbohydrate metabolism, lipid metabolism, and acid-base balance. The examined groups consisted exclusively of prepubertal children with drug-resistant epilepsy. In contrast to previous studies, we compared the results of adipokine levels in children with drug-resistant epilepsy treated with the KD to those of not only healthy children, but also children with epilepsy who were receiving VPA.

CONCLUSIONS

The results demonstrated that serum proinflammatory adipokines leptin and chemerin were significantly lower in in children with drug-resistant epilepsy treated with KD than patients treated with VPA and the control group. We also showed their relationship to the parameters of acid-base balance and insulin resistance. Therefore, our results suggest that one of the potential mechanisms of the KD in children with drug-resistant epilepsy might be a decrease in pro-inflammatory adipokine levels.

Tab. 2. Analysis of correlation between anthropometric parameters and biochemical assays and the concentrations of tested adipokines in all examined children (n=72)

Parameter	leptin [ng/ml]	chemerin [ng/ml]	resistin [ng/ml]
age (years)	r=0.15 p=0.24	r=0.07 p=0.54	r=0.20 p=0.10
body mass (kg)	**r=0.26** **p=0.03**	r=0.08 p=0.53	r=-0.02 p=0.88
body mass SDS	**r=0.28** **p=0.02**	r=0.09 p=0.44	r=-0.16 p=0.19
height (cm)	**r=0.25** **p=0.04**	r=0.08 p=0.50	r=0.06 p=0.64
height SDS	r=0.23 p=0.05	r=0.03 p=0.82	r=-0.18 p=0.15
BMI (kg/m$^{2)}$	**r=0.24** **p=0.04**	r=0.07 p=0.59	r=-0.13 p=0.27
BMI SDS	**r=0.28** **p=0.02**	r=0.12 p=0.34	r=-0.21 p=0.08
waist circ. (cm)	**r=0.26** **p=0.03**	r=0.11 p=0.37	r=-0.05 p=0.66
waist circ. SDS	**r=0.29** **p=0.02**	r=0.15 p=0.22	r=-0.16 p=0.20
hip circ. (cm)	r=0.23 p=0.05	r=0.11 p=0.39	r=-0.03 p=0.79
hip circ. SDS	r=0.22 p=0.07	r=0.12 p=0.32	r=-0.23 p=0.06
WHR	r=-0.04 p=0.70	r=-0.02 p=0.90	r=-0.02 p=0.85
total cholesterol (mg/dl)	r=-0.16 p=0.20	r=-0.07 p=0.56	r=0.05 p=0.66
LDL-cholesterol (mg/dl)	r=-0.11 p=0.36	r=-0.04 p=0.73	r=0.03 p=0.79
HDL-cholesterol (mg/dl)	r=-0.01 p=0.91	r=0.12 p=0.31	r=0.07 p=0.58
triglycerides (mg/dl)	**r=-0.36** **p<0.01***	**r=-0.29** **p=0.02***	r=0.18 p=0.13*
AST (IU/l)	r=-0.19 p=0.12*	r=0.08 p=0.54*	r=-0.15 p=0.20*
ALT (IU/l)	r=-0.13 p=0.30*	r=-0.17 p=0.17	r=-0.03 p=0.83*
fasting glucose (mg/dl)	**r=0.48** **p<0.01**	**r=0.43** **p<0.01**	r=-0.23 p=0.06
insulin (mU/ml)	**r=0.66** **p<0.01**	**r=0.51** **p<0.01**	**r=-0.34** **p<0.01**
HOMA-IR	**r=0.66** **p<0.01**	**r=0.54** **p<0.01**	**r=-0.34** **p<0.01**
CRP (mg/l)	r=-0.07 p=0.55	r=0.09 p=0.49*	r=0.09 p=0.49*
HCO^{3-} (mmol/l)	**r=0.40** **p=0.01**	**r=0.36** **p<0.01**	r=-0.22 p=0.08
BE (mEq/l)	**r=0.37** **p<0.01**	**r=0.34** **p=0.01**	r=-0.22 p=0.07
BHBA (mmol/l)	**r=-0.72** **p<0.01***	**r=-0.50** **p<0.01***	r=0.32 p=0.01*

BMI – body mass index; WHR – waist/hip ratio; SDS – standard deviation score; AST – aspartate aminotransferase; ALT – alanine aminotransferase; HOMA-IR – insulin resistance score; CRP – C-reactive protein; HCO3- – bicarbonate level; BE – base excess; BHBA – beta-hydroxybutyrate; * Spearman correlation; significant correlations are marked in bold

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