Iran J Pediatr. 2018 October; 28(5):e63642. doi: 10.5812/ijp.63642.

Published online 2018 July 25. **Research Article**

An Investigation of Serum Irisin Levels in Children Experiencing Convulsive Seizures

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Received 2017 November 04; **Revised** 2018 February 25; **Accepted** 2018 May 15.

Abstract

Background: Seizures are classified as febrile or afebrile in children according to the body temperature and age. The hormone irisin is known to increase with exercise and thermogenesis. The purpose of this study is to investigate the effect of type and duration of seizures involving muscular contraction due to neuronal stimuli in children with seizures on irisin levels.
Methods: The physical, demographic and seizure characteristics of 45 patients and 40 control cases were recorded. Sera were collected from patients and control cases, initially during application for admission to hospital and then secondly within 8 - 24 hours while subjects were experiencing seizures.
Results: Twenty of the patients with seizures were febrile and 25 were afebrile. There was no difference between the patient and control groups in terms of sex or weight and height percentiles. There was no statistically significant difference in irisin levels between the patient and control groups ($P > 0.05$) but a significant difference was observed between initial and second irisin values in the patient group ($P = 0.03$).
Conclusions: Irisin has been reported to increase after aerobic exercise lasting 45 minutes. The mean duration of seizure was 9.3 minutes in our patient group and the suggestion that lactate elevation in patients undergoing seizures is used by the anaerobic pathway may explain why irisin did not increase in these patients. Irisin does not increase in the early stage in patients undergoing seizures, but significant increases occur in 8 - 24 hours. More comprehensive studies are needed on this subject.

Keywords: Irisin, Convulsion, Fever, Children

1. Background

Seizure is occurrence of transient symptoms and findings caused by synchronized abnormal neuronal activities with overstimulation in the brain. It is classified as generalized or focal, depending on localization, and as febrile or afebrile in children under 5 years old depending on whether or not the body temperature is above 38°C (1). Muscle contractions occur during these seizures resulting from the electrical activity in the brain and nervous stimuli. Energy is consumed in this process, and body temperature increases and sweating may occur.

The 112-amino acid protein irisin was first obtained from striated muscle by Bostrom et al. in 2012. This forms during the cleavage of fibronectin type III domain containing protein 5 (FNDC 5) by an unknown protease (2). The major tissue, in which irisin is synthesized, is striated muscle and an association is shown with exercise. Apart from striated muscle tissue, irisin is also shown in neurons and neuroganglia in the brain, cardiac muscle, cutaneous sebaceous glands, perimysium, endomysium, epineurium, axonal and nerve sheaths, the testis, liver, spleen and gut in immuno histochemical studies (3).

Irisin, a glycopeptide hormone belonging to the myokine class, is known to facilitate the conversion of brown adipose tissue that dissipates energy in the form of heat into white adipose tissue that serves as an energy depot (2). In contrast to white adipose tissue, brown adipose tissue is rich in mitochondria and due to the uncoupling protein-1 (UCP1) in the mitochondrial inner membrane that pumps protons into the interior, adenosine three phosphate (ATP) is not synthesized, and fats are converted

into energy (2).

Some studies have reported that irisin levels increase during acute and chronic exercise (4), while other studies have reported that acute exercise does not increase irisin levels (5) or that it even reduces them (6). One recent study reported that acute exercise increased irisin levels and that this increase was not correlated with any specific time of the day (7).

The purpose of this study was to investigate the effect of type and duration of seizures involving muscular contraction due to neuronal stimuli in children undergoing seizures on irisin levels.

2. Methods

In a single-center prospective, cross - sectional trial, 54 children with seizures admitted to Haseki Training and Research Hospital Pediatric Emergency Department, during May 17th - June 30th, 2017 were included in the study. Venous blood samples were collected on arrival (irisin 1) at the emergency department and after 8 - 24 hours (irisin 2) and stored at -80°C for blood irisin examination. Forms eliciting data such as the patient's physical and seizures characteristics were collected. 1 - 5 pubertal stages according to Tanner pubertal staging of patient and control groups were recorded. Nine of the 54 patients were excluded from the study due to frozen sera samples being hemolytic and insufficient when thawed. The control group consisted of 40 random and healthy patients, who were admitted to the general pediatric department at the same dates and agreed to take part. Care was taken to ensure that these patients had no flu-like or other infections, inflammation in the muscles, metabolic, respiratory or cardiac diseases or diseases affecting the skeletal system, diabetes, allergy or myocardial insufficiency. Physical characteristics were recorded, and venous serum was collected and stored at -80°C. Pre-study power analysis revealed a figure of 52 patients for 95% power. Sera were studied using ELISA at the Haseki Hospital Biochemistry Laboratory.

Human Irisin ELISA kit (Catalog No. CK-E90905, Hangzhou Eastbiopharm Co. Ltd.) was used to determine Irisin levels, following the manufacturer's instructions. Sample absorbance was determined on Biotek ELX800 (Biotek, Winooski, VT, ABD) microplate reader at a wavelength of 450 nm. The results were expressed in μg/mL. The minimum detectable level was 0.05 μg/mL.

SPSS 15.0 for Windows program was used for statistical analysis. Descriptive statistics are provided as mean values for numerical variables and as numbers and percentages for standard deviation, minimum, maximum and categorical variables. In the two independent groups, when numerical variable met the normal distribution condition, it was analyzed by Student's *t* test and when it did not meet the normal distribution condition, it was analyzed by Whitney U test. Chi-square analysis was used to compare the ratios in groups. The comparison that was adjusted by covariance in the groups was examined with Univariate ANOVA. If the correlations among numerical variables could not meet the parametric test conditions, Spearman Rank Correlation Analysis was used. The statistical alpha significance level was accepted as $P < 0.05$.

3. Results

Age, weight and height measures were significantly lower in the patient group compared to the control group ($P < 0.001$ for all). No statistically significant difference was found between the groups in terms of sex or weight and height percentiles ($P = 0.536$, $P = 0.054$, and $P = 0.162$, respectively) (Table 1).

No significant correlation was found between irisin levels 1-2 and age, weight or height percentiles in the patient and control groups. No significant correlation was also observed in the control group between irisin level 1 and age and weight and height percentiles (Table 2).

Disease and citizenship characteristics of the patient group are summarized in Table 3.

No statistically significant difference was found when irisin levels were compared in the patient and control groups ($P = 0.098$). A significant increase was observed in post-seizure irisin levels in the patient group ($P = 0.033$) (Table 4).

The difference between irisin levels and irisin values in the patient group was not significantly correlated with the time elapsing from seizure to serum collection, duration of seizure or interval between irisin 1 and 2 (hours) (Table 5).

No statistically significant difference was found when irisin levels were compared on the basis of seizure types (febrile and afebrile) in the patient group ($P = 0.437$, $P = 0.431$). No statistically significant difference was observed in febrile and afebrile group mean irisin levels 1 and 2 compared to the control group ($P > 0.05$).

The patient distribution according to Tanner puberty system was as follows: Three patients at Stage 2; one patient at Stage 3, one patient at Stage 4, seven patients at Stage 5. On the other hand, the patient distribution in the patient group was as follows: One patient at Stage 2 and one patient at Stage 4. The remaining participants of the study were found to be at Stage 1 (prepubertal).

There was no statistically significant difference and correlation between iris 1 values and age and puberty in the patient group ($P = 0.420$, rho = -0,123, $P = 0.948$, rho = -0.010, respectively).

Table 1. Demographic and Physical Characteristics of the Patient and Control Groups

	Patient Group		Control Group		P Value
	No.	%	No.	%	
Sex					0.536
Male	24	53.3	24	60.0	
Female	21	46.7	16	40.0	
	Mean ± SD	**Min - Max**	**Mean ± SD**	**Min - Max**	
Age (y)	3.6 ± 3.7	0.2 - 15	8.4 ± 5.0	0.95 - 17	< 0.001
Weight (kg)	15.4 ± 8.9	4.8 - 45	36.3 ± 24.9	9.7 - 129.5	< 0.001
Weight percentile	42.5 ± 31.3	1 - 92	54.8 ± 33.4	4 - 100	0.054
Height (cm)	95.4 ± 27.9	58 - 171	129.9 ± 30.9	75 - 180	< 0.001
Height percentile	46.8 ± 32.4	1 - 99	56.1 ± 33.2	3 - 99	0.162

There was no statistically significant difference and correlation between iris 2 values and age and puberty in the patient group (P = 0.210, rho = -0.191, P = 0.819, rho = 0.035 respectively).

There was no statistically significant difference and correlation between iris values and age and puberty in the control group (P = 0.705, rho = 0.062, P = 0.069, rho = 0.291 respectively).

There was no statistically significant difference in the mean of iris between adolescence level 1 and 2-5 children in the control group (P = 0.077). Because there were 2 patients in the patient group with 2-5 adolescents level, no statistical study could be performed with other patients with adolescence level 1.

There was no statistically significant difference between the mean of irisin levels of patient and control groups (P = 0.098). There was also no statistically significant difference in the mean irisin levels adjusted by puberty levels of the groups (P = 0.281).

In the patient group, only one patient had a history of two seizures with a 5-hour interval whereas the other patients had no history of recurrent seizures. No patient had a history of taking a shower with hot water before being admitted to the hospital.

4. Discussion

Since convulsion is an event that requires energy in striated muscles and involves the nervous system such as axonal transmission of neuronal hyperactivity, it suggests that the changes in irisin levels during seizures are not unreasonable. If one also considers processes requiring energy such as infection and fever, higher irisin levels may be expected in febrile seizures. The main function of irisin is to consume energy reserves and dissipate heat by increasing the release of uncoupling protein 1 (UCP1) found in mitochondria in white adipose tissue (2). In our study, however, we found no statistically significant difference in irisin levels in the febrile and afebrile patients in the seizure group and the control group (P > 0.05). However, we observed a significant difference between initial serum irisin values (irisin 1) and post - seizure values (irisin 2) in the seizure group (P = 0.003) (Table 4). In 2014, Norheim et al. observed an increase following 12-week stamina and resistance exercises (8). However, Kurdiova et al. observed no difference at the end of exercise and at the 60th min in obese and prediabetic subjects. They reported a 40% decrease in individuals with Type 2 diabetes mellitus and an increase in lean individuals (9). We did not investigate serum lactate levels, a marker of anaerobic metabolism in patients undergoing convulsion. However, in general, there has also been reports of serum lactate acid elevation associated with hypoxia in patients with convulsions (10). Irisin levels in patients undergoing seizure due to the brevity of the muscle contraction period or to anaerobic respiratory mechanisms being more involved than aerobic respiratory mechanisms in patients with convulsions. Timmons et al. observed a 30% increase of FNDC mRNA in muscle biopsy after 20-week exercise in the case of 10 elderly subjects. Nevertheless, they emphasized that this could not be generalized to suggest that exercise increases FNDC (11).

Lecker et al. found that FNDC mRNA increased in muscle biopsies with high aerobic performances in 24 patients with systolic heart failure. They found that aerobic exercises increased oxygen consumption in muscle (VO2) and significantly increased FNDC, while irisin levels rose with aerobic exercises (12). This study also shows that irisin levels increase with aerobic exercise.

Twenty of our patients had febrile seizures and 25 patients had afebrile seizures. Mean duration of seizures in

Table 2. Correlations Between Irisin 1-2 and Age, Weight, Height and Associated Percentiles in the Patient and Control Groups

	rho[a]	P Value
Irisin1 (μg/mL)		
Patient group		
Age	-0.123	0.420
Weight	-0.068	0.657
Weight percentile	-0.047	0.759
Height	-0.100	0.512
Height percentile	-0.232	0.125
Control group		
Age	0.062	0.705
Weight	0.064	0.694
Height	0.084	0.605
Weight percentile	-0.104	0.522
Height percentile	0.059	0.716
Irisin2 (μg/mL)		
Patient group		
Age	-0.191	0.210
Weight	-0.176	0.247
Weight percentile	-0.088	0.564
Height	-0.193	0.205
Height percentile	-0.234	0.121
Control group		
Age	0.062	0.705
Weight	0.064	0.694
Weight percentile	-0.104	0.522
Height	0.084	0.605
Height percentile	0.059	0.716

[a]Correlation coefficient.

Table 3. Disease and Citizenship Characteristics of the Patient Group

	No.	%
Seizure		
Febrile	20	44.4
Afebrile	25	55.6
Total	45	100.0
Citizenship		
Turkish	36	80.0
Foreign (Syrian refugees)	9	20.0
	Mean ± SD	**Min - Max**
Duration of seizure in minutes	9.3 ± 6.7	2 - 30
Time elapsed between seizure and blood sample (h)	1.2 ± 1.0	0.2 - 3

the patients was 9.3 (2 - 30) minutes. Mean time between seizure and serum collection was 72 (12 - 180) minutes (Table 3). No significant relation was found between irisin 1 and 2 levels and the control group (P = 0.098, P = 0.549).

The mean time elapsed between irisin 1 and 2 levels and serum collection was 15 (8-24) hours. No correlation was found between the duration of seizure, the interval between seizure and serum collection or the interval between irisin 1 and irisin 2 and the difference between irisin 1 and irisin 2 (P > 0.05). No significant difference was observed between irisin levels in the febrile and afebrile seizure group in our study (P > 0.05). However, the mean irisin level 2 was higher in the febrile group. A short period between the onset of fever and convulsion may be responsible for irisin elevations, which is not statistically significant.

In one study, after obese and normal weight subjects performed 45-min exercise and showered at 50°C, irisin levels increased in both groups. However, higher values of ırisin were found in the group consisting of obese subjects when taking shower at 50°C (13).

Lee et al. reported that exposure to cold in experimental animals increased irisin and Fibroblast Growth Factor (FGF) 21 in the same manner as exercise. The underlying reason is that intense shivering causes an increase in irisin similar to exercise (14). Irisin levels did not rise significantly at the expected extent despite fever in the febrile cases and in all seizure cases in spite of contraction and shivering for reasons such as the brief duration of seizures and the use of the anaerobic mechanism. The difference between irisin 1 and 2 levels may also have been high due to the effect of high body temperature and shivering in post - seizure phase.

There was no difference in terms of sex between our patient and control groups (P = 0.53). All members of the control group were Turkish citizens, while 36 members of the patient group were Turkish citizens and nine were Syrian refugees. Differences were observed between the groups in terms of age, weight and height (P > 0.05), but none in terms of height and weight percentiles (P < 0.05).

There are inconsistent reports in the literature concerning gender differences. Scalzo et al. found decreased irisin levels in males following the application of a 3-week exercise program to 19 young adults, whereas irisin levels increased in females. The authors suggested the presence of sexual dimorphism in irisin secretion (15). In another study on a pediatric group, 65 obese children underwent an exercise program for 1 year. At the end of the program, a 12% increase in irisin levels was observed, but no variation

Table 4. Comparison of Irisin Levels in the Patient and Control Groups

	Patient Group		Control Group		P Value
	Mean ± SD	Min - Max	Mean ± SD	Min - Max	
Irisin 1 (μg/mL)	10.1 ± 8.3	1.8 - 31.4	12.5 ± 9.9	3.6 - 34.1	0.098
Irisin 2 (μg/mL)	11.2 ± 8.6	1.4 - 31.4			0.549
Irisin level difference (1-2)	1.1 ± 5.0	11.1 - 18.5			0.033
Time elapsed between irisin 1 and 2 (h)	15.0 ± 6.4	8 - 24			

Table 5. Comparison of the Difference Between Irisin Levels and Irisin Values and Time Elapsing from Seizure to Serum Collection, Duration of Seizure and Interval Between Irisin 1 and 2 Levels (Hours)

	Irisin 1 (μg/mL)		Irisin 2 (μg/mL)		Irisin difference	
	rho	P Value	rho	P Value	rho	P Value
Time elapsing from seizure to serum collection	0.107	0.486	0.111	0.470	0.044	0.773
Duration of seizure (min)	0.268	0.075	0.259	0.086	-0.017	0.912
The interval between irisin 1 and 2 (h)	-0.153	0.315	-0.107	0.485	-0.093	0.545

was observed in terms of age or sex (16). Another study suggested that irisin levels are more associated with pubertal status than with weight or BMI, and that prepubertal values were lower than those in puberty (17). According to Tanner pubertal staging, puberty started in 12 cases in the control group and 2 cases in the patient group. However, we found no statistically significant correlation between irisin values related to age and puberty of patients (irisin 1) and control group ($P > 0.05$). Therefore, we think that the age difference between the patient and control groups does not affect our study. There was no statistically significant difference between the mean irisin levels of prepubertal and puberty patients in the control patients ($P > 0.05$). There was no statistically significant difference between the irisin level values adjusted by puberty level of the patient and control groups ($P > 0.05$). There was no sex difference between the groups in our study. In addition, no correlation was found between sex and irisin 1 and 2 levels in the patient group (Table 2).

Stengel et al. showed the correlations between plasma irisin levels and fat mass, insulin resistance and BMI (18). Some authors have maintained that irisin levels are inversely correlated with age (19). Sanchis Gomar reported that irisin concentrations were not associated with BMI, age and other biological parameters, diabetes or obesity (20). Tanisawa et al. reported an inverse correlation between irisin concentrations and age (21). Since the ages of our patient group were significantly higher than those of the control group, mean weight and height also showed a significant difference. However, no difference was found in terms of weight and height percentiles. While there are different views in the literature, the general opinion is that the age does not affect irisin levels. If there was an inverse correlation between serum irisin levels and age, as suggested by Tanisiwa et al. (21), mean irisin levels would be expected to be lower in our control group despite the higher mean age of the controls. However, no low irisin levels in control group from patient group were determined. In addition, no correlation was found between irisin levels and age (Table 2).

We determined no correlation between age, weight, height and height and weight percentiles and irisin 1 and 2 values of patients in the patient and control groups (Table 2). There was also no difference in the irisin levels of obese and overweight patients with seizures.

In conclusion, irisin does not increase in seizures in pediatric patients; however, it increases in the post - seizure period. It can therefore be used as a marker in the monitoring of patients with seizures, and more specifically, in the febrile group. This study is the first on this subject and more comprehensive research is needed.

Acknowledgments

This study was performed after approval was obtained from the Ethical Board of Haseki Training and Research Hospital (The chair person: Dr Fuat Sar, No 403, Date of Approval: 17/05/2017). We thank the Haseki Research and Training Hospital thesis and academic activities information and supervision commission for their support to this project (Project number: 33).

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